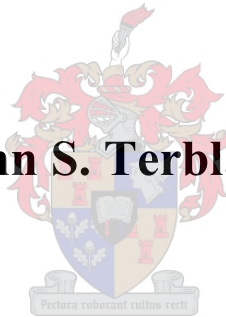


**Variability among individuals and populations:  
implications for arthropod physiology**

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**Promoter: Professor Steven L. Chown**

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## **Declaration**

I, the undersigned, hereby declare that the work presented in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university or institution for a degree.

Signature.....

Date.....

## **Abstract**

Within arthropods, inter-individual and inter-population variation is generally poorly explored for physiological parameters. Such physiological variability is important, as it can provide insight into the capacity for evolutionary adaptation and how animals may cope with anthropogenic climate change. Insect vectors of human and animal diseases, such as tsetse flies (Diptera, Glossinidae) which carry trypanosomes, are of particular interest. Predictions of tsetse fly (*Glossina* spp.) range expansion, possibly paralleled by increased disease occurrence, have been made under future climate scenarios. Moreover, since there are generally strong relationships between abiotic variables (e.g. temperature and moisture availability), population dynamics, distribution and abundance, determining the physiological mechanisms influencing such relationships has utility for predictive modelling of spatial and temporal changes in tsetse fly distributions. I investigated physiological variation among individuals and populations, focusing mainly on *Glossina* spp. but using other arthropods to address certain issues. Specifically, I show the following: i) metabolic rate is repeatable (intra-class correlation coefficient) within individuals, and may be influenced by several factors such as age, gender, body mass and pregnancy; ii) while metabolic rate is repeatable within individuals, it remains unaltered with acclimation to laboratory conditions from field collection; iii) within a population, metabolic rate-temperature relationships are surprisingly invariant among physiological states such as age, gender, feeding status, pregnancy and temperature acclimation; iv) the magnitude of the effect of temperature acclimation differs among traits, in that upper thermal tolerances respond less than lower thermal tolerances, while metabolic rate responds to heat but not to cold. Water loss rate is relatively unresponsive to either treatment; v) plasticity can account for most inter-population variation in physiological traits related to climatic stress resistance (e.g. thermotolerances and desiccation rates) in the natural environment; vi) adult physiological performance responds differently to developmental and adult acclimation, such that marked trait-specific variation occurs, and combinations of both developmental and adult plasticity can result in further alteration of adult performance. These results have implications for the evolution of stress resistance to abiotic factors in these and other arthropods. I conclude by discussing the potential physiological patterns linking population dynamics and abiotic factors, with particular reference to tsetse flies.

## Opsomming

Binne arthropoda, is tussen-individu en tussen-bevolking variasie in die algemeen sleg bestudeer vir fisiologiese parameters. Hierdie fisiologiese variasie is belangrik want dit kan insig gee in die mate van evolusionêre aanpassing en hoe diere antropogenies klimaatsverandering kan hanteer. Dit is veral belangrik in die geval van insekte wat mens- en diersiektes kan dra, soos tsetse vlieë (Diptera, Glossinidae) wat trypanosome oordra. In die geval van tsetse vlieë (*Glossina* spp.) is voorspellings vir verhoogde verspreiding, en gevolglik verhoogde siekte voorkoms, gemaak onder toekomstige voorkoms klimaat senario's. Verder, aangesien daar in die algemeen sterk verwantskappe tussen abiotiese parameters (bv. temperatuur en water beskikbaarheid), bevolkingsdynamika, verspreiding en voorkoms is, is die bepaling van die fisiologiese meganisme, wat 'n invloed het op hierdie verwantskappe, nuttig vir die voorspelling van tsetse oor spasio en tyd. Hier, met hoofsaaklik die gebruik van *Glossina* spp., maar ook ander arthropoda wanneer dit nodig is, word navorsing wat fisiologiese variasie tussen individue en bevolkings aanspreel voorgelê. Spesifiek, word dit gewys dat i) metaboliese tempo is herhaalbaar (intra-klas korrelasie koëffisient) binne individue, alhoewel metaboliese tempo kan beïnvloed word deur faktore soos ouderdom, geslag, liggaamsmassa en swangerskap, ii) terwyl metaboliese tempo binne individue herhaalbaar is, verander dit nie met akklimasie na laboratorium toestande vanaf versameling in die veld, iii) binne 'n bevolking metaboliese tempo-temperatuur verwantskappe is verbasend eenders tussen fisiologiese toestande soos ouderdom, geslag, voeding staat, swangerskap en temperatuur akklimasie, iv) die grootte van die effek van temperatuur akklimasie verskil tussen eienskappe, en die boonste termiese limiete reageer minder as laer termiese limiete, terwyl metaboliese tempo reageer op hitte maar nie koue nie, en waterverlies tempo is relatief terughoudend tot enige temperatuur toestand, v) buigbaarheid kan die meeste van inter-bevolkingsvariasie in fisiologiese eienskappe van klimaatstres weerstandsvermoë (termiese toleransie en uitdrogingstempo) in die natuurlike omgewing verklaar, vi) volwasse fisiologiese prestasie reageer verskillend op ontwikkelende en volwasse akklimasie, soveel dat eienskap-spesifieke variasie kan voorkom, en 'n kombinasie van beide ontwikkelende en volwasse buigbaarheid 'n verskillende volwasse prestasie tot gevolg kan hê. Hierdie resultate het implikasies vir die evolusie van weerstandsvermoë tot abiotiese faktore in hierdie en ander arthropoda. Hierdie tesis word afgesluit met 'n bespreking van die moontlike fisiologiese patrone wat bevolkingsdinamika en abiotiese faktore kan verbind, met 'n fokus op tsetse vlieë.

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## Chapter 1

### General Introduction

*“Variation is not just noise; it is also the stuff of evolution and a central attribute of living systems...The physiological differences between individuals in the same species or population, and also the patterns of variation in different groups, must not be ignored.”*

**G.A. Bartholomew, 1987** (*in* New Directions in Ecological Physiology, pp 32-33).

Our planet contains an astounding range of biodiversity, particularly when viewed from a morphological perspective. However, this biodiversity also includes an alternative, and arguably just as interesting form of variation, namely that of physiological diversity. Physiological diversity within individuals arises as a consequence of variation associated with ontogeny, age, season, size and innate variation in the trait of interest. Physiological variation may be a consequence of genetic or environmental variation, or the interaction of genes with local environments. To understand the levels at which physiological variation can occur, hierarchical levels may be considered (Fig. 1). Typically, the individual, cohort, population, or species is the unit of interest, although higher taxonomic classification may also be useful to cope with substantial variation. Spicer & Gaston (1999) suggest two reasons why investigation of the hierarchical nature of physiological variation may be important. First, from a heuristic perspective, if physiological variation is a basic fact of life it would seem important to understand how this diversity is distributed. Second, an understanding of physiological diversity would give guidance as to the circumstances in which physiological variation at levels other than that which is being explicitly studied may or may not be of major concern. In insects, few studies have attempted to document physiological variation at three or more hierarchical levels (Fig. 1, but see Chown et al., 1999; Chown, 2001; Hoffmann et al., 2001).

At the individual whole-organism level, physiological variation associated with ontogeny has been well documented (Spicer & Gaston, 1999; Chown & Nicolson, 2004). For example, desiccation and thermal physiology changes markedly from larval to adult stage in kelp flies: adults are less capable of functioning at low temperature and have lower rates of water loss than larvae (Klok & Chown, 2001). However, with the obvious exception of senescence, physiological variation within life-stages has received little attention (but see Queathem & Full, 1995; Gray & Bradley, 2003). Moreover, the degree to which factors such as age, gender, feeding and reproductive status influence the state of a physiological trait within individuals in a population has generally been poorly documented in insects (Chown & Nicolson, 2004).

Individuals can also show physiological variation in response to daily or seasonal changes in abiotic factors. This has been demonstrated in insects mainly in response to temperature (e.g. Hoffmann et al., 2003), desiccation (e.g. Parsons, 1982; Gibbs et al., 1997; Gibbs & Matzkin, 2001) and metabolic rate (e.g. Davis et al., 2000). Many of these changes are discussed under the rubric of hardening or acclimation (see below).

Among-individual differences are generally related to differences in genotype between individuals and differences resulting from genotype-environment interactions, such as acclimation, and

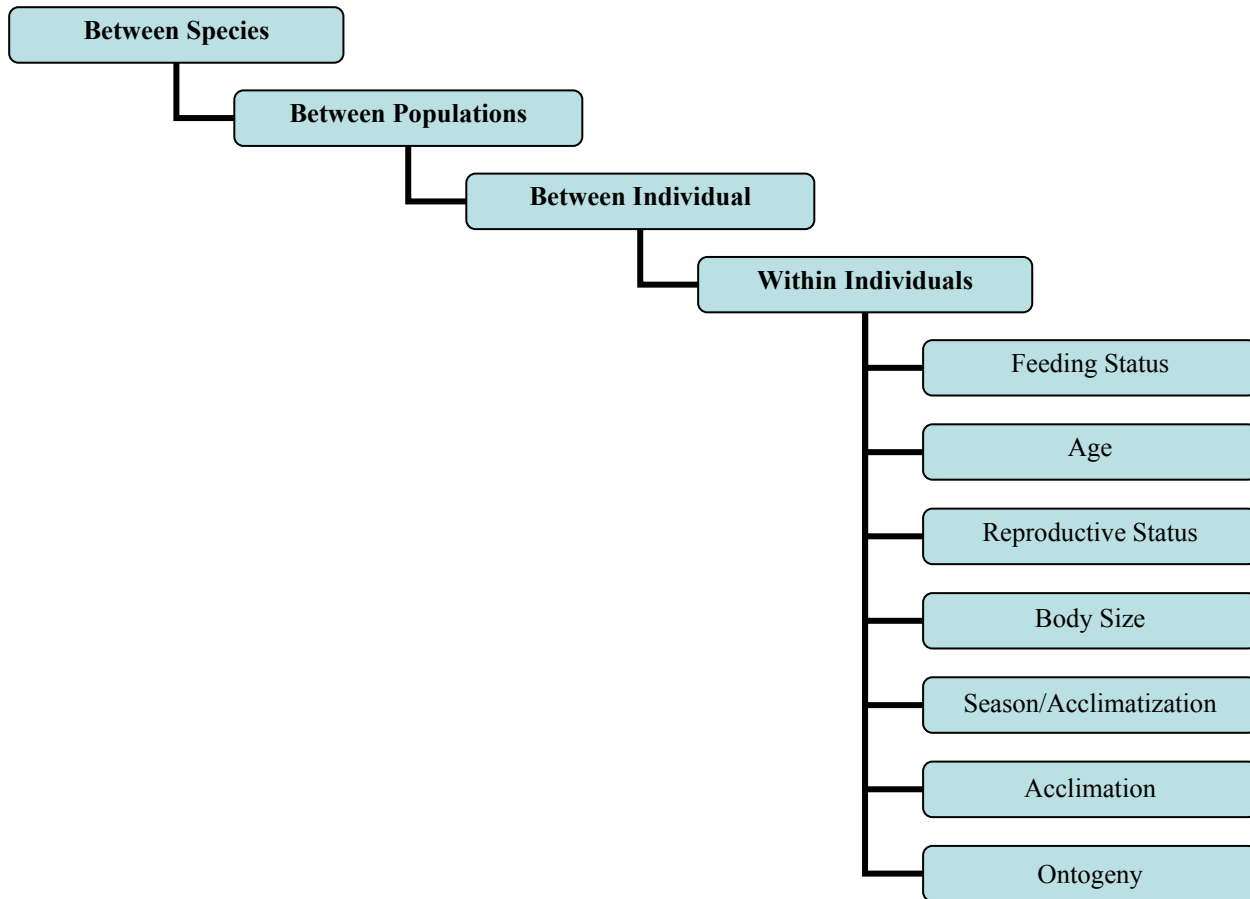
‘maternal’ or ‘parental’ effects (discussed in Falconer & Mackay, 1996). Recent advancements in molecular techniques, in particular the sequencing of the *Drosophila* genome, has greatly enhanced the possibility of linking variation in, for example, temperature resistance to nucleotide variation (Hoffmann et al., 2003). Genotype-environment interactions can explain inter-population variation in, for example, cold tolerance and desiccation resistance (Hoffmann et al., 2001). Parental effects and common environment effects are now accepted as a common cause of among-individual differences in phenotypes within a population (Mousseau & Fox, 1998). For insect physiology, apart from a handful of studies investigating such effects undertaken on model organisms, it is generally unclear to what degree traits are affected by parental conditions and how this varies among species (but see Huey et al., 1995; Watson & Hoffmann, 1995; Magiofoglou & Hoffmann, 2003). Clearly, a mother’s experience of the environment can lead to variation in her growth (i.e. size), condition and physiological state that can be transmitted to her offspring via cytoplasmic factors (e.g. hormones or mRNA) in the egg that may directly or indirectly control progeny development. In many insects, the photoperiod, temperature or host availability experienced by an ovipositing female will determine the probability of diapause in her offspring (Denlinger, 2002).

When considering the substantial within-population physiological variation apparent in traits, it is therefore unsurprising that differences typically exist between populations and species (Spicer & Gaston, 1999). Generally, it is assumed that between-population variation can be accounted for by differences associated with geography otherwise known as local environmental adaptation (Spicer & Gaston, 1999). In insects, among-population variation in physiological traits has been widely documented (e.g. Ayres & Scriber, 1994; Watson & Hoffmann, 1996; David et al., 2003). This variation might result from genetic drift or natural selection. However, in some cases, geographic variation may be a relatively small component of among-population variation (see Hoffmann et al., 2001; Klok & Chown, 2003). It has been argued that among-population variation could also be a consequence of adaptation to past, rather than contemporary, environments. Consequently, the role that inertia plays in physiological and behavioural variation is receiving increased attention (see discussions in Huey & Berrigan, 1996; Huey et al., 2003; Klok & Chown, 2005a). Moreover, because a substantial proportion of geographic variation can be accounted for by plasticity, Ayrinhac et al. (2004) have argued that phenotypic plasticity may be more important than genetic variability in determining geographic variation of physiological traits, although this opinion is not necessarily broadly held among evolutionary and ecological physiologists (Hoffmann et al., 2005; and see Wilson, 2001; Du et al., 2005). Nevertheless, phenotypic plasticity is widespread in nature and may speed up, slow down, or

have little effect on evolutionary change (Price et al., 2003). Moderate levels of plasticity may facilitate genetic evolution but careful analyses of individual traits are needed to ascertain whether plasticity has been essential or merely incidental to population differentiation (Price et al., 2003).

Alternatively, a lack of physiological difference between geographically distinct populations (Spicer & Gaston, 1999) can be attributed to patterns of gene-flow (such as ‘swamping effects’) within species (Haldane, 1956; Kirkpatrick & Barton, 1997) and/or the manner in which genetic variation is maintained, such as a balance between mutation and selection (see Feder & Mitchell-Olds, 2003), tradeoffs between traits within a given population (see e.g. Frankino & Juliano, 1999; Lankford et al., 2001) and a lack of variability (Hoffmann & Hercus, 2000). At intermediate dispersal rates, patterns of gene flow can lead to local adaptation and may increase variation in adaptation through time (Forde et al., 2004). However, at high or low rates of gene flow, a population’s ability to adapt may be restricted. Regardless, some patterns might not reflect adaptation at all and may merely reflect ‘developmentally inevitable’ (i.e. biophysical) consequences of the effects of environmental factors on physiological systems (Huey & Berrigan, 1996).

At the higher levels of the physiological variation hierarchy (Fig. 1), interspecific and interpopulation differences have been shown in cold hardiness (Chen et al., 1990; Sinclair et al., 2003a), desiccation tolerance/resistance (Zachariassen et al., 1987; Chown, 1993; Le Lagadec et al., 1998; Addo-Bediako et al., 2001), upper lethal temperature limits (Roberts et al., 1991; Kimura et al., 1994) metabolic rates (MacKay, 1982; Schultz et al., 1992; Addo-Bediako et al., 2002) and cuticular hydrocarbons (Gibbs et al., 1991) of insects. In general, however, much of the insect ecophysiology literature deals with variation in states of traits between a few closely related species, or populations within a species (Chown, 2001), rather than with comparisons of many species or populations. While data for model organisms provides useful insight and continues rapidly, comparisons among higher, poorly-represented taxa representing broader ranges of terrestrial habitats may add valuable insight to understanding adaptive physiological variation (Kingsolver & Huey, 1998).



**Figure 1.** Schematic diagram of the hierarchical classification for commonly accepted levels of variability in physiological traits (see Spicer & Gaston, 1999 for discussion). Included in the figure are several of the more common factors affecting within-individual variability.

## Physiological Variability and Adaptation

Evolution, defined as descent with modification, is a result of natural selection. Morphological, behavioural, or physiological adaptations are a consequence of natural selection, which in turn requires heritability, fitness and variability. Without all three factors present simultaneously for a particular trait, adaptation via natural selection cannot take place.

Inter-individual variability is the raw material upon which natural selection acts. In consequence, a better understanding of individual variation will assist in understanding the evolution of physiological traits (Bennett, 1987; Spicer & Gaston, 1999; McNab, 2003). Inter-individual differences in physiology represent biologically-relevant variation, not just random noise. However, if intra-individual variation does not result in differential survival, it may have little evolutionary significance, and consequently, in that particular instance it may be considered as noise. Thus, fundamental, repeatable differences between individuals are the signal of interest because these may underlie differential survival and reproduction, and hence evolutionary change. Measures of both intra- and inter-individual variation are clearly of importance if the nature of the variation upon which natural selection acts is to be comprehended.

Repeatability differs considerably depending on the nature of the character in question, whether the underlying genes controlling the phenotypic trait are linked in a similar fashion among all individuals, and the extent of the influence of the local environmental conditions experienced by that population's individuals (discussed in Dohm, 2002). Estimation of variability can also provide important information for understanding the extent to which an experimental technique provides precise estimates of a physiological parameter (Berteaux et al., 1996; Terblanche et al., 2004). In cases where the parameter of interest is unlikely to vary, repeatability can partition measurement- as well as observer-related error (Krebs, 1999).

Repeatability estimation may be obtained through two different approaches, which differ dramatically in their utility. In several cases, 'repeatability' has been determined using a correlational approach (e.g. Nespolo et al., 2003). This method identifies the degree to which two consecutive measurements are similar over a given time interval within a group of individuals, usually expressed as the correlation co-efficient (e.g. Pearson's product-moment  $r$ ). However, this calculation does not provide a measure of the relationship of within- to between-individual variation, and therefore may have little evolutionary significance. For the purposes of this discussion, only repeatability calculated from variance analyses of within *and* among individuals is referred to, as it is this variation that natural selection is most likely to operate on.



Repeatability assessments exist for mammalian physiological traits (e.g. Berteaux et al., 1996; Chappell et al., 1996; Hayes et al., 1998; Fyhn et al., 2001) and are common in the behavioural ecology literature (e.g. Johnsen & Zuk, 1996; Jennions & Petrie, 1997; Aragaki & Meffert, 1998; Hoffmann, 1999; Dingemanse et al., 2002). In insects, repeatability studies are typically focused on genetic and evolutionary investigations of fluctuating asymmetry as a measure of stress in e.g. *Drosophila* (van Dongen, 1998; Whitlock, 1998; van Dongen et al., 1999a, b; Woods et al., 1999) as opposed to physiological traits from diverse taxa. Thus, repeatability assessments are commonly made for other fields, but what is apparent is that repeatability information, and therefore inter-individual variation in measures of physiology within arthropod taxa, is restricted (but see Shaffer & Formanowicz, 2000; Schaus & Sakaluk, 2002; Marais & Chown, 2003). Indeed, few studies have attempted to quantify intra- and inter-individual variation (the lower levels of the hierarchy presented in Fig. 1) in physiological traits, particularly in arthropods (see Chown, 2001; Marais & Chown, 2003).

### **Physiological Plasticity and Phenotypic Flexibility**

Physiological plasticity (*sensu* Spicer & Gaston, 1999) or flexibility (*sensu* Piersma & Drent, 2003) has attracted increasing attention in recent years (reviewed in Huey & Berrigan, 1996; Nylin & Gotthard, 1998; Huey et al., 1999; De Witt & Scheiner, 2004; Badyaev, 2005). Acclimation is broadly considered as the changes in physiology in response to an abiotic factor (Huey et al., 1999) within the laboratory. By contrast, acclimatization is regarded as physiological changes that can occur in the natural environment (in the field) (Schmidt-Nielsen, 1997; Chown & Nicolson, 2004). Adaptive patterns may be identified by means of ‘common garden’ experiments in which organisms are reared under similar conditions prior to comparisons. When common garden experiments involve different environments, the impacts of plastic changes in traits can be assessed relative to genetic differences among populations (see Hoffmann et al., 2005).

Huey & Berrigan (1996) recognize several forms of phenotypic plasticity:

- 1) *Acclimation responses*. These are physiological examples which occur on temporal scales from minutes to months and may be reversible within the organism’s lifetime. For example, acclimatory shifts have been demonstrated in thermal tolerance and metabolic rate (Chown & Nicolson, 2004). The phenotypic responses may be either in reaction to or in anticipation of a change (e.g. to summer conditions, as cued by increasing photoperiod).
- 2) *Developmental switches* (*‘polyphenisms’*). These refer to plastic responses in which the phenotype is irreversibly fixed by environmental conditions experienced during a critical stage

of development. An example of this is the effect of developmental temperature on gender determination in reptiles. Developmental switches have been demonstrated in continuous as well as discrete traits.

- 3) *Developmental pathologies*. These are environmentally induced pathological modifications that may occur during development. Amongst the examples of this are developmental asymmetry in *Drosophila* exposed to heat stress.
- 4) *Labile effects (transient or acute)*. These are rapid alterations in phenotypic performance as a function of the organism's immediate environment, be it physical or physiological. These effects are graphically presented as performance curves, for example, as a function of temperature. Performance curves may be modified by acclimation or by developmental switches.
- 5) *Cross-generational effects* are parentally transmitted phenotypic changes. Such effects have been demonstrated in several insect species, most notably *Drosophila*, and are potentially important in their influence on phenotypic plasticity.

Knowledge of the magnitude of acclimation and factors controlling and/or affecting acclimation are important for understanding the permanent limitations to an organism's existence. If physiological differences between individuals or populations can be reversed under controlled laboratory conditions, the original differences between populations in the field can be attributed to local physiological acclimatization, or reversible non-genetic differences (Spicer & Gaston, 1999). However, the magnitude of acclimation can vary substantially between traits (Chown, 2001), although some degree of plasticity itself appears to be ubiquitous among organisms and phenotypes in general (Piersma & Drent, 2003; Price et al., 2003; Badyaev, 2005). The underlying mechanisms setting the upper limits to the flexibility inherent in physiological traits, and why the magnitude of performance can be affected by acclimation, are generally poorly understood (Kingsolver & Huey, 1998; Angilletta et al., 2003; Badyaev, 2005; but see Denlinger & Lee, 1998).

While it is commonly assumed that acclimation is beneficial, and similar alterations in phenotypic traits in the field should benefit the organism of interest (this hypothesis has been termed the Beneficial Acclimation Hypothesis, BAH (Leroi et al., 1994)), it may not always be the case (Huey & Berrigan, 1996; Huey et al., 1999; Deere, 2005). Specifically, the BAH predicts that acclimation to a particular environment gives a performance advantage (e.g. reproductive success) to an organism, relative to individuals that have not had the opportunity to acclimate to that same environment. Tests of this

hypothesis have rejected the generality of the predictions. For example, using *Escherichia coli*, Leroi et al. (1994) found that 41.5°C acclimated bacteria performed worse at 41.5°C than those acclimated at 32°C and tested at 41.5°C. Several other studies have also challenged the BAH on empirical and/or theoretical grounds (Krebs & Loeschcke, 1994; Hoffmann, 1995; Huey et al., 1995; Zamudio et al., 1995; Padilla & Adolph, 1996; Bennett & Lenski, 1997; Woods, 1999; Woods & Harrison, 2001). For example, Woods & Harrison (2002) have argued that uncertainty arises when interpreting rejections of the BAH because the hypothesis focuses on the relative performance of organisms exposed to one environment relative to another, whereas the raw material available for evolution is variation in acclimation responses of individual traits. Indeed, they argue that a better match between the evolutionary pressures shaping acclimation and the tests used to examine them can be achieved by focusing on the fitness consequences of acclimation changes in individual traits (Woods & Harrison, 2002). Subsequently, Wilson & Franklin (2002) argued that tests of the BAH have actually examined the effects of developmental plasticity, rather than adult, non-developmental acclimation. Therefore the BAH deserves further attention, but care should be taken to separate the effects of developmental and post-developmental plasticity.

Phenotypic flexibility can be demonstrated in the laboratory as well as the natural environment. For example, phenotypic changes to laboratory conditions have been demonstrated over several generations for traits of stress resistance (Hoffmann et al., 2001) or cold and heat tolerance (Hoffmann et al., 2005), and may even occur relatively quickly, as demonstrated in *Drosophila melanogaster* (Sgrò & Partridge, 2000). Most studies of acclimation focus on effects at the level of the population. Few studies, especially within insects, have investigated laboratory adaptation or acclimation effects on within- and among-individual variation of physiological traits (discussed in Rezende et al., 2004).

In insects, acclimatization is typically demonstrated as changes in response to season. For example, Sinclair et al. (2003b) found diurnal and seasonal changes in supercooling points in various Antarctic Collembola species. At longer time scales, acclimatization may be important because it allows individuals to survive seasonal variation in environmental temperatures (Sinclair et al., 2003a). At shorter time scales, evidence for rapid cold hardening among several species (Lee et al., 1987; Chen & Denlinger, 1992; Kelty & Lee, 1999) has led to the notion that tracking daily changes in temperature variation may be important for survival of extreme conditions (Sinclair & Chown, 2005). Because the time scales at which the phenotypic changes occur can vary markedly, and it is commonly assumed that there will be a benefit to survival in some way (but see discussion of the BAH above), understanding plasticity of physiological traits could result in improved predictive ability for when and where the

effects of climate change are likely to occur (Helmuth et al., 2005). Indeed, Kingsolver & Huey (1998) propose that studies of plasticity in physiological traits, such as thermal tolerances, may be fundamentally valuable in understanding the role of environmental variation in the evolution of plasticity, in physiological and mechanistic ways, but particularly when the variability and predictability of the thermal environment itself can be quantified on ecologically relevant time-scales (see also Berteaux et al., 2004; Badyaev, 2005).

## Thesis Goals

In this thesis I address the following key questions mainly using tsetse flies (*Glossina* species) for reasons outlined below, but also using more readily available alternative species when necessary:

- 1) Is metabolic rate repeatable within individuals and what factors influence metabolic rate at this hierarchical level (Fig. 1)?
- 2) If metabolic rate is repeatable within individuals, does it change with acclimation to laboratory conditions from capture in the field?
- 3) Do metabolic rate-temperature (R-T) relationships differ among various physiological states within a population (e.g. between acclimation treatments, hungry and fed states, pregnant and virgin females, or between genders)?
- 4) To what extent does the magnitude of the effect of temperature acclimation differ among traits such as in metabolic rate, thermal tolerance, and water loss rate?
- 5) To what degree does plasticity (induced e.g. by temperature acclimation) account for inter-population variation in physiological traits in the natural environment?
- 6) Does adult physiological performance respond similarly to developmental and adult acclimation?

Thus, variation in metabolic rate associated with gender, age, feeding-status, and pregnancy, and the variation of the repeatability of metabolic rate with age and gender is examined in *Glossina pallidipes* (Diptera, Glossinidae). Owing to their status as vectors of trypanosomes, there is much interest in understanding the factors influencing the distribution and abundance of tsetse (Rogers & Randolph, 1986; 1991; Rogers & Williams, 1994; Robinson et al., 1997a, b) (and discussed below). Metabolic rates are important in this regard because they are used to estimate daily energy budgets (Bursell & Taylor, 1980), which subsequently provide information regarding daily flight time and thus both dispersal and survival capabilities. However, before exploring geographic variation in tsetse

metabolic rates, understanding of the intrinsic factors influencing variation in metabolic rate is required. This can be readily illustrated by considering the repeatability of metabolic rate.

If metabolic rate is not repeatable (i.e. there is greater within- than among- individual variation) then it is unlikely that variation in metabolic rate would translate into variation in fitness (for further discussion see Endler, 1986; Bech et al., 1999; Marais & Chown, 2003). In consequence, tsetse would be unable to respond to selection for a change in metabolic rate. Alternatively, if metabolic rate is repeatable, then selection could act on this trait (see discussion in *Physiological Variability and Adaptation*), meaning either that a population could respond to selection, or that if it did not, other factors, such as gene flow should be sought to explain the lack of response (Case & Taper, 2000). Comprehending intrinsic variation in metabolic rate in tsetse is particularly important because previous investigations have suggested that feeding status, age and reproductive status are important contributors to variation in metabolic rate in these flies (Rajagopal & Bursell, 1966; Taylor, 1977c).

Second, using a field-collected whip-spider, *Damon annulatipes* (Chelicerata: Amblypigi) changes in metabolic rate with acclimation to constant laboratory (holding) conditions are explored. Although it has been shown that phenotypic traits in insects can rapidly adapt to rearing conditions across several generations (e.g. Matos et al., 2000; Sgrò & Partridge, 2000; 2001; Houle & Rowe, 2003), it has not been well determined how rapidly changes can take place within a single generation from collection in the field, especially for measures of physiological performance. As such, this may influence the application of repeatability obtained from laboratory studies to field populations. Thus, I specifically ask if the repeatability of metabolic rate changes with laboratory-acclimation within a single generation.

Third, the effects of gender, feeding status, pregnancy and age on metabolic rate-temperature (R-T) relationships in *G. morsitans morsitans* are explored. In addition, using three acclimation temperatures, the influence of acclimation on metabolic rate and R-T relationships is investigated. The aim is not simply to determine the extent of metabolic rate variation with these factors, but more importantly to determine whether they influence the R-T relationship. Although metabolic rate in tsetse has received considerable attention, both in the laboratory (Rajagopal & Bursell, 1966; Taylor, 1977a; b; 1978a; b) and in the field (Taylor, 1978b; Hargrove & Coates, 1990), variation in the temperature-dependence of tsetse metabolic rate is relatively poorly understood. Recent models of tsetse population dynamics typically include the effects of temperature on various life-history parameters (see e.g. Hargrove, 2004), many of which (at least those associated with production – see Kozłowski & Gawelczyk, 2002) are influenced by metabolic rate. These models necessarily simplify temperature

effects across various physiological stages and cohorts, although the extent to which such assumptions are valid is not known.

Fourth, to better understand how insects in general may respond physiologically to temperature acclimation, variation in thermotolerance, desiccation and metabolic rates with acclimation to several temperatures is investigated using *Chirodica chalconota*, a Chrysomelid beetle from the local (South African) Fynbos Biome. Since this region is predicted to be affected severely by changing climates in the near future (Midgley et al., 2003; Rouget et al., 2003), and in general relatively little is known about insect responses in these areas, understanding *C. chalconota*'s responses to temperature variation has practical implications for understanding the ways in which local ecosystem function may be affected by climatic variability.

Fifth, in order to understand the potential for adaptation (or lack thereof) in determining spatial physiological variation within species, geographic variation in water loss rates, heat and cold tolerance and the role of plasticity in this variation are examined in *G. pallidipes*. Furthermore, the likelihood that acclimation responses of water loss rates and thermotolerances to heat and cold may have influenced the geographic distributions of the species investigated is addressed. Moreover, the potential implications for climate change upon these species distributions are discussed based on the degree of plasticity inherent in these thermal limits.

The final research goal of this dissertation is to separate the effects of developmental and adult plasticity, and examine how these interact to determine adult physiological performance. Here, this is done using a laboratory colony of *G. pallidipes*. Although several studies have examined the separate effects of acclimation on either developmental or adult plasticity (Gibert et al., 2001; Fischer et al., 2003; Zeilstra & Fischer, 2005), few studies have attempted to separate the effects of within-generation developmental and adult phenotypic plasticity or to address possible interactions between these two forms of plasticity, specifically for physiological traits. Therefore, these two distinct forms of plasticity (developmental and adult), and the contribution of developmental plasticity to adult plasticity, using thermotolerances, water loss rates and metabolic rates are deliberately addressed. Furthermore, to my knowledge no studies have investigated the effects of acclimation on thermal tolerances in any life stage for tsetse.

This dissertation is concluded with a discussion of the potential physiological mechanisms that could link abiotic factors, population dynamics and biogeography in tsetse flies. Specifically, these latter two avenues of research that have undisputed utility in aiding the prediction of animal responses to the environment have been drawn together and reviewed. This has been done not simply because



predicting responses of species can benefit from understanding the mechanisms underlying environmental tolerances (see e.g. discussions in Helmuth et al., 2005; Somero, 2005), but rather because these two distinct approaches which address spatial and temporal distribution and abundance of insects have yielded several biological predictions that can be tested using comparative techniques. For example, Rogers and Randolph (1986) have shown that *G. morsitans*, *G. palpalis* and *G. tachinoides* differ markedly in their responses to a similar range of climatic conditions, and therefore one may predict that some form of climatic (temperature and/or moisture) adaptation has taken place. In addition, *G. morsitans* sub-species in Zambia appear to respond very differently to climatic variables (Robinson et al., 1997a, b), which suggests that flies show adaptations to regional climate (Rogers & Robinson, 2004; see also discussion on p. 130 in Hargrove, 2004). Addressing environmental adaptation from an experimental physiological perspective may thus improve predictions of the changing risks of trypanosome transmission with predicted climate change.

### **Tsetse Flies as a Model Organism for Understanding Vectors of Disease in a World of Changing Climate**

Tsetse flies are the only vectors of the trypanosomes that cause sleeping sickness in people and ‘nagana’ in cattle and, as such, have profound medical and economic importance. In Africa, human sleeping sickness can become epidemic and cause regional depopulation (Ford, 1971; Jordan, 1986). Nearly 45 000 cases were reported in 1999, but more than 45 million people are considered to be at risk (WHO, 2001). The 45 000 case figure is thought not to reveal the true situation, but rather probably reflects the lack of screening in many foci (WHO, 2001). Approximately 48 000 deaths were attributed to trypanosomiasis in the most recent World Health Report (WHO, 2004). There are about 200 identified foci in Africa and there is a continuous threat of severe epidemics. Indeed, epidemics have occurred within the last decade in southern Sudan, Angola, Uganda and the Congo (WHO, 2001), where the problem is thought to be exacerbated by civil unrest and human population movements (Welburn et al., 1999). In certain villages of many provinces of Angola, the Democratic Republic of Congo and southern Sudan, the prevalence of sleeping sickness lies between 20 and 50 %. In these areas, sleeping sickness has become the first or second greatest cause of mortality, ahead of HIV/AIDS (WHO, 2001). Two forms of the human disease have been described. A chronic form occurs in western and central Africa, caused by *Trypanosoma brucei gambiense*. A more acute form occurs in east and southern Africa, caused by *T. b. rhodesiense* infection. Unless treated by trained medical personnel with costly drugs, human trypanosomiasis is invariably fatal. As many as one million people were

estimated to be infected in a recent epidemic (Barrett, 1999). These flies are therefore of major medical and veterinary importance because of their adverse influences on social and economic development in Africa.

Animal trypanosomosis remains a gigantic barrier to husbandry and therefore agricultural and economic development. Domestic animals cannot be maintained in about 11.5 million km<sup>2</sup> of tropical Africa (an area about the size of continental USA) because they are rapidly killed by trypanosomes (Rogers & Randolph, 1991). As well as a source of wealth and security in themselves, livestock provide food, manure and draft power. The inability to keep livestock is a major constraint to rural households. Adequate nutrition is basic to public health. Because drug prophylaxis and treatment is uncertain, expensive, and unavailable to the vast majority of Africans at risk, and because trypanosomes are becoming resistant to existing drugs, vector control is the only realistic way to reduce greatly or to eliminate altogether trypanosomiasis in the long term.

In consequence, understanding the factors influencing the distribution and abundance of the vectors is of considerable importance. It is this goal that is being addressed in the present study, especially because of the close relationship between climatic variables and tsetse abundance and distribution (Rogers & Randolph, 1986; Robinson et al., 1997a, b; Hargrove, 2001b; Rogers & Robinson, 2004). As global warming continues, there is concern for increased transmission for some tropical diseases and potential for their expansion into temperate regions (Patz et al., 2000). Moreover, concerns for the effects of climate change impacting on species' distributions and abundances via physiological tolerances are becoming realized (e.g. Berteaux et al., 2004; Somero, 2005) and thus warrant urgent investigation, particularly in the context of vectors of diseases such as trypanosomosis (see Rogers & Packer, 1993; Patz et al., 2000; Beniston, 2002). However, in tsetse there is relatively poor evidence of causal changes in disease transmission and climate within Africa (IPCC, 1997). This lack of evidence does not mean that these changes do not exist, but rather, probably reflect the lack of available epidemiological data as a result of poor or absent surveillance and health information systems. Within Africa, 71.3% of the burden of disease is attributed to infectious diseases, with malaria given the credit for the single greatest contributor (10.8%) (IPCC, 1997). All other vector-borne, helminthic, and environmentally related diseases that are affected by climate contribute about 2% of the total burden of disease (IPCC, 1997).

Environmental change, whether occurring as a natural phenomenon or through human intervention, changes the ecological state and context within which disease hosts or vectors and parasites breed, develop, and transmit diseases (Patz et al., 2000). Indeed, the temporal and spatial



changes in temperature, precipitation and humidity that are expected to occur under different climate change scenarios will affect the biology and ecology of vectors and intermediate hosts, and consequently the risk of disease transmission (Githeko et al., 2000; Patz et al., 2000). African trypanosomiasis also could proliferate because higher temperature would increase the range of the vector in areas prone to this infection (IPCC, 1997). Specifically, it has been predicted that the distribution of tsetse flies may increase in east Africa (Rogers & Packer, 1993). Alternative mechanisms which may be influenced by climate change are, for example, that within tsetse populations increases in temperature could result in increased metabolic energy requirements (Rajagopal & Bursell, 1966) which may result in increased frequency of feeding, and, in consequence, increased disease transmission rates. Moreover, increases in temperature may lead to a reduction in time to maturity (Bursell, 1960) which may lead to increases in reproductive capacity, resulting in larger populations. These latter temperature-dependent physiological processes serve as warning signs for the potential impacts that temperature variation associated with climate change may have on vector transmission. In sum, areas of traditionally low risk may become more vulnerable. However, temperature-dependent processes may ultimately affect various life stages differently (Hargrove, 2004) and may thus represent a balance between costs and benefits to the species in question, potentially resulting in little or no significant changes in disease transmission rates. For example, increased environmental temperature may cause a reduction in time to maturity, but this may be offset by increased heat-induced sterility (Leegwater-van der Linden, 1984) or a reduction in survival (Hargrove, 2001a). By contrast, the ability of vectors to change or adapt to changing environments may alter the outcome of the factors determining disease transmission. If, for example, metabolic processes are highly flexible, over short time scales they may be an initial increase in metabolic rate in response to temperature variation, which over longer periods, may decline to the original baseline values. Such a process has been demonstrated in several insect species in response to cold acclimation (see Hoffmann, 1985).

Generally, the physiological relationships among climate, vectors, and pathogens are only partially understood. Malaria provides an example of how potential climate change may affect vector-borne diseases (see e.g. Massad & Forattini, 1998; Hoshen & Morse, 2004; Pascual & Dobson, 2005; Zhou et al., 2005) although predictions show a low probability for a change in distribution under future climate scenarios (Rogers & Randolph, 2000). Surveillance systems and epidemiological data on malaria exist in some of the regions most susceptible to climate change, allowing future monitoring to move from speculative to causal relationships (e.g. Abeku et al., 2004). However, in the case of

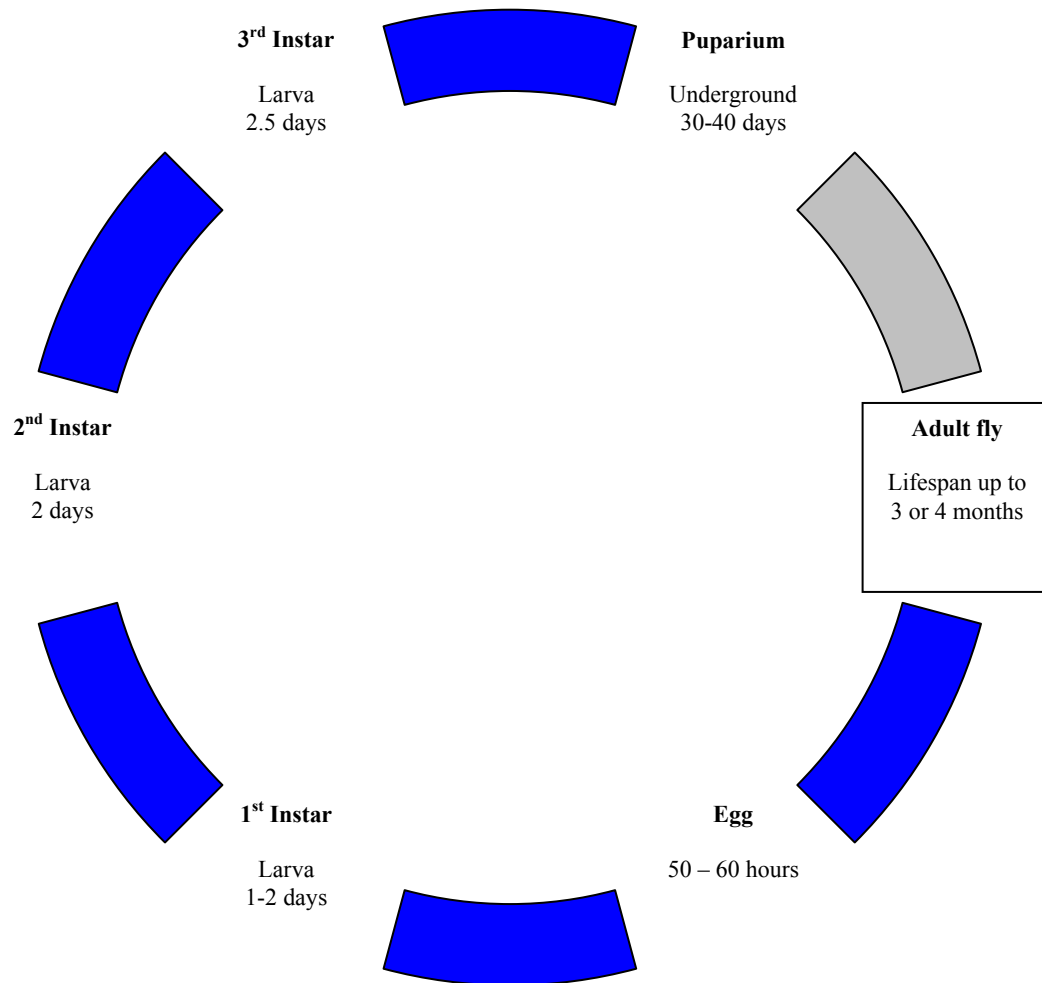
trypanosomiasis transmitted by tsetse flies, these relationships are poorly understood and many aspects of the physiological link(s) between abiotic factors and population dynamics, distribution and abundance and have not been elucidated. For example, Robinson et al's (1997a, b) work specifically predicts biological differences between some tsetse species and sub-species (see also Rogers & Robinson, 2004). Physiological variation may be a means through which differences in the environmental response of tsetse species and populations have arisen, so resulting in pronounced differences in geographic distribution (Rogers & Randolph, 1991; Rogers & Williams, 1994; Robinson et al., 1997a, b). Climate change, especially for human and animal populations that lack immunity, may thus have severe consequences (individual, social, and economic) for exposed individuals and health authorities (IPCC, 1997). Consequently, understanding basal and inducible physiological performances at several time scales deserves attention.

## **Tsetse Biology**

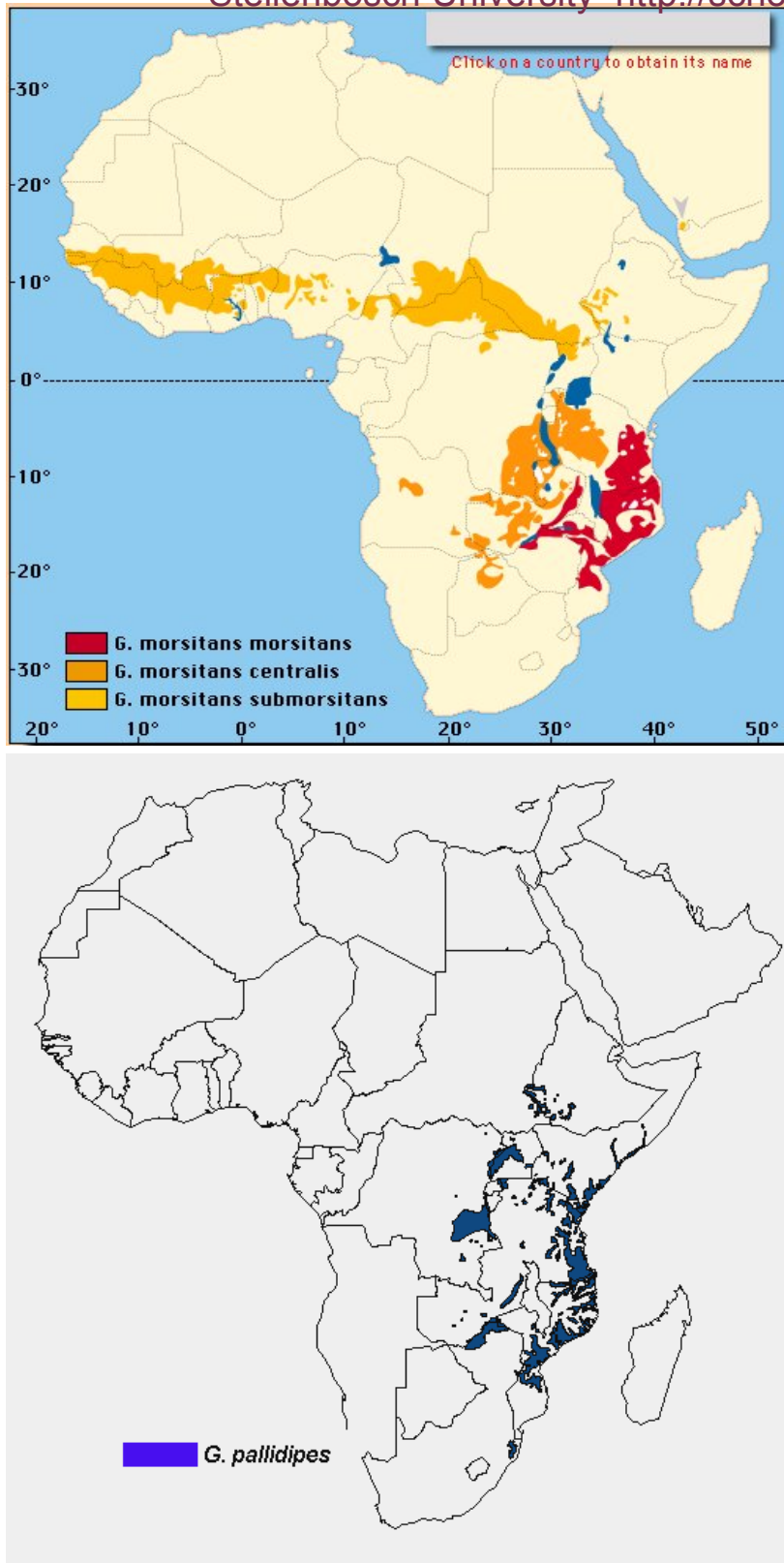
Leak (1999) has provided a thorough review of tsetse biology and ecology, while Langley (1977) has reviewed tsetse physiology with respect to reproduction and development. Tsetse population dynamics have been reviewed by Hargrove (2004), while distribution and modelling is examined in detail by Rogers & Robinson (2004). In consequence, here only a brief overview will be given, with some focus on the aspects of the flies' biology most relevant to the major questions posed in this project.

Tsetse reproduction and feeding are unique by comparison with other insects, and especially other Dipterans. Unlike most flies, tsetse have a low reproductive rate with a high energetic investment per individual offspring (K-strategy) (Leak, 1999). Tsetse reproduction is thus classified as adentrophic viviparity, and is characterised by eggs which hatch in the uterus prior to larviposition (the life cycle of tsetse flies is outlined in Fig. 2). A tsetse female gives birth to a single third-instar larva every 9-10 days. Tsetse flies are blood feeders, and unlike several other haematophagous groups (e.g. mosquitoes (Culicidae)), are restricted to feeding exclusively on blood taken only from vertebrate prey. The haemoglobin from the blood is digested into its haem and amino acid components. The amino acids are used to synthesise and store fat, from which the amino acid proline is generated for use in rapid energy production, particularly during flight (Bursell, 1963). The majority of metabolic energy is obtained from proline, and the magnitude of this amino acid's pools is regarded as a limitation on flight performance (Bursell & Slack, 1968).

Tsetse are relatively large flies and generally easy to recognise. The adult flies display considerable size-dimorphism, with females usually much larger than males. They are widely distributed in Africa (Fig. 3), occurring in a variety of habitats ranging from mesic forests to more xeric savannahs. Due to their haematophagous nature and unusual mode of reproduction they exhibit a remarkable range of host preferences and other unique behavioural characteristics.



**Figure 2.** Life cycle of the tsetse fly (*Glossina* spp.; adapted from Leak, 1999). (■ life-stages that occur within the female's uterus.)



**Figure 3.** Geographic distribution of *G. morsitans sensu lato* and *G. pallidipes*.

## Desiccation and Water Balance in Tsetse

It is generally accepted that insects employ three physiological mechanisms to survive dehydration. Either animals can maintain high resistance to water loss (desiccation resistance), survive low water content (desiccation tolerance), or finally, they may increase their body water content (dubbed the “canteen hypothesis”; reviewed in Danks, 2000 and Chown & Nicolson, 2004).

Terrestrial arthropods exhibit considerable intra- and inter-specific variation in tolerances of water loss (Hadley, 1994), and water contents may vary from 17 % to more than 90 % of fresh mass in different life stages of different species. Generally, this basic water content is related to fat content within individuals. Insect species inhabiting environments that differ in their aridity show marked variation in their ability to resist and tolerate water loss (Edney, 1977; Hadley, 1994). This variation can occur between populations and species, and extends also to larger geographic scales (Addo-Bediako et al., 2001; Hoffmann et al., 2001; Chown, 2002; Marron et al., 2003).

Desiccation of adult tsetse was once considered to be an important cause of death in natural populations (Buxton & Lewis, 1934; Mellanby, 1936; and see Leak, 1999). However, desiccation-related mortality is now more commonly attributed to the pupal stages (see e.g. Hargrove, 2001a; 2004). In one of the earliest accounts of tsetse ecological physiology, Mellanby (1936) showed that more severely desiccating conditions caused more rapid mortality in *G. palpalis*, and that fed flies survived these same conditions longer than unfed flies. Additionally, males were suggested to be more hardy and survived desiccating conditions better than females, but whether this was a merely consequence of size remained undetermined until Bursell’s classic studies (see below) in which accounting for size in the analyses removed gender-related differences in water loss rates (Bursell, 1959). Mellanby (1936) concluded that flies experiencing low humidity were dying from desiccation rather than starvation, which was the case in more moderate humidity levels. Moderate humidity levels did not have any harmful desiccating effect, and an optimum humidity environment of between 65-80% relative humidity (r.h.) was estimated. Currently, the optimum humidity of laboratory colonies is in the region of 75% (A. Parker, IAEA, pers. comm.).

Drier conditions do not lead to increased feeding, and thus, no clear “thirst” effect has been observed in tsetse flies (Mellanby, 1936; Bursell, 1959). At the inter-specific level, Bursell (1959) found little variation in body water content relative to fat-free dry mass among *Glossina* spp. (~76.2% body water). In addition, a distinct positive relationship exists between dry mass (fat-free) and water content in teneral and non-teneral (post-blood meal) flies (Bursell, 1959). The relative body water

content proved constant across various species of differing size classes, with the exception of *G. morsitans* at 77.0 %, a value accounted for by higher pupal water content (Bursell, 1958).

No differences in rates of water loss between teneral versus non-teneral flies have been found and increased activity is usually accompanied by an increase in water loss rates (Bursell, 1959), at least when expressed as percentages of mass loss relative to fat-free mass per hour (%/hr). However, Bursell (1959) found little evidence for differences in rates of water loss between genders within a species when size was factored into the analyses. Nevertheless, interspecific differences were found, in which *G. austeni* had the highest rates of water loss at 3.26%/hr, and this parameter varied significantly to a lower limit of 2.18%/hr in *G. morsitans* (Bursell, 1959). Bursell (1959) proposed that such differences may be accounted for by the species' habitat preference and geographic distribution (see also Mellanby, 1936). Somewhat surprisingly, Bursell (1959) ultimately concluded that no relation existed between habitat and resistance to desiccation, as it seemed more likely that tsetse would die from starvation before desiccation in the wild (Bursell, 1961). To my knowledge, no studies have investigated temperature and/or humidity effects on water balance in tsetse with respect to acclimation responses.

### **Critical Thermal Limits in Tsetse**

Only a single climatic variable appears to determine tsetse distribution at the edge of its range, that of maximum mean monthly temperature, although more climatic variables are required to describe their distributions suitably within the continental range (Rogers & Robinson, 2004). Indeed, mortality rates and population density are well correlated with meteorological data in tsetse, usually as a combination of temperature and saturation deficit variables (Rogers & Randolph, 1991; see also Rogers & Randolph, 1986). Temperature is an important abiotic environmental factor because it directly affects the kinetic energy of reactants and therefore the biochemical and physiological processes within organisms (Hochachka & Somero, 2002) and is thought to be one of the most important variables determining distribution and abundance of species (Cossins & Bowler, 1987; Chown & Nicolson, 2004). While temperature may be relatively easy to measure, the study of thermal physiology can be complex. This is due in part to the interactive role of behavioural and phenotypic plasticity in the regulation of body temperature (Huey et al., 2003) and also to interactive (linear and/or non-linear) effects of time and duration of exposure to temperature on ectotherm survival (Chown & Nicolson, 2004). In insects in general, large-scale inter-specific comparisons of upper thermal limits show little

association with latitude, while lower thermal limits have been shown to vary more strongly with latitude (Addo-Bediako et al., 2000; Hoffmann et al., 2002; Kimura, 2004).

Among tsetse flies, the upper temperature tolerance of adult *G. palpalis* has been assessed (Buxton & Lewis, 1934; Mellanby, 1936). In *G. tachinoides* and *G. submorsitans*, Buxton & Lewis (1934) demonstrated that exposure to 43 °C was fatal, but an hour at 40 °C could be survived. At slightly longer time scales, a three hour exposure to 40 °C was fatal, while 38 °C did not result in mortality. The general conclusion reached by both Buxton & Lewis (1934) and Mellanby (1936) was that high temperatures which cause excessive desiccation would be harmful, and may influence the distribution of these flies. Although many studies have demonstrated that lethal temperatures (above ~40 °C) occur in the field, microclimate conditions in refugia frequented by adult flies remain substantially (~5 °C) cooler (Torr & Hargrove, 1999). Nevertheless, the literature on tsetse flies is replete with inferences regarding temperature tolerances and distribution (Robinson et al., 1997a, b; Leak, 1999; Rogers & Robinson, 2004). Both Robinson et al. (1997a) and Hargrove (2001a) demonstrated that temperature has a significant influence on tsetse distribution and survival probability, respectively (see also Rogers & Randolph, 1991). However, in retrospect, what is clearly absent from the tsetse physiology literature is a thorough assessment of adult temperature tolerances for a variety of species and physiological conditions, potentially linking the information obtained from meteorological data and population dynamics with physiological mechanisms.

Also prominent for tsetse is the relationship of mortality and survival with temperature, particularly with reference to pupal fat content and reproductive performance (e.g. Bursell, 1960; Leegwater-van der Linden, 1984; Zdarek et al., 1992; Zdarek & Denlinger, 1995). In, for example, *G. morsitans* pupal fat content is highest when reared at 24-25 °C (Bursell, 1960), a temperature which corresponds closely with optimal rearing conditions for laboratory maintenance. The relationships for pupae between temperature and development, and between temperature and fat consumption are such that the size-specific fat contents of emerging tsetse flies are highest for temperatures in the region of 26 °C. Most importantly, however, temperature causes mortality in various life stages (e.g. pupae, immature teneral, adult flies) in different ways and can also change within a life stage (e.g. within mature females) and, for several reasons discussed in Hargrove (2004), there is considerable difficulty attaining accurate estimates of population growth and decline parameters (Hargrove, 2004).

Generally, population dynamics represents a complex interaction between growth rates (immigration + birth rates) and loss rates (emigration + mortality). In all life stages of tsetse, estimates of loss rates are generally positive and linearly related to mean temperature across the range of 15-33



°C, except for pupae, which have a u-shaped relationship. By contrast, growth rates are all non-linear (a-shaped) with optima in the region of 26-28 °C (Hargrove, 2004). In consequence, the effects of temperature on tsetse biology are diverse, with a variety of proposed underlying mechanisms. For example, temperature has been hypothesized to affect demographics directly via mating performance (Mutika et al., 2001), reproductive abnormalities and abortions (Hargrove, 2004), and indirectly through effects on adult mortality by temperature-dependent interactions on pupal duration (Torr & Hargrove, 1999; see also Hargrove, 2004). Temperature may also affect adult mortality via indirect effects on the critical lipid mass of teneral flies and/or puparia (Bursell, 1960; Phelps, 1973; Phelps & Clarke, 1974), and even indirectly through prey-density via effects on vegetation structure or hunger-related deaths resulting from an increase in feeding rate with increasing temperature (Hargrove, 2001a). Mortality in the field may also be affected by increased losses of teneral flies due to the combined effects of high temperatures and low fat at emergence (Phelps & Clarke, 1974), or increased parasitic levels (Hargrove & Langley, 1993).

Most of the population dynamics work has been done on a few key species for which adequate time-series data have been acquired. Nevertheless, inter-specific variation has been observed for relationships between mortality and temperature. For *G. m. morsitans*, the calculated daily instantaneous mortality rates are linear and positively correlated with mean temperature (Hargrove, 2001a). By contrast, in *G. pallidipes* this relationship is strongly non-linear and has a definite breakpoint in the 22-24 °C range, with mortality generally increasing sharply above 25 °C (Hargrove, 2004).

Lower lethal or critical thermal limits have rarely been investigated, probably because it is assumed that tsetse flies would seldom encounter these in the field (see discussion in Phelps & Burrows, 1969 on puparia), although some studies have made inferences that lower critical growth thresholds probably lie in the range of 14-18 °C based on regression techniques (e.g. Bursell, 1960). Of the few studies of cold temperature tolerance, Macfie (1912) demonstrated that in *G. palpalis* a few minutes at 2.5 °C was harmful, while about 8 °C resulted in a complete lack of activity which may be assumed to approximate the lower critical thermal limit (see also Mellanby, 1936). At slightly longer timescales, Bursell (1960) showed in pupae that temperatures ranging from 6 to -1 °C increased mortality relative to controls over a period of one to five days (see also Phelps & Burrows, 1969). In general, however, there is poor understanding of cold-tolerance physiology of tsetse, especially in comparison to other laboratory-reared flies (e.g. *Drosophila*, see Hoffmann et al., 2003). Although it is clear that tsetse are capable of sensing (Reinouts van Haga & Mitchell, 1975; Denotter & Vannaters,



1992) and responding to temperature variation (Huyton & Brady, 1975), few studies have directly examined thermal tolerances, and apparently none have investigated responses to acclimation within these traits in any *Glossina* species.

### **Metabolic Rate in Tsetse**

From a physiological perspective, oxygen uptake is regarded as a measure of ATP demand, and is considered to be very different to the vast array of cellular, sub-cellular and organismal chemical reactions, which together constitute whole animal metabolism (see Hochachka et al., 2003; Chown & Nicolson, 2004). Oxygen uptake may be regarded as the summation of the ATP costs of these processes (Clarke, 1993), but does not necessarily equate directly to them.

The respiratory gas exchange system of insects faces the simultaneous challenge of maximising the flow of respiratory gases through the spiracles while minimizing water loss. In insects at rest, gas exchange can take place by means of diffusion, convection or a combination thereof (Chown & Nicolson, 2004) and has been reviewed several times (see e.g. Kestler, 1985; Chown & Nicolson, 2004 and references therein). Because the modification of spiracle-opening can significantly reduce whole-animal metabolic rate and also the contribution of respiratory water loss to total water loss (for discussion of principles and mechanisms see Kestler, 1985), variation in gas exchange patterns and metabolic rate have been suspected as a means of altering water loss in an adaptive fashion (reviewed in Chown, 2002). Indeed, it has been demonstrated in *Drosophila* that adaptive spiracle-closing behaviour could reduce the risk of desiccation for animals flying in arid conditions (Lehmann et al., 2000) and in several dung beetle species that modulation of gas exchange could be an adaptation for the reduction of spiracular water loss (Chown & Davis, 2003). Several lines of evidence in ecological physiology suggest that organisms may be able to cope with dry conditions by lowering their metabolic rate (reviewed in Chown & Gaston, 1999), but the issue remains complex and unresolved. For example, several authors have argued that a reduction in CO<sub>2</sub> production is probably not a mechanism for resisting desiccation (Williams et al., 1998; see also Rourke, 2000; Shelton & Appel, 2001a, b; Johnson & Gibbs, 2004). By contrast, tsetse flies appear to regulate spiracle closure more carefully when partially dehydrated (Bursell, 1957), while in *Aedes* (Diptera, Culicidae) spiracles can respond to the relative humidity of ambient air (Krafsur, 1971).

Techniques for measuring respiratory gas-exchange have also received criticism (Chown & Gaston, 1999; Addo-Bediako et al., 2002). Due to the potential overestimation inherent in other respirometry methods, several authors have emphasized the importance of using flow-through

respirometry for the measurement of metabolic rates (Chown & Gaston, 1999; Klok & Chown, 2005b). Previously, metabolic rates in *Glossina* have been estimated using mainly one of two techniques, either via the release of radioactive caesium (Hargrove & Coates, 1990) or by means of conventional closed-system respirometry (Rajagopal & Bursell, 1966; Taylor, 1977b). However, no studies have yet investigated metabolic rates, even at the family level, using flow-through respirometry. Moreover, metabolic rates have not been estimated in either field populations or in laboratory-colonies using the latter method. Therefore, an aim of this study is to use flow-through respirometry to obtain estimates of resting metabolic rate within individual flies under a variety of conditions.

Metabolic rate and its temperature dependence (or rate-temperature (R-T) relationships) are also responsive to environmental variation, and the metabolic response to temperature can differ between populations and between species (Scholander et al., 1953; Sømme & Block, 1991; Sibly & Atkinson, 1994; Chown et al., 1997; Addo-Bediako et al., 2002). In general, however, the patterns in and processes underlying variation in R-T relationships have not been systematically explored for insects, although variation of metabolic rate with temperature has been widely documented (Keister & Buck, 1964; Chown & Nicolson, 2004). For tsetse flies, few studies have investigated the metabolic R-T relationship (but see Rajagopal & Bursell, 1966; Taylor, 1977b) but perhaps more importantly, no studies have investigated variation in R-T relationships for various life-stages and biological classes (e.g. fed vs fasted, acclimated to several temperatures) in order to verify the simplifying assumptions made by population dynamics models (e.g. Hargrove, 2001a).

## Summary

In summary, the primary aim of this thesis is to gain insight into physiological variation within and between populations (see Fig. 1) both under laboratory and field conditions, in order to understand adaptive processes and hence evolution in the natural environment. A more general secondary aim of this work is to attempt to provide a physiological basis for the patterns of distribution and abundance in tsetse as provided by population dynamics and distribution models. As the former goal is more easily attained, the latter goal may seem somewhat bold, and in consequence is probably not fully realised in this dissertation. Nevertheless, it provides a starting point from which to progress, as is customary in the scientific method. Hopefully, physiological ecologists will find these aims as worthwhile, rewarding and enlightening as my colleagues and I have, and ultimately that this information will contribute in a positive way to tsetse control and eradication programmes.

## References

- Abeku, T.A., De Vlas, S.J., Borsboom, G.J.J.M., Tadege, A., Gebreyesus, Y., Gebreyohannes, H. et al., 2004. Effects of meteorological factors on epidemic malaria in Ethiopia: a statistical modelling approach based on theoretical reasoning. *Parasitology* 128, 585-593.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* 267, 739-745.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2001. Revisiting water loss in insects: a large scale view. *Journal of Insect Physiology* 47, 1377-1388.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology* 16, 332-338.
- Angilletta, M.J., Wilson, R.S., Navas, C.A., James, R.S., 2003. Tradeoffs and the evolution of thermal reaction norms. *Trends in Ecology and Evolution* 18, 234-240.
- Aragaki, D.L.R., Meffert, L.M., 1998. A test of how well the repeatability of courtship predicts its heritability. *Animal Behaviour* 55, 1141-1150.
- Ayres, M.P., Scriber, J.M., 1994. Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera: Papilionidae). *Ecological Monographs* 64, 465-482.
- Ayrinhac, A., Debat, V., Gibert, P., Kister, A.-G., Legout, H., Moreteau, B. et al., 2004. Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology* 18, 700-706.
- Badyaev, A.V., 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proceedings of the Royal Society of London B* 272, 877-886.
- Barrett, M.P., 1999. The rise and fall of sleeping sickness. *Lancet* 353, 113-114.
- Bech, C., Langseth, I., Gabrielsen, G.W., 1999. Repeatability of basal metabolism in breeding female kittiwakes *Rissa triactyla*. *Proceedings of the Royal Society of London B* 266, 2161-2167.
- Beniston, M., 2002. Climate change: possible impacts on human health. *Swiss Medical Weekly* 132, 332-337.
- Bennett, A.F., 1987. Interindividual variability: an underutilized resource. In: Feder, M.E., Bennett, A.F., Burggren, W., Huey, R.B. (eds), *New Directions in Ecological Physiology*. Cambridge University Press, Cambridge, pp. 147-169.
- Bennett, A.F., Lenski, R.E., 1997. Evolutionary adaptation to temperature. VI. Phenotypic acclimation and its evolution in *Escherichia coli*. *Evolution* 51, 36-44.

- Berteaux, D., Thomas, D.W., Bergeron, J.M., Lapierre, H., 1996. Repeatability of daily field metabolic rate in female Meadow Voles (*Microtus pennsylvanicus*). *Functional Ecology* 10, 751-759.
- Berteaux, D., Reale, D., McAdam, A.G., Boutin, S., 2004. Keeping pace with fast climate change: can Arctic life count on evolution? *Integrative and Comparative Biology* 44, 140-151.
- Bursell, E., 1957. Spiracular control of water loss in the tsetse fly. *Proceedings of the Royal Entomological Society of London A* 32, 21-29.
- Bursell, E., 1958. The water balance of Tsetse pupae. *Philosophical Transactions of the Royal Society of London B* 241, 179-210.
- Bursell, E., 1959. The water balance of tsetse flies. *Transactions of the Royal Entomological Society of London* 111, 205-235.
- Bursell, E., 1960. The effect of temperature on the consumption of fat during pupal development in *Glossina*. *Bulletin of Entomological Research* 51, 583-598.
- Bursell, E., 1961. Starvation and desiccation in tsetse flies (*Glossina*). *Entomologia Experimentalis et Applicata* 4, 301-310.
- Bursell, E., 1963. Aspects of the metabolism of amino acids in the tsetse fly *Glossina* (Diptera). *Journal of Insect Physiology* 9, 439-452.
- Bursell, E., Slack, E., 1968. Indications concerning the flight activity of tsetse flies (*Glossina morsitans* Westw.) in the field. *Bulletin of Entomological Research* 58, 575-579.
- Bursell, E., Taylor, P., 1980. An energy budget for *Glossina*. *Bulletin of Entomological Research* 70, 187-197.
- Buxton, P.A., Lewis, D.J., 1934. Climate and tsetse flies: Laboratory studies upon *Glossina submorsitans* and *tachinoides*. *Philosophical Transactions of the Royal Society of London B* 224, 175-242.
- Case, T.J., Taper, M.L., 2000. Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. *American Naturalist* 155, 583-605.
- Chappell, M.A., Zuk, M., Johnsen, T.S., 1996. Repeatability of aerobic performance in Red Junglefowl: effects of ontogeny and nematode infection. *Functional Ecology* 10, 578-585.
- Chen, C.-P., Denlinger, D.L., 1992. Reduction of cold injury in flies using an intermittent pulse of high temperature. *Cryobiology* 29, 138-143.
- Chen, C.-P., Lee Jr, R.E., Denlinger, D.L., 1990. A comparison of the responses of tropical and temperate flies (Diptera: Sarcophagidae) to cold and heat stress. *Journal of Comparative Physiology* 160, 543-547.

- Chown, S.L., 1993. Desiccation resistance in six sub-Antarctic weevils (Coleoptera: Curculionidae): humidity as an abiotic factor influencing assemblage structure. *Functional Ecology* 7, 318-325.
- Chown, S.L., 2001. Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* 47, 649-660.
- Chown, S.L., 2002. Respiratory water loss in insects. *Comparative Biochemistry and Physiology A* 133, 791-804.
- Chown, S.L., Davis, A.L.V., 2003. Discontinuous gas exchange and the significance of respiratory water loss in scarabaeine beetles. *Journal of Experimental Biology* 206, 3547-3556.
- Chown, S.L., Gaston, K.J., 1999. Exploring links between physiology and ecology at macro scales: the role of respiratory metabolism in insects. *Biological Reviews* 74, 87-120.
- Chown, S.L., Nicolson, S.W., 2004. *Insect Physiological Ecology. Mechanisms and Patterns*. Oxford University Press, Oxford.
- Chown, S.L., Van Der Merwe, M., Smith, V.R., 1997. The influence of habitat and altitude on oxygen uptake in sub-antarctic weevils. *Physiological Zoology* 70, 116-124.
- Chown, S.L., Le Lagadec, M.D., Scholtz, C.H., 1999. Partitioning variance in a physiological trait: desiccation resistance in keratin beetles (Coleoptera, Trogidae). *Functional Ecology* 13, 838-844.
- Clarke, A., 1993. Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? *Functional Ecology* 7, 139-149.
- Cossins, A.R., Bowler, K., 1987. *Temperature Biology of Animals*. Chapman and Hall, London.
- Danks, H.V., 2000. Dehydration in dormant insects. *Journal of Insect Physiology* 46, 837-852.
- David, J.R., Gibert, P., Moreteau, B., Gilchrist, G.W., Huey, R.B., 2003. The fly that came in from the cold: geographic variation in recovery time from low-temperature exposure in *Drosophila subobscura*. *Functional Ecology* 17, 425-430.
- Davis, A.L.V., Chown, S.L., McGeoch, M.A., Scholtz, C.H., 2000. A comparative analysis of metabolic rate in six *Scarabaeus* species (Coleoptera : Scarabaeidae) from southern Africa: further caveats when inferring adaptation. *Journal of Insect Physiology* 46, 553-562.
- De Witt, T.J., Scheiner, S.M., 2004. *Phenotypic Plasticity: Functional and Conceptual Approaches*. Oxford University Press, New York.
- Deere, J.A. (2005) Acclimation effects on thermal tolerance in ameronothrid mites at sub-Antarctic Marion Island. M.Sc. thesis, University of Stellenbosch Printers, Stellenbosch.
- Denlinger, D.L., 2002. Regulation of diapause. *Annual Review of Entomology* 47, 93-122.

- Denlinger, D.L., Lee, R.E. Jr, 1998. Physiology of cold sensitivity. In: Hallman, G.J., Denlinger, D.L. (eds), *Temperature Sensitivity in Insects and Application in Intergrated Pest Management*. Westview Press, Boulder, pp. 55-95.
- Denotter, C.J., Vannaters, W.M.V., 1992. Single cell recordings from tsetse (*Glossina morsitans*) antennae reveal olfactory, mechano and cold receptors. *Physiological Entomology* 17, 33-42.
- Dingemans, N.J., Both, C., Drent, P.J., Van Oers, K., Van Noordwijk, A.J., 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour* 64, 929-938.
- Dohm, M.R., 2002. Repeatability estimates do not always set an upper limit to heritability. *Functional Ecology* 16, 273-280.
- Du, W.G., Ji, X., Zhang, Y.P., Xu, X.F., Shine, R., 2005. Identifying sources of variation in reproductive and life-history traits among five populations of a Chinese lizard (*Takydromus septentrionalis*, Lacertidae). *Biological Journal of the Linnean Society* 85, 443-453.
- Edney, E.B., 1977. *Water Balance in Land Arthropods*. Springer, Berlin.
- Endler, J.A., 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton.
- Falconer, D.S., Mackay, T.F.C., 1996. *Introduction to Quantitative Genetics*, 4th edn. Prentice Hall, Harlow.
- Feder, M.E., Mitchell-Olds, T., 2003. Evolutionary and ecological functional genomics. *Nature Reviews* 4, 649-655.
- Fischer, K., Eenhorn, E., Bot, A.N.M., Brakefield, P.M., Zwaan, B.J., 2003. Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. *Proceedings of the Royal Society of London B* 270, 2051-2056.
- Ford, J., 1971. *The Role of Trypanosomiases in African Ecology: A Study of the Tsetse Fly Problem*. Clarendon, Oxford.
- Forde, S.E., Thompson, J.N., Bohannan, B.J.M., 2004. Adaptation varies through space and time in a coevolving host-parasitoid interaction. *Nature* 431, 841-844.
- Frankino, W.A., Juliano, S.A., 1999. Costs of reproduction and geographic variation in the reproductive tactics of the mosquito *Aedes triseriatus*. *Oecologia* 120, 59-68.
- Fyhn, M., Gabrielsen, G.W., Nordoy, E.S., Moe, B., Langseth, I., Bech, C., 2001. Individual variation in field metabolic rate of kittiwakes (*Rissa tridactyla*) during the chick-rearing period. *Physiological and Biochemical Zoology* 74, 343-355.
- Gibbs, A.G., Matzkin, L.M., 2001. Evolution of water balance in the genus *Drosophila*. *Journal of Experimental Biology* 204, 2331-2338.

- Gibbs, A., Mousseau, T.A., Crowe, J.H., 1991. Genetic and acclimatory variation in biophysical properties of insect cuticle lipids. *Proceedings of the National Academy of Sciences of the USA* 88, 7257-7260.
- Gibbs, A.G., Chippendale, A.K., Rose, M.R., 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology* 200, 1821-1832.
- Gibert, P., Moreteau, B., Pétavy, G., Karan, D., David, J.R., 2001. Chill-coma tolerance, a major climatic adaptation among *Drosophila* species. *Evolution* 55, 1063-1068.
- Githeko, A.K., Lindsay, S.W., Confalonieri, U.E., Patz, J.A., 2000. Climate change and vector-borne diseases: a regional analysis. *Bulletin of the World Health Organisation* 78, 1136-1147.
- Gray, E.M., Bradley, T.J., 2003. Metabolic rate in female *Culex tarsalis* (Diptera : Culicidae): Age, size, activity, and feeding effects. *Journal of Medical Entomology* 40, 903-911.
- Hadley, N.F., 1994. *Water Relations of Terrestrial Arthropods*. Academic Press, San Diego.
- Haldane, J.B.S., 1956. The relation between density regulation and natural selection. *Proceedings of the Royal Society of London B* 145, 306-308.
- Hargrove, J.W., 2001a. The effect of temperature and saturation deficit on mortality in populations of male *Glossina m. morsitans* (Diptera: Glossinidae) in Zimbabwe and Tanzania. *Bulletin of Entomological Research* 91, 79-86.
- Hargrove, J.W., 2001b. Factors affecting density-independent survival of an island population of tsetse flies in Zimbabwe. *Entomologia Experimentalis et Applicata* 100, 151-164.
- Hargrove, J.W., 2004. Tsetse population dynamics. In: Maudlin, I., Holmes, P.H., Miles, M.A. (eds), *The Trypanosomiases*. CABI Publishing, Wallingford, pp. 113-138.
- Hargrove, J.W., Coates, T.W., 1990. Metabolic rates of tsetse flies in the field as measured by the excretion of injected cesium. *Physiological Entomology* 15, 157-166.
- Hargrove, J.W., Langley, P.A., 1993. A field trial of pyriproxyfen-treated targets as an alternative method for controlling tsetse (Diptera: Glossinidae). *Bulletin of Entomological Research* 83, 361-368.
- Hayes, J.P., Bible, C.A., Boone, J.D., 1998. Repeatability of mammalian physiology: Evaporative water loss and oxygen consumption of *Dipodomys merriani*. *Journal of Mammalogy* 79, 475-485.
- Helmuth, B., Kingsolver, J.G., Carrington, E., 2005. Biophysics, physiological ecology, and climate change: Does mechanism matter? *Annual Review of Physiology* 67, 177-201.
- Hochachka, P.W., Somero, G.N., 2002. *Biochemical Adaptation. Mechanism and Process in Physiological Evolution*. Oxford University Press, New York.



- Hochachka, P.W., Darveau, C.A., Andrews, R.D., Suarez, R.K., 2003. Allometric cascade: a model for resolving body mass effects on metabolism. *Comparative Biochemistry and Physiology A* 134, 675-691.
- Hoffmann, A.A., 1995. Acclimation: increasing survival at a cost. *Trends in Ecology and Evolution* 10, 1-2.
- Hoffmann, A.A., 1999. Is the heritability for courtship and mating speed in *Drosophila* (fruit fly) low? *Heredity* 82, 158-162.
- Hoffmann, A.A., Hercus, M.J., 2000. Environmental stress as an evolutionary force. *Bio Science* 50, 217-226.
- Hoffmann, A.A., Hallas, R., Sinclair, C., Mitrovski, P., 2001. Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. *Evolution* 55, 1621-1630.
- Hoffmann, A.A., Anderson, A., Hallas, R., 2002. Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecology Letters* 5, 614-618.
- Hoffmann, A.A., Sorensen, J.G., Loeschke, V., 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology* 28, 175-216.
- Hoffmann, A.A., Shirrifs, J., Scott, M., 2005. Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Functional Ecology* 19, 222-227.
- Hoffmann, K.H., 1985. Metabolic and enzyme adaptation to temperature. In: Hoffmann, K.H. (ed), *Environmental Physiology and Biochemistry of Insects*. Springer-Verlag, Berlin, pp. 1-32.
- Hoshen, M.B., Morse, A.P., 2004. A weather-driven model of malaria transmission. *Malaria Journal* 3, Art. no. 32.
- Houle, D., Rowe, L., 2003. Natural selection in a bottle. *American Naturalist* 161, 50-67.
- Huey, R.B., Berrigan, D., 1996. Testing evolutionary hypotheses of acclimation. In: Johnston, I.A., Bennett, A.F. (eds), *Animals and Temperature. Phenotypic and Evolutionary Adaptation*. Cambridge University Press, Cambridge, pp. 205-237.
- Huey, R.B., Wakefield, T., Crill, W.D., Gilchrist, G.W., 1995. Within- and between-generation effects of temperature on early fecundity of *Drosophila melanogaster*. *Heredity* 74, 216-223.
- Huey, R.B., Berrigan, D., Gilchrist, G.W., Herron, J.C., 1999. Testing the adaptive significance of acclimation: a strong inference approach. *American Zoologist* 39, 323-336.



- Huey, R.B., Hertz, P.E., Sinervo, B., 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. *American Naturalist* 161, 357-366.
- Huyton, P.M., Brady, J., 1975. Some effects of light and heat on feeding and resting behavior of tsetse flies, *Glossina morsitans* Westwood. *Journal of Entomology A - Physiology & Behaviour* 50, 23-30.
- IPCC, 1997. The Regional Impacts of Climate Change: An Assessment of Vulnerability. Intergovernmental Panel on Climate Change
- Jennions, M.D., Petrie, M., 1997. Variation in mate choice and mating preference: A review of causes and consequences. *Biological Reviews of the Cambridge Philosophical Society* 72, 283-327.
- Johnsen, T.S., Zuk, M., 1996. Repeatability of mate choice in female red jungle fowl. *Behavioural Ecology* 7, 243-246.
- Johnson, R.A., Gibbs, A.G., 2004. Effect of mating stage on water balance, cuticular hydrocarbons and metabolism in the desert harvester ant, *Pogonomyrmex barbatus*. *Journal of Insect Physiology* 50, 943-953.
- Jordan, A.M., 1986. Trypanosomiasis Control and African Rural Development. Longman, London.
- Keister, M., Buck, J., 1964. Some endogenous and exogenous effects on rate of respiration. In: Rockstein, M. (ed) *Physiology of Insecta*, Vol 3. Academic Press, New York, pp. 617-658.
- Kelty, J.D., Lee, R.E., 1999. Induction of rapid cold hardening by cooling at ecologically relevant rates in *Drosophila melanogaster*. *Journal of Insect Physiology* 45, 719-726.
- Kestler, P., 1985. Respiration and respiratory water loss. In: Hoffmann, K.H. (ed) *Environmental Physiology and Biochemistry of Insects*. Springer, Berlin, pp. 137-186.
- Kimura, M.T., 2004. Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia* 140, 442-449.
- Kimura, M.T., Ohtsu, T., Yoshida, T., Awasaki, T., Lin, F.-J., 1994. Climatic adaptations and distributions in the *Drosophila takahashii* species subgroup (Dipera: Drosophilidae). *Journal of Natural History* 28, 401-409.
- Kingsolver, J.G., Huey, R.B., 1998. Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist* 38, 545-560.
- Kirkpatrick, M., Barton, N.H., 1997. Evolution of a species' range. *American Naturalist* 150, 1-23.
- Klok, C.J., Chown, S.L., 2001. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *Journal of Insect Physiology* 47, 95-109.

- Klok, C.J., Chown, S.L., 2003. Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* 78, 401-414.
- Klok, C.J., Chown, S.L., 2005a. Inertia in physiological traits: *Embryonopsis halticella* caterpillars (Yponomeutidae) across the Antarctic Polar Frontal Zone. *Journal of Insect Physiology* 51, 87-97.
- Klok, C.J., Chown, S.L., 2005b. Temperature- and body mass-related variation in cyclic gas exchange characteristics and metabolic rate in seven weevil species: broader implications. *Journal of Insect Physiology* 51, 789-801.
- Kozłowski, J., Gawelczyk, A.T., 2002. Why are species' body size distributions usually skewed to the right? *Functional Ecology* 16, 419-432.
- Krafsur, E.S., 1971. Behavior of thoracic spiracles of *Aedes* mosquitoes in controlled relative humidities. *Annals of the Entomological Society of America* 64, 93-97.
- Krebs, C.J., 1999. *Ecological Methodology*, 2nd edn. Addison-Wesley, Menlo Park.
- Krebs, R.A., Loeschcke, V., 1994. Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. *Functional Ecology* 8, 730-737.
- Langley, P.A., 1977. Physiology of tsetse flies (*Glossina* spp.)(Diptera: Glossinidae): a review. *Bulletin of Entomological Research* 67, 523-574.
- Lankford, T.E., Billerbeck, J.M., Conover, D.O., 2001. Evolution of intrinsic growth and energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia menidia*. *Evolution* 55, 1873-1881.
- Le Lagadec, M.D., Chown, S.L., Scholtz, C.H., 1998. Desiccation resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): the influence of body size, habitat and phylogeny. *Journal of Comparative Physiology B* 168, 112-122.
- Leak, S.G.A., 1999. *Tsetse Biology and Ecology. Their Role in the Epidemiology and Control of Trypanosomosis*. CABI Publishing, New York.
- Lee, R.E. Jr, Chen, C.-P., Denlinger, D.L., 1987. A rapid cold-hardening process in insects. *Science* 238, 1415-1417.
- Leegwater-van der Linden, M.E., 1984. Temperature and fertility in *Glossina pallidipes*. *Tropical and Geographical Medicine* 36, 391.
- Lehmann, F.-O., Dickinson, M.H., Staunton, J., 2000. The scaling of carbon dioxide release and respiratory water loss in flying fruit flies (*Drosophila* spp.). *Journal of Experimental Biology* 203, 1613-1624.

- Leroi, A.M., Bennett, A.F., Lenski, R.E., 1994. Temperature acclimation and competitive fitness: an experimental test of the Beneficial Acclimation Assumption. *Proceedings of the National Academy of Sciences of the USA* 91, 1917-1921.
- Macfie, J.W.S., 1912. Experiments and observations upon *Glossina palpalis*. *Bulletin of Entomological Research* 3, 61-72.
- MacKay, W.P., 1982. An altitudinal comparison of oxygen consumption rates in three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). *Physiological Zoology* 55, 367-377.
- Magiofoglou, A., Hoffmann, A.A., 2003. Cross-generation effects due to cold-exposure in *Drosophila serrata*. *Functional Ecology* 17, 664-672.
- Marais, E., Chown, S.L., 2003. Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. *Journal of Experimental Biology* 206, 4565-4574.
- Marron, M.T., Markow, T.A., Kain, K.J., Gibbs, A.G., 2003. Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. *Journal of Insect Physiology* 49, 261-270.
- Massad, E., Forattini, O.P., 1998. Modelling the temperature sensitivity of some physiological parameters of epidemiologic significance. *Ecosystem Health* 4, 119-129.
- Matos, M., Rose, M.R., Pite, M.T.R., Rego, C., Avelar, T., 2000. Adaptation to the laboratory environment in *Drosophila subobscura*. *Journal of Evolutionary Biology* 13, 9-19.
- McNab, B.K., 2003. Sample size and the estimation of physiological parameters in the field. *Functional Ecology* 17, 82-86.
- Mellanby, K., 1936. Experimental work with the tsetse fly *Glossina palpalis* in Uganda. *Bulletin of Entomological Research* 27, 611-633.
- Midgley, G.F., Hannah, L., Millar, D., Thuiller, W., Booth, A., 2003. Developing regional and species-level assessments of climate change impacts on biodiversity in the Cape Floristic Region. *Biological Conservation* 112, 87-97.
- Mousseau, T.A., Fox, C.W., 1998. The adaptive significance of maternal effects. *Trends in Ecology and Evolution* 13, 403-407.
- Mutika, G.N., Opiyo, E., Robinson, A.S., 2001. Assessing mating performance of male *Glossina pallidipes* (Diptera: Glossinidae) using a walk-in field cage. *Bulletin of Entomological Research* 91, 281-287.

- Nespolo, R.F., Lardies, M.A., Bozinovic, F., 2003. Intrapopulation variation in the standard metabolic rate of insects: repeatability, thermal dependence and sensitivity ( $Q_{10}$ ) of oxygen consumption in a cricket. *Journal of Experimental Biology* 206, 4309-4315.
- Nylin, S., Gotthard, K., 1998. Plasticity in life-history traits. *Annual Review of Entomology* 43, 63-83.
- Padilla, D.K., Adolph, S.C., 1996. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. *Evolutionary Ecology* 10, 105-117.
- Parsons, P.A., 1982. Evolutionary ecology of Australian *Drosophila*: a species analysis. *Evolutionary Biology* 14, 297-350.
- Pascual, M., Dobson, A., 2005. Seasonal patterns of infectious diseases. *PLoS Medicine* 2, 18-20.
- Patz, J.A., Graczyk, T.K., Geller, N., Vittor, A.Y., 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* 30, 1395-1405.
- Phelps, R.J., 1973. The effect of temperature on fat consumption during the puparial stages of *Glossina morsitans morsitans* (Diptera: Glossinidae) under laboratory conditions, and its implications in the field. *Bulletin of Entomological Research* 62, 423-438.
- Phelps, R.J., Burrows, P.M., 1969. Lethal temperature for puparia of *Glossina morsitans orientalis*. *Entomologia Experimentalis et Applicata* 22, 23-32.
- Phelps, R.J., Clarke, G.P.Y., 1974. Seasonal elimination of some classes in males of *Glossina morsitans morsitans* (Diptera: Glossinidae). *Bulletin of Entomological Research* 64, 313-324.
- Piersma, T., Drent, J., 2003. Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution* 18, 228-233.
- Price, T.D., Qvarnström, A., Irwin, D.E., 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London B* 270, 1433-1440.
- Queathem, E.J., Full, R.J., 1995. Variation in jump force production within an instar of the grasshopper *Schistocerca americana*. *Journal of Zoology* 235, 605-620.
- Rajagopal, P.K., Bursell, E., 1966. The respiratory metabolism of resting tsetse flies. *Journal of Insect Physiology* 12, 287-297.
- Reinouts van Haga, H.A., Mitchell, B.K., 1975. Temperature receptors on the tarsi of the tsetse fly *Glossina morsitans* West. *Nature* 255, 225-226.
- Rezende, E.L., Chappell, M.A., Hammond, K.A., 2004. Cold-acclimation in *Peromyscus*: temporal effects and individual variation in maximum metabolism and ventilatory traits. *Journal of Experimental Biology* 207, 295-305.

- Roberts, C.S., Seely, M.K., Ward, D., Mitchell, D., Campbell, J.D., 1991. Body temperatures of Namib Desert tenebrionid beetles: their relationship in laboratory and field. *Physiological Entomology* 16, 463-475.
- Robinson, T., Rogers, D., Williams, B., 1997a. Mapping tsetse habitat suitability in the common fly belt of Southern Africa using multivariate analysis of climate and remotely sensed vegetation data. *Medical and Veterinary Entomology* 11, 235-245.
- Robinson, T., Rogers, D., Williams, B., 1997b. Univariate analysis of tsetse habitat in the common fly belt of Southern Africa using climate and remotely sensed vegetation data. *Medical and Veterinary Entomology* 11, 223-234.
- Rogers, D.J., Packer, M.J., 1993. Vector-borne diseases, models, and global change. *Lancet* 342, 1282-1284.
- Rogers, D.J., Randolph, S.E., 1986. Distribution and abundance of tsetse flies (*Glossina* Spp.). *Journal of Animal Ecology* 55, 1007-1025.
- Rogers, D.J., Randolph, S.E., 1991. Mortality rates and population density of tsetse flies correlated with satellite imagery. *Nature* 351, 739-741.
- Rogers, D.J., Randolph, S.E., 2000. The global spread of malaria in a future, warmer world. *Science* 289, 1763-1766.
- Rogers, D.J., Robinson, T.P., 2004. Tsetse distribution. In: Maudlin, I., Holmes, P.H., Miles, M.A. (eds), *The Trypanosomiases*. CABI Publishing, Wallingford, pp. 139-180.
- Rogers, D.J., Williams, B.G., 1994. Tsetse distribution in Africa: seeing the wood *and* the trees. In: Edwards, P.J., May, R.M., Webb, N.R. (eds), *Large-Scale Ecology and Conservation Biology*. Blackwell Science, Oxford, pp. 247-271.
- Rouget, M., Richardson, D.M., Cowling, R.M., Lloyd, J.W., Lombard, A.T., 2003. Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region, South Africa. *Biological Conservation* 112, 63-85.
- Rourke, B.C., 2000. Geographic and altitudinal variation in water balance and metabolic rate in a California grasshopper, *Melanoplus sanguinipes*. *Journal of Experimental Biology* 203, 2699-2712.
- Schaus, J.M., Sakaluk, S.K., 2002. Repeatability of sperm number across multiple matings in three cricket species, *Gryllodes sigillatus*, *Gryllus veletis*, and *Gryllus texensis* (Orthoptera: Gryllidae). *Canadian Journal of Zoology* 80, 582-585.
- Schmidt-Nielsen, K., 1997. *Animal Physiology. Adaptation and Environment*, 5th edn. Cambridge University Press, Cambridge.

- Scholander, P.F., Flagg, W., Walters, V., Irving, L., 1953. Climatic adaptation in Arctic and Tropical poikilotherms. *Physiological Zoology* 26, 67-92.
- Schultz, T.D., Quinlan, M.C., Hadley, N.F., 1992. Preferred body temperature, metabolic physiology, and water balance of adult *Cicindela longilabris*: A comparison of populations from boreal habitats and climatic refugia. *Physiological Zoology* 65, 226-242.
- Sgrò, C.M., Partridge, L., 2000. Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *American Naturalist* 156, 341-353.
- Sgrò, C.M., Partridge, L., 2001. Laboratory adaptation of life history in *Drosophila*. *American Naturalist* 158, 657-658.
- Shaffer, L.R., Formanowicz, D.R., 2000. Sprint speeds of juvenile scorpions: Among family differences and parent offspring correlations. *Journal of Insect Behaviour* 13, 45-54.
- Shelton, T.G., Appel, A.G., 2001a. Carbon dioxide in *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* (Kollar): effects of caste, mass, and movement. *Journal of Insect Physiology* 47, 213-224.
- Shelton, T.G., Appel, A.G., 2001b. Cyclic CO<sub>2</sub> release and water loss in alates of the eastern subterranean termite (Isoptera: Rhinotermitidae). *Annals of the Entomological Society of America* 94, 420-426.
- Sibly, R.M., Atkinson, D., 1994. How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology* 8, 486-493.
- Sinclair, B.J., Chown, S.L., 2005. Climatic variability and hemispheric differences in insect cold tolerance: support from southern Africa. *Functional Ecology* 19, 214-221.
- Sinclair, B.J., Addo-Bediako, A., Chown, S.L., 2003a. Climatic variability and the evolution of insect freeze tolerance. *Biological Reviews* 78, 181-195.
- Sinclair, B.J., Klok, C.J., Scott, M.B., Terblanche, J.S., Chown, S.L., 2003b. Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. *Journal of Insect Physiology* 49, 1049-1061.
- Somero, G.N., 2005. Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Frontiers in Zoology* 2.
- Sømme, L., Block, W., 1991. Adaptations to alpine and polar environments. In: Lee, R.E., Denlinger, D.L. (eds), *Insects at Low Temperature*. Chapman and Hall, London, pp. 318-359.
- Spicer, J.I., Gaston, K.J., 1999. *Physiological Diversity and its Ecological Implications*. Blackwell Science, Oxford.

- Taylor, P., 1977a. A continuously recording respirometer, used to measure oxygen consumption and estimate locomotor activity in tsetse flies, *Glossina morsitans*. *Physiological Entomology* 2, 241-245.
- Taylor, P., 1977b. The respiratory metabolism of the tsetse (Diptera: Glossinidae) in relation to temperature, blood-meal size and the pregnancy cycle. *Physiological Entomology* 2, 317-322.
- Taylor, P., 1977c. The respiratory metabolism of tsetse flies, *Glossina* spp., in relation to temperature, blood-meal size and pregnancy cycle. *Physiological Entomology* 2, 317-322.
- Taylor, P., 1978a. Radioisotopes as metabolic labels for *Glossina* (Diptera: Glossinidae). I. Laboratory determination of the relationship between radioisotope metabolism, respiration and temperature. *Bulletin of Entomological Research* 68, 1-9.
- Taylor, P., 1978b. Radioisotopes as metabolic labels for *Glossina* (Diptera: Glossinidae). II. The excretion of  $^{137}\text{Cs}$  under field conditions as a means of estimating energy utilisation, activity and temperature regulation. *Bulletin of Entomological Research* 68, 331-340.
- Terblanche, J., Fahlman, A., Myburgh, K.H., Jackson, S., 2004. Measurement reliability of highly variable physiological responses to experimentally-manipulated gas fractions. *Physiological Measurement* 25, 1189-1197.
- Torr, S.J., Hargrove, J.W., 1999. Behaviour of tsetse (Diptera: Glossinidae) during the hot season in Zimbabwe: the interaction of micro-climate and reproductive status. *Bulletin of Entomological Research* 89, 365-379.
- van Dongen, S., 1998. How repeatable is the estimation of developmental stability by fluctuating asymmetry? *Proceedings of the Royal Society of London B* 265, 1423-1427.
- van Dongen, S., Lens, L., Molenberghs, G., 1999a. Mixture analysis of asymmetry: modelling directional asymmetry, antisymmetry and heterogeneity in fluctuating asymmetry. *Ecology Letters* 2, 387-396.
- van Dongen, S., Sprengers, E., Lofstetd, C., Matthysen, E., 1999b. Heritability of tibia fluctuating asymmetry and developmental instability in the winter moth (*Operophtera brumata* L.)(Lepidoptera, Geometridae). *Heredity* 82, 535-542.
- Watson, M.J.O., Hoffmann, A.A., 1995. Cross-generation effects for cold resistance in tropical populations of *Drosophila melanogaster* and *D. simulans*. *Australian Journal of Zoology* 43, 51-58.
- Watson, M.J.O., Hoffmann, A.A., 1996. Acclimation, cross-generation effects, and the response to selection for increased cold resistance in *Drosophila*. *Evolution* 50, 1182-1192.
- Welburn, S.C., Fevre, E., Coleman, P., 1999. Sleeping sickness rediscovered. *Parasitology Today* 15, 303-305.



- Whitlock, M., 1998. The repeatability of fluctuating asymmetry: a revision and extension. *Proceedings of the Royal Society of London B* 265, 1429-1431.
- WHO, 2001. Fact Sheet No. 259: African trypanosomes or sleeping sickness. World Health Organization, pp. 1-4.
- WHO, 2004. The World health report: 2004: changing history. World Health Organization, Geneva, p. 120.
- Williams, A.E., Rose, M.R., Bradley, T.J., 1998. Using laboratory selection for desiccation resistance to examine the relationship between respiratory pattern and water loss in insects. *Journal of Experimental Biology* 201, 2945-2952.
- Wilson, R.S., 2001. Geographic variation in thermal sensitivity of jumping performance in the frog *Limnodynastes peronii*. *Journal of Experimental Biology* 204, 4227-4236.
- Wilson, R.S., Franklin, C.E., 2002. Testing the beneficial acclimation hypothesis. *Trends in Ecology & Evolution* 17, 66-70.
- Woods, H.A., 1999. Patterns and mechanisms of growth of fifth-instar *Manduca sexta* caterpillars following exposure to low- or high-protein food during early instars. *Physiological and Biochemical Zoology* 72, 445-454.
- Woods, H.A., Harrison, J.F., 2001. The beneficial acclimation hypothesis versus acclimation of specific traits: physiological change in water-stressed *Manduca sexta* caterpillars. *Physiological and Biochemical Zoology* 74, 32-44.
- Woods, H.A., Harrison, J.F., 2002. Interpreting rejections of the beneficial acclimation hypothesis: when is physiological plasticity adaptive? *Evolution* 56, 1863-1866.
- Woods, R.E., Sgrò, C.M., Hercus, M.J., Hoffmann, A.A., 1999. The association between fluctuating asymmetry, trait variability, trait heritability, and stress: a multiply replicated experiment on combined stresses in *Drosophila melanogaster*. *Evolution* 53, 493-505.
- Zachariassen, K.E., Anderson, J., Maloiy, G.M.O., Kamau, J.M.Z., 1987. Transpiratory water loss and metabolism of beetles from arid areas in East Africa. *Comparative Biochemistry and Physiology A* 86, 403-408.
- Zamudio, K.R., Huey, R.B., Crill, W.D., 1995. Bigger isn't always better: body size, temperature and male territorial success in *Drosophila melanogaster*. *Animal Behaviour* 49, 671-677.
- Zdarek, J., Denlinger, D.L., Otieno, L.H., 1992. Does the tsetse parturition rhythm have a circadian basis? *Physiological Entomology* 17, 305-307.



- Zdarek, J., Denlinger, D.L., 1995. Changes in temperature, not photoperiod, control the pattern of adult eclosion in the tsetse, *Glossina morsitans*. *Physiological Entomology* 20, 362-366.
- Zeilstra, I., Fischer, K., 2005. Cold tolerance in relation to developmental and adult temperature in a butterfly. *Physiological Entomology* 30, 92-95.
- Zhou, G.F., Minakawa, N., Githeko, A.K., Yan, G.Y., 2005. Climate variability and malaria epidemics in the highlands of East Africa. *Trends in Parasitology* 21, 54-56.

## Chapter 2

### Metabolic rate variation in *Glossina pallidipes* (Diptera: Glossinidae): gender, ageing and repeatability\*

*"There must be some way out of here," said the joker to the thief,  
There's too much confusion, I can't get no relief.  
Businessmen, they drink my wine, plowmen dig my earth,  
None of them along the line know what any of it is worth."*

**Bob Dylan**

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## Introduction

Metabolic rate is one of the most significant characteristics of organisms. It provides an estimate of the costs of living, and can therefore provide insight into the evolution of life histories across a range of environments (Weiner, 1992; Lighton, 1996; Chown & Gaston, 1999). For this reason, and because estimates of metabolic rate can be readily obtained there has been considerable attention paid to metabolic rate variation in animals for many years (Buck, 1962; Keister & Buck, 1964; Addo-Bediako et al., 2002). Much of this interest has concerned the allometry of, and effects of temperature on metabolic rate (Keister & Buck, 1964; Peters, 1983; Schmidt-Nielsen, 1984; Cossins & Bowler, 1987). Indeed, with the development of nutrient supply network models that purport not only to explain the scaling of metabolic rates from cellular to whole-organismal levels (West et al., 1997, 1999), but also to show how the mass- and temperature-dependence of metabolic rates can fundamentally influence the distribution of diversity (Gillooly et al., 2001; Allen et al., 2002), there has been a resurgence of interest in the causes of metabolic rate variation.

Although the major causes of metabolic rate variation in insects are activity, size, and temperature, metabolic rate varies for other reasons too. Age, gender, feeding status, season, and time of day can have an influence on metabolic rate (Denlinger et al., 1972; Taylor, 1977a; McEvoy, 1984; Takahashi-Del-Bianco et al., 1992; Bennett et al., 1999; Davis et al., 2000; Rogowitz & Chappell, 2000). However, the extent to which these intrinsic and extrinsic factors have an influence on metabolic rate has not been extensively explored for a wide variety of insects, despite its obvious importance (see Spicer & Gaston, 1999 for further discussion).

Metabolic rate is also thought to vary in an adaptive fashion over larger scales in two ways. First, in many insects, species or populations from colder environments show elevated metabolic rates relative to those from warmer environments at the same temperature. This conservation of rate is thought to be important to enable insects to complete growth, development and reproduction at comparatively low temperatures (Chown & Gaston, 1999; Addo-Bediako et al., 2002). On the other hand, metabolic rate can be lowered as an adaptive response to dry conditions in xeric species (Edney, 1977; Juliano, 1986; Hoffmann & Parsons, 1991; Davis et al., 2000). A reduction in metabolic rate is thought to contribute to the conservation of water because at least some water is lost from the spiracles during gas exchange (Lighton, 1994; Chown, 2002). In both cases the alteration in metabolic rate is assumed to be adaptive, although thorough demonstrations that natural selection has been responsible for the change, by either comparative or laboratory selection methods, are often not provided (though this increasingly being done for *Drosophila* – see Hoffmann & Parsons, 1989a, b; Gibbs et al., 1997,

2003; Gibbs & Matzkin, 2001; Gibbs, 2002). Indeed, it has been suggested by several authors (e.g. Lighton, 1998; Chown, 2001; Marais & Chown, 2003) that there is considerable scope not only for improving demonstrations of adaptive variation for a range of species, but also for demonstrating that the prerequisites for selection, such as repeatability, heritability and a consistent relationship between the trait of interest and fitness (Endler, 1986), are met. Repeatability of metabolic rate in particular, is relatively straightforward to ascertain and has been thoroughly examined in several vertebrates (e.g. Chappell et al., 1996; Bech et al., 1999). However, it has been studied in only a handful of insect species, including a lepidopteran (Buck & Keister, 1955), two boring beetles (Chappell & Rogowitz, 2000), an orthopteran (Nespolo et al., 2003), and a highly variable montane cockroach (Marais & Chown, 2003).

In this study, I therefore examine variation in metabolic rate associated with gender, age, feeding-status, and pregnancy, and the variation of the repeatability of metabolic rate with age and gender in the tsetse fly, *Glossina pallidipes*. This species was chosen for several reasons, of which three are most significant. First, owing to their status as vectors of trypanosomes, which are exclusively responsible for human and animal trypanosomiasis (Leak, 1999), there is much interest in understanding the factors influencing the distribution and abundance of tsetse (Rogers & Randolph, 1986, 1991; Rogers & Williams, 1994; Robinson et al., 1997a, b). Metabolic rates are important in this regard because they are used to estimate daily energy budgets (Bursell & Taylor, 1980), which subsequently provide information regarding daily flight time and thus both dispersal and survival capabilities, so influencing demography and fitness. Thus, understanding variation in metabolic rate in tsetse could provide insight into the ways in which such temperature variation influences mortality, and therefore affects abundance and determines range limits. However, before this is done, an understanding of the intrinsic factors influencing variation in metabolic rate is required. This can be readily illustrated by considering the repeatability of metabolic rate. If metabolic rate is not repeatable (i.e. there is greater within- than among- individual variation) then it is unlikely that variation in metabolic rate would translate into variation in fitness (for further discussion see Endler, 1986; Bech et al., 1999). In consequence, tsetse would be unable to respond to selection for a change in metabolic rate, and therefore the relationship between metabolic rate variation and adult mortality would be invariant between populations. Alternatively, if metabolic rate is repeatable, then selection could act on this trait, meaning either that a populations' response to selection could take place, or that, if it did not, other factors, such as gene flow should be sought to explain the lack of response (Case & Taper, 2000). Comprehending intrinsic variation in metabolic rate in tsetse is particularly important because previous

investigations have suggested that feeding status, age and reproductive status are important contributors to variation in metabolic rate in these flies (Rajagopal & Bursell, 1966; Taylor, 1977a).

Second, whilst estimates of metabolic rate in various tsetse species have been made previously, these have relied on two methods, both of which require additional verification. In the first instance, closed system respirometry has been used to investigate metabolic rates in laboratory-reared *Glossina morsitans* pupae (Rajagopal & Bursell, 1965) and in both wild-caught and laboratory-reared *G. morsitans* adults (Rajagopal & Bursell, 1966; Taylor, 1977a, b), and in wild-caught *G. pallidipes* adults (Taylor, 1977a). Whilst closed system methods are intrinsically accurate (Sláma, 1984), in all but the most quiescent of stages it is difficult to distinguish activity metabolism from standard metabolic rate owing mostly to the poor temporal resolution and the integrative nature of the technique (Lighton & Fielden, 1995). In consequence, it is often difficult to make comparisons between groups and to ascertain the nature of both intrinsic and extrinsic variation in metabolic rates. In the second instance, although radioisotopic cesium decay-rate methods might at present be the only a reliable means of estimating field metabolic rates for tsetse (Taylor 1978a, b, though see Wolf et al., 1996), they also make several assumptions. Requirements for re-capture may make the method prone to trapping bias (Hargrove, 1999), and changes in environmental temperature, which may affect excretion rates of radioisotopes from metabolic pathways in a non-linear fashion (Hargrove & Coates, 1990) might also confound estimates. By contrast, flow-through respirometry techniques, which cannot readily be applied to the field (though see Lighton & Duncan, 2002), overcome many of these problems. Thus, it is essential to verify estimates of metabolic rate that have been made previously using closed system techniques.

Finally, metabolic rate estimates for flies are not as common as those for beetles and hymenopterans (Chown et al., 2002). Bias in the numbers of species from different taxa (or of different body sizes) might have considerable consequences for estimates of the allometry of metabolic rate (Duncan et al., 2002), and this is likely to be especially true if significant variation in metabolic rate is partitioned at the order level, which it is (Addo-Bediako et al., 2002). In consequence, adding well-verified data to that available for poorly represented insects is important.

## Materials and methods

All experiments were performed on *Glossina pallidipes*. Flies were received as pupae, shipped within one week of larviposition, from large, well-established colonies in the Seibersdorf Entomology Unit, IAEA, Austria. These colonies have been maintained in the laboratory for approximately 25

years, and were originally derived from flies caught in Tororo, Uganda. Genetic variation is similar in laboratory-reared and wild-type flies (Krafsur & Wohlford, 1999), and the laboratory-reared flies are considered representative of flies from field populations.

On their arrival at the laboratory, pupae were transferred to cages ( $n = 10$  cages,  $\leq 20$  pupae per cage) for emergence. Eclosion levels were high ( $>90\%$ ) and after emergence from pupae, teneral flies were sorted into cages representative of experimental categories. The flies were maintained in the laboratory in climate chambers regulated at  $24 \pm 1^\circ\text{C}$  with a 12L:12D photoperiod. Cage locations within climate chambers were randomised daily. The flies were fed every alternative day on screened bovine blood (supplied by Onderstepoort Veterinary Institute, South Africa) using pre-heated trays covered with silicone membranes, until experiments were completed (Gooding et al., 1997). Animals were given 20 minutes to feed, after which cages were removed from the membranes and replaced in the climate chamber. Feeding took place at approximately the same time of day (09h00) on each occasion. The few flies that did not feed were replaced with equal age/gender animals from surplus stocks of the same batch of flies. On feeding days, one hour passed after feeding was completed, to allow for the excretion of excess fluid from the blood-meal, before flies were transferred into cuvettes for respirometry.

The experimental categories were males, virgin females, and pregnant females, of various age and feeding classes (Table 1). For each category, seven individuals were weighed (to 0.01 mg, on a Mettler Toledo AX504 (Columbus, OH, USA), electronic microbalance), and each placed in a 5 ml cuvette. The cuvettes were connected to a Sable Systems (Sable Systems, Las Vegas, Nevada) eight-channel multiplexer (channel 8 was used for baseline measurements) located inside a darkened Sable Systems PTC1 Peltier-controlled temperature cabinet which maintained a constant temperature of  $24 \pm 0.2^\circ\text{C}$ . The flies were allowed to settle for 20 minutes, after which respirometry commenced. A Sable Systems flow-through  $\text{CO}_2$  respirometry system was used to measure  $\text{CO}_2$  production and its variation. Synthetic air (21%  $\text{O}_2$ , balance  $\text{N}_2$ ) was passed through sodalime, silica gel and Drierite columns to remove  $\text{CO}_2$  and  $\text{H}_2\text{O}$  residues. The flow rate of the scrubbed air was regulated to  $100\text{ml}\cdot\text{min}^{-1}$  with a mass flow controller. To prevent excessive desiccation during the respirometry recordings the airflow from the mass flow controller was connected to a LiCor 610 dew point generator to rehumidify the scrubbed air at  $18^\circ\text{C}$  dew point (equivalent to  $\sim 70\%$  R.H. at  $24^\circ\text{C}$ ). The dew point generator was connected to the master inlet of the multiplexer. The multiplexer was programmed using DATACAN V to sample respiration of each fly (from channel 1 to 7) for 12 minutes during an 84 minute recording session and channel 8 took baseline measurements at both the start and end of each recording session.

**Table 1.** Age and feeding classes for males and females as used in the repeated metabolic rate recordings for *G. pallidipes* adults

Age (hours)	24 H	48 H	96 H	120 H	168 H	192 H
Feeding status	(unfed)	(1 <sup>st</sup> feed)	(starved)	(3 <sup>rd</sup> feed)	(starved)	(5 <sup>th</sup> feed)
Females (Virgin)	X	X	X	X		
Females (Mated)					X	X
Males	X	X	X	X		

The non-active cuvettes were flushed with the same rehumidified air to prevent the build-up of excessive CO<sub>2</sub> inside the cuvettes (flow rate = 100 ml.min<sup>-1</sup>). The active cuvette's airflow was directed through the multiplexer's master outlet to a LiCor 6262 CO<sub>2</sub>/H<sub>2</sub>O infra-red gas analyzer. Cuvette 1 was housed in a Sable Systems AD1 activity detector to record all motor activity during the respirometry recording. This ensured that sections of the recording showing increased CO<sub>2</sub> production associated with activity could be detected and discarded. Similar CO<sub>2</sub> patterns recorded for the other cuvettes were also discarded from the analyses to ensure that only CO<sub>2</sub> recordings representing standard metabolic rate were used. The LiCor gas analyzer and other peripheral equipment were connected with a Sable Systems Universal Interface to a desktop computer using DATACAN V software for data capture and control of the respirometry system. DATACAN V was programmed to automatically repeat the recording five times. The entire system was stored within an air-conditioned laboratory that held temperature at 24 ± 1°C. At the end of each experiment, flies were weighed, transferred to individual, labelled cages and returned to the climate chamber.

On completion of the experiments, flies were dried at 60°C to constant mass (< 72 h) and weighed to 0.01 mg (dry mass). Solvent-based lipid extractions were performed using a chloroform:methanol (2:1) solution. The flies were perforated gently to allow successful penetration of the solvent solution to all tissues. Care was taken not to lose any of the fly parts. After three solvent washes (approximately 24 h per wash), the flies' remains were dried at 60°C for < 48 h to constant mass and weighed to estimate lipid-free dry mass.

Metabolic rate data were extracted from the recorded data files using DATACAN V. To investigate metabolic rate variation, analyses of variance (ANOVA) and analyses of covariance (ANCOVA), with body mass as the covariate, were used (Zar, 1996). Data are presented as means ± standard error unless otherwise stated, and significance was set at 0.05. Repeatability and its confidence limits were calculated using the intra-class correlation coefficient approach (Lessells &

Boag, 1987; Krebs, 1999) within the specified experimental categories. Repeatability of standard metabolic rate (SMR) was calculated from the repeated tests on each day, for females and males separately, and for each of the various age classes.

## Results

Metabolic rate data were only extracted for resting or 'quiet' intervals across all flies, by comparison with the activity of the fly recorded in cuvette 1. Summary statistics for body mass, SMR expressed as CO<sub>2</sub> production (mlCO<sub>2</sub>.hr<sup>-1</sup>) and re-calculated as microwatts using an R.Q. = 0.84 (owing to no change in lipid mass (see below) and protein metabolism documented by Bursell & Taylor (1980)), for the different ages, genders and feeding status of *G. pallidipes* adults are presented in Table 2.

For flies up to 120 H only (i.e. excluding mated females), standard metabolic rate varied significantly with age and feeding-status in *G. pallidipes* adult males and females (ANCOVA  $F_{(3, 46)} = 20.86$ ,  $P < 0.01$ ; Fig. 1). However, there was no difference in metabolic rate between the genders (ANCOVA  $F_{(1, 46)} = 0.8$ ,  $P > 0.4$ ), and no interaction between age and gender (ANCOVA  $F_{(3; 46)} = 0.5787$ ,  $P > 0.6$ ). In all cases, fresh mass was a better predictor of metabolic rate than dry mass (multiple regression of metabolic rate against fresh mass,  $F_{(1, 53)} = 69.98$ ,  $r^2 = 0.5490$ ,  $P < 0.0001$ ; when using dry mass,  $F_{(1, 53)} = 39.13$ ,  $r^2 = 0.4247$ ,  $P < 0.0001$ ). In the females, there was a significant effect of age and mating status on metabolic rate, which showed a continuous increase late into the first pregnancy cycle (ANCOVA  $F_{(5, 33)} = 19.58$ ,  $P < 0.01$ ; Fig. 1).

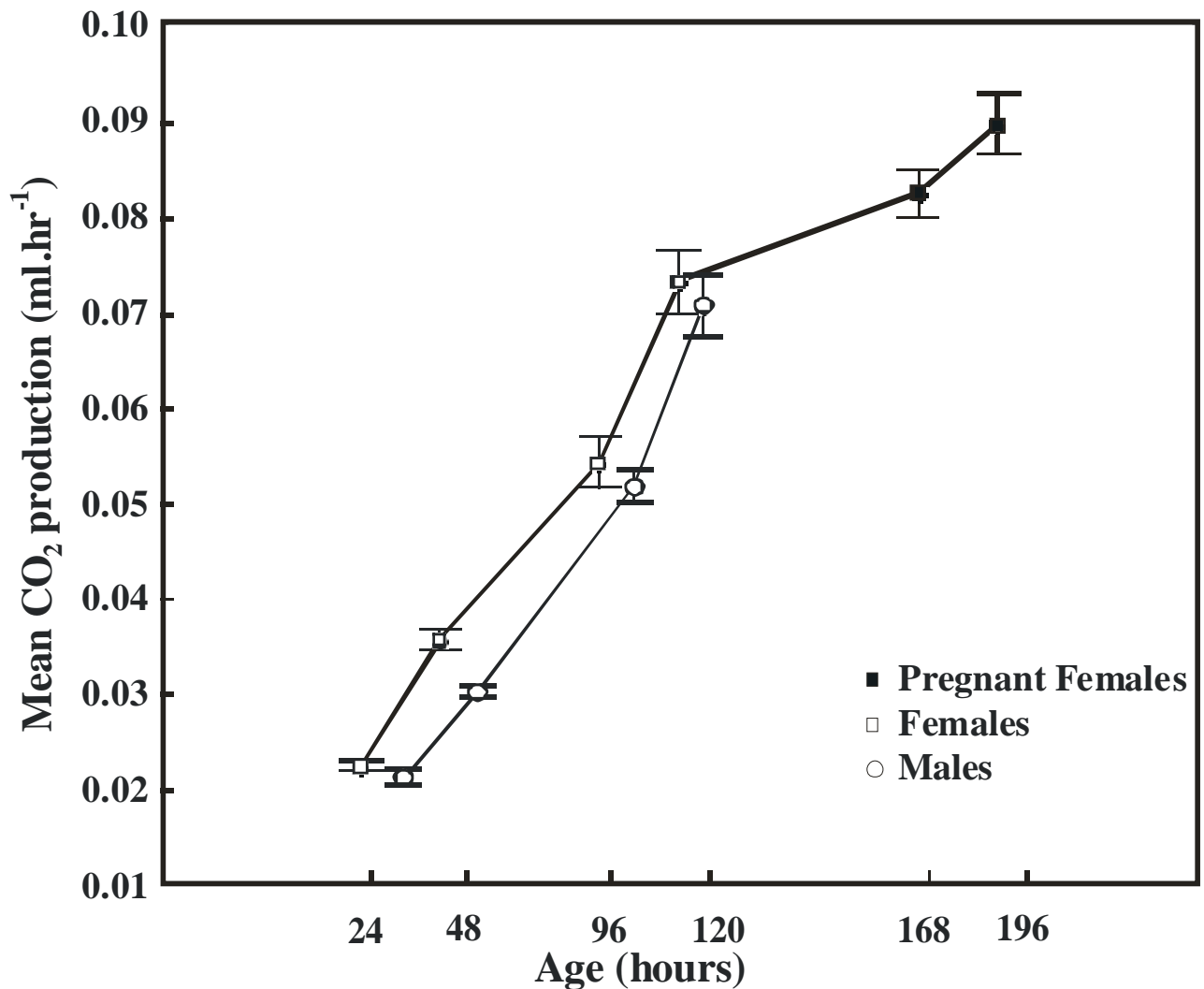
There was a significant increase in dry mass with age in males (24 H – 120 H) ( $F_{(3, 24)} = 6.72$ ,  $P < 0.01$ ) and females (24 H – 192 H) ( $F_{(3, 34)} = 6.46$ ,  $P < 0.01$ ). By contrast, there was no significant effect of age on lipid mass ( $F_{(3, 46)} = 0.39$ ,  $P > 0.76$ ), although females contained more lipids than males ( $F_{(1; 46)} = 12.66$ ,  $P < 0.01$ ). There was no interaction between gender and age ( $F_{(3, 46)} = 0.257$ ,  $P > 0.86$ ).

In all of the experimental categories investigated, except the 48 H female group, repeatability of standard metabolic rate was high and significant (Fig. 2; Table 3). In both genders, repeatability increased with age, and feeding appeared to have little effect on the repeatability of metabolic rate (Fig. 2). Repeatability declined slightly, but non-significantly, in the mated females at 168 H and 192 H.



**Table 2.** Summary statistics (means  $\pm$  S.E.) of body size, and standard metabolic rate expressed as CO<sub>2</sub> production (ml CO<sub>2</sub>.h<sup>-1</sup>) and re-calculated as microwatts (using an R.Q. = 0.84) for different ages, genders and feeding status of *G. pallidipes* adults. All measurements made at 24°C and 70% R.H. in complete darkness.

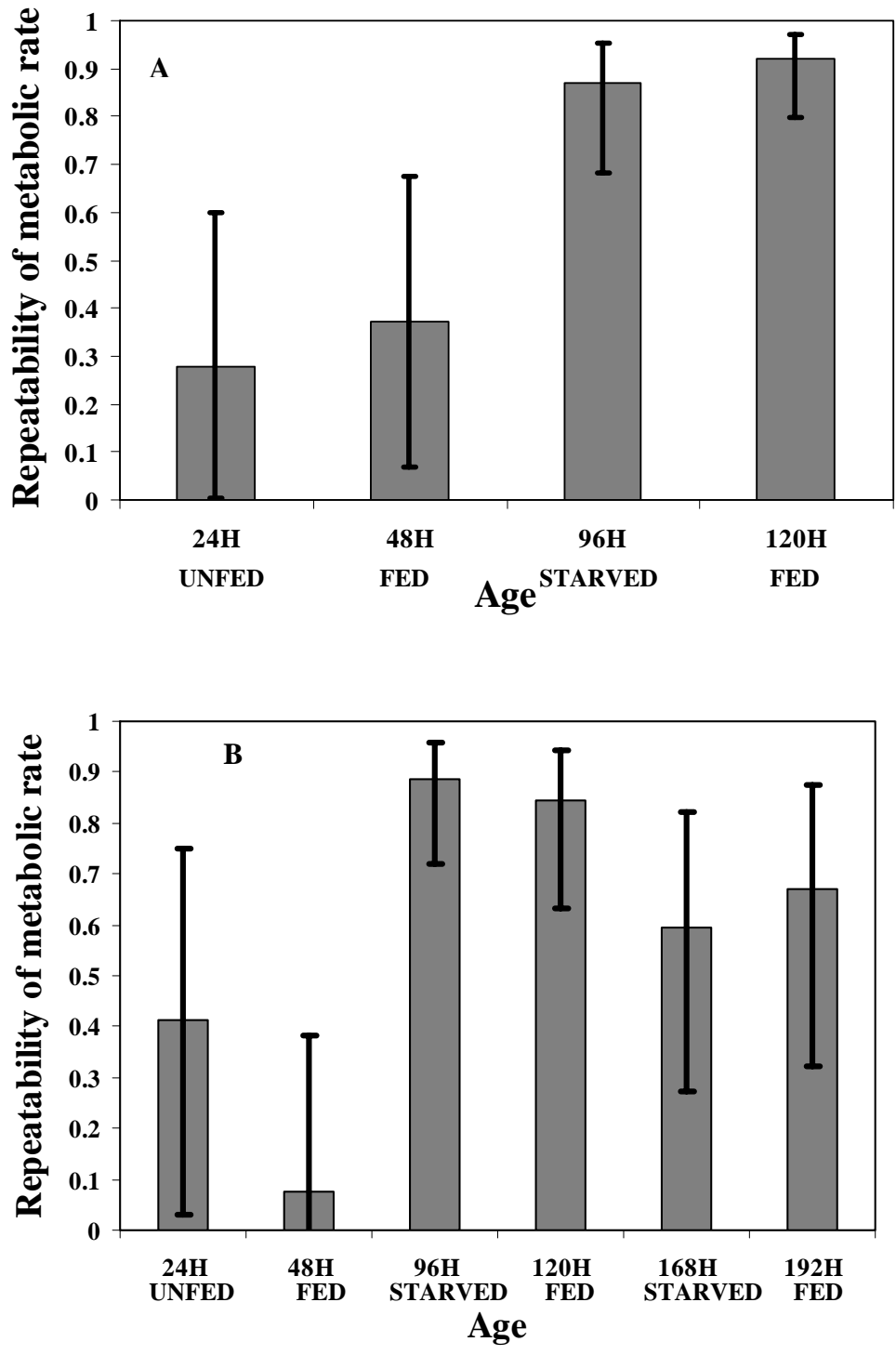
Class	Body size (mg)	Metabolic rate (ml CO <sub>2</sub> .h <sup>-1</sup> )	Metabolic rate ( $\mu$ W)	N
<b>Adult males</b>				
24 H (unfed)	23.16 $\pm$ 0.91	0.0214 $\pm$ 0.0012	143.9 $\pm$ 21.2	7
48 H (1 <sup>st</sup> feed)	39.49 $\pm$ 2.72	0.0303 $\pm$ 0.0009	203.2 $\pm$ 16.4	7
96 H (starved)	41.49 $\pm$ 2.30	0.0515 $\pm$ 0.0037	345.5 $\pm$ 66.0	7
120 H (3 <sup>rd</sup> feed)	53.07 $\pm$ 2.06	0.0701 $\pm$ 0.0073	471.0 $\pm$ 130.2	7
<b>Adult females</b>				
<b>Virgins</b>				
24 H (unfed)	26.72 $\pm$ 0.96	0.0224 $\pm$ 0.0010	150.3 $\pm$ 15.8	6
48 H (1 <sup>st</sup> feed)	45.35 $\pm$ 1.67	0.0356 $\pm$ 0.0011	239.4 $\pm$ 19.7	7
96 H (starved)	35.41 $\pm$ 4.00	0.0525 $\pm$ 0.0061	352.6 $\pm$ 108.2	7
120 H (3 <sup>rd</sup> feed)	51.14 $\pm$ 3.99	0.0730 $\pm$ 0.0071	490.2 $\pm$ 125.9	7
<b>Mated</b>				
168 H (starved)	52.37 $\pm$ 1.75	0.0823 $\pm$ 0.0048	552.4 $\pm$ 32.1	7
192 H (5 <sup>th</sup> feed)	59.38 $\pm$ 2.09	0.0946 $\pm$ 0.0049	635.2 $\pm$ 32.7	6



**Figure 1.** Variation of standard metabolic rate with age in *G. pallidipes* adult males and females.

## Discussion

In *Glossina pallidipes* there was little effect of gender on metabolic rate, at least for the first five days of adult life. Thereafter, it was not so much gender that caused differences between males and females, but a combination of age and reproductive status of the females. As might be expected, given the additional cost of *in utero* larval development (Denlinger & Ma, 1974) metabolic rate continued to increase into pregnancy. These findings are in keeping with those for *G. morsitans*, where males and females show no difference in metabolic rate for the first two weeks of adult life (Rajagopal & Bursell, 1966), but metabolic rate varies through the pregnancy cycle (Taylor, 1977a; Denlinger et al., 1983). However, a comparison of virgin and pregnant flies is required to fully disentangle the effects of age and pregnancy on metabolic rate.



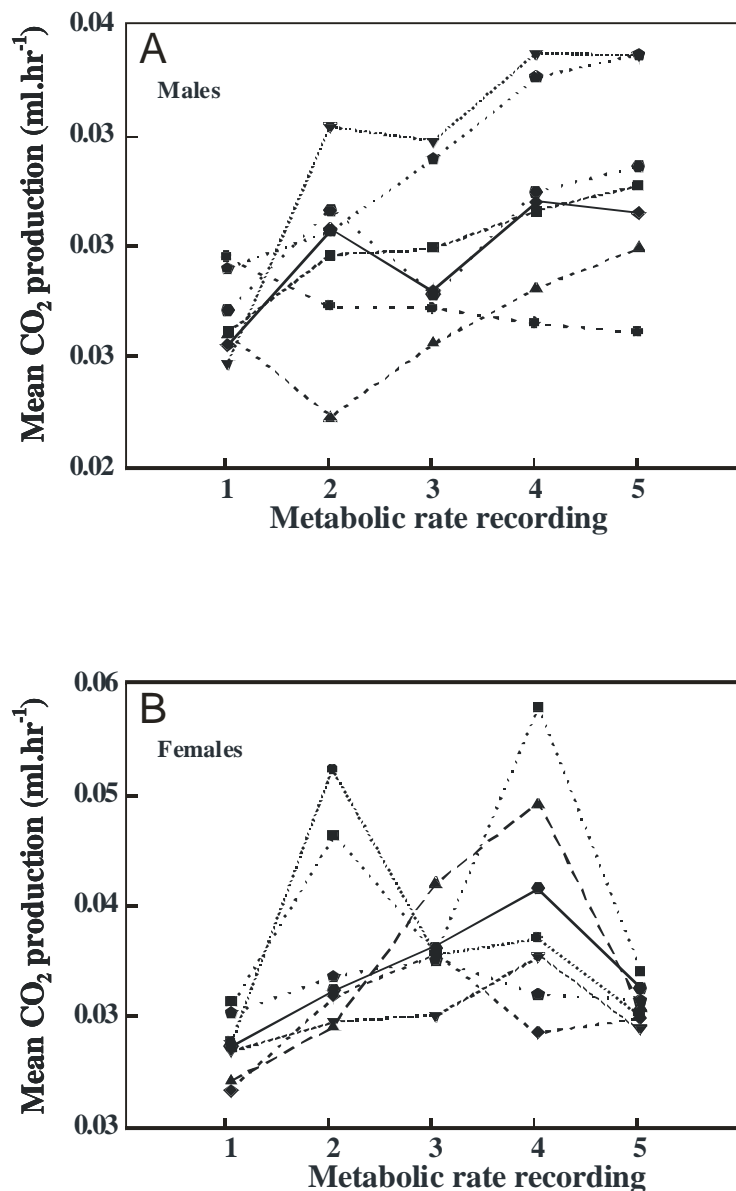
**Figure 2.** Repeatability (with upper and lower 95 % confidence limits) of standard metabolic rate for various experimental age and feeding classes in (a) male and (b) virgin and mated female *G. pallidipes*. In all cases, repeatability was both significant ( $P < 0.05$ ) and high, except in the case of 48 H females.

By contrast with gender, age had a significant effect on metabolic rate in *G. pallidipes*, even after body size differences were taken into account, with the mean CO<sub>2</sub> production increasing steadily for the first five days of adult life, in similar ways in both males and females. Increases in SMR with age are probably a result of increases in the ratio of flight muscle to body mass (Bursell, 1961, 1973; Hargrove, 1975; Marden, 2000) and the maturation of sexual reproductive organs, and of pregnancy in mated females. Age-related changes in SMR probably continue for about the first 8-10 days of adult life given that development occurs for approximately the same amount of time (Bursell & Kuwengwa, 1972). Certainly in this particular study, the absence of an increase in lipid reserves but an increase in overall mass suggests that the observed increases in metabolic rate were at least partly a consequence of increases in flight muscle mass (Bursell & Kuwengwa, 1972). Several authors have noted that the metabolic costs of maintaining flight muscle are high (Zera & Denno, 1997; Reinhold, 1999; Harrison & Roberts, 2000; Addo-Bediako et al., 2002), and this might also be true for *G. pallidipes*. Although specific dynamic action probably also contributed to increases in metabolic rate, it is unlikely to have been responsible for the ongoing elevation of rates in unfed flies. Of the energy obtained from a blood meal, only 10-19% is used in digestion (Taylor, 1977a, b; Bursell & Taylor, 1980), and Taylor (1977a, b) demonstrated that the peak of oxygen consumption in mature flies occurs at 5–10 hours post-feeding, rather than 25-35 hours later (the time at which unfed flies were measured here). Therefore, the effects of specific dynamic action alone could not have accounted for the substantial (> 70%) increase in metabolic rates of *G. pallidipes* with age (Table 2).

In this study, I found that the repeatability of metabolic rate generally increased with age. It was low in newly emerged flies, remained low after the first blood meal, and then increased substantially, only to decline again during pregnancy. It seems likely that low repeatability in young flies is a consequence of rapid temporal change in muscle morphology, and indeed, development is rapid post-eclosion (Bursell & Kuwengwa, 1972). This is also shown clearly in unfed *G. pallidipes* by the generally increasing trend in metabolic rate over the five repeats in each of these periods (Fig. 3). The small, but insignificant decline in repeatability with pregnancy supports this contention. Larval development is likely to take place rapidly (e.g. within nine days in *G. morsitans* – Denlinger & Ma, 1974), and therefore metabolic rates are likely to be somewhat more variable within individuals over time. Few investigations of age-related variation in the repeatability of metabolic rate have been undertaken generally, and none in insects. Nonetheless, the fact that metabolic rate is generally highly repeatable in older flies suggests that it is characteristic of metabolic rates in *G. pallidipes*, as is the case in the few other insects that have been investigated (Rogowitz & Chappell, 2000; Marais &

Chown, 2003; Nespolo et al., 2003). Significant and high repeatability of metabolic rate suggests that selection could act on it. Therefore, variation in metabolic rate might be one mechanism through which differences in the environmental response of tsetse species and populations have arisen, so resulting in pronounced differences in geographic distribution (Rogers & Randolph, 1991; Rogers & Williams, 1994; Robinson et al., 1997a, b). However, simply documenting repeatability in metabolic rate is not sufficient evidence to support this proposed causal chain. Rather, what is also required are substantial data on metabolic rate variation in different species and populations of tsetse from the field, in association with both genetic markers and georeferenced climatic data (see e.g. Bettencourt et al., 2002; Hoffmann et al., 2003). That metabolic rate is repeatable makes the collection of these data both more straightforward and more meaningful.

The metabolic rates I measured for inactive *G. pallidipes* were, in general, comparable to those measured for other tsetse species, particularly once the effects of size had been taken into account (Fig. 4). Comparison with the estimates made by Taylor (1977a) of metabolic rate in laboratory-reared and wild-caught *G. pallidipes* suggests that my values are slightly (~22 %) lower, as might be expected for open vs. closed system respirometry. Nonetheless, the differences were not large, providing confidence in previous metabolic rate estimates for tsetse made using closed system techniques (Rajagopal & Bursell, 1966; Taylor, 1977a, b). Moreover, my estimates of standard metabolic rate for *G. pallidipes* and those for flies active in the field also differ by approximately 22 % (Taylor, 1978a, b). This is in keeping with Taylor's (1978a) suggestion that flies are only likely to be active for a few minutes a day.



**Figure 3.** Changes in metabolic rate of individual, 24-hour old (unfed) flies during five consecutive recordings of (a) males and (b) females.

These data also support the idea that fly metabolic rates scale interspecifically with an exponent that cannot be distinguished from 0.75 ( $t_{(11)} = 1.754$ ;  $P > 0.05$ ), and in a way similar to that found for most insects (Lighton & Fielden's (1995) consensus scaling relationship for arthropods is  $SMR (\mu W) = 906M^{0.825}$  (mass in g)) (Table 4). Lighton & Fielden (1995) argued that the slope of the consensus scaling relationship for arthropods differs from the 0.75 value that has previously been proposed, and that is once again being widely supported on theoretical grounds (West et al., 1997, 1999). However, the relationship I derived for flies differs statistically from neither value, and Duncan et al. (2002)

suggested that the consensus scaling relationship derived by Lighton & Fielden (1995) could be biased by an absence of data on taxa other than ants and beetles. Therefore, it seems likely that additional data on other insect orders could well bring the slope of the insect metabolic rate-size relationship closer to a value of 0.75 than the previous relationships provided by Lighton & Fielden (1995) and Lighton et al. (2001) have indicated. For the moment, the jury will have to remain out on this matter, especially because so few of the studies have specifically set out to investigate scaling of metabolic rate, and therefore are confounded by specific dynamic action, activity, and other potential sources of variation.

Be that as it may, my estimates of resting metabolic rate for *G. pallidipes* and those obtained for other Diptera suggest that whilst the scaling of metabolic rate in flies and other insects might be similar, flies have considerably higher metabolic rates than other insects (Table 4). The intercept of the consensus scaling relationship for arthropods is 906  $\mu\text{W}$ , whilst for flies this value is 6987  $\mu\text{W}$ . Undoubtedly, this is due in part to the overestimates of metabolic rate associated with closed system respirometry, as comparisons of the intercepts of the scaling equations for ant and beetle metabolic rates derived using open and closed system data show (Table 4, see also Lighton & Fielden, 1995). However, my data on *G. pallidipes* also suggest that the difference between flies and other insects is not just an artefact of measurement technique. One reason for this difference may be that most ants and beetles investigated using flow through systems are flightless (e.g. most ant workers and many tenebrionid beetles, see Lighton & Fielden (1995) for data sources), whereas dipterans are generally very active and sophisticated fliers, and the maintenance of a flight machinery is metabolically costly (Zera & Denno, 1997; Reinhold, 1999; Harrison & Roberts, 2000). Alternatively, highly active flies might well have a higher resting metabolic rate than winged beetles owing to the metabolic rate trade-offs that are likely to be associated with maintenance of high activity levels (Reinhold, 1999). Clearly, there is much scope for more careful comparison of the slopes and intercepts of the scaling of metabolic rate in insects.

**Table 4.** Statistics for the mass scaling equations for insect metabolic rates in the form of  $SMR = a M^b$  (SMR in  $\mu W$  and M in g) indicating the differences in metabolic rate and relationships with size. I distinguish clearly between alate and apterous species and metabolic rate data derived using flow-through or closed system techniques.

Taxon	Intercept $\pm$ SE	Slope $\pm$ SE	F <sub>(df)</sub>	P	Adjusted r <sup>2</sup>	Respirometry
<i>Interspecific mass-scaling</i>						
<i>Apterous insects</i>						
Coleoptera	891 $\pm$ 1.3	0.899 $\pm$ 0.204	19.5 <sub>(1, 13)</sub>	<0.0007	0.569	Flow-through
Formicidae	982 $\pm$ 4.5	0.830 $\pm$ 0.360	5.3 <sub>(1, 8)</sub>	0.0503	0.323	Flow-through
Coleoptera	1059 $\pm$ 1.3	0.494 $\pm$ 0.077	40.6 <sub>(1, 33)</sub>	<0.0001	0.538	Closed system
Formicidae	6452 $\pm$ 1.8	0.961 $\pm$ 0.087	121.4 <sub>(1, 31)</sub>	<0.0001	0.797	Closed system
<i>Alate insects</i>						
Odonata	4436 $\pm$ 1.4	0.734 $\pm$ 0.202	13.2 <sub>(1, 11)</sub>	<0.004	0.504	Closed system
Coleoptera	743 $\pm$ 1.2	0.641 $\pm$ 0.141	20.7 <sub>(1, 4)</sub>	<0.0104	0.800	Flow-through
Coleoptera	1560 $\pm$ 1.1	0.725 $\pm$ 0.041	311.4 <sub>(1, 57)</sub>	<0.0001	0.843	Closed system
Lepidoptera	2377 $\pm$ 1.1	0.802 $\pm$ 0.053	226.2 <sub>(1, 35)</sub>	<0.0001	0.862	Closed system
Diptera	8472 $\pm$ 1.7	0.899 $\pm$ 0.156	33.4 <sub>(1, 6)</sub>	<0.0012	0.822	Closed system
Diptera including <i>G. pallidipes</i> * and <i>G. morsitans</i> †	6987 $\pm$ 1.6	0.840 $\pm$ 0.124	45.6 <sub>(1, 11)</sub>	<0.0001	0.788	Mixed
<i>Intraspecific mass-scaling of G. pallidipes</i>						
	25119 $\pm$ 3.2	1.386 $\pm$ 0.350	15.7 <sub>(1, 6)</sub>	<0.0075	0.677	Flow-through

Data from Addo-Bediako et al. (2002).

\* *G. pallidipes* this study (flow-through respirometry) and Taylor (1978a) (cesium excretion method)

† *G. morsitans* Rajagopal and Bursell (1966) (closed system respirometry) and Taylor (1978a) (cesium excretion method)



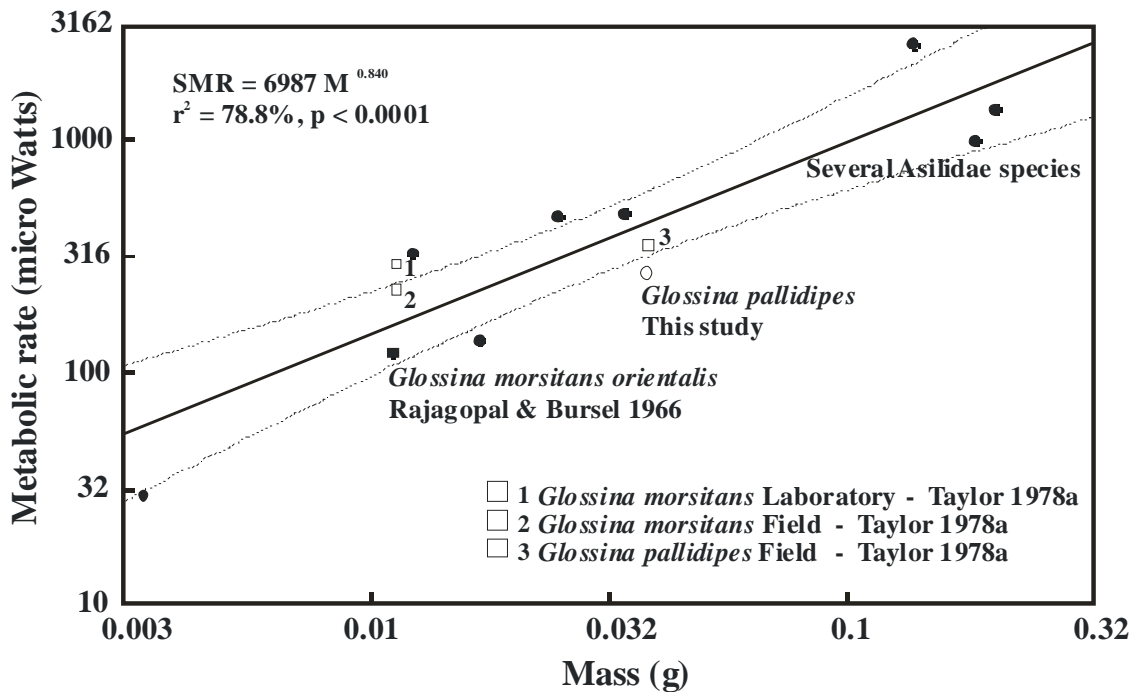


Fig. 4. Mass scaling regression plot for various fly species including metabolic rate data points for *Glossina pallidipes* and *G. morsitans* from several studies.

In conclusion, I have shown that in *G. pallidipes* metabolic rate varies with age but not gender, and is, in general, highly repeatable except in young, developing flies. Therefore, there is merit in examining variation in metabolic rate in different species and populations as a means to understanding the relationships between environmental variation and the distribution and abundance of tsetse species.

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## References

- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology* 16, 332-338.
- Allen, A.P., Brown, J.H., Gillooly, J., 2002. Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science* 297, 1545-1548.
- Bech, C., Langseth, I., Gabrielsen, G.W., 1999. Repeatability of basal metabolism in breeding female kittiwakes *Rissa tridactyla*. *Proceedings of the Royal Society of London B* 266, 2161-2167.
- Bennett, V.A., Kukal, O., Lee, R.E., 1999. Metabolic opportunists: feeding and temperature influence the rate and pattern of respiration in the high Arctic woollybear caterpillar *Gynaephora groenlandica* (Lymantriidae). *Journal of Experimental Biology*. 202, 47-53.
- Bettencourt, B.R., Kim, I., Hoffmann, A.A., Feder, M.E., 2002. Response to natural and laboratory selection at the *Drosophila HSP70* genes. *Evolution* 56, 1796-1801.
- Buck, J., 1962. Some physical aspects of insect respiration. *Annual Review of Entomology* 7, 27-56.
- Buck, J., Keister, M., 1955. Cyclic CO<sub>2</sub> release in diapausing *Agapema* pupae. *Biological Bulletin* 109, 144-163.
- Bursell, E., 1961. Post-teneral development of the thoracic musculature in tsetse flies. *Proceedings of the Royal Entomological Society of London A* 36, 69-74.
- Bursell, E. 1973. Development of mitochondrial and contractile components of the flight muscle in adult tsetse flies, *Glossina morsitans*. *Journal of Insect Physiology* 19, 1079-1086.
- Bursell, E., Kuwengwa, T., 1972. The effect of flight on the development of flight musculature in the tsetse fly (*Glossina morsitans*). *Entomologia Experimentalis et Applicata* 15, 229-237.
- Bursell, E., Taylor, P., 1980. An energy budget for *Glossina*. *Bulletin of Entomological Research* 70, 187-197.
- Case, T.J., Taper, M.L., 2000. Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. *American Naturalist* 155, 583-605.
- Chappell, M.A., Rogowitz, G.L., 2000. Mass, temperature and metabolic effects on discontinuous gas exchange cycles in *Eucalyptus*-boring beetles (Coleoptera: Cerambycidae). *Journal of Experimental Biology* 203, 3809-3820.
- Chappell, M.A., Zuk, M., Johnsen, T.S., 1996. Repeatability of aerobic performance in Red Junglefowl: effects of ontogeny and nematode infection. *Functional Ecology* 10, 578-585.
- Chown, S.L., 2001. Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* 47, 649-660.

- Chown, S.L., 2002. Respiratory water loss in insects. *Comparative Biochemistry and Physiology A* 133, 791-804.
- Chown, S.L., Addo-Bediako, A., Gaston, K.J., 2002. Physiological variation in insects: large-scale patterns and their implications. *Comparative Biochemistry and Physiology B* 131, 587-602.
- Chown, S.L., Gaston, K.J., 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews* 74, 87-120.
- Cossins, A.R., Bowler, K., 1987. *Temperature Biology of Animals*. Chapman and Hall, London.
- Davis, A.L.V., Chown, S.L., McGeoch, M.A., Scholtz, C.H., 2000. A comparative analysis of metabolic rate in six *Scarabaeus* species (Coleoptera: Scarabaeidae) from southern Africa: further caveats when inferring adaptation. *Journal of Insect Physiology* 46, 553-562.
- Denlinger, D. L., Ma, W.C., 1974. Dynamics of the pregnancy cycle in the tsetse *Glossina morsitans*. *Journal of Insect Physiology* 20, 1015-1026.
- Denlinger, D.L., Saini, R.K., Chaudhury, M.F.B., 1983. Parturition in the tsetse fly *Glossina morsitans*: pattern of activity, sound production and evidence for control by the mother's brain. *Journal of Insect Physiology* 29, 715-721.
- Denlinger, D.L., Willis, J.H., Fraenkel, G., 1972. Rates and cycles of oxygen consumption during pupal diapause in *Sarcophaga* flesh flies. *Journal of Insect Physiology* 18, 871-882.
- Duncan, F.D., Krasnov, B., McMaster, M., 2002. Metabolic rate and respiratory gas-exchange-patterns in tenebrionid beetles from the Negev Highlands, Israel. *Journal of Experimental Biology* 205, 791-798.
- Edney, E. B. 1977. *Water Balance in Land Arthropods*. Springer, Berlin.
- Endler, J.A., 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton.
- Gibbs, A.G., 2002. Water balance in desert *Drosophila*: lessons from non-charismatic microfauna. *Comparative Biochemistry and Physiology A* 133, 781-789.
- Gibbs, A.G., Chippindale, A.K., Rose, M.R., 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology* 200, 1821-1832.
- Gibbs, A.G., Fukuzato, F., Matzkin, L.M., 2003. Evolution of water conservation mechanisms in *Drosophila*. *Journal of Experimental Biology* 206, 1183-1192.
- Gibbs, A.G., Matzkin, L.M., 2001. Evolution of water balance in the genus *Drosophila*. *Journal of Experimental Biology* 204, 2331-2338.
- Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. *Science* 293, 2248-2251.

- Gooding, R.H., Feldmann, U., Robinson, A.S., 1997. Care and maintenance of tsetse colonies. In: Crampton, J.M., Beard, C.B., Louis, C. (eds), *The Molecular Biology of Insect Disease Vectors*. Chapman & Hall, London, pp. 41-55.
- Hargrove, J.W., 1975. Some changes in the flight and apparatus of tsetse flies during maturation. *Journal of Insect Physiology* 21, 1485-1489
- Hargrove, J.W., 1999. Nutritional levels of female tsetse *Glossina pallidipes* from artificial refuges. *Medical and Veterinary Entomology* 13, 150-164.
- Hargrove, J.W., Coates, T.W., 1990. Metabolic rates of tsetse flies in the field as measured by the excretion of injected caesium. *Physiological Entomology* 15, 157-166.
- Harrison, J.F., Roberts, S.P., 2000. Flight respiration and energetics. *Annual Review of Physiology* 62, 179-205.
- Hoffmann, A.A., Hallas, R.J., Dean, J.A., Schiffer, M., 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science*, 301, 100-102.
- Hoffmann, A.A., Parsons, P.A., 1989a. An integrated approach to environmental stress tolerance and life history variation. Desiccation tolerance in *Drosophila*. *Biological Journal of the Linnean Society* 37, 117-136.
- Hoffmann, A.A., Parsons, P.A., 1989b. Selection for increased desiccation resistance in *Drosophila melanogaster*: Additive genetic control and correlated responses for other stresses. *Genetics* 122, 837-845.
- Hoffmann, A.A., Parsons, P.A., 1991. *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford.
- Juliano, S.A., 1986. Resistance to desiccation and starvation of two species of *Brachinus* (Coleoptera: Carabidae) from southeastern Arizona. *Canadian Journal of Zoology* 64, 73-80.
- Keister, M., Buck, J., 1964. Some endogenous and exogenous effects on rate of respiration. In: Rockstein, M. (ed) *Physiology of Insecta*, Vol 3. Academic Press, New York, pp. 617-658.
- Krafsur, E.S., Wohlford, D.L., 1999. Breeding structures of *Glossina pallidipes* populations evaluated by mitochondrial variation. *Journal of Heredity*, 90, 635-642.
- Krebs, C.J., 1999. *Ecological Methodology*, 2<sup>nd</sup> edn. Addison-Welsey, Menlo Park.
- Leak, S.G.A., 1999. *Tsetse Biology and Ecology. Their Role in the Epidemiology and Control of Trypanosomosis*. CABI Publishing, New York.
- Lessells, C.M., Boag, P.T., 1987. Unrepeatable repeatabilities: a common mistake. *Auk* 104, 116-121.

- Lighton, J.R.B., 1994. Discontinuous ventilation in terrestrial insects. *Physiological Zoology* 67, 142-162.
- Lighton, J.R.B., 1996. Discontinuous gas exchange in insects. *Annual Review of Entomology* 41, 309-324.
- Lighton, J.R.B., 1998. Notes from underground: towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods. *American Zoologist* 38, 483-491.
- Lighton, J.R.B., Brownell, P.H., Joos, B., Turner, R.J., 2001. Low metabolic rate in scorpions: implications for population biomass and cannibalism. *Journal of Experimental Biology* 204, 607-613.
- Lighton, J.R.B., Duncan, F.D., 2002. Energy cost of locomotion: validation of laboratory data by *in situ* respirometry. *Ecology* 83, 3517-3522.
- Lighton, J.R.B., Fielden, L.J., 1995. Mass scaling of standard metabolism in ticks: A valid case of low metabolic rates in sit-and-wait strategists. *Physiological Zoology* 68, 43-62.
- Marais, E., Chown, S.L., 2003. Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. *Journal of Experimental Biology* 206, 4565-4574.
- Marden, J.H., 2000. Variability in the size, composition, and function of insect flight muscles. *Annual Review of Physiology* 62, 157-178.
- McEvoy, P.B., 1984. Increase in respiratory rate during feeding in larvae of the cinnabar moth *Tyria jacobaeae*. *Physiological Entomology* 9, 191-195.
- Nespolo, R.F., Lardies, M.A., Bozinovic, F., 2003. Intrapopulation variation in the standard metabolic rate of insects: repeatability, thermal dependence, and sensitivity ( $Q_{10}$ ) of oxygen consumption in a cricket. *Journal of Experimental Biology* 206, 4309-4315.
- Peters, R.H., 1983. *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge.
- Rajagopal, P.K., Bursell, E., 1965. The effect of temperature on the oxygen consumption of tsetse pupae. *Bulletin of Entomological Research* 56, 219-225.
- Rajagopal, P.K., Bursell, E., 1966. The respiratory metabolism of resting tsetse flies. *Journal of Insect Physiology* 12: 287-297.
- Reinhold, K., 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology* 13, 217-224.

- Robinson, T., Rogers, D., Williams, B., 1997a. Univariate analysis of tsetse habitat in the common fly belt of Southern Africa using climate and remotely sensed vegetation data. *Medical and Veterinary Entomology* 11, 223-234.
- Robinson, T., Rogers, D., Williams, B., 1997b. Mapping tsetse habitat suitability in the common fly belt of Southern Africa using multivariate analysis of climate and remotely sensed vegetation data. *Medical and Veterinary Entomology* 11, 235-245.
- Rogers, D.J., Randolph, S.E., 1986. Distribution and abundance of tsetse flies (*Glossina* spp.). *Journal of Animal Ecology* 55, 1007-1025.
- Rogers, D.J., Randolph, S.E., 1991. Mortality rates and population density of tsetse flies correlated with satellite imagery. *Nature* 351, 739-741.
- Rogers, D.J., Williams, B.G., 1994. Tsetse distribution in Africa: seeing the wood *and* the trees. In: Edwards, P.J., May, R.M., Webb, N.R. (eds.), *Large-Scale Ecology and Conservation Biology*. Blackwell Science, Oxford, pp. 247-271.
- Rogowitz, G.L., Chappell, M.A., 2000. Energy metabolism of eucalyptus-boring beetles at rest and during locomotion: gender makes a difference. *Journal of Experimental Biology* 203, 1131-1139.
- Schmidt-Nielsen, K., 1984. *Scaling. Why is Animal Size so Important?* Cambridge University Press, Cambridge.
- Sláma, K., 1984. Microrespirometry in small tissues and organs. In: Bradley, T.J., Miller, T.A. (eds.) *Measurement of Ion Transport and Metabolic Rates in Insects*. Springer, Berlin, pp. 101-129.
- Spicer, J.I., Gaston, K.J., 1999. *Physiological Diversity and its Ecological Implications*. Blackwell Science, Oxford.
- Takahashi-Del-Bianco, M., Benedito-Silva, A.A., Hebling, M.J.A., Marques, N., Marques, M.D., 1992. Circadian oscillatory patterns of oxygen uptake in individual workers of the ant *Camponotus rufipes*. *Physiological Entomology* 17, 377-383.
- Taylor, P., 1977a. The respiratory metabolism of tsetse flies, *Glossina* spp., in relation to temperature, blood-meal size and pregnancy cycle. *Physiological Entomology* 2, 317-322.
- Taylor, P., 1977b. A continuously recording respirometer, used to measure oxygen consumption and estimate locomotor activity in tsetse flies, *Glossina morsitans*. *Physiological Entomology* 2, 241-245.
- Taylor, P., 1978a. Radioisotopes as metabolic labels for *Glossina* (Diptera: Glossinidae). II. The excretion of  $^{137}\text{Cs}$  under field conditions as a means of estimating energy utilization, activity and temperature regulation. *Bulletin of Entomological Research* 68, 331-340.

- Taylor, P., 1978b. Radioisotopes as metabolic labels for *Glossina* (Diptera: Glossinidae). I. Laboratory determination of the relationship between radioisotope metabolism, respiration and temperature. *Bulletin of Entomological Research* 68, 1-9.
- Weiner, J., 1992. Physiological limits of sustainable energy budgets in birds and mammals: ecological implications. *Trends in Ecology and Evolution* 7, 384-388.
- West, G.B., Brown, J.H., Enquist, B.J., 1997. A general model for the origin of allometric scaling laws in biology. *Science* 276, 122-126.
- West, G.B., Brown, J.H., Enquist, B.J., 1999. The fourth dimension of life: fractal geometry and allometric scaling of organisms. *Science* 284, 1677-1679.
- Wolf, T.J., Ellington, C.P., Davis, S., Feltham, M.J., 1996. Validation of the doubly labeled water technique for *Bombus terrestris* (L.). *Journal of Experimental Biology* 199, 959-972.
- Zar, J.H., 1996. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River.
- Zera, A.J., Denno, R.F., 1997. Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology* 42, 207-231.

## Chapter 3

### Metabolic rate in the whip-spider, *Damon annulatipes* (Arachnida: Amblypygi)\*

*"Don't gain the world and lose your soul, wisdom is better than silver or gold..."*

**Bob Marley**

\* Published as: Terblanche, J.S., Klok, C.J., Marais, E., Chown, S.L., 2004. Metabolic rate in the whip-spider *Damon annulatipes* (Arachnidae: Amblypygi). *J. Insect Physiol.* 50: 637-645.



## Introduction

Metabolic rate is a measure of the energetic cost of living, which in turn has a major influence on the fitness of organisms. Understanding the causes and consequences of metabolic rate variation can therefore provide substantial insight into the evolution of life histories across a range of environments (Chown & Gaston, 1999; Hochachka & Somero, 2002; Kozłowski & Gawelczyk, 2002). In consequence, much attention has been focussed on comprehending the magnitude and sign of those factors that cause variation in metabolic rate, and the temporal scales over which they are significant. It is widely appreciated that over relatively short periods, metabolic rate in arthropods varies as a consequence of activity (Bartholomew et al., 1985; Lighton, 1991), feeding status (Taylor, 1977; McEvoy, 1984; Gouveia et al., 2000), gender (Rogowitz & Chappell, 2000), time of day (Crozier, 1979; Takahashi-Del-Bianco et al., 1992), and fluctuations in temperature (Keister & Buck, 1964; Bartholomew et al., 1981; Cossins & Bowler, 1987). It also varies over longer time scales with age (Hack, 1997) and with season (acclimatization) (Forlow & MacMahon, 1988; Bennett et al., 1999; Davis et al., 2000) or particular laboratory treatments (acclimation) (Stamou et al., 1995; Berrigan, 1997; Djawdan et al., 1997). Over the longest periods, there are evolved differences in metabolic rates between populations and species that reflect not only differences in the environments the species occupy (Hoffmann & Parsons, 1991; Berrigan & Partridge, 1997; Gibbs, 2002), but also their phylogenetic membership (Harvey & Pagel, 1991; Feder et al., 2000) and the outcome of the dynamic process that determines the body size of individuals and the species to which they belong (Schmidt-Nielsen, 1984; Lighton & Fielden, 1995; Kozłowski & Weiner, 1997; Kozłowski & Gawelczyk, 2002). Nonetheless, many aspects of metabolic rate variation in arthropods remain poorly understood (Chown & Nicolson, 2004). Most significant amongst these are the extent to which adaptation has resulted in the evolved differences between species, the form of the influence of phylogenetic constraint and body size on metabolic rates, and the rate at which unintentional laboratory adaptation (across generations), or indeed acclimation (within generations), takes place, if it does so at all.

Whilst several studies have demonstrated metabolic responses to laboratory selection, and therefore that metabolic rate does show adaptive responses, these have mostly concerned *Drosophila* (Berrigan, 1997; Djawdan et al., 1997; Williams et al., 1997; Gibbs et al., 1997, 2003). Few other studies have determined whether the conditions required for selection to take place (heritability, consistent variation, links of variation to fitness, see Endler, 1986; Bech et al., 1999), and thus for adaptation, are satisfied. One of the most straightforward of these requirements to assess is consistent variation (or repeatability – Lessells & Boag, 1987; Falconer & Mackay, 1996; Krebs, 1999) of the trait

of interest. Amongst arthropods it has only been studied in a handful of insect species - a lepidopteran (Buck & Keister, 1955), two boring beetles (Chappell & Rogowitz, 2000), an orthopteran (Nespolo et al., 2003), a highly variable cockroach (Marais & Chown, 2003), and in the tsetse fly *Glossina pallidipes* (Terblanche et al., 2004). All of these studies have shown relatively high repeatability values for metabolic rate ( $r = 0.38-0.60$ ), and presumably this would be the case in other arthropods too. However, no studies have investigated repeatability of metabolic rates in non-insect arthropods.

Laboratory selection studies are also partly confounded by the effects of laboratory adaptation (Harshman & Hoffmann, 2000; Sgrò & Partridge, 2000, 2001; Hoffmann et al., 2001). This adaptation to holding conditions typically involves either selection, or mutation accumulation as a result of relaxation of selection, on individual life-history traits (Sgrò & Partridge, 2000). Indeed, Hoffmann et al. (2001) regard the marked decline in resistance to environmental stress during adaptation to laboratory culture a result of reduced intensity of selection for resistance under laboratory conditions that occurs over several generations. However, responses to the laboratory can also be much faster than that, and involve adjustment to a new suite of conditions. Such laboratory acclimation can take a variety of forms and might also be transient (reviewed in Spicer & Gaston, 1999). Typically, investigations of phenotypic flexibility (Huey & Berrigan, 1996; Huey et al., 1999; Piersma & Drent, 2003) of metabolic rates in arthropods compare responses to several different environments after a fixed period (Stamou et al., 1995; Berrigan, 1997; Berrigan & Partridge, 1997; Djawdan et al., 1997; Fields et al., 1998; Bennett et al., 1999; Sokolova & Pörtner, 2003), and are less concerned about the time course of such adjustments. However, it is clear from investigations of other physiological traits, such as desiccation and heat and cold resistance (David et al., 1997; Berrigan & Hoffman, 1998; Fields et al., 1998; Kostal et al., 1998; Sinclair et al., 2003; Topp, 2003) that responses (either intended by the experimenter or inadvertent) change rapidly through time. Surprisingly few studies have investigated the extent to which variation in metabolic rate, or its repeatability, changes following maintenance under fixed laboratory conditions (Spicer & Gaston, 1999).

Over and above studies of repeatability and laboratory selection, comparative studies of arthropod metabolic rates have provided much support for the idea that these are likely to vary in an adaptive fashion (Lighton & Duncan, 1995; Lighton & Fielden 1995, 1996; Chown & Gaston, 1999; Lighton et al., 2001). However, they have also shown that there is considerable phylogenetic constraint associated with metabolic rate, independent of the effects of body size. Indeed, it appears that there are consistent differences in metabolic rate associated with higher arthropod taxa that reflect the overall life histories of these groups. Ticks have the lowest metabolic rates, followed by scorpions, followed by

centipedes (Klok et al., 2002), followed by ‘typical arthropods’ (which include spiders, ants, beetles, solifuges and mites; Lighton et al., 2001). This variation, largely in the constant of the scaling of metabolic rate, has been attributed to the sit-and-wait life style of ticks (Lighton & Fielden, 1995), cannibalism of scorpions (Lighton et al., 2001), and differences between apterous and pterygote (highly active) insects (Reinhold, 1999; Addo-Bediako et al., 2002). Whether this phylogenetic variation is reflected in other arthropod taxa is not known, mostly because so few studies have been undertaken on metabolic rates in these groups, and because at least some of the work that has been done is confounded by the problems associated with closed-system respirometry (Lighton, 1991, 1996; Addo-Bediako et al., 2002).

Here, I repeatedly measure the standard metabolic rate of individuals of an amblypygid species, *Damon annulatipes* (Wood) (Arachnida: Amblypygi), to address these issues. Specifically, I determine the extent of repeatability of metabolic rate immediately after capture, and then again following 14 days maintenance under constant laboratory conditions, to determine whether this variation is partitioned mostly among individuals (i.e. is repeatable) and whether both the extent of the variation and repeatability change with laboratory acclimation. Although an assessment of the metabolic rates of the Amblypygi, or whip-spiders, has previously been made (in *Tarantula marginemaculata* (Koch) (Anderson, 1970)), as far as I could ascertain metabolic rates have not been measured in any other Amblypygid species. Furthermore, the closed-system respirometry technique (as employed for estimating metabolic rate in *T. marginemaculata* (Anderson, 1970)) may require confirmation of its accuracy (see discussions by Lighton, 1991; 1996 and Addo-Bediako et al., 2002). The amblypygids are poorly represented in the physiological literature (see also Hebets & Chapman, 2000), and I therefore compare my results to metabolic rates of other Chelicerata, to determine whether there is any indication of the likely metabolic strategy to which the group might be constrained by their foraging behaviour.

## Materials and methods

We collected *Damon annulatipes* whip-spiders from Salt Rock, Kwa-Zulu Natal, South Africa. These whip-spiders live in and among leaf litter in forested areas but have been reported to enter houses and other human constructions (Lawrence, 1953). After collection the specimens were placed in plastic containers with moist filter paper and leaf litter and they were transported to the laboratory within three days of collection. Upon arrival in the laboratory the animals were transferred to individual containers and housed within a regulated climate chamber ( $21.0 \pm 1.0$  °C; mean ambient humidity,  $40 \pm$

15 % R.H.; photoperiod: 12H:12H, L:D). The whip-spiders were allowed to feed *ad libitum* off moistened dog food pellets and freely available water (B.A. Bouricius, personal communication). Cage locations within the climate chamber were randomized daily during experiments, and every second to third day during the acclimation period.

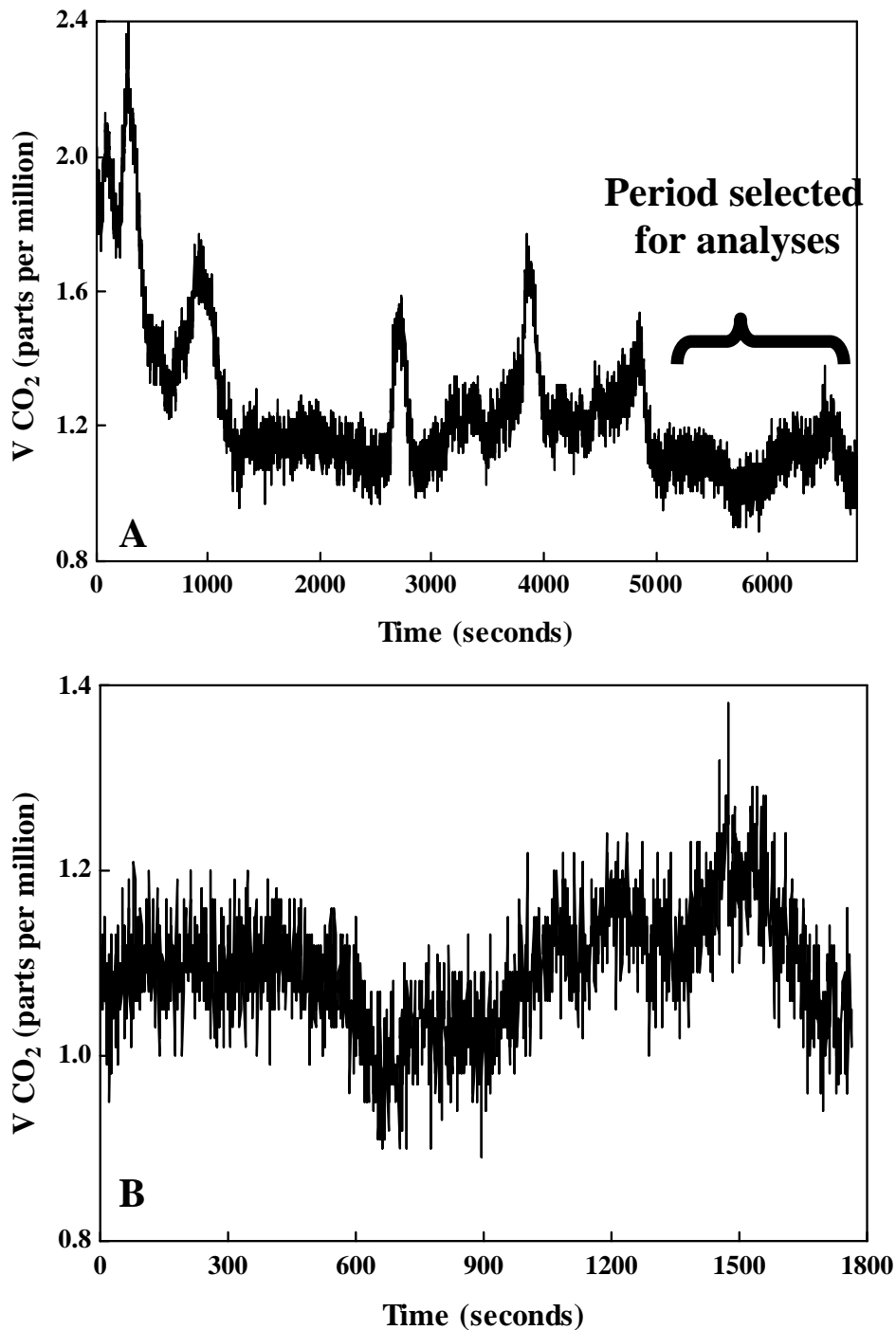
Metabolic rate recordings were performed on field-fresh (FF, n = 9) and acclimated (ACC, n = 7) whip-spiders once each day for five consecutive days. After metabolic rate recordings had been performed, FF animals were then acclimated for two weeks in the climate chamber with no experimental interference (except feeding), and then re-used once per day for another five days of metabolic rate recordings (ACC, n = 7). In most cases, metabolic rates were recorded in an individual at the same time of day to eliminate any possible diurnal effects. Whip-spiders were placed into a cuvette of either ~120 ml or ~60 ml in volume, corresponding to the animal's size, and weighed on an electronic microbalance (to 0.01 mg on a Mettler Toledo AX504, Columbus, OH, USA). The same cuvette size was always used for an individual whip-spider in all tests. The cuvette was connected to a LI-7000 infra-red gas analyser (LiCor, Lincoln, Nevada) using Sable Systems flow-through respirometry equipment to regulate gas flow (Sable Systems, Las Vegas, Nevada) at 100 ml.min<sup>-1</sup>. Synthetic air (21% O<sub>2</sub>, balance N<sub>2</sub>) was passed through sodalime, silica gel and Drierite columns to remove CO<sub>2</sub> and H<sub>2</sub>O residues. The LI-7000 was connected to a desktop computer using LI-7000 software for instrument calibration and data capture. The gas analyser and experimental animal housed in the cuvette were stored within a darkened climate chamber at 21.0 ± 1.5 °C. The entire system was stored within an air-conditioned laboratory that held temperature at 24.0 ± 3.0 °C. I used two LI-7000 gas analyser systems (set up as described above) to perform experiments simultaneously on two spiders. Care was taken to ensure that a spider was always recorded using the same gas analyser setup in every test (i.e. there was no switching of animals between recording equipment during the entire protocol). At the end of each experiment, whip-spiders were weighed, transferred to individual, labelled cages and returned to the climate chamber. In two cases, individuals died of unknown causes.

Metabolic rate data were extracted from the recorded LI-7000 data files, via Microsoft Excel, using DATACAN V (corrected to standard temperature and pressure). Data from the first 30 minutes were usually discarded and corresponded to a period of settling ('activity bursts' in the metabolic rate of the spider were often visible), until a comfortable position had been adopted. Metabolic rate data used in analyses were usually from the 30-120 minute periods of experimental runs. In preliminary experiments (not used in the analyses) performed with a light on, I observed that after a restless period

of approximately ten minutes, individuals settle down and remain immobile for most of the remainder of the experiment (Fig. 1). Large size precluded the use of an electronic activity detector.

To investigate metabolic rate variation between groups, repeated measures analyses of variance (ANOVA) and analyses of covariance (ANCOVA; Zar, 1996) (i.e. mass-independent metabolic rate comparisons), as appropriate, were used. Data are presented as means  $\pm$  standard error unless otherwise stated, and significance was set at  $P < 0.05$ . Repeatability and its confidence limits were calculated using the intra-class correlation coefficient approach (Lessells & Boag, 1987; Krebs, 1999) within the specified experimental categories. Repeatability of metabolic rate was calculated from the repeated tests within each group (tests 2-5; 7-10), for the field-fresh and acclimated groups respectively, thus representing repeatability over two short periods (i.e. four days in each case). Because repeatability generally declines notably as the period over which it is examined is increased (Chappell et al., 1995, 1996), repeatability was also calculated over the two-week period by investigating the intraclass correlation coefficient for trials 3 and 8.

To compare the metabolic rates of this whip-spider species with other arthropods I extracted data on metabolic rate and body mass from the literature (Anderson, 1970; Greenstone & Bennett, 1980; Anderson & Prestwich, 1982; Punzo, 1991; Kalarani et al., 1992; Lighton & Fielden, 1995; Anderson, 1996; Lighton & Fielden, 1996; Magano, 2000; Lighton et al., 2001).



**Figure 1.** **A)** A typical metabolic rate recording for a *Damon annulatipes* whip-spider (mass = 122.6 mg), clearly showing an initial activity period during which the animal adopts a comfortable position in the respirometry cuvette. The obvious respiratory ‘spike’ (centre and left) represents an activity burst. The period after this peak would be selected for raw data transformation (from CO<sub>2</sub> parts per million to  $\mu\text{l CO}_2\cdot\text{hr}^{-1}$ ) and analysis of standard metabolic rate. **B)** The period of the respirometry trace selected for data transformation and analysis of metabolic rate clearly showing continuous ventilation (metabolic rate =  $6.341 \mu\text{l CO}_2\cdot\text{hr}^{-1}$ ).

## Results

*Damon annulatipes* does not show cyclic or discontinuous gas exchange at rest, and the effects of activity on metabolic rate are readily discernible in the gas exchange traces (Fig. 1). Nonetheless, the first trial was always used to observe the behaviour of the whip-spiders in the cuvettes during respirometry recordings (in some cases with a light on). For this reason, and to reduce handling and “training effects” of the respirometry protocol, these first trials were not used in subsequent analyses. Rather, the last four trials for each individual were used for all analyses (field-fresh group, tests 2-5; acclimated group, tests 7-10). Corresponding test data were used for measures of water loss rates and body mass. Summary statistics for body mass, SMR expressed as CO<sub>2</sub> production ( $\mu\text{lCO}_2\cdot\text{hr}^{-1}$ ) and recalculated as microwatts using an R.Q. = 0.72, and rate of water loss ( $\text{mgH}_2\text{O}\cdot\text{hr}^{-1}$ ) for individuals of *Damon annulatipes*, are presented in Table 1. There were no differences in body size between FF and ACC groups (Table 1). Because there were no systematic changes in metabolic rate with repeated recordings (Fig. 2), metabolic rates were pooled across the four tests in each individual when comparing the mass-independent metabolic rates in the FF and ACC groups (Table 1).

In *D. annulatipes* the ACC group’s mass-independent metabolic rate was significantly reduced compared to the FF group (when calculated as either microwatts or CO<sub>2</sub> production, (general linear model (GLM), with body size as covariate, Table 1)). However, when calculated across individual whip-spiders that completed all FF and ACC experiments only (i.e. animals that died were discarded), this result became marginally non-significant ( $P = 0.0513$ ). In *D. annulatipes*, mass-independent rates of water loss did not change after two weeks of acclimation (Table 1). Even with correction for CO<sub>2</sub> loss during the recording interval, rates of water loss did not differ between FF and ACC groups (least-squares adjusted mean ( $\pm$  S.E.), [FF: 6.3 ( $\pm$  2.5), ACC: 11.5 ( $\pm$  2.7)  $\text{mg H}_2\text{O}\cdot\text{hr}^{-1}$ ;  $F_{1, 12} = 1.95$ ,  $P > 0.19$ ]).

It was not possible to detect any consistent patterns of change in metabolic rate (Fig. 2) or body mass with increasing test number within either the FF group or the ACC group (repeated measures ANOVA,  $P > 0.4$ ). Likewise, the decline in the ratio of within-individual variation to between-individual variation caused by acclimation, as demonstrated by an increase of ~12 % in the repeatability of metabolic rate, was not statistically significant ( $P > 0.05$ ; Table 2).

**Table 1.** Summary statistics for body size, metabolic rate, and rates of water loss in *Damon annulatipes*. All experiments performed in the dark at 21.0 °C and 0 % relative humidity. Analyses were performed for all individuals in each group (FF<sub>All</sub> vs. ACC), and separately only among those that completed the entire experimental protocol, i.e. all trials, (FF vs. ACC)). Comparisons of metabolic rate (GLM, covariate: body mass) allow specifically for mass-independent analyses between groups, thereby accounting for the relatively broad mass range of these whip-spiders.

<i>Group</i>	<i>Mean ± S.E.M.</i>	<i>Min</i>	<i>Max</i>	<i>N</i>	<i>Individuals x test</i>
<i>Mass (mg)</i>					
<b>FF<sub>All</sub><sup>1</sup></b>	640.4 ± 66.1	115.5	1592.1	9	9 x 4
<b>FF</b>	632.4 ± 0.20	119.3	1579.1	7	7 x 4
<b>ACC</b>	629.0 ± 0.20	118.7	1714.6	7	7 x 4
<i>Metabolic rate (μlCO<sub>2</sub>.hr<sup>-1</sup>)</i>					
<b>FF<sub>All</sub><sup>2</sup></b>	30.22 ± 2.87	4.48	67.43	9	9 x 4
<b>FF<sup>3</sup></b>	25.54 ± 1.19	5.52	58.54	7	7 x 4
<b>ACC</b>	21.75 ± 1.19	4.25	52.16	7	7 x 4
<i>Metabolic rate (μW)</i>					
<b>FF<sub>All</sub><sup>2</sup></b>	229.6 ± 21.8	34.1	521.2	9	9 x 4
<b>FF<sup>3</sup></b>	194.0 ± 9.06	42.0	444.6	7	7 x 4
<b>ACC</b>	165.2 ± 9.06	32.3	396.2	7	7 x 4
<i>Rate of water loss (mgH<sub>2</sub>O.hr<sup>-1</sup>)</i>					
<b>FF<sub>All</sub><sup>4</sup></b>	3.63 ± 0.45	1.00	12.09	9	9 x 4
<b>FF<sup>5</sup></b>	3.17 ± 0.54	1.00	7.73	7	7 x 4
<b>ACC</b>	3.66 ± 0.54	1.47	7.63	7	7 x 4

1 FF<sub>All</sub> vs. ACC - No difference (repeated measures ANOVA: F<sub>1, 67</sub> = 0.000; P > 0.99)

2 FF<sub>All</sub> vs. ACC - GLM, (covariate: body mass); F<sub>1, 13</sub> = 4.753; P < 0.05

3 FF vs. ACC - GLM, (covariate: body mass); F<sub>1, 13</sub> = 4.78; P = 0.0513

4 FF<sub>All</sub> vs. ACC - No difference (GLM, covariate body mass: F<sub>1, 13</sub> = 0.29; P > 0.60)

5 FF vs. ACC - No difference (GLM, covariate body mass: F<sub>1, 13</sub> = 0.37; P > 0.56)

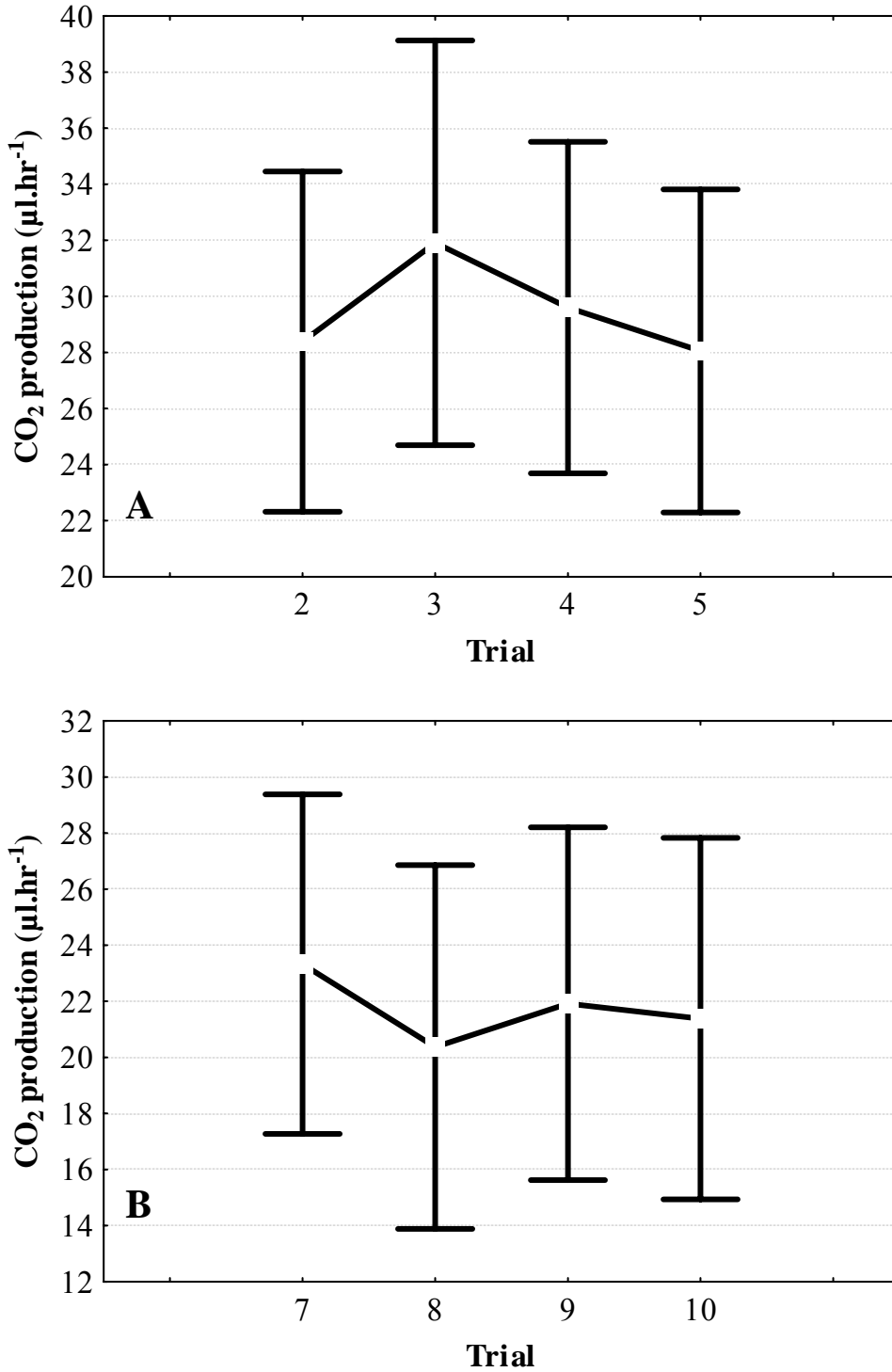


**Table 2.** Results of analyses of variance to calculate repeatability ( $r$ ) of metabolic rate in *Damon annulatipes*. Overlap in the 95% confidence limits ( $LCL_{0.95}$  and  $UCL_{0.95}$ ) demonstrates that repeatability was consistent across the field fresh (FF) and acclimated (ACC) individuals, and that it did not differ from these estimates when calculated over the two-week period.

	Source of variation	DF	MS	F-ratio	P	Repeatability ( $r$ , %)	$LCL_{0.95}$	$UCL_{0.95}$
FF	Among	6	$1.28 \times 10^{-3}$	30.16	< 0.001	76.4	43.6	90.8
	Within	21	$4.25 \times 10^{-3}$					
ACC	Among	6	$1.06 \times 10^{-3}$	55.22	< 0.001	88.6	65.0	95.9
	Within	21	$1.92 \times 10^{-5}$					
Longer-term <sup>1</sup>	Among	6	$4.34 \times 10^{-3}$	20.60	< 0.001	73.7	38.8	89.7
	Within	7	$2.46 \times 10^{-4}$					

<sup>1</sup> Repeatability calculated from test 3 and 8 (two weeks apart) only in whip-spiders that completed all experiments

The intraspecific scaling relationship of body mass and metabolic rate was investigated using least squares linear regression. In *D. annulatipes*, the scaling relationships of log metabolic rate on log body mass, in the form of  $\mu W = a M^b$ , was highly significant for the FF group ( $\mu W_{FF} = 342 M^{0.857}$ ;  $F_{1,7} = 55.91$ ;  $r^2 = 0.889$ ;  $P < 0.001$ ) and the ACC group ( $\mu W_{ACC} = 258 M^{0.897}$ ;  $F_{1,5} = 137.72$ ;  $r^2 = 0.965$ ;  $P < 0.001$ ; Fig. 3a). The slopes of the relationships of metabolic rate on mass between the FF and ACC group did not differ from each other ( $t_{1,7} = 0.349$ ;  $P > 0.5$ ). In addition, they did not differ from a slope of 0.75 (FF:  $t_{1,7} = 0.931$ ;  $P > 0.2$ ; ACC:  $t_{1,5} = 1.922$ ;  $P > 0.05$ ) nor from a slope of 1 (FF:  $t_{1,7} = 1.250$ ;  $P > 0.2$ ; ACC:  $t_{1,5} = 1.351$ ;  $P > 0.2$ ).



**Figure 2.** Mean metabolic rate ( $\pm$  S.E.) in *Damon annulatipes* showed no systematic changes with experimental observation (test) in either the field-fresh (A) or acclimated (B) group. Metabolic rate was recorded at 21.0 °C, 0 % R.H. and in total darkness.

## Discussion

A major concern for investigations of repeatability of standard metabolic rate (SMR) is the extent to which movement by the animals might confound estimates of the intraclass correlation coefficient (Marais & Chown, 2003). Whilst routine, electronic activity detection was not possible for this species, initial observations, and inspections of the respirometry trace (Fig. 1) enabled us to select periods representative of SMR. Therefore, it is unlikely that movement confounded the present estimates of repeatability. Indeed, I found that the repeatability of standard metabolic rate in *Damon annulatipes* whip-spiders is both significant and high compared with that reported for insects ( $r$ : 0.4 - 0.6; Chappell & Rogowitz, 2000; Nespolo et al., 2003; Marais & Chown, 2003; Terblanche et al., 2004), a finding unlikely were movement to have had an unpredictable effect on estimates of metabolic rate. High repeatability of metabolic rate in this species confirms that one of the prerequisites for natural selection (Endler, 1986; Bech et al., 1999) is met not only in insects (Nespolo et al., 2003; Marais & Chown, 2003; Terblanche et al., 2004), but in other arthropods too. Thus adaptive metabolic rate variation in arthropods is not unlikely (see discussion in Chown & Gaston, 1999; Chown & Nicolson, 2004).

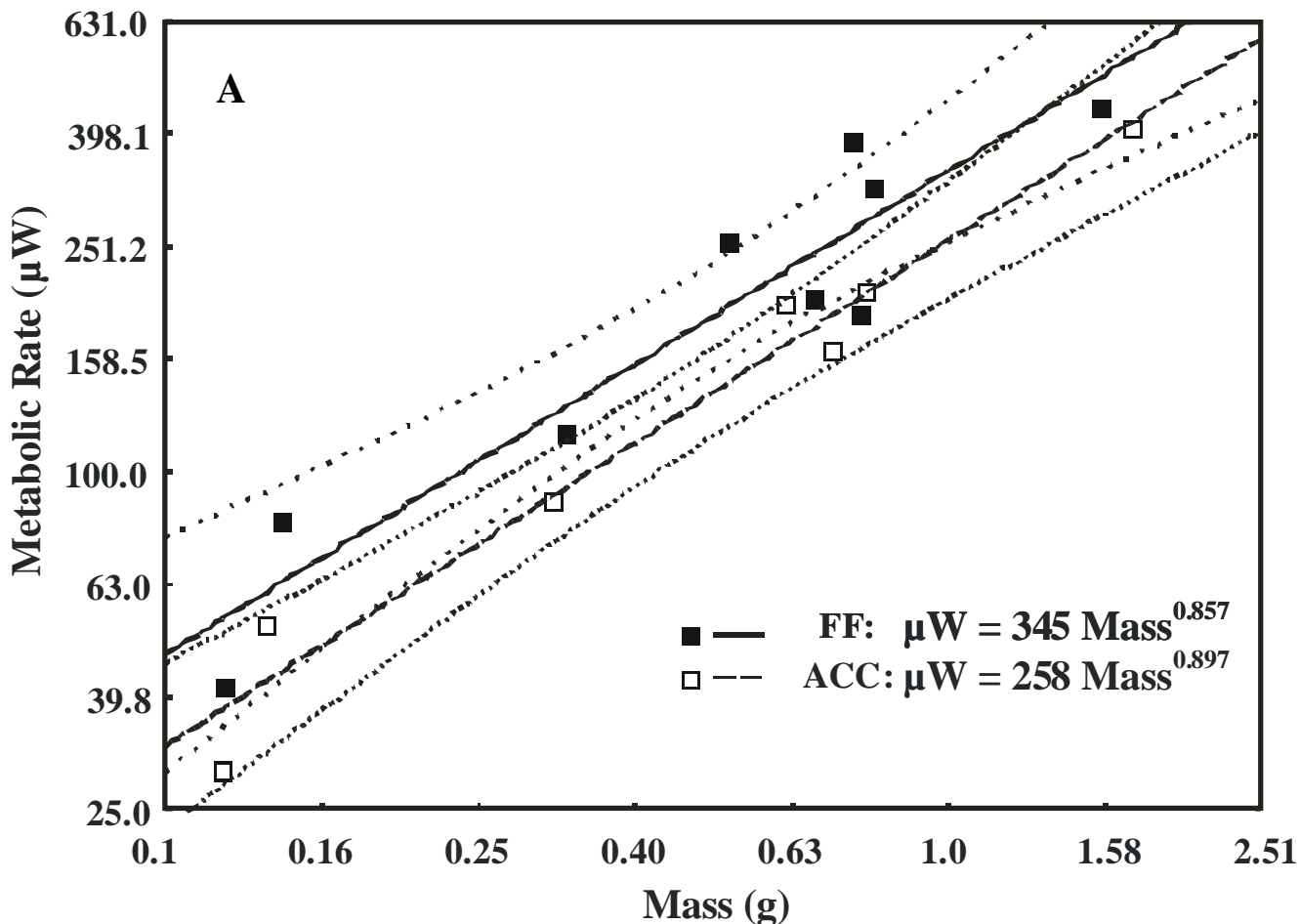
The finding that metabolic rate repeatability remained unchanged with two weeks of laboratory acclimation (Table 2), over both the short and longer-term estimates, suggests that, at least for this species, laboratory acclimation effects on the repeatability of standard metabolic rate can be disregarded. Previous studies have assumed that this is the case (Buck & Keister, 1955; Chappell & Rogowitz, 2000; Nespolo et al., 2003; Marais & Chown, 2003), and my results suggest that this assumption is justified.

By contrast, the absolute value of mass-independent metabolic rate declined, and significantly so (although the reduced data set was marginal), with two weeks of laboratory acclimation. This was not the case for mass-independent rates of water loss, even when corrected for CO<sub>2</sub> loss during the course of an experiment, nor for body mass. It therefore seems likely that the decline in mass-independent metabolic rate was a response to the stable laboratory environment, probably mediated through reduced temperature variation (see Harshman & Hoffmann, 2000; Sgrò & Partridge, 2000, 2001; Hoffmann et al., 2001 for a discussion of similar, though longer-term responses in *Drosophila*). Presumably, the decline in mass-independent metabolic rate might also have been a function of less demanding foraging requirements (Kuntner et al., 1999), and a reduction in the need to avoid predation, but these longer-term changes seem less likely over the short period examined here. Whilst the animals might also have become more accustomed to handling stress, the absence of a systematic decline in

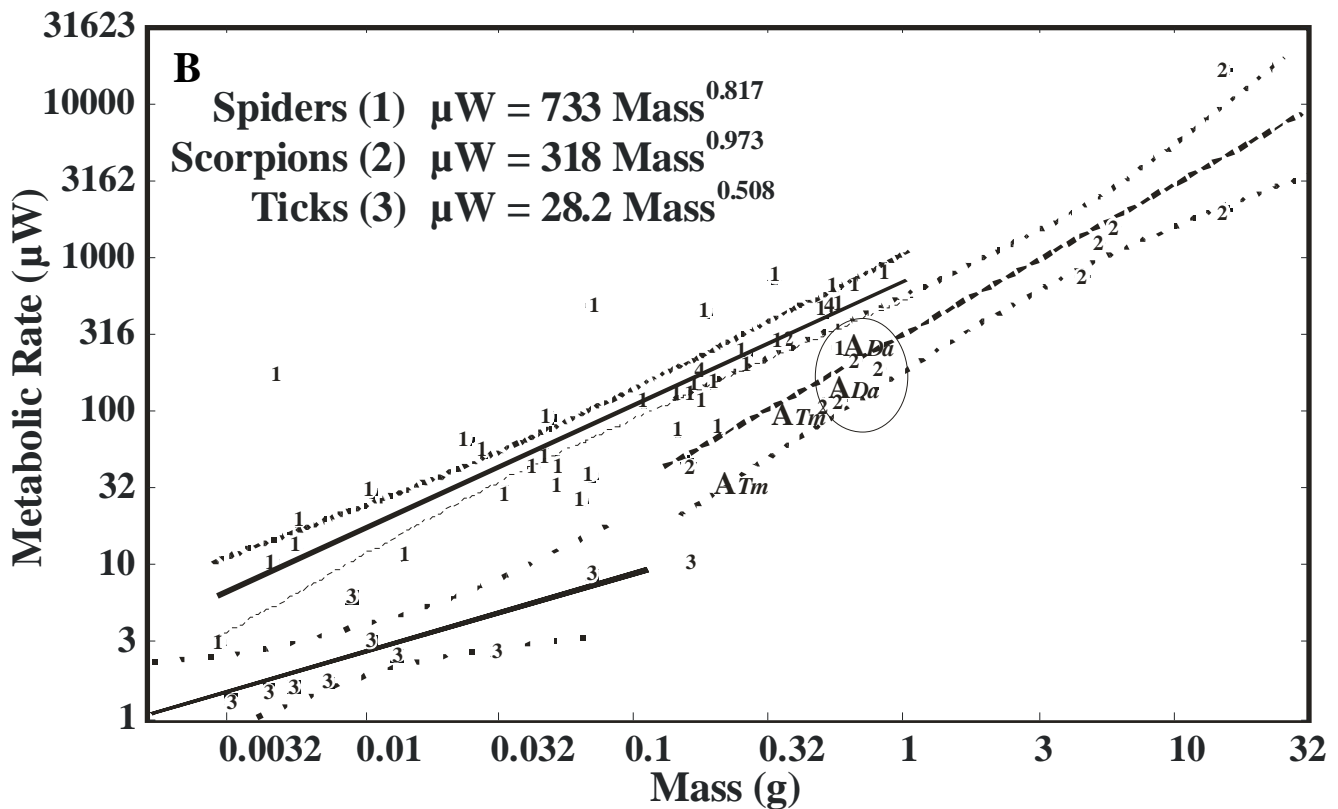
metabolic rate with test number in the repeatability assays suggests that this is not the case. Whatever the reason for the significant and fairly substantial decline in metabolic rate (c. 16-30%), it is in keeping with expectations based on the relaxation of various stressors (Stamou et al., 1995; Berrigan, 1997; Djawdan et al., 1997; Spicer & Gaston, 1999). Finally, the similar intraspecific mass-scaling of metabolic rate in the FF and ACC animals (Fig. 3a) implies that metabolic rate changes proportionally with acclimation across all animals of a given body size. Because the slopes of these intraspecific scaling relationships could not be distinguished from either 0.75 or from 1.0, I have refrained from additional interpretation thereof. However, I note that these values are also not distinguishable from the interspecific equation for chelicerates (FF:  $t_{1,7} = 0.156$ ,  $P > 0.5$ ; ACC:  $t_{1,5} = 0.275$ ,  $P > 0.5$ ), owing mostly to substantial variance of the former.

It is not only variation in metabolic rates of *D. annulatipes* with acclimation that are of interest, but also the absolute values. As far as I could ascertain, this is the first recording of the metabolic rate of *D. annulatipes*, and probably the second species of whip-spider recorded (see Anderson, 1970). Amblypygids are usually inactive, generalist feeders on dead organic matter (B. A. Bouricius, personal correspondence), although they will also catch live prey, striking out rapidly (Lawrence, 1953, personal observation, J.S.T. and C.J.K.). This behaviour is similar to other sit-and-wait predators like some scorpions and spiders. Therefore, on the basis of Lighton & Fielden's (1995) arguments regarding the relationships between life-history and metabolic rate, it might be predicted that the metabolic rate of *D. annulatipes* should be low by comparison with other arthropods, or at least the chelicerates. This certainly seems to be the case for *D. annulatipes* and also for Anderson's (1970) estimates of *T. marginemaculata* (Fig. 3b). Although the metabolic rates of this taxon are not as low as those of ticks, given their body size, they appear to be lower than those of spiders, but similar to the values recorded for scorpions. Unfortunately, without assessments of additional amblypygid species it is not possible to determine the generality of this result. Nonetheless, it does appear to be likely that there is considerable constraint in the evolution of arthropod metabolic rates that is associated with life-history, and probably to a lesser extent with phylogeny, given that much life-history variation is partitioned at higher taxonomic levels. In this particular instance, Wheeler & Hayashi's (1998) phylogeny suggests that the amblypygids are the sister-group of the spiders, rather than of the scorpions. However, metabolically, the whip-spiders are more like scorpions, and this may be a consequence of their sedentary life-style coupled with the absence of energetically-expensive web construction (see Lighton & Fielden 1995; Lighton et al., 2001).

In conclusion, I have demonstrated that this whip-spider species has a metabolic rate in keeping with its sedentary lifestyle, that its gas exchange is continuous, as might be expected for an arthropod with book lungs (see also Klok et al., 2004), and that repeatability of metabolic rate does not decline with laboratory acclimation, although this is the case for absolute mass-independent metabolic rate. All of these findings have implications for further understanding of the evolution of arthropod metabolic rates.



**Figure 3A.** Intraspecific mass scaling relationship for metabolic rate in field fresh (FF: filled squares) and acclimated (ACC: open squares) *Damon annulatipes* whip-spiders.



**Figure 3B.** Interspecific scaling relationship of metabolic rate for 1) spiders (Araneae), 2) scorpions (Scorpiones) and 3) ticks (Acari) taken from the literature (Anderson, 1970; Greenstone & Bennett, 1980; Anderson & Prestwich, 1982; Punzo, 1991; Kalarani et al., 1992; Lighton & Fielden, 1995; Anderson, 1996; Magano, 2000; Lighton et al., 2001). Solifugids (Solifugae) (4) are indicated (from Lighton & Fielden, 1996). *Damon annulatus* (Amblypygi) (encircled,  $A_{Da}$ ) include field-fresh (upper symbol) and acclimated (lower symbol) respirometry recordings from this study, while  $A_{Tm}$  represents *Tarantula marginemaculata* whip-spiders as obtained from Anderson's (1970) closed system respirometry estimates. (The regression equation describing the relationship including all the above data is  $\mu W = 513 M^{0.875}$ , mass (M) in g).

## Acknowledgements

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## References

- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology* 16, 332-338.
- Anderson, J.F., 1970. Metabolic rates of spiders. *Comparative Biochemistry and Physiology* 33, 51-72.
- Anderson, J.F., 1996. Metabolic rates of resting Salticid and Thomisid spiders. *Journal of Arachnology* 24, 129-134.
- Anderson, J.F., Prestwich, K.N., 1982. Respiratory gas exchange in spiders. *Physiological Zoology* 55, 72-90.
- Bartholomew, G.A., Lighton, J.R.B., Louw, G.N., 1985. Energetics of locomotion and patterns of respiration in tenebrionid beetles from the Namib Desert. *Journal of Comparative Physiology B* 155, 155-162.
- Bartholomew, G.A., Vleck, D., Vleck, C.M., 1981. Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *Journal of Experimental Biology* 90, 17-32.
- Bech, C., Langseth, I., Gabrielsen, G.W., 1999. Repeatability of basal metabolism in breeding female kittiwakes *Rissa tridactyla*. *Proceedings of the Royal Society of London B* 266, 2161-2167.
- Bennett, V.A., Kukul, O., Lee, R.E., 1999. Metabolic opportunists: feeding and temperature influence the rate and pattern of respiration in the high Arctic woollybear caterpillar *Gynaephora groenlandica* (Lymantriidae). *Journal of Experimental Biology* 202, 47-53.
- Berrigan, D., 1997. Acclimation of metabolic rate in response to developmental temperature in *Drosophila melanogaster*. *Journal of Thermal Biology* 22, 213-218.
- Berrigan, D., Hoffmann, A.A., 1998. Correlations between measures of heat resistance and acclimation in two species of *Drosophila* and their hybrids. *Biological Journal of the Linnean Society* 64, 449-462.
- Berrigan, D., Partridge, L., 1997. Influence of temperature and activity on the metabolic rate of adult *Drosophila melanogaster*. *Comparative Biochemistry and Physiology A* 118, 1301-1307.
- Buck, J., Keister, M., 1955. Cyclic CO<sub>2</sub> release in diapausing *Agapema* pupae. *Biological Bulletin* 109, 144-163.
- Chappell, M.A., Bachman, G.C., Odell, J.P., 1995. Repeatability of maximal aerobic performance in Belding's Ground Squirrels, *Spermophilus beldingi*. *Functional Ecology* 9, 498-504.

- Chappell, M.A., Rogowitz, G.L., 2000. Mass, temperature and metabolic effects on discontinuous gas exchange cycles in *Eucalyptus*-boring beetles (Coleoptera: Cerambycidae). *Journal of Experimental Biology* 203, 3809-3820.
- Chappell, M.A., Zuk, M., Johnsen, T.S., 1996. Repeatability and aerobic performance in Red Junglefowl: effects of ontogeny and nematode infection. *Functional Ecology* 10, 578-585.
- Chown, S.L., Gaston, K.J., 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews* 74, 87-120.
- Chown, S.L., Nicolson, S.W., 2004. *Insect Physiological Ecology. Mechanisms and Patterns*. Oxford University Press, Oxford.
- Cossins, A.R., Bowler, K., 1987. *Temperature Biology of Animals*. Chapman and Hall, London.
- Crozier, A.J.G., 1979. Supradian and infradian cycles of oxygen uptake in diapausing pupae of *Pieris brassicae*. *Journal of Insect Physiology* 25, 575-82.
- David, J.R., Gibert, P., Gravot, E., Petavy, G., Morin, J.P., Karan, D., Moreteau, B., 1997. Phenotypic plasticity and developmental temperature in *Drosophila*: analysis and significance of reaction norms of morphometrical traits. *Journal of Thermal Biology* 22, 441-451.
- Davis, A.L.V., Chown, S.L., McGeoch, M.A., Scholtz, C.H., 2000. A comparative analysis of metabolic rate in six *Scarabaeus* species (Coleoptera: Scarabaeidae) from southern Africa: further caveats when inferring adaptation. *Journal of Insect Physiology* 46, 553-562.
- Djawdan M., Rose, M., Bradley, T.J., 1997. Does selection for stress resistance lower metabolic rate? *Ecology* 78, 828-837.
- Endler, J.A., 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton.
- Falconer, D.S., Mackay, T.F.C., 1996. *Introduction to Quantitative Genetics*, 4<sup>th</sup> edn. Prentice Hall, Harlow.
- Feder, M.E., Bennett, A.F., Huey, R.B., 2000. Evolutionary physiology. *Annual Review of Ecology and Systematics* 31, 315-341.
- Fields, P.G., Fleurat-Lessard, F., Lavanseau, L., Febvay, G., Peypelut, L., Bonnot, G., 1998. The effect of cold acclimation and deacclimation on cold tolerance, trehalose and free amino acid levels in *Sitophilus granarius* and *Cryptolestes ferrugineus* (Coleoptera). *Journal of Insect Physiology* 44, 955-965.
- Forlow, L.J., MacMahon, J.A., 1988. A seasonal comparison of metabolic and water loss rates of three species of grasshoppers. *Comparative Biochemistry and Physiology A* 89, 51-60.



- Gibbs, A.G., 2002. Water balance in desert *Drosophila*: lessons from non-charismatic microfauna. *Comparative Biochemistry and Physiology A* 133, 781-789.
- Gibbs, A.G., Chippindale, A.K., Rose, M.R., 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology* 200, 1821-1832.
- Gibbs, A.G., Fukuzato, F., Matzkin, L.M., 2003. Evolution of water conservation mechanisms in *Drosophila*. *Journal of Experimental Biology* 206, 1183-1192.
- Gouveia, S.M., Simpson, S.J., Raubenheimer, D., Zanotto, F.P., 2000. Patterns of respiration in *Locusta migratoria* nymphs when feeding. *Physiological Entomology* 25, 88-93.
- Greenstone, M.H., Bennett, A.F., 1980. Foraging strategy and metabolic rate in spiders. *Ecology* 61, 1255-1259.
- Hack, M.A., 1997. The effects of mass and age on standard metabolic rate in house crickets. *Physiological Entomology* 22, 325-331.
- Harshman, L.G., Hoffmann, A.A., 2000. Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends in Ecology and Evolution* 15, 32-36.
- Harvey, P.H., Pagel, M.D., 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.
- Hebets, E.A., Chapman, R.F., 2000. Surviving the flood: plastron respiration in the non-tracheate arthropod *Phrynus marginemaculatus* (Amblypygi: Arachnida). *Journal of Insect Physiology* 46, 13-19.
- Hochachka, P.W., Somero, G.N., 2002. *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford University Press, Oxford.
- Hoffmann, A.A., Hallas, R.J., Sinclair, C., Mitrovski, P. 2001. Level of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. *Evolution* 55, 1621-1630.
- Hoffmann, A.A., Parsons, P.A., 1991. *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford.
- Huey, R.B., Berrigan, D., 1996. Testing evolutionary hypotheses of acclimation. In: Johnstone, I.A., Bennett, A.F. (eds), *Animals and Temperature. Phenotypic and Evolutionary Adaptation*. Cambridge University Press, Cambridge.
- Huey, R.B., Berrigan, D., Gilchrist, G.W., Herron, J.C., 1999. Testing the adaptive significance of acclimation: A strong inference approach. *American Zoologist* 39, 323-336.

- Kalarani, V., Mohan, P.M., Davies, R.W., 1992. Thermal-acclimation and metabolism of the hepatopancreas in the tropical scorpion, *Heterometrus fulvipes*. *Journal of Thermal Biology* 17, 141-146.
- Keister, M., Buck, J., 1964. Some endogenous and exogenous effects on rate of respiration. In: Rockstein, M. (ed), *Physiology of Insecta*, Vol. 3. Academic Press, New York, pp. 617-658.
- Klok, C.J., Mercer, R.D., Chown, S.L., 2002. Discontinuous gas-exchange in centipedes and its convergent evolution in tracheated arthropods. *Journal of Experimental Biology*, 205, 1019-1029.
- Klok, C.J., Sinclair, B.J., Chown, S.L., 2004. Upper thermal tolerance and oxygen-limitation in terrestrial arthropods. *Journal of Experimental Biology*, 207, 2361-2370.
- Kostal, V., Sula, J., Simek, P., 1998. Physiology of drought tolerance and cold hardiness of the Mediterranean tiger moth *Cymbalophora pudica* during summer diapause. *Journal of Insect Physiology* 44, 165-173.
- Kozłowski, J., Gawelczyk, A.T., 2002. Why are species' body size distributions usually skewed to the right? *Functional Ecology* 16, 419-432.
- Kozłowski, J., Weiner, J., 1997. Interspecific allometries are by-products of body size optimization. *American Naturalist* 149, 352-380.
- Krebs, C.J., 1999. *Ecological Methodology*, 2<sup>nd</sup> edn. Addison-Welsey, Menlo Park.
- Kuntner, M., Sket, B., Blejec, A., 1999. A comparison of the respiratory systems in some cave and surface species of spiders (Aranae, Dysderidae). *Journal of Arachnology* 27, 142-148.
- Lawrence, R.F., 1953. *The Biology of the Cryptic Fauna of Forests*. Balkema, Cape Town.
- Lessells, C.M, Boag, P.T., 1987. Unrepeatable repeatabilities: a common mistake. *Auk* 104, 116-121.
- Lighton, J.R.B., 1991. Measurement on insects. In: Payne, P.A. (ed), *Concise Encyclopedia on Biological and Biomedical Measurement Systems*. Pergamon, Oxford, pp. 201-208.
- Lighton, J.R.B., 1996. Discontinuous gas exchange in insects. *Annual Review of Entomology* 41, 309-324.
- Lighton, J.R.B., Brownell, P.H., Joos, B., Turner, R.J., 2001. Low metabolic rate in scorpions: implications for population biomass and cannibalism. *Journal of Experimental Biology* 204, 607-613.
- Lighton, J.R.B., Duncan, F.D., 1995. Metabolic effects on temperature, mass and activity in an unsegmented arthropod, the velvet mite *Dinothrombium pandorae*. *Journal of Insect Physiology* 41, 877-884.
- Lighton, J.R.B., Fielden, L.J., 1995. Mass scaling of standard metabolism in ticks: A valid case of low metabolic rates in sit-and-wait strategists. *Physiological Zoology* 68, 43-62.

- Lighton, J.R.B., Fielden, L.J., 1996. Gas exchange in wind spiders (Arachnida, Solpugidae): Independent evolution of convergent control strategies in solpugids and insects. *Journal of Insect Physiology* 42, 347-357.
- Magano, S.R., 2000. Tick-host Interactions in *Hyalomma* Species. Ph.D. Thesis, University of Pretoria.
- Marais, E., Chown, S.L., 2003. Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. *Journal of Experimental Biology* 206, 4565-4574.
- McEvoy, P.B., 1984. Increase in respiratory rate during feeding in larvae of the cinnabar moth *Tyria jacobaeae*. *Physiological Entomology* 9, 191-195.
- Nespolo, R.F., Lardies, M.A., Bozinovic, F., 2003. Intrapopulation variation in the standard metabolic rate of insects: repeatability, thermal dependence, and sensitivity ( $Q_{10}$ ) of oxygen consumption in a cricket. *Journal of Experimental Biology* 206, 4309-4315.
- Piersma, T., Drent, J., 2003. Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution* 18, 228-233.
- Punzo F., 1991. The effects of temperature and moisture on survival capacity, cuticular permeability, hemolymph osmoregulation and metabolism in the scorpion, *Centruroides hentzi* (Banks) (Scorpiones, Buthidae). *Comparative Biochemistry and Physiology A* 100, 833-837.
- Reinhold, K., 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology* 13, 217-224.
- Rogowitz, G.L., Chappell, M.A., 2000. Energy metabolism of eucalyptus-boring beetles at rest and during locomotion: gender makes a difference. *Journal of Experimental Biology* 203, 1131-1139.
- Schmidt-Nielsen, K., 1984. *Scaling. Why is Animal Size so Important?* Cambridge University Press, Cambridge.
- Sgrò, C.M., Partridge, L., 2000. Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *American Naturalist* 156, 341-353.
- Sgrò, C.M., Partridge, L., 2001. Laboratory adaptation of life history in *Drosophila*. *American Naturalist* 158, 657-658.
- Sinclair, B.J., Klok, C.J., Scott, M.B., Terblanche, J.S., Chown, S.L., 2003. Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. *Journal of Insect Physiology* 49, 1049-1061.
- Spicer, J.I., Gaston, K.J., 1999. *Physiological Diversity and its Ecological Implications*. Blackwell Science, Oxford.

- Sokolova, I.M., Pörtner, H.-A., 2003. Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda: Littorinidae) from different latitudes. *Journal of Experimental Biology* 206, 195-207.
- Stamou, G.P., Asikidis, M.D., Argyropoulou, M.D., Iatrou, G.D., 1995. Respiratory responses of Oribatid mites to temperature changes. *Journal of Insect Physiology* 41, 229-233.
- Takahashi-Del-Bianco, M., Benedito-Silva, A.A., Hebling, M.J.A., Marques, N., Marques, M.D., 1992. Circadian oscillatory patterns of oxygen uptake in individual workers of the ant *Camponotus rufipes*. *Physiological Entomology* 17, 377-383.
- Taylor, P., 1977. The respiratory metabolism of tsetse flies, *Glossina* spp., in relation to temperature, blood-meal size and pregnancy cycle. *Physiological Entomology* 2, 317-322.
- Terblanche, J.S., Klok, C.J., Chown, S.L., 2004. Metabolic rate variation in *Glossina pallidipes* (Diptera: Glossinidae): gender, ageing and repeatability. *Journal of Insect Physiology* 50, 419-428.
- Topp, W., 2003. Phenotypic plasticity and development of cold-season insects (Coleoptera: Leiodidae) and their response to climatic change. *European Journal of Entomology* 100, 233-243.
- Wheeler, W.C., Hayashi, C.Y., 1998. The phylogeny of the extant Chelicerate orders. *Cladistics* 14, 173-192.
- Williams, A.E., Rose, M.R., Bradley, T.J., 1997. CO<sub>2</sub> release patterns in *Drosophila melanogaster*: the effect of selection for desiccation resistance. *Journal of Experimental Biology* 200, 615-624.
- Zar, J.H., 1996. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River.

## Chapter 4

### **Temperature-dependence of metabolic rate in *Glossina morsitans morsitans* (Diptera, Glossinidae) does not vary with gender, age, feeding, pregnancy or acclimation\***

*"Complex, statistically improbable things are by their nature more difficult to explain than simple, statistically probable things"*

**Richard Dawkins**

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## Introduction

Metabolic rate may be defined as the summation of sub-cellular, cellular, and organism-level processes which require ATP (Hochachka et al., 2003). As such, metabolic rate represents the costs of living in a specific environment and may in turn provide insight into the evolution of life histories across a range of environments (Chown & Gaston, 1999). For this reason, and because metabolic rate estimates can be readily obtained, there has been considerable attention paid to metabolic rate and its variation for many years (reviewed in McNab, 2002; Chown & Nicolson, 2004). Much of this work has been concerned with allometry (scaling) and the effects of temperature on metabolic rate. The recent development of a 'metabolic theory of ecology' (Brown et al., 2004), which is a general theory apparently explaining the scaling of metabolic rate across all taxa, much of the variation in life histories of organisms, and global-scale variation in diversity, has stimulated renewed interest in metabolic rate and its variation.

In insects, metabolic rate may vary for several reasons, of which the most commonly accepted are size (Lighton & Fielden, 1995), activity (Bartholomew & Lighton, 1985; Reinhold, 1999), and ambient temperature (Keister & Buck, 1964). However, metabolic rate can vary for other reasons too. For example, age and ontogeny, diapause, gender, feeding status, reproductive status, time of day and season can all exercise a considerable influence on metabolic rate (Chown & Nicolson, 2004). Over larger scales, metabolic rate can also vary adaptively, principally in two possible ways. First, a reduction in metabolic rate is often found as a response to xeric conditions, and is thought to function as a water conservation mechanism through a reduction in respiratory water loss (Edney, 1977; Chown, 2002). Second, cold climate (usually high latitude or altitude) populations may show elevated metabolic rates relative to warm climate populations when tested at similar temperatures, which is widely known as temperature compensation (Hazel & Prosser, 1974) or metabolic cold adaptation (MCA, Chown & Gaston, 1999). Although controversial (e.g. Clarke, 2003) MCA may be beneficial to insects by enabling them to complete growth, development and reproduction at relatively cooler temperatures (Chown & Gaston, 1999).

Metabolic rate and its temperature dependence (or rate-temperature (R-T) relationships) are also responsive to environmental variation, and the metabolic response to temperature can differ between populations and between species (Scholander et al., 1953; Sømme & Block, 1991; Addo-Bediako et al., 2002). In general, however, the patterns in and processes underlying variation in R-T relationships have not been systematically explored for insects, although variation of metabolic rate with temperature has been widely documented (Keister & Buck, 1964; Chown & Nicolson, 2004). Nonetheless, there have been some comparative studies. At the intraspecific level, Berrigan & Partridge (1997) found no relationship between the slope of the R-T relationship and latitude in *Drosophila melanogaster*. By contrast, Chown et al. (1997) documented systematic changes in the

R-T relationship among populations of weevils on sub-Antarctic Marion Island such that the higher elevation populations tended to have reduced slopes than those at lower elevations. At the interspecific level, Addo-Bediako et al. (2002) found an increase in the slope of the R-T relationship with increasing latitude in northern (but not southern) hemisphere species. They suggested that hemispheric variation in the extent of latitudinal change of the R-T relationship might be a consequence of more variable environments in the northern than in the southern hemispheres.

Thus, it is clear that comprehension of the nature and extent of variation in R-T relationships can provide considerable insight into the responses of organisms to their environments and how these responses influence variation in diversity at a variety of hierarchical levels. Nowhere has this become more clear than in the recent debate regarding the underlying causes of temperature dependence of metabolic rate and their consequences for the metabolic theory of ecology (Gillooly et al., 2001; Clarke, 2004; Clarke & Fraser, 2004). Because variation in the temperature dependence of metabolic rate must, at least to some extent, be a function of natural selection (Hochachka & Somero, 2002; Clarke, 2004), understanding this variation at a population level is of considerable importance in the context of the way in which the environment influences R-T relationships. Indeed, Bennett (1987) pointed out that, despite its importance for understanding the evolution of physiological responses, inter-individual variation is surprisingly poorly investigated.

In this chapter I therefore explore the effects of gender, feeding status, pregnancy and age on metabolic rate and its temperature dependence in the tsetse fly *Glossina morsitans morsitans* (Diptera, Glossinidae). In addition, using three acclimation temperatures, I investigate the influence of temperature on metabolic rate and R-T relationships. My aim is not simply to determine the extent of metabolic rate variation with these factors, but more importantly to determine whether they influence the R-T relationship. In choosing a tsetse fly as a model organism, I recognize that it is one of a group of vectors of trypanosomes that can infect both humans and animals, which are therefore of considerable medical and socio-economic importance (Leak, 1999). Although metabolic rate in tsetse has received considerable attention, both in the laboratory (Rajagopal & Bursell, 1966; Taylor, 1977a; b; 1978a; b; Terblanche et al., 2004) and in the field (Taylor, 1978b; Hargrove & Coates, 1990), variation in the temperature-dependence of tsetse metabolic rate is relatively poorly understood. Recent models of tsetse population dynamics typically include the effects of temperature on various life-history parameters (Hargrove, 2004), many of which (at least those associated with production – see Kozłowski & Gawelczyk, 2002) are influenced by metabolic rate. These models necessarily simplify temperature effects across various physiological stages and cohorts, although the extent to which such assumptions are valid is not known. Thus an additional goal of this work is to determine whether these simplifying assumptions are realistic.

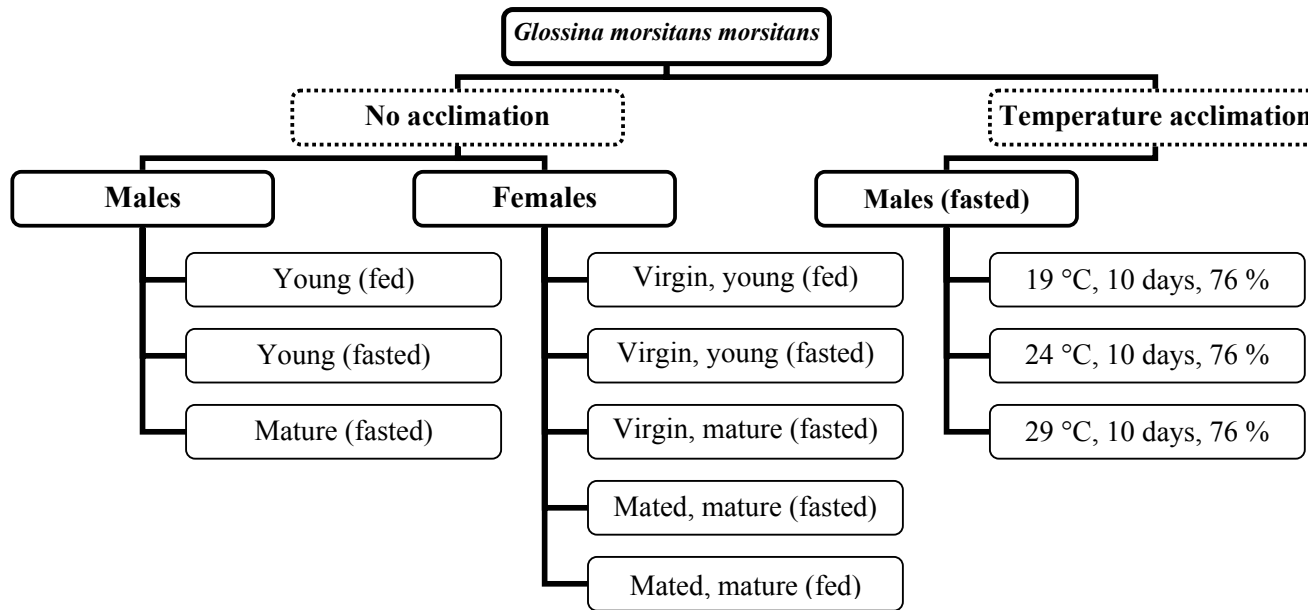
## Materials and methods

### *Study animals and laboratory conditions*

Pupae of *Glossina morsitans morsitans* Westwood (Diptera, Glossinidae) were obtained from the laboratory colony maintained at the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, International Atomic Energy Agency, Vienna, Austria. Gene diversities over four mitochondrial loci in these laboratory flies are within the range of six field populations (Wohlford et al., 1999). Similarly, microsatellite diversities are homogeneous among two laboratory strains and six field populations (Krafsur & Endsley, 2002). On receipt, pupae were immediately placed inside plastic containers and transferred to a climate chamber set to 24 °C ( $24.6 \pm 2.5$  °C, photoperiod: 12H L: 12H D). Relative humidity (R.H.) was regulated to 76 % by means of saturated salt (NaCl) solutions located within each container (Winston & Bates, 1960). Flies were transferred to mesh cages (10 cm diameter, n ~ 25-35 per cage) after eclosion for use in various gender, age and feeding categories (Fig. 1). In acclimation experiments, similar procedures were used (4-5 mesh cages per acclimation treatment, total n ~ 100 per treatment). All cages were located within closed, non-airtight plastic containers which also held 150 ml jars containing saturated salt solution to maintain a constant relative humidity (~76% R.H.). Feeding took place using a membrane-tray system (Gooding et al., 1997) every alternative day (similar to the methods described in Terblanche et al., 2004), at which point container locations were randomized within climate chambers. Care was taken to ensure that all treatment groups were handled for the same duration during transfer from the climate chamber to the feeding area, and spent a similar amount of time outside the climate chambers during feeding (~25 min per acclimation group).

Flies used for respirometry approximately one hour after a bloodmeal were considered 'fed', whilst flies which had not fed for at least 36 hours were considered 'fasted'. Females separated upon eclosion were used in the 'virgin' female category, and mixed sex cages were used for selection of 'mated' females (Fig. 1). Dissection of five 'mated' females confirmed that these flies contained developing larvae and were appropriately classified as pregnant. All flies used in these experimental classes were reared in climate chambers set at 24-25 °C with the same photoperiod and humidity described for pupal rearing conditions (above). A comparison of seven and seventeen-day old fasted flies was also performed to test for an effect of age in males and females separately.





**Figure 1.** Schematic diagram of the experimental categories used to investigate the temperature-dependence of metabolic rate and its variation in *Glossina morsitans morsitans*. No acclimation = reared at 24 °C, 76 % relative humidity (12L:12D photoperiod) and fed every alternative day on bovine blood. Temperature acclimation = all conditions were the same as described for the “no acclimation” experimental categories except that flies were exposed to one of three temperature treatments (19, 24, 29 °C) lasting 10 days. All acclimated experiments were performed on fasted flies (i.e. non-feeding days).

Male flies were acclimated at 19, 24 and 29 °C temperature regimes for ten days. Temperatures were maintained by climate chambers (Labocon, South Africa) with the same synchronized photoperiod (12L: 12D) and constant 76 % humidity levels used for rearing pupae (the resulting mean ( $\pm$  SD) temperatures as recorded from ThermoChron iButtons (Dallas Semiconductors, Dallas, USA; sampling rate = 15 min) were  $18.6 \pm 0.4$ ;  $23.3 \pm 3.1$ ;  $28.1 \pm 2.0$  °C). All experimental and rearing facilities were housed in air-conditioned laboratories. Due to equipment constraints only male *G. m. morsitans* were used for acclimation treatments.

### *Metabolic rates*

Metabolic rates were recorded as carbon dioxide production using flow-through respirometry. A LI-6262 (LiCor, Lincoln, USA) infra-red gas analyzer (IRGA) was connected to a Sable Systems International (Las Vegas, USA) eight channel multiplexer inside a Sable Systems PTC-1 temperature-controlled cabinet (similar to the methods described in Terblanche et al., 2004). The first seven channels regulated the flow-through respirometry for individual flies and channel eight was used as an empty reference channel for CO<sub>2</sub> and H<sub>2</sub>O baseline measurements. All electronic units were connected to a desktop computer and Sable Systems DATACAN V software was used for instrument control and data acquisition. These recordings were performed for males (n = 7) and females (n = 7) separately at various temperatures (20, 24, 28 and 32 °C) among the various experimental categories (Fig. 1). Flies were selected at random from all available cages for use in an experimental category to minimize any cage by treatment effect. Mass was recorded before and after respirometry recordings using a calibrated electronic balance (0.1 mg; FA 304T, Avery Berkel, EU). Airflow was regulated to 100 ml.min<sup>-1</sup> and the compressed air was passed through sodalime, silica-gel and Drierite columns to give 0 % R.H. and 0 % CO<sub>2</sub>. The multiplexer was programmed to regulate airflow through channels 1 to 7 consecutively for 14 minutes at a time during one recording (sampling rate = 1 sec). While the active channel was recorded, an additional airflow line was set up to flush the other six inactive channels to prevent the build-up of CO<sub>2</sub> and water vapour in the cuvettes. A 30 sec interval was programmed between each channel switching event to allow the active cuvette's airflow to stabilize before recording continued. A thermocouple, connected to a Sable Systems TC-1000 Thermocouple meter, was inserted into the first cuvette to measure the temperature inside a respirometry chamber containing an individual fly. The PTC-1 temperature was manually set at 20, 24, 28, 32 °C at the start of each test temperature's respirometry recording. The test temperatures were fixed in increasing order during all experiments to remove possible confounding time-temperature interactions between experimental groups. Respirometry recording was only started once the cuvette temperature was within 0.5 °C of the target test temperature. A Sable Systems AD-1 electronic activity detector was connected to the first

cuvette only and used to identify CO<sub>2</sub> traces representative of stable, resting metabolic rate. Similar traces were then selected for all other flies for analysis of standard metabolic rate at a given temperature. Sable Systems DATACAN V software was used to extract and analyze standard (resting) metabolic rate (SMR) data.

### *Statistical analyses*

Data were checked for normality using a Shapiro-Wilks test, and in most cases log<sub>10</sub> transformation corrected, or at least improved, metabolic rate data which were not normally distributed (Zar, 1999). The effects of feeding status, gender, test temperature, mass, and interactions of the categorical variables on log<sub>10</sub> metabolic rate were assessed using general linear models (GLM). Mean experimental mass of each individual was included as a covariate to ensure that its strong effect on metabolic rate was accounted for. To further explore differences in R-T relationships among groups, especially because I could not obtain an interaction effect between treatment temperature (continuous variable) and the categorical variables, multiple regressions (ordinary least-squares, Type III) were used to determine slopes of log<sub>10</sub> metabolic rate-temperature relationships including mass as a covariate. The R-T slopes were then compared within various categories (age, feeding status, gender, pregnancy, acclimation treatment) using a GT-2 multiple pair-wise comparison (Sokal & Rohlf, 1995). General linear models, including mass as a covariate, were also used to investigate the effects of age on log<sub>10</sub> metabolic rate for each gender separately, and to investigate the effect of acclimation treatment on log<sub>10</sub> metabolic rate in male flies. Data are presented as means ± standard error of the mean (S.E.) unless otherwise stated and significance was set at  $P = 0.05$ .

## **Results**

### **Non-acclimated flies**

#### *Gender and feeding status effects in young flies*

Body mass and test temperature both had a significant positive influence on metabolic rate (Tables 1 & 2; Fig. 2). Furthermore, metabolic rate (throughout I use this term to mean metabolic rate corrected for mass in the GLM) was significantly affected by gender in *G. m. morsitans* at all test temperatures when flies were in the fasted state, although in the fed state males had lower metabolic rates than females only at 24 and 28 °C. Thus, there were significant interactions between gender and feeding status (Table 2). The slopes of the metabolic R-T relationship remained constant among all categories (Table 3).

**Table 1.** Summary statistics for metabolic rate and mass in the categories of *Glossina morsitans morsitans* investigated in this study. Standard metabolic rate data are presented as recorded at 20 °C using flow-through respirometry. ‘Fasted’ and ‘Fed’ classes were all reared at 24 °C. Only male flies in a fasted state were used in the assessment of acclimated metabolic rate-temperature relationships. Mass presented as mean experimental mass ( $\pm$  S.E.).

Class	Gender	Mating status	Age (days)	Mass $\pm$ S.E. (mg)	Metabolic rate $\pm$ S.E. ( $\mu\text{LCO}_2\cdot\text{hr}^{-1}$ )	<i>n</i>
Fasted						
	Male		6-7	18.09 $\pm$ 1.71	10.37 $\pm$ 0.93	7
	Female	Virgin	6-7	30.09 $\pm$ 1.90	26.78 $\pm$ 2.12	7
	Female	Virgin	19-20	31.61 $\pm$ 2.88	20.27 $\pm$ 2.84	7
Fed						
	Male		6-7	30.08 $\pm$ 1.58	19.74 $\pm$ 1.45	6
	Female	Virgin	6-7	32.41 $\pm$ 1.12	20.61 $\pm$ 1.77	7
	Female	Virgin	19-20	48.99 $\pm$ 1.34	40.63 $\pm$ 2.97	7
	Female	Mated	19-20	46.51 $\pm$ 3.57	36.49 $\pm$ 4.48	7
Acclimated						
19 °C	Male		16-17	27.95 $\pm$ 1.00	22.07 $\pm$ 2.29	7
24 °C	Male		16-17	20.94 $\pm$ 1.12	12.03 $\pm$ 0.48	7
29 °C	Male		16-17	22.49 $\pm$ 0.82	13.90 $\pm$ 0.54	7

#### *Reproductive and feeding status effects in mature female flies*

In mature female *G. m. morsitans*, metabolic R-T relationships were unaffected by mating status (GLM  $F_{1, 49} = 1.82$ ;  $P = 0.184$ ), although statistically significant effects of body size and temperature remained. By contrast with younger flies (Table 2), feeding status had no effect on the metabolic R-T relationships of mature females (Table 3). In mature female flies, metabolic rate was unaffected by feeding status (GLM  $F_{1, 49} = 0.89$ ;  $P = 0.35$ ), and the slopes of the relationships were statistically identical among these categories (Table 3).

#### *Effects of ageing on metabolic rate-temperature relationships*

Whilst metabolic rate increased in mature (17 days) fasted male flies relative to younger (7 days) flies acclimated at 24 °C (Table 4), there was no effect of these age groups on the metabolic R-T relationship (Table 3). By contrast, in fasted virgin females’ metabolic rate declined with age

**Table 2.** Results of a general linear model (GLM) testing for the effects of gender, feeding status, test temperature, mass, and the interaction of the categorical factors, on metabolic rate (log mlCO<sub>2</sub>.hr<sup>-1</sup>) in early adult (6-7 days old, 3 bloodmeals) *Glossina morsitans morsitans* (Diptera, Glossinidae). Temperature and mass treated as continuous variables in the GLM. (SS = sums of squares; DF = degrees of freedom; MS = mean squares).

Effect	SS	DF	MS	F-ratio	P
Intercept	14.941	1	14.941	3485.46	< 0.0001
Covariate: test temperature	2.241	1	2.241	522.86	< 0.0001
Covariate: mean experimental mass	1.290	1	1.290	300.98	< 0.002
Gender	0.044	1	0.044	10.16	< 0.0001
Feeding status	0.120	1	0.120	27.92	< 0.0001
Gender x Feeding status	0.078	1	0.078	18.13	< 0.0001
Error	0.433	101	0.004		

(Table 4), whilst as in the case of male *G. m. morsitans* there was no effect of age on the temperature sensitivity (slope) of metabolic rate (Table 3).

### Acclimated flies

#### *High and low temperature acclimation*

Acclimation resulted in a significant increase in metabolic rate measured at 20, 24, 28 and 32 °C in male *G. m. morsitans* from the cold, but not in the warm-acclimated group relative to the mid-temperature group (Table 5; Fig. 3). Within each acclimation group, ordinary least-squares regressions (Type III) of log<sub>10</sub> metabolic rate against both temperature and body mass were highly significant (Table 3). There was no effect of acclimation treatment on metabolic R-T relationships (Table 3 & 5). Metabolic R-T relationships in 29 °C-acclimated male *G. m. morsitans* remained the same as in 24 °C-acclimated flies (*Post hoc* tests). By contrast, metabolic rate was up-regulated in the 19 °C-acclimated flies at all test temperatures, constituting an overall increase in metabolism rather than a change in temperature sensitivity as such (Table 3 & 5; Fig. 3). That is, while the slope of the relationship between metabolic rate and temperature did not change, the intercept for this relationship increased substantially with cold acclimation.

**Table 3.** Outcomes of the multiple regression (Type III) analyses testing for the effects of temperature (holding mass constant) on metabolic rate in various experimental categories of *Glossina morsitans morsitans*. Comparisons of slopes within various *Effect* groups performed using GT-2 analyses for multiple pair-wise comparisons of slopes (Sokal and Rohlf, 1995). (DF = degrees of freedom, Adj. R<sup>2</sup> = adjusted R<sup>2</sup>.) *P*<sub>Among</sub> refers to comparisons between groups, *P*<sub>Within</sub> is significance of rate-temperature regression within an experimental category.

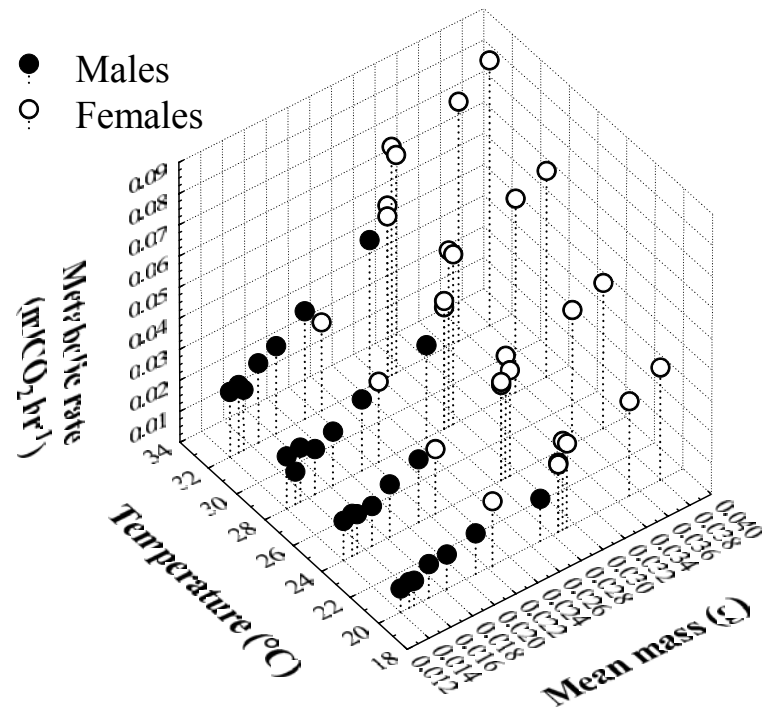
Effect	Gender	Category	Slope ± SE	DF	F-ratio	<i>P</i> <sub>Within</sub>	Adj. R <sup>2</sup>	Critical value	<i>P</i> <sub>Among</sub>
Feeding	Males	Fasted	0.03332 ± 0.002660	2, 24	114.555	< 0.0001	0.897		
		Fed	0.03076 ± 0.002770	2, 21	158.993	< 0.0001	0.932		
	Females	Fasted	0.03035 ± 0.002734	2, 25	98.724	< 0.0001	0.833		
		Fed	0.03428 ± 0.003002	2, 25	68.488	< 0.0001	0.833	3.041	> 0.05
Ageing	Males (Fasted)	Young	0.03332 ± 0.002660	2, 24	114.555	< 0.0001	0.897		
	Males (Fasted)	Mature	0.03834 ± 0.004077	2, 24	45.101	< 0.0001	0.772		
	Females (Fasted, Virgin)	Young	0.03035 ± 0.002734	2, 25	98.724	< 0.0001	0.833		
	Females (Fasted, Virgin)	Mature	0.03179 ± 0.005521	2, 25	29.417	< 0.0001	0.678	3.041	> 0.05
Pregnancy	Females (Fed)	Mated	0.03046 ± 0.005205	2, 22	25.041	< 0.0001	0.640		
	Females (Fed)	Virgin	0.03556 ± 0.004355	2, 22	34.111	< 0.0001	0.734		
	Females (Fasted)	Virgin	0.03179 ± 0.005521	2, 25	29.417	< 0.0001	0.678	2.454	> 0.05
Acclimation	Males (Fasted)	19 °C	0.03704 ± 0.004930	2, 25	30.314	< 0.0001	0.685		
	Males (Fasted)	25 °C	0.03834 ± 0.004077	2, 24	45.101	< 0.0001	0.772		
	Males (Fasted)	29 °C	0.03403 ± 0.003130	2, 25	60.107	< 0.0001	0.814	2.454	> 0.05

**Table 4.** Outcome of a general linear model of the effect of age (7 vs. 17 days old) on metabolic rate ( $\log_{10}$  mlCO<sub>2</sub>.hr<sup>-1</sup>) in fasted male and female *Glossina morsitans morsitans* reared at 24 °C.

Effect	SS	DF	MS	F-ratio	P
<i>Males</i>					
Intercept	7.603	1	7.603	1056.74	< 0.0001
Covariate: test temperature	1.432	1	1.432	199.00	< 0.0001
Covariate: mean experimental mass	0.229	1	0.229	31.82	< 0.0001
Age	0.068	1	0.068	9.48	< 0.004
Error	0.360	50	0.007		
<i>Females</i>					
Intercept	7.411	1	7.411	710.74	< 0.0001
Covariate: test temperature	1.081	1	1.081	103.69	< 0.0001
Covariate: mean experimental mass	0.739	1	0.739	70.82	< 0.0001
Age	0.255	1	0.255	24.49	< 0.0001
Error	0.542	52	0.010		

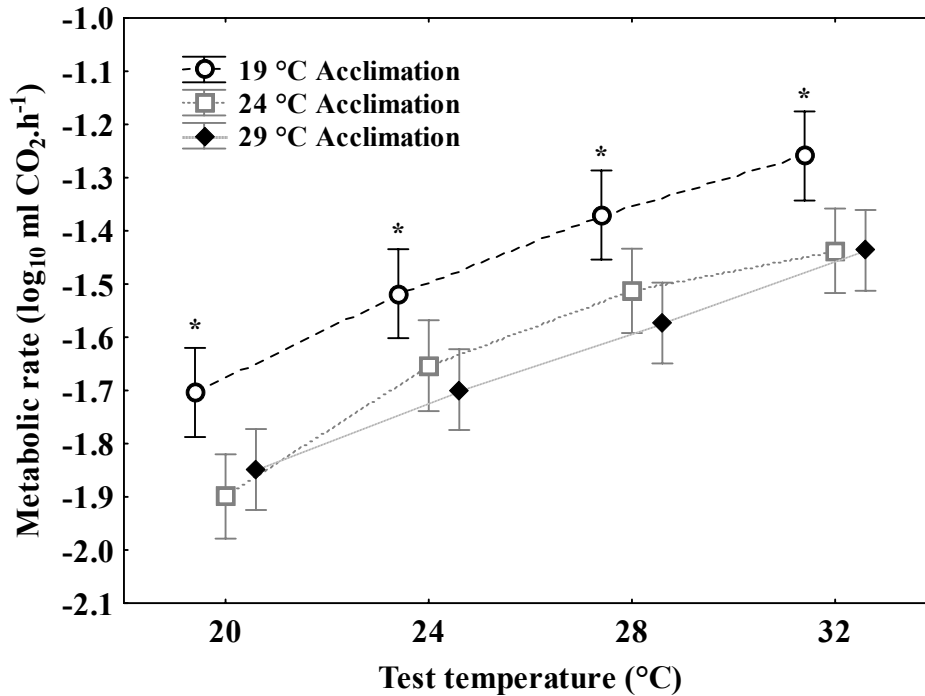
**Table 5.** Outcome of a general linear model testing for the effect of ten days of acclimation at three temperature treatments (either 19, 24 or 29 °C) on A) metabolic rate ( $\log_{10}$  mlCO<sub>2</sub>.hr<sup>-1</sup>) and B) homogeneity of slopes of metabolic rate-temperature relationships in *Glossina morsitans morsitans* (Diptera, Glossinidae).

Effect	SS	DF	MS	F-ratio	P
A)					
Intercept	4.699	1	4.699	483.29	< 0.0001
Covariate: test temperature	2.228	1	2.228	229.20	< 0.0001
Covariate: mean experimental mass	0.029	1	0.029	3.00	0.087
Acclimation	0.240	2	0.240	12.32	< 0.0001
Error	0.758	78	0.010		
B)					
Intercept	0.187	1	0.187	19.033	< 0.0001
Covariate: mean experimental mass	0.002	1	0.002	0.24	0.624
Acclimation x Temperature	0.0001	2	0.00004	0.004	0.996
Acclimation x Temp x Mass	0.0002	2	0.0001	0.011	0.989
Error	0.698	71	0.010		



**Figure 2.** 3D scatterplot showing mean metabolic rate in *Glossina morsitans morsitans* (Diptera, Glossinidae) for fasted post-developmental males and females (reared at 24 °C and 76 % relative humidity) at four experimental test temperatures at mean experimental mass.





**Figure 3.** The effect of ten days of laboratory acclimation at 19, 24 and 29 °C on mass-independent metabolic rate in male *Glossina morsitans morsitans* (Diptera, Glossinidae). Symbols are least-squares adjusted means ( $\pm$  95% confidence limits). Asterisks represent significant differences (see Table 5 for statistics) at a particular test temperature between acclimation treatments (*Post-hoc* unequal sample Honest Significant Difference).

## Discussion

Several previous studies have investigated metabolic rate in adult *Glossina* species (Rajagopal & Bursell, 1966; Taylor, 1977b, 1978a; Hargrove & Coates, 1990; Terblanche et al., 2004). In *G. morsitans* and *G. pallidipes*, feeding, pregnancy and maturation elicit increases in resting metabolic rate (Rajagopal & Bursell, 1966; Taylor, 1977b; Terblanche et al., 2004). Fasted teneral male *G. m. orientalis* (mean individual mass of approximately 16.6 mg, estimated from Rajagopal & Bursell, 1966) have a metabolic rate of 124  $\mu$ W (at 25 °C and converted following Lighton, 1991). At a similar temperature and using a similar closed-system respirometer Taylor (1977b) found metabolic rates of about 178  $\mu$ W in laboratory-reared male *G. m. morsitans* of an unspecified mean mass. By comparison with these studies, my values for *G. m. morsitans* are relatively low, and ranged from 75  $\mu$ W in young fasted males (mass: 18.1 mg) to 87  $\mu$ W in mature fasted males (mass: 20.1 mg; metabolic rate converted to 25 °C using an assumed  $Q_{10} = 2.0$ ). However, these lower metabolic rate values are not unexpected because it has been shown that

many kinds of closed-system respirometry methods (as opposed to flow-through methods) tend to overestimate metabolic rate in insects, largely because activity cannot be adequately taken into account (see discussion in Lighton & Fielden, 1995; Addo-Bediako et al., 2002). In addition, it has been shown that developing teneral flies can have elevated metabolic rate relative to more mature flies, probably due to increased costs of developing flight muscle and reproductive organs (Bursell, 1961; Hargrove, 1975; Taylor, 1977b; Terblanche et al., 2004). Therefore it is unsurprising that the more mature flies in my study had a lower metabolic rate at a similar mass and temperature than the teneral flies in the studies of Rajagopal and Bursell (1966) and Taylor (1977b).

Although metabolic rate-temperature (R-T) relationships in *G. morsitans* have been determined on two previous occasions (Rajagopal & Bursell, 1966; Taylor, 1977b), no studies have specifically examined the effects of gender, feeding, reproductive status and age, nor of acclimation, on metabolic R-T relationships within this or any other tsetse species. Indeed, as far as I can ascertain, no studies have previously explored the effects of temperature acclimation on any physiological traits among *Glossina* species. Taylor (1977b) found that in *G. m. morsitans* the slope of the R-T relationship was  $0.036 (\pm 0.002)$  and  $0.042 (\pm 0.001)$  for wild-caught and laboratory-reared males, respectively. My estimates for R-T slopes appear more similar to the wild-caught than the laboratory-reared flies in Taylor's (1977b) study. Indeed, young and mature fasted male flies in my study did not differ from Taylor's (1977b) estimates of wild flies (*t*-method for *post-hoc* comparison of slopes, (Zar, 1999); young vs. wild:  $t = 0.89$ ;  $P > 0.1$ ; mature vs. wild:  $t = 0.59$ ;  $P > 0.2$ ). Although my estimate for mature fasted male *G. m. morsitans* did not differ from Taylor's (1977b) wild-caught flies ( $t = 0.92$ ;  $P > 0.2$ ), young fasted males in my study were, however, different from the laboratory-reared flies in Taylor's (1977b) study ( $t = 2.891$ ;  $P < 0.05$ ). Regardless, the range of values for R-T relationships found in my study (0.03035-0.03834) virtually encompassed the values found by Taylor (1977b).

Whilst ontogenetic variation in metabolic rate is reasonably well explored in insects (e.g. Hamilton, 1964; Hack, 1997), age-related variation has not been well explored (but see Ernsting & Isaaks, 1991). Where age-related changes in metabolic rate have been observed, such as in *G. morsitans* and *G. pallidipes*, they are usually considered a consequence of developmental changes or changing reproductive performance (Terblanche et al., 2004; see also Langley & Clutton-Brock, 1998). In the case of *G. m. morsitans*, there appears to be no change in the R-T relationship between post-developmental adults (7 day old) and more mature flies (17 day old) reared at 25 °C in both genders, although this was not explored in very mature age classes (e.g. 45-60 days old). However, it is not clear what to expect of the variation in R-T relationships with ageing and maturation in insects. In *Drosophila*, Fleming & Miquel (1983) found different effects of temperature on

metabolic rate in young and old flies with temperature sensitivity increasing by ~1.5 times in the older flies.

In tsetse, females usually have a higher metabolic rate than males. However, it is relatively well established that, at least in *G. pallidipes* and *G. m. morsitans*, metabolic rate does not differ between males and females once the effects of body size have been taken into account (Rajagopal & Bursell, 1966; Taylor, 1977b; Terblanche et al., 2004). In insects, sex-related variation in metabolic rate is not uncommon, usually as a consequence of activity levels and lifestyle (see e.g. Morgan, 1987; Kolluru et al., 2004), and it is also known that metabolic rates in males and females respond differently to temperature acclimation (discussed in Hoffmann, 1985; and see Chaabane et al., 1999). Why this should be the case has not been well established. However, it is known that key metabolic enzymes such as LDH (lactate dehydrogenase) and aldolase respond differently to temperature acclimation in males and females (Das & Das, 1982a; b) possibly as a consequence of sex-specific isozymes. There might also be interactive effects of sex-specific hormones with acclimation, and sex-related differences in protein synthesis requirement (a process known to be energetically expensive (Clarke & Fraser, 2004)) resulting from sex-related differences in body composition (Hoffmann, 1985). Thus, it would not seem unreasonable to expect that the sensitivity of metabolic rate to temperature should differ between the sexes. In my study, however, male and female R-T sensitivity did not differ, but clearly more work is required in order to determine the generality of this outcome for insects.

Feeding can have a dramatic effect on insect metabolic rate (Chown & Nicolson, 2004; Nespolo et al., 2005), and usually causes an increase in rate largely owing to the costs of digestion (heat increment of feeding or specific dynamic action, SDA; Chown & Nicolson, 2004; Nespolo et al., 2005). However, it is not directly evident to what degree temperature affects these costs within or between species. Before comparisons of SDA can be made at various temperatures, peak SDA values must be estimated which, in turn, require frequently-logged, continuously recorded data within resting individuals to correctly observe the post-prandial peak in respiration rate (Bradley et al., 2003). Although here feeding has an effect on the metabolic rate of *G. m. morsitans*, as has been shown previously for *G. pallidipes* (Taylor, 1977b; Terblanche et al., 2004) and *G. morsitans* (Rajagopal & Bursell, 1966; Taylor, 1977b), and the effect of temperature on metabolic rate is similar in fed and unfed individuals, I was not able to determine specifically if the peak in SDA was different at various temperatures.

In *G. m. morsitans*, cold-acclimation increases metabolic rate relative to warm-acclimation (29 °C) and the conditions at which the flies are typically reared (24 °C). Here, the flies that were acclimated to cold conditions also achieved larger body mass, possibly as a consequence of increased feeding requirements or lipid storage. The elevation in metabolic rate at low temperatures

found here is comparable to the findings of Dehnel & Segal (1956) in which significant up-regulation of metabolic rate to cool (10 °C) temperatures occurred within 1-3 weeks in *Periplaneta americana* under similar conditions. In insects, evidence for up-regulation of metabolic rate in response to cold acclimation (or metabolic cold adaptation, MCA) has mainly come from beetles (Schultz et al., 1992; Chown et al., 1997), grasshoppers (Chappell, 1983; Massion, 1983; Rourke, 2000), flies (Chown, 1997) and cockroaches (Dehnel & Segal, 1956). However, not all studies support MCA (e.g. Nylund, 1991; Berrigan & Partridge, 1997), and both the phenomenon and the level at which it should be investigated remain controversial (Chown & Gaston, 1999; Chown et al., 2003; Hodkinson, 2003).

By contrast, there are fewer studies that consider variation in R-T relationships, either as a consequence of geography or acclimation (but see e.g. Berrigan, 1997; Berrigan & Partridge, 1997; Chown, 1997). Addo-Bediako et al. (2002) undertook one of the few recent synthetic analyses of R-T variation with latitude. They found that the R-T relationship steepened towards the pole in the northern hemisphere, but that this did not take place in the south. Their analysis included few tropical species, but comparison of the R-T slopes for the southern hemisphere flies they included (data drawn from Chown, 1997) with the present data show that the slopes of the R-T relationships in flies from Marion and South Georgia Islands are typically steeper than the slopes recorded in *G. m. morsitans*. For example, *Antrops truncipennis*, *Paractora dreuxi* and *P. trichosterna* had slopes ranging from 0.049-0.077, 0.075-0.095 and 0.074-0.085 respectively, which were significantly steeper than the R-T relationships found in *G. m. morsitans* (*Post-hoc t*-method,  $P < 0.001$  in all cases except when *A. truncipennis*' slope = 0.049, then  $P > 0.05$ ). This suggests that there is an increase in the slope of the R-T relationship towards the south, but it may well be gentler than the increase towards the north. This conclusion awaits confirmation using a more detailed set of data.

In conclusion, the present data indicate that irrespective of age, gender, feeding status, pregnancy and acclimation, there is little variation in R-T relationships in *G. m. morsitans*. This finding is robust. A post-hoc power analysis for effect size of  $d = 1.9597$  (calculated from the maximum slope obtained (fasted mature male) vs. minimum slope obtained (fasted young female) with the respective errors) at  $\alpha = 0.05$ , found  $t_{12} = 2.1788$ ,  $\delta = 3.6663$ , and therefore power = 0.9193. This is a relatively high power (see Di Stefano, 2003 for discussion), and it therefore seems unlikely that I have erroneously failed to reject the null hypothesis (i.e. made a Type II error). This finding is important for two reasons. First, it constitutes an explicit and extensive exploration of the effects of age, feeding status, pregnancy, gender and acclimation on R-T relationships in insects, demonstrating that these factors do not influence R-T relationships within a given population, at least in this species. Whilst the direct effects of these factors on metabolic rate are well known (see Chown & Nicolson, 2004), their effects on R-T relationships have not been widely explored.

Second, this finding confirms that tsetse population dynamic models, which typically make simplifying assumptions regarding variation in temperature effects on performance among physiological stages can use a single parameter for these effects, although age, feeding status, mass and acclimation history still need to be taken into account in terms of their absolute effects on metabolic rate. How general these findings are in both contexts remains to be explored in future work.

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## References

- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology* 16, 332-338.
- Bartholomew, G.A., Lighton, J.R.B., 1985. Ventilation and oxygen consumption during rest and locomotion in a tropical cockroach, *Blaberus giganteus*. *Journal of Experimental Biology* 118, 449-454.
- Bennett, A.F. 1987. Interindividual variability: an underutilized resource. In: Feder, M.E., Bennett, A.F., Burggren, W.W., Huey, R.B. (eds), *New Directions in Ecological Physiology*. Cambridge University Press, Cambridge, pp. 147-169.
- Berrigan, D., 1997. Acclimation of metabolic rate in response to developmental temperature in *Drosophila melanogaster*. *Journal of Thermal Biology* 22, 213-218.
- Berrigan, D., Partridge, L., 1997. Influence of temperature and activity on the metabolic rate of adult *Drosophila melanogaster*. *Comparative Biochemistry and Physiology A* 118, 1301-1307.
- Bradley, T.J., Brethorst, L., Robinson, S., Hetz, S., 2003. Changes in the rate of CO<sub>2</sub> release following feeding in the insect *Rhodnius prolixus*. *Physiological and Biochemical Zoology* 76, 302-309.
- Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. *Ecology* 85, 1771-1789.
- Bursell, E., 1961. Post-teneral development of the thoracic musculature in tsetse flies. *Proceedings of the Royal Entomological Society of London A* 36, 69-74.

- Chaabane, K., Josens, G., Loreau, M., 1999. Respiration of *Abax ater* (Coleoptera, Carabidae): a complex parameter of the energy budget. *Pedobiologia* 43, 305-318.
- Chappell, M.A., 1983. Metabolism and thermoregulation in desert and montane grasshoppers. *Oecologia* 56, 126-131.
- Chown, S.L., 1997. Thermal sensitivity of oxygen uptake of Diptera from sub-Antarctic South Georgia and Marion Island. *Polar Biology* 17, 81-86.
- Chown, S.L., 2002. Respiratory water loss in insects. *Comparative Biochemistry and Physiology A* 133, 791-804.
- Chown, S.L., Gaston, K.J., 1999. Exploring links between physiology and ecology at macro scales: the role of respiratory metabolism in insects. *Biological Reviews* 74, 87-120.
- Chown, S.L., Nicolson, S.W., 2004. *Insect Physiological Ecology. Mechanisms and Patterns*. Oxford University Press, Oxford.
- Chown, S.L., Van Der Merwe, M., Smith, V.R., 1997. The influence of habitat and altitude on oxygen uptake in sub-antarctic weevils. *Physiological Zoology* 70, 116-124.
- Chown, S.L., Addo-Bediako, A., Gaston, K.J., 2003. Physiological diversity: listening to the large-scale signal. *Functional Ecology* 17, 568-572.
- Clarke, A., 2003. Costs and consequences of evolutionary temperature adaptation. *Trends in Ecology and Evolution* 18, 573-581.
- Clarke, A., 2004. Is there a Universal Temperature Dependence of metabolism? *Functional Ecology* 18, 252-256.
- Clarke, A., Fraser, K.P.P., 2004. Why does metabolism scale with temperature? *Functional Ecology* 18, 243-251.
- Das, A.K., Das, A.B., 1982a. Compensations for temperature in the activities of digestive enzymes of *Periplaneta americana* (L.). *Comparative Biochemistry and Physiology A* 71, 255-263.
- Das, A.K., Das, A.B., 1982b. Patterns of enzymatic compensations for temperature in the fat body of male and female *Periplaneta americana* (Linn.). *Proceedings of the Indian National Science Academy B* 48, 44-54.
- Dehnel, P.A., Segal, E., 1956. Acclimation of oxygen consumption to temperature in the American cockroach (*Periplaneta americana*). *Biological Bulletin* 111, 53-61.
- Di Stefano, J., 2003. How much power is enough? Against the development of an arbitrary convention for statistical power calculations. *Functional Ecology* 17, 707-709.
- Edney, E.B., 1977. *Water Balance in Land Arthropods*. Springer, Berlin.
- Ernsting, G., Isaaks, J.A., 1991. Accelerated aging - a cost of reproduction in the carabid beetle *Notiophilus biguttatus* F. *Functional Ecology* 5, 299-303.

- Fleming, J.E., Miquel, J., 1983. Effects of temperature on the metabolic rate of young and old *Drosophila*. *Experientia* 39, 267-268.
- Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. *Science* 293, 2248-2251.
- Gooding, R.H., Feldmann, U., Robinson, A.S., 1997. Care and maintenance of tsetse colonies. In: Crampton, J.M., Beard, C.B., Louis, C. (eds), *The Molecular Biology of Insect Disease Vectors*. Chapman & Hall, London, pp. 41-55.
- Hack, M.A., 1997. The effects of mass and age on standard metabolic rate in house crickets. *Physiological Entomology* 22, 325-331.
- Hamilton, A.G., 1964. The occurrence of periodic or continuous discharge of carbon dioxide by male desert locusts (*Schistocerca gregaria* Forskål) measured by an infra-red gas analyser. *Proceedings of the Royal Society of London B* 160, 373-395.
- Hargrove, J.W., 1975. Some changes in the flight apparatus of tsetse flies during maturation. *Journal of Insect Physiology* 21, 1485-1489.
- Hargrove, J.W., 2004. Tsetse population dynamics. In: Maudlin, I., Holmes, P.H., Miles, M.A. (eds), *The Trypanosomiases*. CABI Publishing, Wallingford. pp. 113-138.
- Hargrove, J.W., Coates, T.W., 1990. Metabolic rates of tsetse flies in the field as measured by the excretion of injected cesium. *Physiological Entomology* 15, 157-166.
- Hazel, J.R., Prosser, C.L., 1974. Molecular mechanisms of temperature compensation in poikilotherms. *Physiological Reviews* 54, 620-677.
- Hochachka, P.W., Darveau, C.A., Andrews, R.D., Suarez, R.K., 2003. Allometric cascade: a model for resolving body mass effects on metabolism. *Comparative Biochemistry and Physiology A* 134, 675-691.
- Hochachka, P. W., Somero, G. N., 2002. *Biochemical Adaptation. Mechanism and Process in Physiological Evolution*. Oxford University Press, New York.
- Hodkinson, I.D., 2003. Metabolic cold adaptation in arthropods: a smaller-scale perspective. *Functional Ecology* 17, 562-567.
- Hoffmann, K.H., 1985. Metabolic and enzyme adaptation to temperature. In: Hoffmann, K.H. (ed), *Environmental Physiology and Biochemistry of Insects*. Springer-Verlag, Berlin, pp. 1-32.
- Keister, M., Buck, J., 1964. Some endogenous and exogenous effects on rate of respiration. In: Rockstein, M. (ed), *Physiology of Insecta*, Vol 3. Academic Press, New York, pp. 617-658.
- Kolluru, G.R., Chappell, M.A., Zuk, M., 2004. Sex differences in metabolic rates in field crickets and their dipteran parasitoids. *Journal of Comparative Physiology B* 174, 641-648.
- Kozłowski, J., Gawelczyk, A.T., 2002. Why are species' body size distributions usually skewed to the right? *Functional Ecology* 16, 419-432.



- Krafsur, E.S., Endsley, M.A., 2002. Microsatellite diversities and gene flow in the tsetse fly, *Glossina morsitans s.l.* Medical and Veterinary Entomology 16, 292-300.
- Langley, P.A., Clutton-Brock, T.H., 1998. Does reproductive investment change with age in tsetse flies, *Glossina morsitans morsitans* (Diptera : Glossinidae)? Functional Ecology 12, 866-870.
- Leak, S.G.A., 1999. Tsetse Biology and Ecology. Their Role in the Epidemiology and Control of Trypanosomosis. CABI Publishing, New York.
- Lighton, J.R.B., 1991. Measurements on insects. In: Payne, P.A. (ed), Concise Encyclopedia on Biological and Biomedical Measurement Systems. Pergamon Press, Oxford, pp. 201-208.
- Lighton, J.R.B., Fielden, L.J., 1995. Mass scaling of standard metabolism in ticks: A valid case of low metabolic rates in sit-and-wait strategists. Physiological Zoology 68, 43-62.
- Massion, D.D., 1983. An altitudinal comparison of water and metabolic relations in two acridid grasshoppers (Orthoptera). Comparative Biochemistry and Physiology A 74, 101-105.
- McNab, B.K., 2002. The Physiological Ecology of Vertebrates. Cornell University Press, Ithaca.
- Morgan, K.R., 1987. Temperature regulation, energy metabolism and mate-searching in rain beetles (*Pleocomma* spp.), winter-active, endothermic scarabs (Coleoptera). Journal of Experimental Biology 128, 107-122.
- Nespolo, R.F., Castaneda, L.E., Roff, D.A., 2005. The effect of fasting on activity and resting metabolism in the sand cricket, *Gryllus firmus*: a multivariate approach. Journal of Insect Physiology 51, 61-66.
- Nylund, L., 1991. Metabolic rates in *Calathus melanocephalus* (L) (Coleoptera, Carabidae) from alpine and lowland habitats (Jeloy and Finse, Norway and Drenthe, The Netherlands). Comparative Biochemistry and Physiology A 100, 853-862.
- Rajagopal, P.K., Bursell, E., 1966. The respiratory metabolism of resting tsetse flies. Journal of Insect Physiology 12, 287-297.
- Reinhold, K., 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. Functional Ecology 13, 217-224.
- Rourke, B.C., 2000. Geographic and altitudinal variation in water balance and metabolic rate in a California grasshopper, *Melanoplus sanguinipes*. Journal of Experimental Biology 203, 2699-2712.
- Scholander, P.F., Flagg, W., Walters, V., Irving, L., 1953. Climatic adaptation in Arctic and Tropical poikilotherms. Physiological Zoology 26, 67-92.
- Schultz, T.D., Quinlan, M.C., Hadley, N.F., 1992. Preferred body temperature, metabolic physiology, and water balance of adult *Cicindela longilabris*: A comparison of populations from boreal habitats and climatic refugia. Physiological Zoology 65, 226-242.



- Sokal, R.R., Rohlf, F.J., 1995. Biometry. The Principles and Practice of Statistics in Biological Research. W. H. Freeman, New York.
- Sømme, L., Block, W., 1991. Adaptations to alpine and polar environments. In: Lee, R.E., Denlinger, D.L. (eds), *Insects at Low Temperature*. Chapman and Hall, London, pp. 318-359.
- Taylor, P., 1977a. A continuously recording respirometer, used to measure oxygen consumption and estimate locomotor activity in tsetse flies, *Glossina morsitans*. *Physiological Entomology* 2, 241-245.
- Taylor, P., 1977b. The respiratory metabolism of the tsetse (Diptera: Glossinidae) in relation to temperature, blood-meal size and the pregnancy cycle. *Physiological Entomology* 2, 317-322.
- Taylor, P., 1978a. Radioisotopes as metabolic labels for *Glossina* (Diptera: Glossinidae). I. Laboratory determination of the relationship between radioisotope metabolism, respiration and temperature. *Bulletin of Entomological Research* 68, 1-9.
- Taylor, P., 1978b. Radioisotopes as metabolic labels for *Glossina* (Diptera: Glossinidae). II. The excretion of  $^{137}\text{Cs}$  under field conditions as a means of estimating energy utilisation, activity and temperature regulation. *Bulletin of Entomological Research* 68, 331-340.
- Terblanche, J.S., Klok, C.J., Chown, S.L., 2004. Metabolic rate variation in *Glossina pallidipes* (Diptera : Glossinidae): gender, ageing and repeatability. *Journal of Insect Physiology* 50, 419-428.
- Winston, P.W., Bates, D.H., 1960. Saturated solutions for the control of humidity in biological research. *Ecology* 41, 232-237.
- Wohlford, D.L., Krafur, E.S., Griffiths, N.T., Marquez, J.G., Baker, M.D., 1999. Genetic differentiation of some *Glossina morsitans morsitans* populations. *Medical and Veterinary Entomology* 13, 377-385.
- Zar, J.H., 1999. *Biostatistical Analysis*. Prentice-Hall, New Jersey.

## Chapter 5

### **The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalcoptera* (Coleoptera: Chrysomelidae)\***

*“The important thing is not to stop questioning.”*

**Albert Einstein**

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## Introduction

The ways in which physiological responses vary through space and the extent to which phenotypic plasticity might effect a change in these responses over time are enjoying renewed interest, most notably because of concerns about climate change effects on biota, and an increase in the sophistication of molecular and genomic tools to investigate these questions (Feder & Mitchell-Olds, 2003; Hoffmann et al., 2003; Chown & Nicolson, 2004). In insects, spatially explicit work has revealed several significant patterns. First, responses to high and low temperature are decoupled, lower lethal temperature limits typically respond more strongly to acclimation and to natural selection than upper lethal limits, and it appears that phenotypic plasticity can account for much of the response shown by insects (reviewed in Chown & Nicolson, 2004; see also Ayriñac et al., 2004; Hoffmann et al., 2005). Second, metabolic rate does not seem to vary with environmental temperature (Addo-Bediako et al., 2002), nor does it always scale as  $\text{mass}^{0.75}$  (Lighton & Fielden, 1995). Third, desiccation resistance generally varies in a direction that is consistent with adaptive responses to environmental water availability (Edney, 1977; Gibbs et al., 1997; Addo-Bediako et al., 2001), and responds predictably to acclimation at given humidity conditions (Hoffmann, 1990; Sjørnsen et al., 2001).

These patterns are all controversial for different reasons. In the first case, several studies have shown that decoupling of upper and lower lethal limits is not typical of all species (Hoffmann et al., 2002; Sgrò & Blows, 2004), and most investigations are undertaken using model species, leaving several groups and geographic regions under-represented (Chown et al., 2002; Klok & Chown, 2003). As far as metabolic rate variation is concerned, the metabolic theory of ecology predicts that metabolic rate should covary with environmental temperature and should scale as  $\text{mass}^{0.75}$  (Gillooly et al., 2001; Brown et al., 2004). In insects, this does not seem to be the case, although the data are equivocal (Lighton & Fielden, 1995; Chown & Gaston, 1999; Addo-Bediako et al. 2002), largely because several groups and areas are again under-represented amongst the information that is available (e.g. Duncan et al., 2002). In the last instance, the controversy concerns not so much the fact that desiccation resistance should vary with water availability, but rather which routes of water loss are likely to vary the most and how strong a temperature acclimation response is likely to be, if it is at all extant (Chown, 2002; Gibbs et al., 1998). Indeed, thermal acclimation effects on desiccation resistance have been investigated in only a few instances (Hoffmann, 1990; Hoffmann & Watson, 1993; Hoffmann et al., 2005).

A theme common to the debate in each of these fields is that whilst useful data on model organisms continue to accumulate apace, broader scale studies, which can usefully complement these investigations (see Kingsolver & Huey, 1998), are compromised by an absence of information from

many areas and taxa, and by an absence of information on a variety of traits for the same species (Chown et al., 2002). Indeed, information on the full range of ecophysiological traits is available for only a handful of species (Chown & Nicolson, 2004). There are several ways in which these problems might be addressed (see e.g. Zachariassen et al., 1987), of which the accumulation of information on a variety of traits for poorly-represented taxa from little studied environments is a significant one. This is particularly true for environments that are not considered “stressful”, and so have had little attention paid to their constituent species because “clear-cut”, significant responses are unexpected (for additional discussion see Feder et al., 1987).

In this chapter I therefore provide data on the responses of critical thermal limits, metabolic rate and desiccation resistance to temperature acclimation from a species that is a member of a diverse higher taxon, the Chrysomelidae, which is poorly represented in the physiological literature. This species also occurs in a geographic area that is not considered particularly climatically stressful – the Fynbos Biome of South Africa. Specifically, I determine: i) whether the strength of the acclimation response is the same for both critical thermal minima and maxima; ii) what, if any, is the extent of the response of desiccation resistance to treatment temperature; iii) whether there is conservation of metabolic rate with given different treatment temperatures, and how the metabolic rate of this species compares with other similar-sized beetles. Rapid cold hardening in insects from environments that may not be thermally ‘extreme’ has not been widely investigated (Chown & Nicolson, 2004). Therefore, I include investigations of both cold hardiness and rapid cold hardening. Information on the microclimate of this species is provided because comprehension of the survival strategies of insects cannot be achieved without it (Baust & Rojas, 1985; Bale, 1991).

## Materials and methods

### *Study site, collection and animal maintenance*

Adult *Chirodica chalconota* Germar (Coleoptera: Chrysomelidae, Alticinae), which are winged and flight capable, were obtained from *Protea nerifolia* inflorescences. One or two inflorescences per plant (total plants sampled 10-14) were removed from Swartboskloof (18.58°E 33.60°S, 350 m a.s.l.) in the Cape Floral Kingdom (Fynbos Biome), Jonkershoek Nature Reserve near Stellenbosch, South Africa. Each inflorescence contained in excess of 50 beetles and five inflorescences were used per temperature treatment, giving a total of 30 inflorescences. ‘Field-fresh’ beetles were returned to the laboratory and kept as for acclimations (see below), but at room temperature (18-21 °C) under ambient light. The cut inflorescences were placed in jars of water in

groups of five inside mesh-topped buckets. For acclimation groups, the buckets were then randomly assigned to one of three incubators (Labocon, South Africa) that regulated temperature at either 12 °C (Range: 12.0-12.5 °C), 19 °C (Range: 19.0-19.5 °C), or 24 °C (22.5-25 °C) (all with 12:12 H L:D) and kept there for seven days. Within two days of collection from the field, or upon completion of the acclimation period, the beetles were then shaken from the inflorescences and used immediately. For logistic reasons, the acclimation treatments and experiments were conducted in two blocks approximately two weeks apart, each with a 19 °C and a second temperature treatment. All analyses were preceded by a comparison of the two 19 °C acclimation treatments to ensure that there was no experimental block effect on the physiological parameters. Because no block effect was found (e.g. critical thermal minima onset:  $P > 0.9$ ), all data for the 19 °C treatment were pooled for analyses. A further acclimation at 29 °C was abandoned due to high mortality in the beetles. Mortality in acclimation cages at other temperatures was negligible and was mostly a result of beetles drowning in the water in which the inflorescences were kept. The large volume of free water inside each container resulted in high (> 80%) relative humidity, and it is therefore unlikely that the beetles experienced desiccation stress during acclimation.

Initially, as part of a pilot trial that would allow sex identification, fresh and dry body masses in a group of 124 beetles were determined, before proceeding with field-fresh physiology. Fresh mass was determined within two days of collection from the field (0.1 mg, Mettler Toledo AX 504; Columbus, OH, USA). Beetles were dried in an oven at 55-60°C for three days to constant mass, re-weighed, and sexed.

All live beetles used for experimental purposes in respirometry recording, desiccation and thermal tolerance determination were carefully handled only by manipulation with plastic cuvettes to reduce the likelihood of cuticular abrasion (see Machin et al., 1991). With the exception of supercooling point (SCP) and rapid cold hardening (RCH) determination, beetles were not handled with forceps or by hand.

### *Critical Thermal Limits*

Methods similar to those described by Klok & Chown (2003) were used. Individual beetles were placed in one of ten water-jacketed chambers connected to a programmable water bath (Grant LTC12, Grant Instruments, Cambridge, UK). The beetles were allowed to equilibrate for ten minutes at either 6 or 35 °C before minimum and maximum critical thermal limit assessments, respectively, commenced. After equilibration, the temperature of the chambers was raised or lowered at 0.25 °C.min<sup>-1</sup>

<sup>1</sup>. The temperature of onset of critical thermal minimum (CTMin<sub>Onset</sub>) was recorded for each individual when coordinated muscle function was lost during the decline in temperature. Thereafter the chamber temperature was lowered 0.5 °C below the CTMin<sub>Onset</sub> recorded for the last beetle and held for 5 minutes at this temperature before being raised again at 0.25 °C.min<sup>-1</sup>. As the temperatures increased, the recovery of the beetles from cold stupor was recorded as CTMin<sub>Recovery</sub> until all beetles had regained full motor coordination. During the CTMin<sub>Recovery</sub> assessment the beetles were vigorously agitated by probing them with a non-conducting plastic rod at least once every 0.1 °C per individual to elicit an escape response and to ensure that the beetles did not remain inactive beyond their actual CTMin<sub>Recovery</sub> temperature. Critical thermal maximum (CTMax) was recorded at the onset of muscle spasms during increasing temperatures (see Lutterschmidt & Hutchison, 1997). Groups of ten beetles were assessed at once, and wherever possible, two CTMin and CTMax trials were conducted for each acclimation treatment and for the field-fresh group at similar times of the day.

#### *Cold Hardiness, Rapid Cold Hardening and Supercooling Points*

The cold hardiness of *C. chalcoptera* and its capacity for rapid cold hardening were determined only in field-fresh individuals. For the former, field-fresh beetles were individually placed into 200 µl pipette tips in contact with a 44-SWG type-T thermocouple connected to a computer via two 8-channel Pico Tech TC-08 thermocouple interfaces (Pico Technology Ltd, Cambridge, UK), and data were logged using the associated software. The pipette tips were placed in an aluminium block cooled by Peltier modules and equilibrated for 15 minutes at 5 °C before being cooled to a predetermined test temperature at 0.1 °C min<sup>-1</sup>. After being held at the test temperature for 2 h, the beetles were re-warmed and placed individually into pierced 1.5 ml microcentrifuge tubes over distilled water for survival assessment after 24 hours. Beetles were classified as alive if they displayed a righting response and were capable of coordinated walking: in practice, all surviving beetles would cling to the lid of the microcentrifuge tube. Each beetle could be associated with its temperature trace, and the presence of an exotherm indicated that a beetle had frozen. In those individuals that froze, the temperature recorded immediately prior to the exotherm was noted as the supercooling point (SCP) (Lee, 1991). Fifteen beetles were used at each of four test temperatures (-1.6, -5.8, -9.9 and -14.4 °C), and an LLT50 (lower lethal temperature at which 50% mortality is predicted) was calculated using PROC LOGISTIC in SAS 9.1 (SAS Institute Inc, Cary, NC, USA).

Rapid Cold Hardening (RCH) was assessed using a mortality assay, following Lee et al. (1987), at a discriminating temperature of -10.1 °C. This discriminating temperature was determined by rapidly

exposing field-fresh beetles, placed in groups of five into 1.5 ml microcentrifuge tubes, for two hours in a water bath (Grant LTD20, Grant Instruments, Cambridge, UK) set to six temperatures between 0 and -12.5 °C. The beetles ( $n = 5$  groups per assessment) were then removed from the water bath and placed in their groups into each of five Petri dishes per temperature treatment, with wet cotton wool at 19 °C (12:12 L:D). The discriminating temperature was chosen as the temperature at which survival declined below 30% following assessment after 24 h. To determine whether the species showed RCH, field-fresh beetles were placed in groups of five into 1.5 ml microcentrifuge tubes ( $n = 5$  tubes per treatment) and held for a two hour pre-treatment period either in the Peltier cooling device, which regulated temperature either at 0 °C or at -2.1 °C, or in an incubator at 19 °C (control). The beetles were then exposed to the discriminating temperature following the protocol above (2 h at -10.1 °C) and removed to Petri dishes with wet cotton wool for assessment of recovery following 24 h at 19 °C (12:12 L:D). Because the improvement of survival over the control group was of interest (rather than comparisons between treatments), survival was compared independently between each treatment and the control using a two-sample t-statistic (Sokal & Rohlf, 1995), the significance of which was tested in a resampling-without-replacement protocol on Resampling Stats for Excel (Resampling Stats Inc., Arlington, Virginia, USA).

The effect of the acclimation treatments on supercooling points was determined as follows. Following acclimation for seven days, beetles were placed in pipette tips in contact with a thermocouple, and then placed in the Peltier-cooled device as described above. They were allowed to equilibrate for 15 min at 5 °C before being cooled at 0.1 °C min<sup>-1</sup> to -30 °C. SCP distributions were examined for bimodality and absolute (i.e. positivized) SCP values were compared between treatment temperatures using Generalized Linear models (GLZ) assuming a Poisson distribution, because the data are bounded by 0 at the upper end, and using an identity link function in Statistica Version 6.1 (Statsoft, Tulsa, Oklahoma, USA).

### *Desiccation Rate*

Desiccation rate was determined in flowing air (FAD – flowing air desiccation, < 5 % relative humidity) at  $25.0 \pm 1.0$  °C. Beetles were individually weighed and then placed into each of sixteen 5 ml cuvettes. Room air was pumped through silica gel and Drierite columns using an aquarium pump and then passed through a mass flow controller (Side-Trak, Sierra Instruments Inc., Monterey, CA, USA) to a Sable Systems MF8 airflow manifold (Sable Systems Inc., Las Vegas, NV, USA). Each outflow channel of the manifold was split so that two cuvettes were served per channel. The mass flow

controller was set to produce an air flow rate of  $50 \text{ ml}\cdot\text{min}^{-1}$  per cuvette. Following one hour of desiccation, beetles were re-weighed and water loss rate estimated as the difference between the initial and final masses per unit time.

### *Metabolic Rate*

Metabolic rates were recorded using flow-through respirometry (methods described in Terblanche et al., 2004). In brief, an LI-6262 (LiCor, Lincoln, Nebraska) infra-red gas analyzer was connected to a Sable Systems eight channel multiplexer ( $n = 7$  beetles plus an empty cuvette for baseline recordings), which provided a 14 minute metabolic rate recording period for each beetle consecutively from cuvette 1 to cuvette 7. Cuvette 1 was also placed into a Sable Systems AD-1 activity detector to monitor activity. The entire multiplexer system was housed within a Sable Systems PTC-1 temperature-controlled cabinet set at  $25 \text{ }^\circ\text{C}$ . Active periods and quiet periods were identified on the activity detector output and the corresponding respirometry patterns noted. Quiet periods were clear on the respirometry traces and similar periods on the traces from cuvettes 2-7 were used to obtain standard metabolic rate for all individuals. Carbon-dioxide output was expressed as  $\text{ml CO}_2\cdot\text{hr}^{-1}$  at standard temperature and pressure. The effect of acclimation on standard metabolic rate (SMR) was determined using analyses of covariance (ANCOVA) with mean experimental body mass  $((\text{start mass} + \text{end mass}) / 2)$  as the covariate and acclimation temperature as the grouping variable.

The mass-scaling of metabolic rate for the Coleoptera was determined by extracting standard metabolic rate and body mass data from the modern Anglophone literature with greatest emphasis on the last 50 years. Only studies which reported mass and resting metabolic rate were included. In some cases, the author's equations were used to calculate body mass and/or metabolic rates. When respiratory quotients (RQ) were not presented, and in the case of *C. chalconota*, an RQ of 0.84 was used (following Addo-Bediako et al., 2002). If sex-related metabolic rate variation was reported in a particular study, the sex with the lowest metabolic rate (and its corresponding body mass) was used in the database. In cases where no difference between sexes was reported, metabolic rates presented separately for males and females by the investigators were pooled to obtain a single species' estimate. When only dry body mass was presented, beetle fresh (live) mass was estimated by assuming a body water content of 67 %. In several cases, the same species was represented more than once in the literature, and therefore, the following selection criteria were used to select a single SMR value. First, flow-through respirometry was given the highest priority. Second, if respirometry techniques were similar among studies, the study which recorded metabolic rate closest to  $25 \text{ }^\circ\text{C}$  was selected. Finally,



when all else was equal, and for lack of better *a priori* assumptions, the most recent study was chosen. Experimental temperatures in the data finally used ranged from 15-30 °C. Metabolic rates were therefore all adjusted to a single temperature, 25 °C, assuming a  $Q_{10}$  of 2.5 (when none was provided by the authors). Thus, for the present analyses, a mass-scaling relationship was obtained using temperature-adjusted  $\log_{10} \mu\text{W}$  data.

### *Microclimate Temperatures*

To gain an indication of the thermal environment experienced by beetles in the inflorescence habitat in the field, microclimate temperatures ( $\pm 0.5$  °C) in Swartboskloof were monitored every 30 minutes using calibrated iButton thermochron dataloggers (Dallas Semiconductors, Dallas, TX, USA) from 29 May to 5 July 2004. The iButtons were attached to a 30 cm thin plastic strip (5 mm wide x 1 mm thick), and a hole made through the base of the *Protea* spp. inflorescence. The plastic strip was threaded through the hole from the opening of the flower, and then used to pull the iButton as far into the inflorescence as possible (c. 12-15 cm). Inflorescences of *Protea nerifolia* ( $n = 5$ ) and *P. repens* ( $n = 3$ ) 1.5-1.8 m above the ground were chosen, and inflorescences of the same species mounted with dataloggers were a minimum of 10 m apart. Shaded iButtons ( $n = 5$ ) mounted on *Protea* branches at the same height above the ground, and on the same plant as the inflorescences within which temperature was measured, were used to record outside air temperature.

Mean, minimum and maximum temperatures of air inside the inflorescences and the ambient environment were calculated using an MS-Excel implementation of the microclimate macros developed by Sinclair (2001). The number of events and time spent above 25 °C (maximum treatment temperature), and the number of events and time spent below 12 °C (minimum treatment temperature) were determined for each logger. These were compared between the two species' inflorescences and outside air temperature using Kruskal-Wallis Rank ANOVA on Statistica 6.1 followed by non-parametric multiple comparisons.

**Table 1.** Summary statistics for shaded air temperatures and temperatures recorded in inflorescences of *Protea repens*, *P. nerifolia* over 38 days in Swartboskloof, Jonkershoek Nature Reserve, Stellenbosch, South Africa during May-July 2004. *Chirodica chalcoptera* (Coleoptera: Chrysomelidae) are abundant in these flowers during the austral late summer and early winter. Similar superscript letters represent statistically homogeneous groups (non-parametric multiple comparisons).

Variable		<i>P. nerifolia</i>	<i>P. repens</i>	Ambient air	Statistics	
					H <sup>1</sup>	P
Mean $\pm$ s.e. ( $^{\circ}$ C)		11.9 $\pm$ 0.2	12.6 $\pm$ 0.4	12.2 $\pm$ 0.1		
Median ( $^{\circ}$ C)		11	11.5	11.5		
Mean Maximum $\pm$ s.e. ( $^{\circ}$ C)		28.7 $\pm$ 1.4	31.8 $\pm$ 0.2	27.8 $\pm$ 0.2		
Absolute Maximum ( $^{\circ}$ C)		32.0	36.5	28.5		
Mean Minimum $\pm$ s.e. ( $^{\circ}$ C)		2.2 $\pm$ 0.2	3.0 $\pm$ 0.3	2.8 $\pm$ 0.3		
Absolute Minimum ( $^{\circ}$ C)		2.0	2.5	2.0		
Events > 25 $^{\circ}$ C	Mean	8.8	14.3	3.0	6.25	< 0.044 <sup>2</sup>
	Max.	19.0	23.0	3.0		
	Min.	0.0	6.0	3.0		
Time > 25 $^{\circ}$ C (h)	Mean	24.8	35.7	10.4	5.29	0.071 <sup>2</sup>
	Max.	50.0	74.0	11.0		
	Min.	0.0	12.5	9.5		
Events < 12 $^{\circ}$ C	Mean	45.8 <sup>A</sup>	51.3 <sup>A</sup>	74.6 <sup>B</sup>	10.61	< 0.005
	Max.	49.0	53.0	80.0		
	Min.	43.0	15.0	66.0		
Time < 12 $^{\circ}$ C (h)	Mean	562.1 <sup>A</sup>	489.3 <sup>B</sup>	493.4 <sup>A, B</sup>	7.31	< 0.026
	Max.	591.0	510.0	534.5		
	Min.	518.0	460.0	411.0		

1 Kruskal-Wallis (DF = 2; n = 13)

2 No difference in non-parametric multiple comparison test.

## Results

### *Microclimate Temperatures*

Microclimate and shaded air temperatures showed much diurnal variation. The absolute maximum field temperatures, as well as the number of events (but not the amount of time) above 25 °C were highest in *Protea repens* inflorescences, followed by *P. nerifolia* inflorescences and then by the shaded air sites (Table 1). By contrast, the two *Protea* microhabitats experienced fewer events below 12 °C than the shaded sites, although *P. nerifolia* habitats spent significantly more time below 12 °C than *P. repens* (Table 1). Sub-zero temperatures were not recorded at any time during the monitoring period.

### *Critical Thermal Limits*

Critical thermal maxima did not differ between the 12 °C-acclimated and field-fresh groups (Table 2). However, CTMax of 19 °C-acclimated beetles was significantly lower by *c.* 0.8 °C than that of the 12 °C-acclimated and field-fresh groups (Table 2). CTMin<sub>Onset</sub> of field-fresh and 12 °C-acclimated beetles did not differ significantly, but were significantly lower than the CTMin<sub>Onset</sub> of beetles acclimated to 19 and 25 °C (Table 2). CTMin<sub>Recovery</sub> tended to be higher in beetles exposed to the higher treatment temperatures, but the patterns were not as clear as they were for CTMin<sub>Onset</sub>, until correction for experimental duration was incorporated into the analyses (Table 2). Overall, CTMax varied by less than 1 °C over the treatment temperatures, whereas variation was slightly higher for CTMin (noting, however, that CTMax values for 25 °C-acclimated beetles are missing because of the death of beetles from the acclimation treatment).

**Table 2.** Physiological variation in field-collected (FF) and laboratory temperature-acclimated *Chirodica chalconota* beetles. Similar superscript letters represent homogeneous groups (*post-hoc* unequal sample HSD comparisons). Mass-independent rates are presented as least-squares means adjusted for body size. Time-independent analyses of recovery temperature from cold exposure are also given for  $CTMin_{Recovery}$  (ANCOVA: duration of experiment (min) was highly significant as a covariate). F and P refer to simultaneous comparisons of FF, 12, 19 and 25 °C acclimation groups.

<i>Physiological variable</i>	<i>FF</i>	<i>12 °C acclimation</i>	<i>19 °C acclimation</i>	<i>25 °C acclimation</i>	<i>F</i>	<i>P</i>
$CTMax^1$ (° C)	41.8 ± 0.2 <sup>A</sup>	41.9 ± 0.3 <sup>A</sup>	41.0 ± 0.3 <sup>B</sup>		3.279	0.049
<i>N</i>	20	10	10			
$CTMin_{Onset}^1$ (° C)	-0.3 ± 0.2 <sup>A</sup>	0.1 ± 0.2 <sup>A</sup>	1.0 ± 0.2 <sup>B</sup>	2.3 ± 0.2 <sup>C</sup>	50.26	< 0.0001
<i>N</i>	20	10	20	20		
$CTMin_{Recovery}^1$ (° C)	7.8 ± 0.3 <sup>A,B</sup>	6.7 ± 0.4 <sup>A</sup>	8.6 ± 0.3 <sup>B</sup>	8.2 ± 0.3 <sup>B</sup>	6.84	< 0.0004
$CTMin_{Recovery}^2$ (° C)	5.9 ± 0.3	5.6 ± 0.3 <sup>A</sup>	8.1 ± 0.2 <sup>B</sup>	11.0 ± 0.4 <sup>C</sup>	36.75	< 0.0001
<i>N</i>	20	10	20	20		
<i>Mass-independent desiccation rate</i> <sup>3</sup> (mg H <sub>2</sub> O.hr <sup>-1</sup> )	0.360 ± 0.059	0.244 ± 0.077	0.324 ± 0.076	0.232 ± 0.086	2.230	0.102
<i>N</i>	12	7	7	6		
<i>Mass-independent metabolic Rate</i> <sup>4</sup> (mlCO <sub>2</sub> .hr <sup>-1</sup> )	2.456 ± 0.661 <sup>A</sup>	3.657 ± 0.871 <sup>A</sup>	4.164 ± 1.019 <sup>A,B</sup>	5.436 ± 0.895 <sup>B</sup>	4.898	< 0.01
<i>N</i>	12	7	5	7		

1 ANOVA

2 ANCOVA, covariate: experimental duration (time in min)

3 ANCOVA, covariate: log<sub>10</sub> mean experimental body mass

4 ANCOVA, covariate: log<sub>10</sub> mean experimental body mass

*Cold Hardiness, Rapid Cold Hardening and Supercooling Points*

All beetles for which freezing events were recorded died. In addition, all the individuals that did not freeze when exposed to -14.4 °C (8/15) died at this temperature, and four of thirteen beetles that remained unfrozen at -9.9 °C also died, demonstrating pre-freeze mortality in this species. The approximate LLT50 of this species was estimated at -10.1 °C from logistic regression. Survival of -10.1 °C (20 %) was significantly improved by a 2 h pre-treatment at 0 °C (54 %;  $P = 0.029$ ) or -2 °C (76 %;  $P = 0.004$ ), indicating a substantial rapid cold hardening response.

Supercooling points were unimodally distributed but right-skewed in some cases (FF: Shapiro-Wilk  $W = 0.963$ ,  $P = 0.28$ ; 12 °C Acclimation: right-skewed,  $W = 0.713$ ,  $P = 0.009$ ; 19 °C Acclimation (8 June): right-skewed,  $W = 0.930$ ,  $P = 0.049$ ; 19 °C Acclimation (21 June):  $W = 0.921$ ,  $P = 0.33$ ; 25 °C:  $W = 0.918$ ,  $P = 0.105$ ). Only six SCP values were obtained from the 12 °C treatment (median  $\pm$  upper, lower quartile:  $-8.7 \pm -8.6$ ,  $-8.8$ ), and they were therefore excluded from the analyses. SCPs did not differ between the two 19 °C treatments (median of pooled treatments  $\pm$  upper, lower quartile (N):  $-13.5 \pm -11.5$ ,  $-14.7$  (41)); comparison: Wald  $\chi^2 = 0.228$ ,  $df = 2$ ,  $P = 0.88$ ), nor between either of those and the field fresh (median  $\pm$  upper, lower quartile (N):  $-14.6 \pm -11.4$ ,  $-17.4$  (35)) and 25 °C treatments (median  $\pm$  upper, lower quartile (N):  $-13.1 \pm -12.4$ ,  $-14.5$  (19); Wald  $\chi^2 = 2.458$ ,  $df = 2$ ,  $P = 0.293$ ).

*Desiccation Rate and Body Size*

In most cases, live and dry body masses of male and female field-fresh *C. chalconota* examined separately were not normally distributed ( $P < 0.10 - 0.0001$ ). However,  $\log_{10}$  transformation normalized these data in most cases (except for female dry mass). Therefore,  $\log_{10}$  transformed fresh body mass data were used to correct analyses of desiccation rate and metabolic rate for individual variation in mass. Males and females differed in their live and dry masses, and inclusion of live mass into the ANCOVA models of body water content and desiccation rate revealed no effect of sex on these variables that was independent of size (Table 3). The acclimation treatments did not affect desiccation rate (Table 2).

*Metabolic Rate*

Discontinuous or cyclic ventilation patterns were not observed in any CO<sub>2</sub> release traces recorded from *Chironomus chalconota*. Metabolic rate did not differ between male and female field-fresh beetles (Table 3). Nevertheless, to avoid potential confounding sex-related acclimation differences (see Das & Das, 1982a, b), only male beetles were used to investigate the effects of

acclimation on metabolic rate. After seven days of acclimation, 25 °C-acclimated beetles had higher mass-independent metabolic rates relative to field-fresh and 12 °C-acclimated beetles, but were similar to the 19 °C acclimation group (Table 2). Metabolic rates of *C. chalconota* were within the range of the variation found in inter-specific  $\log_{10}$  metabolic rate-mass scaling relationships among adult Coleoptera ( $n = 161$ ), and were hardly different from values found for similar-sized chrysomelid beetles (Fig. 1).

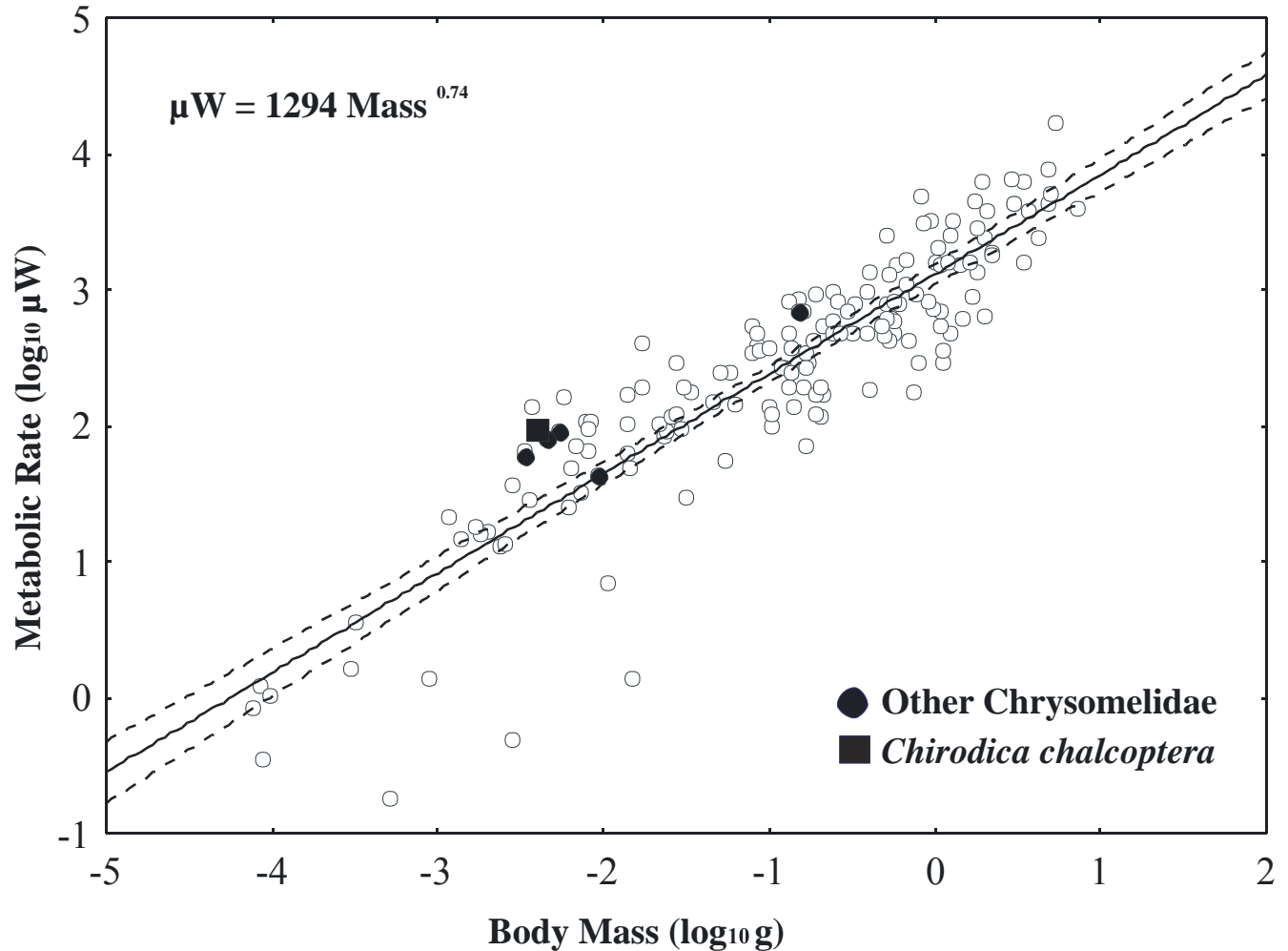
**Table 3.** Sex-related variation in mass, water content, desiccation rate and metabolic rate in field-collected *Chirodica chalconota* chrysomelid beetles (means  $\pm$  s.e.m.). Least-squares adjusted means are presented for mass-independent comparisons (ANCOVA). Desiccation and metabolic rate were measured at 25 °C, 50 ml.min<sup>-1</sup>, 0 % relative humidity.

<i>Physiological variable</i>	<i>Males</i>	<i>Females</i>	<i>F</i>	<i>P</i>
<i>Fresh body mass (mg)</i> <sup>1</sup>	3.1 $\pm$ 1.0	3.7 $\pm$ 0.1	12.32	< 0.001
<i>N</i>	65	59		
<i>Dry body mass (mg)</i> <sup>1</sup>	1.0 $\pm$ 0.1	1.3 $\pm$ 0.1	12.52	< 0.001
<i>N</i>	65	59		
<i>Mass-independent body water content</i> <sup>2</sup> (mg H <sub>2</sub> O)	2.2 $\pm$ 0.1	2.2 $\pm$ 0.1	0.698	0.405
<i>N</i>	65	59		
<i>Mass-independent desiccation rate</i> <sup>3</sup> (mg H <sub>2</sub> O.hr <sup>-1</sup> )	0.258 $\pm$ 0.104	0.526 $\pm$ 0.124	3.14	0.110
	7	5		
<i>Mass-independent metabolic rate</i> <sup>3</sup> (mlCO <sub>2</sub> .hr <sup>-1</sup> )	2.772 $\pm$ 0.471	2.517 $\pm$ 0.560	0.007	0.936
<i>N</i>	7	5		

1 Analysis results given for  $\log_{10}$  transformed data comparisons

2 GLM, covariate:  $\log_{10}$  dry body mass

3 GLM, covariate:  $\log_{10}$  mean experimental body mass



**Figure 1.** The relationship between  $\log_{10}$  standard metabolic rate and  $\log_{10}$  fresh body mass for 162 species of adult Coleoptera (open circles) extracted from published literature, including this study's field-fresh *Chirodica chalconota* (filled square). Filled circles indicate other chrysomelid species.

## Discussion

*Chirodica chalconota* is in many ways a typical temperate-environment beetle species. Although the adults are chill-susceptible (see Bale, 1993), they can survive brief exposures to subzero temperatures and show a significant rapid cold hardening response. Rapid cold hardening could be explained as a mechanism that enables *C. chalconota* to survive unpredictable cold events that are common within their natural thermal habitat: extreme and unpredictable cold front conditions are not uncommon throughout the year in the Western Cape (e.g. Tyson & Preston-Whyte, 2000; Sinclair & Chown, 2005). Rapid cold hardening is thought to play this role in a variety of other insect species, enabling them to survive unexpected and potentially lethal short-term declines in temperature (Lee et

al., 1987; Kelty & Lee, 1999, 2001). The cold hardiness strategy of *C. chalconota* is similar to other chrysomelid species. Adult *Aulacophora nigripennis* (Chrysomelidae) in Japan have only limited chill tolerance (Watanabe & Tanaka, 1999), and adults of the Colorado potato beetle (*Leptinotarsa decemlineata*, Chrysomelidae) cannot survive freezing (Mail & Salt, 1933, Lee et al., 1994). By contrast, the only two adult chrysomelids which may be described as cold tolerant, *Melosoma collaris* and *Phyllodecta laticollis* from Norway, are both strongly freeze tolerant (Sømme & Conradi-Larsen, 1979; van der Laak, 1982).

The upper and lower critical limits of the adults of this species are typical of many temperate species (see Addo-Bediako et al., 2000), and the overall thermal tolerance range (CTMax-CTMin<sub>Onset</sub>) of just over 42 °C is consistent with the pattern of thermal responses shown by temperate insects (Chen et al., 1990; Chown, 2001; Klok & Chown, 2003; Chown & Nicolson, 2004). In *C. chalconota*, CTMax declined significantly with acclimation (Table 2), although no data were available for the highest treatment temperature. Nonetheless, the difference in CTMax between all the treatments was less than 1 °C, whereas the CTMin<sub>Onset</sub> temperatures were significantly affected by acclimation, spanning a range of ~2.6 °C over an acclimation range of 13 °C. Similar, if not greater, responses were found for the temperature of CTMin<sub>Recovery</sub> when comparing means adjusted for experimental duration (Table 2). These findings are broadly consistent with the observations that in insects upper and lower critical thermal limits are typically decoupled, with lower thermal limits showing greater variation and a more plastic response to acclimation than upper limits (Chown, 2001; Chown & Nicolson, 2004; Kimura, 2004; Hoffmann et al., 2005; see also examples in Kingsolver & Huey, 1998).

The standard metabolic rate of adult *C. chalconota* is similar both to other Coleoptera and to other chrysomelid species of the same size (Fig. 1). Although high-carbohydrate nectar probably makes up a significant portion of the diet of *C. chalconota*, suggesting that an RQ of 0.84 may have resulted in erroneous estimates of the SMR of this species, this was not the case. The position of *C. chalconota* in the interspecific scaling relationship is relatively insensitive to changing RQ (RQ = 0.7, log<sub>10</sub> μW = 2.03; RQ = 0.84, log<sub>10</sub> μW = 2.00; RQ = 1.0, log<sub>10</sub> μW = 1.91), at least from the perspective of the scaling relationship. Perhaps of more significance is the fact that metabolic rates and body sizes are available for only an extremely small fraction (<< 0.01 %) of the potential number of extant beetle species (Gaston, 1991), and representative data for only five chrysomelid species could be obtained from the literature. The frequency distribution of log<sub>10</sub> mass (in g) among all the beetle species examined in the metabolic rate-body mass relationship presented in Figure 1 was left-skewed owing to the paucity of small species (Shapiro-Wilk  $W = 0.624$ ,  $P < 0.0001$ ). Therefore, the inter-specific mass-

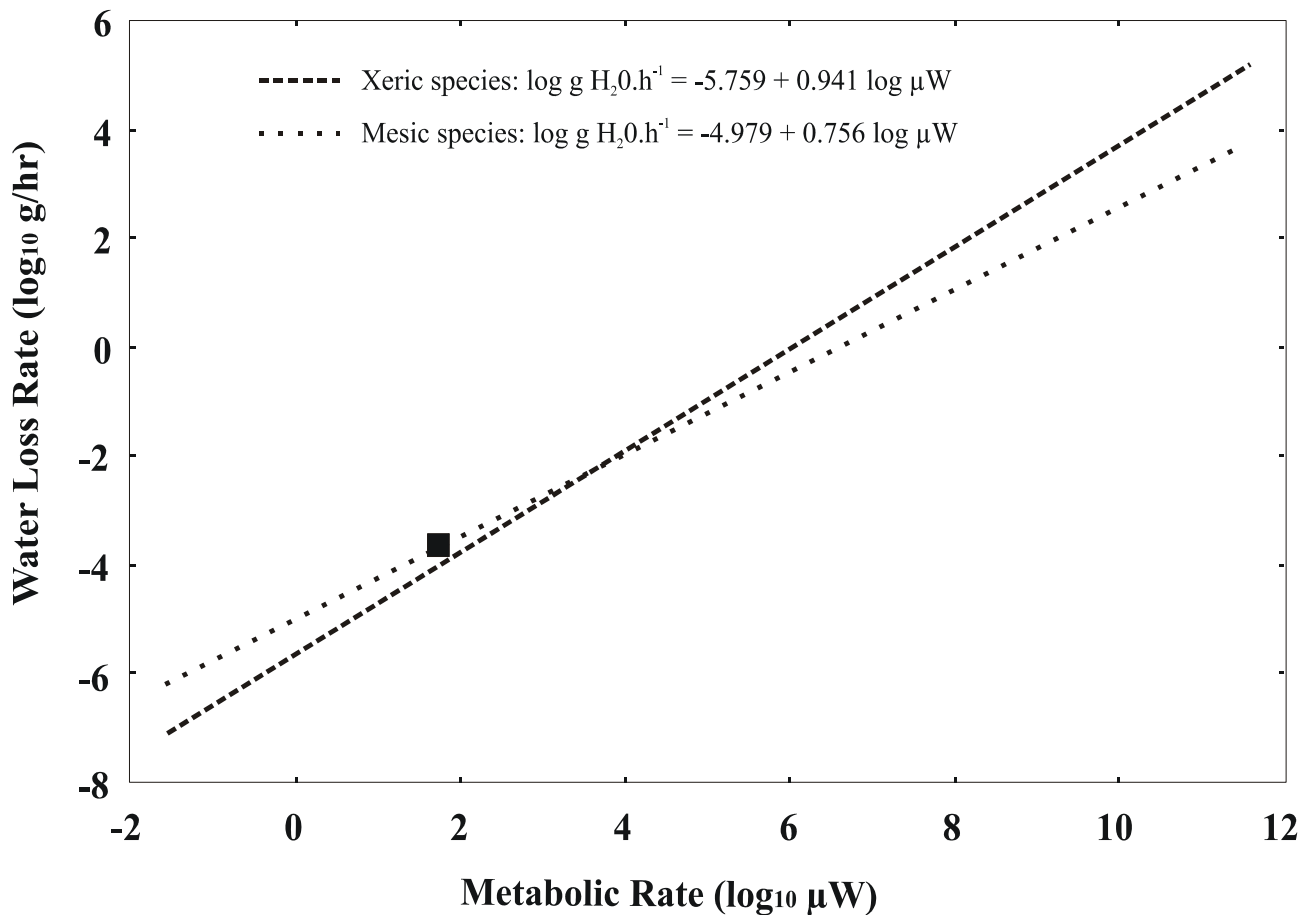


scaling relationship presented in Figure 1 could be altered significantly with the addition of new data, especially if these data fall into the extreme ends of the body size classes. Thus, conclusions regarding the extent to which metabolic rate of this species can be considered typical cannot be made with confidence at this point.

Acclimation had a strong, significant and positive effect on metabolic rate, such that metabolic rates of individuals held at 25 °C were 1.5 times higher than those of individuals held at 12 °C. This is the opposite of what has been termed compensation or metabolic cold adaptation (the conservation of rate in the face of a temperature decline (see Hazel & Prosser, 1974; Chown & Gaston, 1999)). However, the effect was most pronounced at 25 °C, whereas the difference between individuals held at 12 °C and 19 °C was much smaller and not significant. Given that mortality was considerable at the highest acclimation temperature, it may well be that the high metabolic rates are an indication of considerable stress, and should therefore be disregarded (see Woods & Harrison (2002) for rationale). If this were to be done then there would be evidence of compensation as has been found in a variety of other insect species at the intraspecific level (reviewed in Chown & Gaston, 1999). However, the opposite pattern (of elevated metabolic rates at high temperatures) is characteristic of many other ectotherms (Clarke, 1993; Lardies et al., 2004) and therefore should not be dismissed without additional investigation.

The adults of *C. chalconota* have relatively high desiccation rates (which vary depending on size but not on gender), and are more similar to mesic than to xeric species, as is clear from a plot of mean metabolic rate and mean desiccation rate for this species against the consensus equations derived by Zachariassen (1996), and confirmed by Addo-Bediako et al. (2001), for xeric and mesic species (Fig. 2). High water loss rates are perhaps unsurprising given the protected environment within which the adults live, and the time of year at which they are active (autumn-winter, which are both moist and cool). Again, the species appears to be typical of those from temperate environments, and this is true also of the absence of a response of desiccation rate to thermal acclimation. Although Gibbs et al. (1998) found increases in cuticular hydrocarbon melting points (but no changes in hydrocarbon mass) with high temperature acclimation (33 °C) in a desert-dwelling *Drosophila*, desiccation rate remained unaffected by acclimation to either warm or cold temperature (17 °C). Similar results have been documented for woodlice (Isopoda: Oniscoidea) (Cloudsley-Thompson, 1969). However, Hoffmann et al. (2005) showed that a summer acclimation treatment generally improved desiccation resistance (time to death) in several *D. melanogaster* populations relative to a constant temperature regime. Owing to the paucity of studies, it is difficult to determine how general the present results are. Thermal

acclimation effects on desiccation resistance are not commonly investigated, despite the fact that temperature has a substantial effect on the drying power of the air (Chown & Nicolson, 2004).



**Figure 2.** Ordinary least squares regression lines of the relationships between log<sub>10</sub> metabolic rate and log<sub>10</sub> water loss rate obtained from the equations provided by Addo-Bediako et al., (2001) for xeric and mesic insect species. Relative to its metabolic rate, *Chirodica chalconota* (filled square) appears to have a mesic rate of water loss.

In sum, this work has shown that the adults of *C. chalconota* are typically mesic-temperate insects. Although microclimate temperatures in the *Protea* inflorescences they inhabit did not reach the CT<sub>Max</sub>, CT<sub>Min<sub>Onset</sub></sub> or lower lethal temperatures of the beetles, maximum temperature was reasonably close to the CT<sub>Max</sub> of the adults. Moreover, the adults are clearly also much more sensitive to desiccation than species that are typical of the arid environments to the north and east of the Fynbos Biome. Thus, if climates change to the extent predicted for the region, including elevated temperatures and a decline in rainfall as well as a shift of the regime towards summer, rather than winter rainfall (Midgley et al., 2003) (which likely means an increase in sunshine duration and therefore heat budget

of the inflorescences), then the physiological tolerances of this species might well be exceeded, even though there may be no shortage of resources given that it inhabits at least three different *Protea* species (Mostert et al., 1980). If the tolerances of the species were exceeded, and this would not have to take place for a particularly protracted period, that might mean a substantial change to ecosystem functioning of those Fynbos areas in which this beetle is common, especially because it is an important food source for the locally abundant and functionally significant avian pollinator – the Cape Sugarbird (*Promerops cafer*) (Mostert et al., 1980; Johnson, 1992). The outcome of these changes cannot be predicted with any certainty, but the present data suggest that similar investigations need to be undertaken for other insects that are of significance in the Fynbos Biome (see e.g. Johnson & Steiner, 1997, 2003) given the rapid changes that are taking place in the region and that are forecast for the next 50 years (Midgley et al., 2003; Rouget et al., 2003).

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## **References**

- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* 267, 739-745.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2001. Revisiting water loss in insects: a large-scale view. *Journal of Insect Physiology* 47, 1377-1388.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology* 16, 332-338.

- Ayrinhac, A., Debat, V., Gibert, P., Kister, A.-G., Legout, H., Moreteau, B. et al., 2004. Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology* 18, 700-706.
- Bale, J.S., 1991. Insects at low temperature: a predictable relationship? *Functional Ecology* 5, 291-298.
- Bale, J.S., 1993. Classes of insect cold hardiness. *Functional Ecology* 7, 751-753.
- Baust, J.G., Rojas, R.R., 1985. Insect cold hardiness: facts and fancy. *Journal of Insect Physiology* 31, 755-759.
- Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Towards a metabolic theory of ecology. *Ecology* 85, 1771-1789.
- Chen, C.-P., Lee Jr, R.E., Denlinger, D.L., 1990. A comparison of the responses of tropical and temperate flies (Diptera: Sarcophagidae) to cold and heat stress. *Journal of Comparative Physiology B* 160, 543-547.
- Chown, S.L., 2001. Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* 47, 649-660.
- Chown, S.L., 2002. Respiratory water loss in insects. *Comparative Biochemistry and Physiology A* 133, 791-804.
- Chown, S.L., Gaston, K.J., 1999. Exploring links between physiology and ecology: the role of respiratory metabolism in insects. *Biological Reviews* 74, 87-120.
- Chown, S.L., Nicolson, S.W., 2004. *Insect Physiological Ecology. Mechanisms and Patterns*. Oxford University Press, Oxford.
- Chown, S. L., Addo-Bediako, A., Gaston, K.J., 2002. Physiological variation in insects: large-scale patterns and their implications. *Comparative Biochemistry and Physiology* 131 B, 587-602
- Clarke, A., 1993. Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? *Functional Ecology* 7, 139-149.
- Cloudsley-Thompson, J.L., 1969. Acclimation, water and temperature relations of the woodlice *Metoponorthus pruinosus* and *Periscyphis jannonei* in the Sudan. *Journal of Zoology* 158, 267-276.
- Das, A.K., Das, A.B., 1982a. Patterns of enzymatic compensations for temperature in the fat body of male and female *Periplaneta americana* (Linn.). *Proceedings of the Indian National Science Academy B* 48, 44-54.
- Das, A.K., Das, A.B., 1982b. Compensations for temperature in the activities of digestive enzymes of *Periplaneta americana* (L.). *Comparative Biochemistry and Physiology* 71A, 255-263.

- Duncan, F.D., Krasnov, B., McMaster, M., 2002. Metabolic rate and respiratory gas-exchange in tenebrionid beetles from the Negev Highlands, Israel. *Journal of Experimental Biology* 205, 791-798.
- Edney, E.B., 1977. *Water Balance in Land Arthropods*. Springer, Berlin.
- Gaston, K.J., 1991. The magnitude of global insect species richness. *Conservation Biology* 5, 283-296.
- Feder, M.E., Bennett, A.F., Burggren, W.W., Huey, R.B., 1987. *New Directions in Ecological Physiology*. Cambridge University Press, Cambridge.
- Feder, M.E., Mitchell-Olds, T. 2003. Evolutionary and ecological functional genomics. *Nature Reviews Genetics* 4, 649-655.
- Gibbs, A.G., Chippindale, A.K., Rose, M.R., 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology* 200, 1821-1832.
- Gibbs, A.G., Louie, A.K., Ayala, J.A., 1998. Effects of temperature on cuticular lipids and water balance in a desert *Drosophila*: is thermal acclimation beneficial? *Journal of Experimental Biology* 201, 71-80.
- Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. *Science* 293, 2248-2251.
- Hazel, J.R., Prosser, C.L., 1974. Molecular mechanisms of temperature compensation in poikilotherms. *Physiological Reviews* 54, 620-677.
- Hoffmann, A.A., 1990. Acclimation for desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses and genetic variation. *Journal of Insect Physiology* 36, 885-891.
- Hoffmann, A.A., Watson, M., 1993. Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. *American Naturalist* 142, S93-S113.
- Hoffmann, A.A., Anderson, A., Hallas, R., 2002. Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecology Letters* 5, 614-618.
- Hoffmann, A.A., Hallas, R.J., Dean, J.A., Schiffer, M., 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science* 301, 100-102.
- Hoffmann, A.A., Shirriffs, J., Scott, M., 2005. Relative importance of plastic versus genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Functional Ecology* 19, 222-227.
- Johnson, S.D. 1992. Plant-animal relationships. In: Cowling, R.M. (ed), *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Oxford University Press, Cape Town, pp. 175-205..

- Johnson, S.D., Steiner, K.E., 1997. Long-tongued fly pollination and evolution of floral spur length in the *Disa draconis* complex (Orchidaceae). *Evolution* 51, 45-53.
- Johnson, S.D., Steiner, K.E., 2003. Specialized pollination systems in southern Africa. *South African Journal of Science* 99, 345-348.
- Kelty, J.D., Lee, R.E., 1999. Induction of rapid cold-hardening by cooling at ecologically relevant rates in *Drosophila melanogaster*. *Journal of Insect Physiology* 45, 719-726.
- Kelty, J.D., Lee, R.E., 2001. Rapid cold-hardening of *Drosophila melanogaster* (Diptera: Drosophilidae) during ecologically based thermoperiodic cycles. *Journal of Experimental Biology* 204, 1659-1666.
- Kimura, M.T., 2004. Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia* 140, 442-449.
- Kingsolver, J.G., Huey, R.B., 1998. Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist* 38, 545-560.
- Klok, C.J., Chown, S.L., 2003. Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* 78, 401-414.
- Lardies, M.A., Catalán, T.P., Bozinovic, F., 2004. Metabolism and life history correlates in a lowland and highland population of a terrestrial isopod. *Canadian Journal of Zoology* 82, 677-687.
- Lee, R.E., Chen, C.-P., Denlinger, D.L., 1987. A rapid cold-hardening process in insects. *Science* 238, 1415-1417.
- Lee, R.E., 1991. Principles of insect low temperature tolerance. In: Lee, R.E., Denlinger, D.L. (eds), *Insects at Low Temperature*, Chapman and Hall, New York, pp. 17-46.
- Lee, R.E., Costanzo, J.P., Kaufman, P.E., Lee, M.R., Wyman, J.A., 1994. Ice-nucleating active bacteria reduce the cold-hardiness of the freeze-intolerant Colorado potato beetle (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 87, 377-381.
- Lighton, J.R.B., Fielden, L.J., 1995. Mass scaling of standard metabolism in ticks: a valid case of low metabolic rates in sit-and-wait strategists. *Physiological Zoology* 68, 43-62.
- Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: history and critique. *Canadian Journal of Zoology* 75, 1561-1574.
- Machin, J., Kestler, P., Lampert, G.J., 1991. Simultaneous measurements of spiracular and cuticular water losses in *Periplaneta americana*: implications for whole-animal mass loss studies. *Journal of Experimental Biology* 161, 439-453.

- Mail, G.A., Salt, R.W., 1933. Temperature as a possible limiting factor in the northern spread of the Colorado potato beetle. *Journal of Economic Entomology* 26, 1068-1075.
- Midgley, G.F., Hannah, L., Millar, D., Thuiller, W., Booth, A. 2003. Developing regional and species-level assessments of climate change impacts on biodiversity in the Cape Floristic Region. *Biological Conservation* 112, 87-97.
- Mostert, D.P., Siegfried, W.R., Louw, G.N., 1980. Protea nectar and satellite fauna in relation to the food requirements and pollinating role of the Cape sugarbird. *South African Journal of Science* 76, 409-412.
- Rouget, M., Richardson, D. M., Cowling, R. M., Lloyd, J. W., Lombard, A. T. 2003. Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region, South Africa. *Biological Conservation* 112, 63-85.
- Sgrò, C.M., Blows, M.W., 2004. The genetic covariance among clinal environments after adaptation to an environmental gradient in *Drosophila serrata*. *Genetics* 167, 1281-1291.
- Sinclair, B.J., 2001. Biologically relevant environmental data: Macros to make the most of microclimate recordings. *CryoLetters* 22, 125-134.
- Sinclair, B.J., Chown, S.L., 2005. Climatic variability and hemispheric differences in insect cold tolerance: Support from southern Africa. *Functional Ecology* 19, 214-221.
- Sjursen, H., Bayley, M., Holmstrup, M., 2001. Enhanced drought tolerance of a soil-dwelling springtail by pre-acclimation to a mild drought stress. *Journal of Insect Physiology* 47, 1021-1027.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry: The principles and practice of statistics in biological research*, 3rd edn. Freeman, San Francisco, CA.
- Sømme, L., Conradi-Larsen, E.-M., 1979. Frost resistance in alpine, adult *Melasoma collaris* (Coleoptera). *Oikos* 33, 80-84.
- Terblanche, J.S., Klok, C.J., Chown, S.L., 2004. Metabolic rate variation in *Glossina pallidipes* (Diptera: Glossinidae): gender, ageing and repeatability. *Journal of Insect Physiology* 50, 419-428.
- Tyson, P.D., Preston-Whyte, R.A., 2000. *The Weather and Climate of Southern Africa*. Oxford University Press, Cape Town.
- van der Laak, S., 1982. Physiological adaptations to low temperature in freezing-tolerant *Phyllodecta laticollis* beetles. *Comparative Biochemistry and Physiology A* 73, 613-620.
- Watanabe, M., Tanaka, K., 1999 Cold tolerance strategy of the freeze-intolerant chrysomelid, *Aulacophora nigripennis* (Coleoptera: Chrysomelidae), in warm-temperate regions. *European Journal of Entomology* 96, 175-181.

- Woods, H.A., Harrison, J.F., 2002. Interpreting rejections of the beneficial acclimation hypothesis: When is physiological plasticity adaptive? *Evolution* 56, 1863-1866.
- Zachariassen, K.E., 1996. The water conserving physiological compromise of desert insects. *European Journal of Entomology* 93, 359-367.
- Zachariassen, K.E., Andersen, J., Maloiy, G.M.O., Kamau, J.M.Z., 1987. Transpiratory water loss and metabolism of beetles from arid areas in East Africa. *Comparative Biochemistry and Physiology A* 86, 403-408.



## Chapter 6

### **Phenotypic flexibility and geographic variation in thermal tolerance and water loss of the tsetse fly *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modelling\***

*“The most erroneous stories are those we think we know best – and therefore never scrutinize or question.”*

**Stephen Jay Gould**

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## Introduction

Climate change and its interaction with human land use patterns are amongst the most significant threats facing biodiversity and society (Sala et al., 2000; Walther et al., 2002; Watson, 2002). Major alterations to species ranges and activities (such as migration) have occurred (Parmesan & Yohe, 2003; Root et al., 2003; Edwards & Richardson, 2004) and are predicted to continue (Thomas et al., 2004), because in many taxa range limits and life history cues are set by temperature and, in terrestrial systems, by water availability (Tauber et al., 1998; Gaston, 2003; Chown & Nicolson, 2004). Therefore, forecasts of the interactive effects of climate change and land use are of considerable importance for predicting the future distribution and abundance of species. They are also essential for enabling society to mitigate and cope with the effects of these changes (Watson, 2002; Opdam & Wascher, 2004).

Typically, predictions of climate change effects on species ranges are based on bioclimatic models (Jeffree & Jeffree, 1996; Erasmus et al., 2002; Thomas et al., 2004), although there is substantial variety in the way in which this modelling is done. Nonetheless, abiotic explanatory variables are usually sought for species ranges using coarse-grained, spatially explicit data, the abiotic variables altered according to general circulation model predictions of future climate, and the species ranges mapped back into geographic space (Jeffree & Jeffree, 1996; Pearson & Dawson, 2003). Bioclimatic models are often able to explain much of the variation in species distributions, and abundances (Rogers & Randolph, 1991; Robinson et al., 1997a, b; Rogers, 2000; Tatem et al., 2003; Huntley et al., 2004), and they are therefore considered to be one of the most effective ways to obtain a rapid estimate of likely alterations in species distributions (Erasmus et al., 2002; Randolph & Rogers, 2003; Thomas et al., 2004). Nonetheless, they have been criticized on a variety of grounds (Davis et al., 1998; Samways et al., 1999).

From a physiological perspective, the bioclimatic modelling approach raises concerns from three principal perspectives: spatial variation in population responses to the environment is often not considered (Davis & Shaw, 2001); the rapid alterations to phenotypes that might take place via phenotypic plasticity in the form of developmental plasticity, acclimation, and hardening are typically ignored (Helmuth et al., 2005); and the likely outcome of covariation among abiotic variables is often not adequately assessed (Rogers & Randolph, 2000). Spatial and temporal variability in phenotypes might substantially alter predicted responses to change (Stillman, 2003), especially if the responses themselves vary among traits (Chown, 2001; Hoffmann et al., 2003). In consequence, it has been proposed that physiological investigations and biophysical modelling should be used in concert with

large scale bioclimatic investigations of species responses to understand what the future might hold for various taxa under a changing climate (Helmuth et al., 2005).

These concerns are equally pertinent to understanding changes in the distributions of arthropod-borne diseases (Kovats et al., 2001). The strength and complexity of the influence of climate change on disease distributions are likely to be very different from their influence on most other taxa, owing to interactions between the disease and human populations; the effects of other disease reservoirs and their environmental requirements; vector distributions; landscape change; and drug resistance (Patz et al., 2000; Hay et al., 2002a). Nonetheless, it has been demonstrated that climate change is likely to alter, or already has altered, the geographic distribution of at least some of these diseases (Githeko et al., 2000; Kovats et al., 2001; Randolph & Rogers, 2003; Burse et al., 2005). In other cases the effects are likely to be small in scope or more difficult to interpret (Rogers & Randolph, 2000; Hay et al., 2002a, b; Patz et al., 2002; Rogers et al., 2002). Given that the distributions of vector species are important in influencing changing patterns of disease transmission (Kovats et al., 2001; Burse et al., 2005), considerable attention has been given to the likely effects of environmental change on vectors. For Africa, much of the attention has focussed on malaria and its mosquito vectors (Hay et al., 2002a; Rogers et al., 2002). However, tsetse flies (*Glossina* spp.), which are long-lived, haematophagous flies, with low reproductive rates, have also been the subject of consideration because they carry trypanosomes that have serious implications for human and animal health, and are considered a major limitation to the socio-economic development of the continent (WHO, 1998; Leak, 1999; Rogers, 2000).

The Intergovernmental Panel on Climate Change has predicted that the ranges of African trypanosomiasis and its tsetse vectors are likely to change under forecast climate scenarios for Africa (Watson et al., 1998). Likewise, it has been suggested that in southern Africa the range of *Glossina morsitans* will contract with climate change (Hulme, 1996). Other forecasts have been more qualitative, but most imply that the distributions of tsetse and trypanosomiasis will change in the future (Beniston, 2002; WHO, 2004). These predictions arise from the fact that abiotic variables that will be affected by climate change, such as land surface temperature and saturation deficit, and other integrated environmental variables (e.g. the Normalized Difference Vegetation Index (NDVI)), are excellent correlates of tsetse distributions and abundances (Rogers & Randolph, 1991; Rogers & Williams, 1994; Robinson et al., 1997a, b; Rogers, 2000), explaining much of their variation. In some cases the mechanisms underlying these correlations are likely to be indirect. Different tsetse species have different habitat requirements, and the relationships between tsetse, wild host species, and human

population and livestock distributions are dynamic (Leak, 1999; Rogers, 2000; Bourn et al., 2001; Rogers & Robinson, 2004). However, the direct role of abiotic environmental variables in affecting tsetse life history parameters is well established. The role of temperature and water availability in the population biology of tsetse has been summarized previously (Hargrove, 2004). Temperature has major effects on birth rates, development, and mortality, and there are especially strong relationships between adult mortality and ambient temperature, which in some species are markedly non-linear (Hargrove, 2001; 2004). Water availability is also known to affect mortality rates; the immature stages are most sensitive to dry conditions, whilst adults are most sensitive to temperature (Hargrove, 2004). Nonetheless, there is substantial variation among tsetse species (Rogers & Randolph, 1986; Rogers, 2000; Rogers & Robinson, 2004).

Despite the foregoing information on how responses to climate might vary among different tsetse species and subspecies, based both on remote sensing work (Robinson et al., 1997a, b), and laboratory-based physiological studies (Bursell, 1959; 1960; Phelps & Burrows, 1969), among-population physiological variability and the rate of development and extent of phenotypic plasticity in tsetse are poorly understood (although some behavioural variation has been described (Langley et al., 1984)). Nonetheless, it is clear from the foregoing discussion that if the responses of these species to climate change are to be fully comprehended, then such information is essential. Moreover, if combined with an understanding of the gene flow among populations (Krafsur et al., 2001; Krafsur, 2002; 2003; Ouma et al., 2005) and likely dispersal rates (Williams et al., 1992; Hargrove, 2004; Ouma et al., 2005), comprehension of physiological variation could provide considerable insight into likely responses to environmental change (Krafsur, 2003). Here, I therefore undertake such a study by investigating among-population variability in seasonal acclimatization, and the extent and time course of phenotypic plasticity of thermal tolerance and desiccation resistance in *Glossina pallidipes* Austen.

## **Materials and methods**

### *Field sampling and study populations*

Adult flies were sampled from four locations representing different thermal habitats in Kenya, East Africa (Table 1). To determine seasonal variation in physiology, the Nguruman District (mid-altitude) and Lambwe District (high-altitude) populations were sampled twice within the same year in mid-July to mid-August 2003 (the end of the long rainy season) and in mid-November to mid-December 2003 (the end of the short rainy season). The Narok (highest altitude) and Kwale Districts (low-altitude) were sampled in November-December 2003. For each of the field experiments, flies

were collected from odour-baited, biconical traps set out in the respective localities. Following removal from the traps, the flies were transported in an insulated container, lined with moist paper towel, to field laboratories. These were located a maximum of two hours drive away from each of the sampling sites of a given district. At the laboratories, the flies were separated into groups for each of the experimental procedures, after which experiments commenced immediately. Depending on the time of the experiment, flies were collected either in the morning or in the late afternoon, usually from 08h00 to 10h00 and from 17h00 to 18h00. Thermal limit assessments were performed during the day using flies sampled in the morning, while desiccation experiments took place during the night using afternoon collected flies.

**Table 1.** Field sampling sites (coordinates in decimal degrees) and time of sampling of *Glossina pallidipes* for this study.

Region	Coordinates (°)	Altitude (m)	Sampling season
Narok	1.12S, 35.20E	1691	After short rains
Lambwe	0.64S, 34.31E	1353	After long and short rains
Nguruman	1.85S, 26.10E	670	After long and short rains
Kwale	4.18S, 39.46E	388	After short rains

#### *Critical thermal limits*

An insulated system of eleven double-jacketed isolation chambers was connected to a programmable water bath (LTD 20 with PZ1 programmer, Grant Instruments, Cambridge, UK) which regulated water flow around the chambers (Klok & Chown, 2003). A single fly was placed into each chamber and a 40 SWG type-T thermocouple was inserted into the control chamber to measure chamber temperature. The flies were allowed to equilibrate for ten minutes at either 16 or 35 °C before either minimum or maximum critical thermal limit assessments commenced, respectively (reviewed in Lutterschmidt & Hutchison, 1997). Because the flies are small, their body temperature was considered equivalent to chamber temperature. After equilibration, chamber temperature was lowered or raised at 0.25 °C.min<sup>-1</sup>. Preliminary experiments confirmed that body temperature did not lag behind or exceed chamber temperature at this rate of temperature change. Critical thermal minimum (CT<sub>Min</sub>) was defined as the loss of coordinated muscle function, and critical thermal maximum (CT<sub>Max</sub>) was defined as the onset of muscle spasms. The temperature at which either of these reactions occurred was recorded for each individual. These endpoints are readily identifiable for any species once an observer is practiced

(Lutterschmidt & Hutchison, 1997). Typically the variance about the endpoints is very low, and here a single observer (CJK) undertook all of this work. Moreover, this experimental procedure has been verified using thermolimit respirometry (Klok et al., 2004), is widely used to assess thermal limits (Chown & Nicolson, 2004), and the observer typically was not informed which acclimation treatment was being assessed. The procedure was repeated three times for both  $CT_{Min}$  and  $CT_{Max}$  to give a total  $n = 30$  per population and trait. Preliminary, replicated experiments found no effect of gender, age or feeding status on critical thermal limits (unpublished data, C.J. Klok and J.S. Terblanche). Critical thermal maxima were lethal while minima were not. However, even in the case of  $CT_{Min}$  all experimental animals were discarded following a trial.

#### *Water loss rates*

Male flies ( $n = 16$ ), held individually in 5 ml cuvettes were subjected to desiccation in flowing air ( $< 2.5\%$  relative humidity, R.H.) for ten hours at  $24.0 \pm 1.0$  °C (PTC-1 Peltier-controlled temperature cabinet, Sable Systems, Las Vegas, Nevada) (Terblanche et al., 2005). Air was pumped through a scrubbing column, containing silica gel and Drierite to remove residual water, and then into a mass flow controller (MFC). The MFC outlet was connected to a Sable Systems MF8 airflow manifold. Each outflow channel of the manifold was further split into two streams so that two cuvettes, each containing a fly, were attached to a single manifold channel. The air flow rate through each cuvette, tested with a second MFC, was regulated to produce a rate of  $100 \text{ ml}\cdot\text{min}^{-1}$ . Experiments took place during the night (21h00-07h00), a period of minimal activity in tsetse, and were repeated twice per location ( $n = 16$  per experiment), each time on individuals collected in the field on that particular day, to give a total  $n = 32$ . Each fly was weighed before and after a trial (Avery Berkel FA 304T, EU, electronic microbalance, 0.1 mg resolution), and water loss rate expressed as  $\text{mg H}_2\text{O}\cdot\text{h}^{-1}$ . Excretion usually takes place after a bloodmeal and here experiments took place well after such events. In addition, even in field-collected flies where the time since feeding could not be accurately determined, no excretion in the cuvettes was observed. Therefore, mass loss was considered representative of water loss even though a small amount of mass change took place as a consequence of metabolism (Bursell, 1959). After the trials, flies were dried to constant mass ( $\sim 50\text{-}60$  °C for  $\sim 72$  hrs) and re-weighed to determine water content. Solvent-based lipid extractions were used to determine lipid mass. Flies used in desiccation trials were dismembered and soaked in 2 ml chloroform:methanol (2:1) solution for approximately 48 h to dissolve internal (body) lipids. Lipid content was estimated by determining dry

body mass before and after lipid extraction. This method is widely used for assessment of lipid content (Naidu & Hattingh, 1988; Hoffmann et al., 2005a).

#### *Activity levels*

Several authors have suggested that differences in desiccation resistance among taxa might simply reflect divergence in activity levels (Bursell, 1959; Hadley, 1994; Gibbs et al., 1997). Because little is known about inter-population variation in tsetse activity levels, particularly under stressful conditions, the proportion of time individuals were active was estimated over a 30 min period for each population. Time active at 20, 24, 28 and 32 °C was determined using an electronic activity detector (AD-1, Sable Systems) which exported voltage data to a computer via Sable Systems DATACAN V software. These experiments were performed during the same field sampling periods reported above. Voltage data were sliced from the raw Datacan V (Sable Systems) data files, copied to MS-Excel, converted to absolute values and sorted from lowest to highest. Based on preliminary observations, I considered activity as any signal > 0.01 Volt measured by the AD-1 (electronic spikes of 5 Volt were ignored and can easily be distinguished from true activity readings). The total time spent above this level of activity was expressed as a proportion of the total recording time. Whilst it is well known that activity in tsetse is sensitive to feeding state (Brady, 1988), the objective here was to determine whether differences in activity level were a likely cause of any differences in water loss rates among populations, not whether the flies activity overall differs among populations.

#### *Within-generation physiological plasticity*

Logistic constraints precluded the use of field populations for investigations of phenotypic plasticity. Therefore, *G. pallidipes* from a laboratory colony maintained at the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, International Atomic Energy Agency, Vienna, Austria was used. Gene diversities over four mitochondrial loci in these laboratory-reared flies are within the range of six field populations (Wohlford et al., 1999). Similarly, microsatellite diversities are homogeneous among two laboratory strains and six field populations (Krafsur & Endsley, 2002). Therefore, by comparison with neutral markers the laboratory-reared flies may be considered genetically representative of flies from field populations. Puparia were immediately placed inside plastic containers and transferred to a climate chamber set to 24 °C ( $24.6 \pm 2.5$  °C, 12:12 L:D) and 76% relative humidity as described previously (Terblanche et al., 2004a). Feeding of the newly emerged adults took place using a membrane-tray system (Gooding et al., 1997) every



alternative day and at this time container locations were randomized within the climate chamber. Care was taken to ensure that all treatment groups were handled for the same duration in transferral from the climate chamber to the feeding area, and spent a similar amount of time outside the climate chamber whilst feeding (~25 min on each feeding day). Flies were held at 19, 24 and 29 °C for ten days in climate chambers with a synchronized photoperiod and constant 76% relative humidity levels. The 24 and 29 °C groups were stored in temperature-regulated climate chambers ((Labcon, South Africa) mean  $\pm$  SD: 24.0  $\pm$  2.3 [n = 1029]; 28.9  $\pm$  1.7 °C [n = 937]) and the 19 °C group was stored in a Peltier-controlled temperature cabinet (mean  $\pm$  SD: 19.4  $\pm$  0.4 °C [n = 905]). Acclimation temperatures were chosen to represent the mean annual temperatures of the respective geographic locations sampled (reported MAT: warm-temperate midlands (< 1850 m): 18-20 °C; warm lowlands (900-1200 m) 22-24 °C; hot lowlands (0-900 m): 24-30 °C (Sombroek et al., 1980)). All experimental and rearing facilities were contained within a quarantine approved, air-conditioned laboratory. The same system and methods as those used in the field experiments for determining geographic variation were used in the laboratory determination of critical thermal limits and desiccation rate.

A separate batch of colony-reared flies was used to determine whether the temperature acclimation effect observed in the  $CT_{Min}$  of *G. pallidipes* is time- and/or temperature-dependent, and whether these changes in  $CT_{Min}$  are reversible. Flies were allowed to emerge in the laboratory as described above, and were fed twice within four days following eclosion. However, a small number of flies appeared only to feed during the second feeding round. Thus, typically after two bloodmeals, flies were randomly assigned to four temperature acclimation groups (24, 21, 19, and 16 °C, n = 180 ~ 220 per group, 5-6 cages per group). Humidity and photoperiod were regulated as described above, and randomization of cage locations within chambers followed the protocol adopted previously. Therefore, handling times were kept constant across all groups during feeding sessions and experimental treatments. The  $CT_{Min}$  of ten randomly selected flies from each treatment group was then determined on acclimation days 1, 3, 5, 7 and 9. After nine days of acclimation, the remaining flies were all returned to 24 °C for an additional nine-day period, after which another sample of flies was assessed on the tenth day. In this experiment, once a fly had been used for determination of  $CT_{Min}$ , it was discarded by bottling in 90% ethanol (i.e. no animals were re-used). Feeding took place using the same methods described above only on days between experimental investigations (i.e. flies were always in a fasted state during experiments).



*Statistical analyses*

Data were assessed for normality using a Shapiro-Wilk's test prior to analyses. As critical thermal limit data were normally distributed, analyses of variance (ANOVA) using the raw data were used to compare critical thermal limits for both field and laboratory studies. Analyses of covariance (ANCOVA) were used to test for differences in rates of water loss and lipid content between populations and seasons (i.e. mass-independent water loss rates), using mean experimental or dry mass as the covariate. Pearson product-moment correlations were used to assess the relationships between time spent active and temperature. Data are presented as means  $\pm$  standard error of the mean (S.E.) unless otherwise stated. Significance was set at  $P = 0.05$ . In no instances for any trait were experimental assessments performed on the same individual on more than one occasion (i.e. animals were not re-used).

**Results***Seasonal variation*

Season had a significant effect on the majority of the variables examined (Table 2). In the Lambwe and Nguruman populations,  $CT_{Min}$  increased and water loss rate declined after the short rains. Although lipid mass also declined in the Lambwe population, the change was not significant in flies from Nguruman. Seasonal changes in body mass and  $CT_{Max}$  were opposite in sign in the two populations, though the seasonal difference in  $CT_{Max}$  was only 0.4 °C in both populations. Body mass increased in the Lambwe population, but declined at Nguruman, leading to an overall difference in mass between the two populations after the short rains (Table 2;  $F_{1, 91} = 20.35$ ;  $P < 0.0001$ ), but not after the long rains (Table 2;  $F_{1, 126} = 0.414$ ;  $P = 0.52$ ).

**Table 2.** Summary statistics for seasonal variation of physiological traits in *Glossina pallidipes* from the Lambwe and Nguruman populations. The results of either ANOVA or ANCOVA (with dry mass as covariate, indicated with superscript A) are shown.

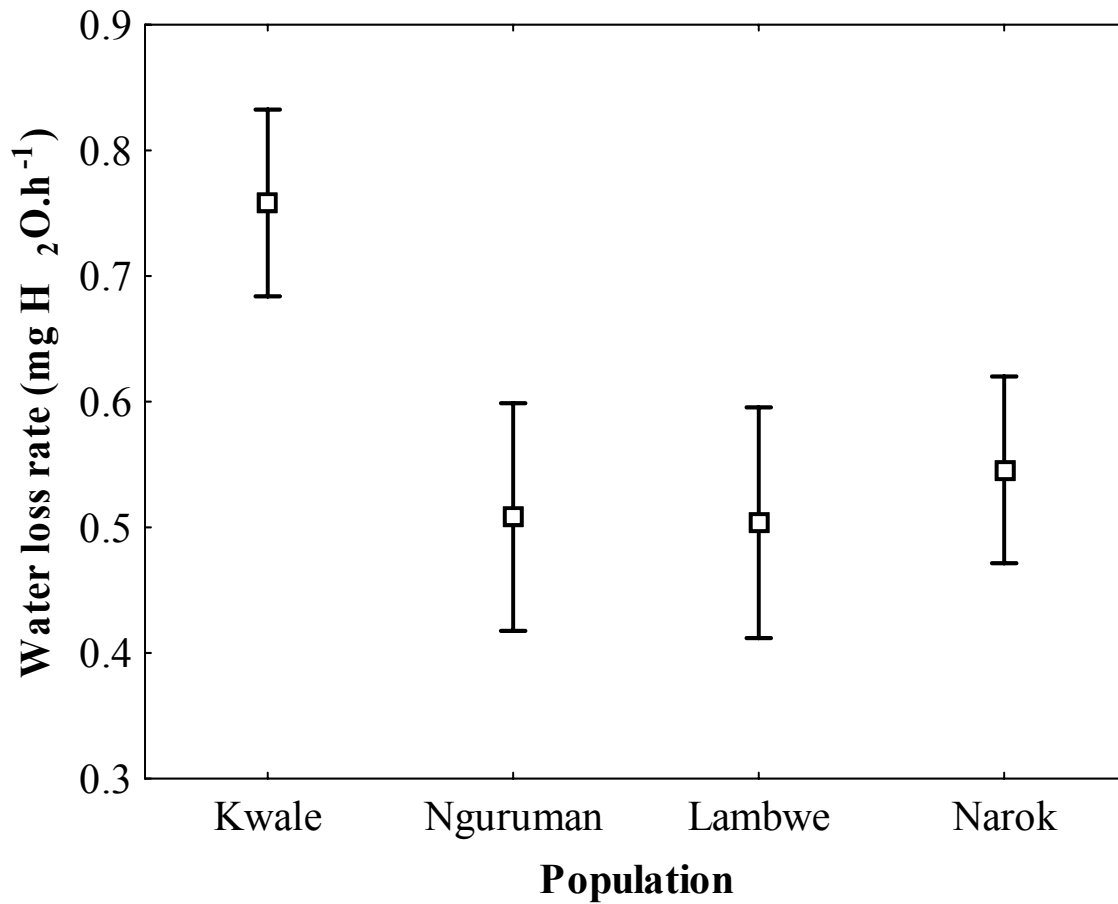
Population	Trait	Season		F-ratio	DF	P
		Long rains	Short rains			
Lambwe	CT <sub>Max</sub> (°C)	45.0 ± 0.1	44.6 ± 0.1	11.18	58	< 0.01
	CT <sub>Min</sub> (°C)	12.3 ± 0.2	13.4 ± 0.3	9.83	58	< 0.01
	Water loss rate (mg H <sub>2</sub> O.h <sup>-1</sup> ) <sup>A</sup>	0.620 ± 0.027	0.536 ± 0.032	6.95	51	< 0.05
	Body (dry) mass (mg)	11.74 ± 0.42	13.73 ± 0.73	6.38	59	< 0.05
	Body lipid content (mg) <sup>A</sup>	2.00 ± 0.07	1.89 ± 0.08	30.03	106	< 0.0001
Nguruman	CT <sub>Max</sub> (°C)	44.6 ± 0.1	45.0 ± 0.1	12.00	56	< 0.01
	CT <sub>Min</sub> (°C)	11.8 ± 0.2	13.8 ± 0.2	74.79	58	< 0.0001
	Water loss rate (mg H <sub>2</sub> O.h <sup>-1</sup> ) <sup>A</sup>	0.789 ± 0.042	0.474 ± 0.033	40.15	54	< 0.0001
	Body (dry) mass (mg)	11.62 ± 0.39	10.84 ± 0.41	7.13	47	< 0.05
	Body lipid content (mg) <sup>A</sup>	1.53 ± 0.07	0.99 ± 0.08	3.308	98	0.072

### Geographic variation

CT<sub>Max</sub> and CT<sub>Min</sub> differed among the four populations (Table 3). The mid-altitude and low altitude populations differed only marginally, whereas both CT<sub>Min</sub> and CT<sub>Max</sub> declined with a further increase in elevation. The slope of the two altitude-critical limit relationships also differed significantly (ANCOVA  $F_{1,234} = 59.42$ ;  $P < 0.0001$ ), with CT<sub>Min</sub> showing a steeper slope and stronger relationship with altitude than CT<sub>Max</sub>. Overall CT<sub>Min</sub> varied by 3.3 °C between the four populations, whereas CT<sub>Max</sub> differed by only 0.4 °C.

Water loss rate also varied significantly between the populations (Fig. 1). It seems unlikely that differences in activity levels among the populations could account for the variation in water loss rate. Total activity time was positively, though in one case weakly, correlated with temperature across the range of 20-32 °C in all four populations (Narok:  $r = 0.64$ ,  $P < 0.01$ ; Lambwe:  $r = 0.47$ ,  $P = 0.068$ ; Nguruman:  $r = 0.64$ ,  $P < 0.01$ ; Kwale:  $r = 0.50$ ,  $P < 0.05$ ). Because there were no sex-related differences in activity time in any of the populations, sexes were pooled for the inter-population comparisons. There were no among-population differences for time spent active (ANCOVA (covariate:

temperature):  $F_{3, 59} = 1.38$ ;  $P = 0.26$ ). Therefore, differences in activity probably did not account for differences in water loss rates.



**Figure 1.** Geographic variation of water loss rate in *Glossina pallidipes*. Note that desiccation rate is significantly higher in the low altitude population from Kwale (388 m) (see text for statistics). All flies were sampled in the same season (Nov-Dec, 2003). (Narok, 1691 m; Lambwe, 1353 m; Nguruman, 670 m.)

**Table 3.** Geographic variation in mean population critical thermal maxima ( $CT_{Max}$ ) and minima ( $CT_{Min}$ ) for *Glossina pallidipes*. All flies were collected in the same season (Nov-Dec, 2003). (ANOVA,  $CT_{Max}$ ,  $F_{3,114} = 14.0$ ;  $P < 0.0001$ ;  $CT_{Min}$ ,  $F_{3,116} = 70.35$ ;  $P < 0.0001$ .)

Trait	Mean ° C ± S.E.	Min	Max	N
$CT_{Max}$				
Narok	44.4 ± 0.1	43.5	45.1	30
Lambwe	44.6 ± 0.1	43.7	45.2	30
Nguruman	45.0 ± 0.1	44.3	45.7	28
Kwale	45.0 ± 0.1	44.2	45.5	30
$CT_{Min}$				
Narok	10.5 ± 0.2	8.9	12.4	30
Lambwe	13.4 ± 0.3	11.8	16.0	30
Nguruman	13.8 ± 0.2	11.8	15.2	30
Kwale	13.8 ± 0.2	12.6	15.9	30

#### *Temperature acclimation of laboratory-bred flies*

Ten days of laboratory acclimation at three different temperatures resulted in significant among treatment variation in  $CT_{Max}$ ,  $CT_{Min}$ , desiccation rate and body water content (Table 4).  $CT_{Max}$  increased significantly with treatment temperature, but the change was less than 0.5 °C. By contrast, at 19 °C  $CT_{Min}$  declined by *c.* 3°C relative to the other treatments. Water loss rate showed similar threshold effects, declining significantly at the highest treatment temperature, whereas body water content showed little change with treatment temperature (Table 4).

In the time-course experiments, changes in  $CT_{Min}$  were induced in the 16 °C and 19 °C treatments only, and the magnitude of the induced change was quite similar (Fig. 2). By contrast, the 21 °C and 24 °C treatments had no effect. In the former treatments,  $CT_{Min}$  had reached its nadir by the fifth day of treatment and typically remained at a similar level for the remainder of the acclimation period (day 9). Ten days at 24 °C were sufficient to completely reverse the effects of the acclimation treatments (i.e. at day 19 no groups differed from the 24 °C group). Thus, significant day\*treatment interactions were found for  $CT_{Min}$  ( $F_{15,216} = 3.99$ ;  $P < 0.0001$ ). The low treatment temperatures clearly came at some cost to normal adult development. Whilst flies gained mass in the 21 °C and 24 °C treatments, and showed low and typically consistent among-individual variation, the flies at the lower

temperatures did not show any steady progression in mass, and variation among individuals was considerable (Fig. 3). The differential effect of treatment on change in mass over the 19 days is evidenced by a significant day\*treatment interaction in a two-way analysis of variance examining the effects of day and treatment on mass ( $F_{15, 120} = 2.7, P = 0.0013$ ).

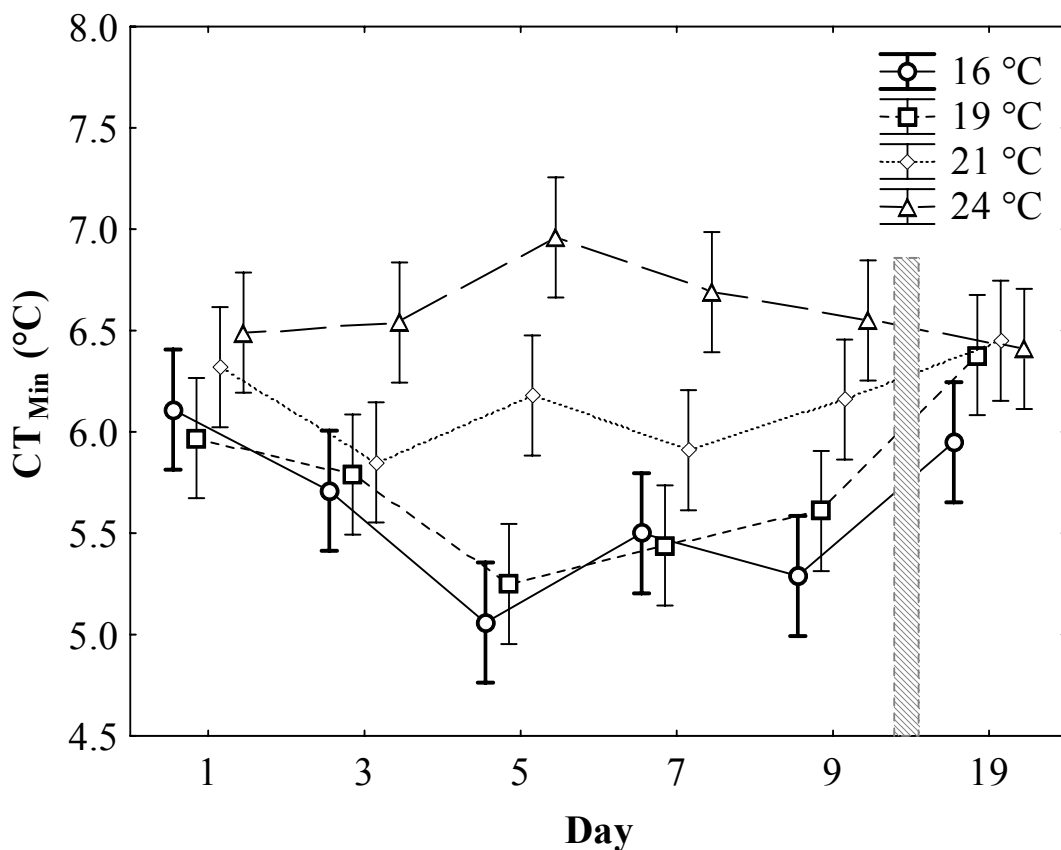
#### *Colony vs. field flies*

Upper and lower critical thermal limits differed among the laboratory-acclimated (colony) flies and field populations (Fig. 4a, b). However, the extent of this difference was only *c.* 1 °C for  $CT_{Max}$ , but from 4 to 10 °C for  $CT_{Min}$ . Water loss rates also varied among the field and laboratory populations, and here the maximum difference among groups (Kwale and the 29 °C treatment group) was substantial, although none of the other groups differed from each other (Fig. 4c). By contrast, dry mass varied little among the populations and groups (Fig. 4d).

**Table 4.** Upper ( $CT_{Max}$ ) and lower ( $CT_{Min}$ ) critical thermal limits, water loss rate at 24 °C and initial body water content after ten days of temperature acclimation in laboratory-reared *Glossina pallidipes* adults. Recorded climate chamber temperatures presented in parentheses (mean  $\pm$  S.D.) following treatment temperature. Similar superscript letters denote statistically homogeneous groups (*Post-hoc* unequal sample HSD). All analyses by means of ANOVA unless otherwise specified.

Acclimation Temperature	$CT_{Max}$ (°C)	$CT_{Min}$ (°C)	Water loss rate <sup>1</sup> (mg H <sub>2</sub> O.hr <sup>-1</sup> )	Body water content (mg H <sub>2</sub> O)
19 °C (19.5 $\pm$ 0.4)	43.9 $\pm$ 0.1 <sup>A</sup> (20)	4.5 $\pm$ 0.1 <sup>A</sup> (20)	0.464 $\pm$ 0.061 <sup>A</sup> (8)	29.9 $\pm$ 0.7 <sup>A</sup> (8)
24 °C (23.4 $\pm$ 0.9)	44.4 $\pm$ 0.1 <sup>B</sup> (10)	7.8 $\pm$ 0.3 <sup>B</sup> (12)	0.393 $\pm$ 0.017 <sup>A</sup> (9)	25.2 $\pm$ 2.8 <sup>A</sup> (9)
29 °C (28.8 $\pm$ 1.7)	44.2 $\pm$ 0.1 <sup>A,B</sup> (20)	7.2 $\pm$ 0.2 <sup>B</sup> (20)	0.213 $\pm$ 0.047 <sup>B</sup> (8)	23.4 $\pm$ 1.5 <sup>A</sup> (8)
F	5.10	97.34	7.89	2.77
P	< 0.01	< 0.001	< 0.003	0.085

<sup>1</sup> ANCOVA with dry body mass as covariate



**Figure 2.** The time-course of acclimation for  $CT_{Min}$  in *Glossina pallidipes* adults. After nine days of acclimation to 16, 19, 21 and 24 °C, all acclimation groups were returned to 24 °C for ten days recovery (tested on Day 19). Within acclimation treatment (between-day) comparisons of the means are presented in Table 5. Between acclimation treatments (within-day) comparisons are presented in Table 6. (Recorded climate chamber temperatures (mean  $\pm$  S.D.), 16, 15.7  $\pm$  0.37; 19, 18.6  $\pm$  0.22; 21, 20.6  $\pm$  0.50; 24, 24.4  $\pm$  1.38 °C.) N = 10 flies for all days and all acclimation temperature groups. There was no effect of acclimation on 21 and 24 °C acclimation groups, but both 16 and 19 °C had significantly reduced  $CT_{Min}$  by Day 5 (ANOVA, 16 °C,  $F_{5, 54} = 8.70$ ;  $P < 0.0001$ ; 19 °C,  $F_{5, 54} = 9.31$ ;  $P < 0.0001$ ; 21 °C,  $F_{5, 54} = 1.99$ ;  $P = 0.095$ ; 24 °C,  $F_{5, 54} = 1.38$ ;  $P = 0.25$ ). By Day 19 all acclimation groups were similar ( $F_{3, 36} = 2.64$ ;  $P = 0.064$ ).

## Discussion

### *Physiological implications*

From a physiological perspective, the current findings are significant in several ways. The temperature and water balance traits differed substantially from each other in the extent of their variability among populations, responses to seasonal changes in the environment, and responses to laboratory acclimation. Of the three traits,  $CT_{Max}$  varied least among populations (0.6 °C), seasons (0.4 °C) and acclimation treatments (0.5 °C), and the full range of variation among all of these samples was only 1.1 °C (maximum value 1.02 x larger than the minimum). By contrast,  $CT_{Min}$  was more variable, differing by 3.3 °C, 1-2 °C, and 3.3 °C, among the populations, seasons and acclimation treatments, respectively, and the full range of variation was 9.3 °C (maximum value = 3.1 x minimum). Water loss rate showed similar, substantial variation, with the overall maximum value being 3.7 x the minimum. These results are consistent with work across a range of insect taxa, including *Drosophila* and other flies, and several coleopteran families. In insects, upper critical temperatures show much less variation than lower critical temperatures among populations and acclimation treatments (Klok & Chown, 2003; Hoffmann et al., 2005b), between selection regimes (Gilchrist et al., 1997; Hoffmann et al., 1997), and among species at a variety of spatial scales (Gaston & Chown, 1999; Addo-Bediako et al., 2000; Kimura, 2004). Indeed, limited phenotypic plasticity in upper compared with lower thermal limits appears to be typical of ectotherms in general (Kingsolver & Huey, 1998). Substantial differences in water loss rates among populations, among seasons within a population, and between humidity acclimation treatments are also common in insects (Hadley, 1994; Chown et al., 1999; Addo-Bediako et al., 2001; Gibbs & Matzkin, 2001; Hoffmann et al., 2001a; Hoffmann et al., 2005b). However, only a few studies have examined temperature acclimation or acclimatization effects on desiccation resistance in insects, with some finding notable effects of temperature (Hoffmann et al., 2005b; Parkash et al., 2005), and others finding no influence at all (Terblanche et al., 2005). Thus, upper thermal critical limits that are not especially variable and water loss rates and lower thermal limits that are more flexible are typical of insects in general. In consequence, their occurrence in *G. pallidipes* is not entirely unexpected (Rogers, 1990), although investigations of tropical sarcophagid flies have demonstrated that these flies have less malleable lower thermal limits than do their temperate counterparts (Chen et al., 1990).

A further significant finding is that the magnitude of the among-population differences was identical to or smaller than the magnitude of the acclimation effects in the laboratory-reared flies. Phenotypic plasticity might therefore account for all of the among-population variation in *G. pallidipes*. However, because of logistic constraints, each population was not examined to determine the extent of its phenotypic plasticity. Therefore, the extent to which among-population



variation really is accounted for by phenotypic plasticity could not be fully determined. Nonetheless, seasonal acclimatization of  $CT_{Min}$ ,  $CT_{Max}$  and water loss rates in the Lambwe and Nguruman populations encompassed 50 – 100% of the among-population variation in these traits. Together with the results from the laboratory acclimation trials, these findings provide substantive evidence for the idea that most of the variation among populations could be accounted for by phenotypic rather than genetic differences. Similar results have been obtained for two independent assessments of *Drosophila melanogaster*. In eastern Australia, comparisons of seasonal and latitudinal (population) variation in thermal tolerance (chill coma recovery and heat knockdown resistance) and desiccation resistance revealed that the magnitude of the seasonal response was equivalent to, or perhaps larger than the clinal variation (Hoffmann et al., 2005b). Across four *D. melanogaster* populations (two from France and two from Kenya) as much as 80% of the variation in chill coma recovery time can be accounted for by phenotypic plasticity (Ayrinhac et al., 2004) (in this case developmental plasticity (Wilson & Franklin, 2002)). Thus, it seems likely that much of the among-population variation in these traits examined in *G. pallidipes* is a consequence of phenotypic plasticity.

In the case of  $CT_{Min}$  these plastic changes take place rapidly. Within five days of exposure to a different temperature, flies held at the lower temperatures had substantially altered their  $CT_{Min}$ , and by nine days after their return to a common, higher temperature environment  $CT_{Min}$  had returned to its original value. Whether the rapid phenotypic change found in *G. pallidipes* is typical of  $CT_{Min}$  or similar traits in other insect species is not clear, largely because the time course of thermal acclimation (rather than developmental plasticity) has not been thoroughly investigated in the group. Most studies concern either rapid cold hardening, which takes place in a matter of hours (although the hardening effect takes longer to be lost), or seasonal, programmed responses to changing environments that develop over several weeks or months (Chown & Nicolson, 2004). Nonetheless, chill coma recovery in *Lycaena tityrus* is modified by only three days of acclimation in the adult stage (Zeilstra & Fischer, 2005), within 10 days acclimation temperature had a much greater effect than development temperature on egg size in the butterfly *Bicyclus anynana* (Fischer et al., 2003), and the interaction between age and test temperature was significant on walking speed in *Drosophila melanogaster* (Gibert et al., 2001). Therefore, reasonably rapid acclimation effects are probably not unusual in insects, at least as far as lower critical limits are concerned.

Finally, whilst  $CT_{Max}$ , water loss rate and dry mass were quite similar across all of the populations and acclimation treatments,  $CT_{Min}$  differed substantially between the field-collected and laboratory-reared flies (Fig. 4). The latter had critical thermal minima that were 3-9 °C lower than those of the field-collected flies (although the magnitude of the difference between populations and between acclimation treatments was the same). In consequence, it seems likely that differences in

the phenotypic plasticity of the upper and lower critical thermal limits extend to the ease with which these traits can evolve. Higher acclimation temperatures in laboratory flies, or lower acclimation temperatures in field flies, might have further altered  $CT_{Min}$ . However, this seems unlikely given that more extreme temperatures are debilitating to flies over the longer term (Rogers, 2000; Hargrove, 2004). Therefore, the differences between the field and laboratory flies might have evolved as a consequence of conditions in the laboratory. The IAEA laboratory colony used in this study was established in 1975 from a natural population in Tororo, Uganda. Assuming linear evolutionary change, approximately 175 generations in the laboratory, and  $CT_{Min}$  values of the original Ugandan field population similar to those found at Lambwe (which is close to the Ugandan border), this would equate to approximately 1 °C change every 29 generations (i.e. 6 °C/175 generations). Whilst the reasons for laboratory evolution are not always clear, substantial changes in animals held in colonies have been documented in several studies (Sgrò & Partridge, 2000; Hoffmann et al., 2001b; Terblanche et al., 2004b). Whatever the reason for the evolved difference, the most noteworthy feature thereof is that this evolution has been restricted to  $CT_{Min}$  and has not taken place in the other traits. In consequence, it seems likely that most of the variation in the other traits is a consequence of phenotypic plasticity, whilst the variation found in  $CT_{Min}$  is a mixture of plasticity and evolved differences between laboratory and field-collected *G. pallidipes*. In addition, it raises questions about the extent to which the laboratory flies are genetically representative of field-collected flies, at least in the case of this trait. Future work exploring the underlying genetic basis of  $CT_{Min}$  would be required to resolve this question (Anderson et al., 2003; Loeschcke et al., 2004; Sgrò & Blows, 2004).

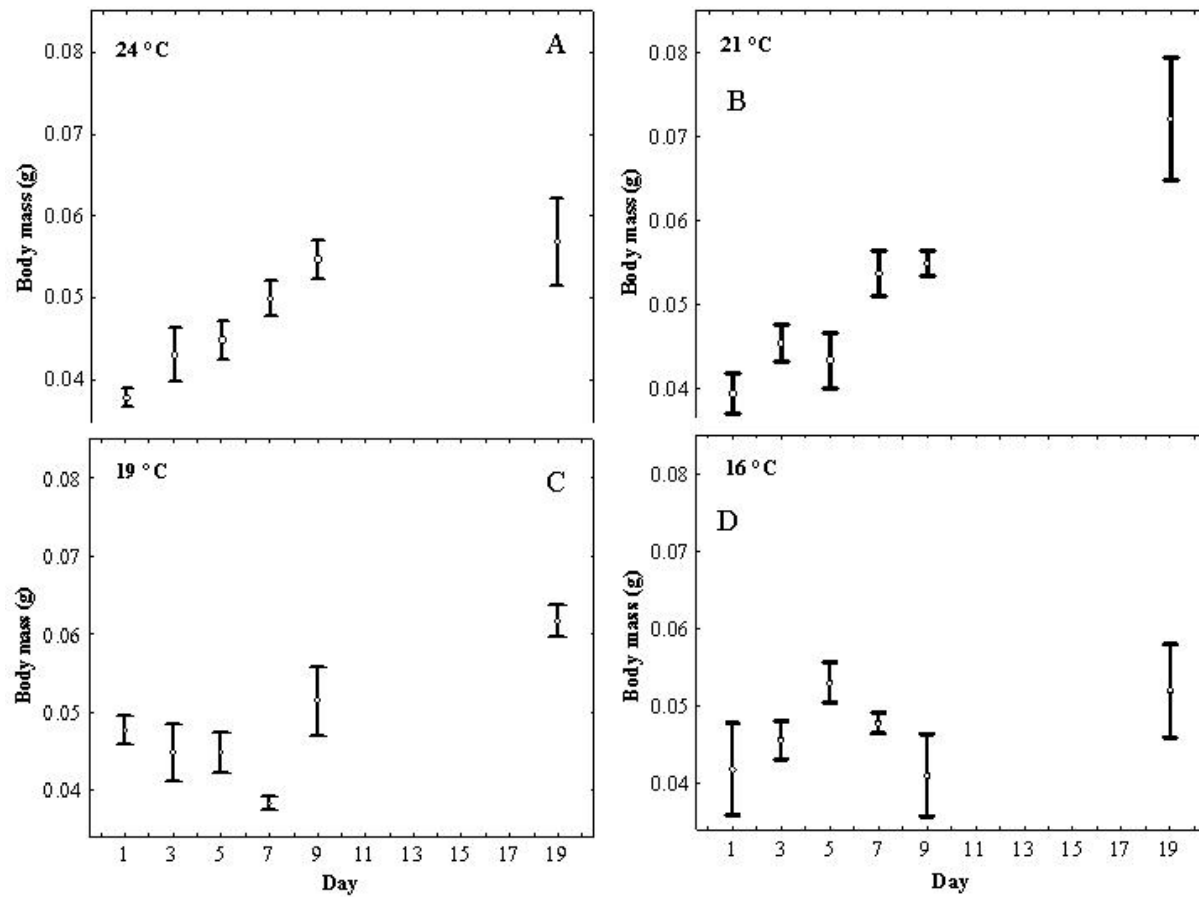
#### *Implications for distributional modelling*

Although the critical thermal maxima of adult *G. pallidipes* documented here are higher than the temperatures that induce mortality in the field (Hargrove, 2004), the present findings nonetheless substantiate correlative work on the determinants of the distribution of this species, and provide several insights into the likely responses of tsetse to climate change. In the first instance, univariate and multivariate analysis of climate and productivity (normalized difference vegetation index, NDVI) variables demonstrated that temperature variables are much better predictors of *G. pallidipes* presence and absence than NDVI variables (Robinson et al., 1997a, b). These results are in keeping with population level analyses that show a strong, though initially non-linear, relationship between increasing temperature and elevated mortality in *G. pallidipes* adults (Hargrove, 2004). These results suggest a causal basis for the determination of *G. pallidipes* distributions by temperature. It appears that in this species tolerance to high temperatures cannot change much either by phenotypic plasticity or by evolution. Such change in tolerance would result

in a weaker relationship between temperature and populations dynamics, and in doing so would reduce the strength of the relationships between temperature and the presence/absence of the fly species (Gaston, 2003; Hoffmann & Blows, 1994; Holt et al., 1997). The limited variation in  $CT_{Max}$  also provides a causal explanation for why, in statistical models of tsetse distribution, the mean temperature difference between areas of fly presence and absence may be less than 1 °C (Rogers & Robinson, 2004).

Limited scope for change in upper critical temperatures might also explain the narrow variation in this trait amongst populations despite the fact that gene flow among *G. pallidipes* populations is typically low (Krafsur & Wohlford, 1999; Krafsur, 2002; Ouma et al., 2005). From a theoretical perspective, limited gene flow among populations should promote diversification so long as gene flow is not so low that novel variants that have successfully survived similar conditions elsewhere are seldom introduced into a population (Kirkpatrick & Barton, 1997; Case & Taper, 2000; Butlin et al., 2003; Forde et al., 2004). However, if the trait itself cannot evolve because it is globally constrained (Hoffmann et al., 2003) then changes in gene flow are unlikely to affect trait diversification. What particular physiological mechanism might be involved in this instance is not clear, but temperature effects on membrane and/or protein structure are likely to be most significant (Chown & Nicolson, 2004; Klok et al., 2004).

From the present data it might seem difficult to discern why flies are not able to colonize lower temperature areas. However, lower temperature thresholds are likely set by development of the larvae, rather than by adult capabilities (Langley, 1977). Moreover, in the acclimation experiments I showed that although flies are able to survive longer-term maintenance at low temperatures, their muscle growth and development (which accounts for the largest change in mass in adult tsetse (Bursell, 1961)) is compromised, given that mass declines substantially by days 7-9, and variance in mass between individuals was larger in the lower than in the high temperature treatments (Fig. 3). Therefore, low temperatures are likely to compromise adults too.

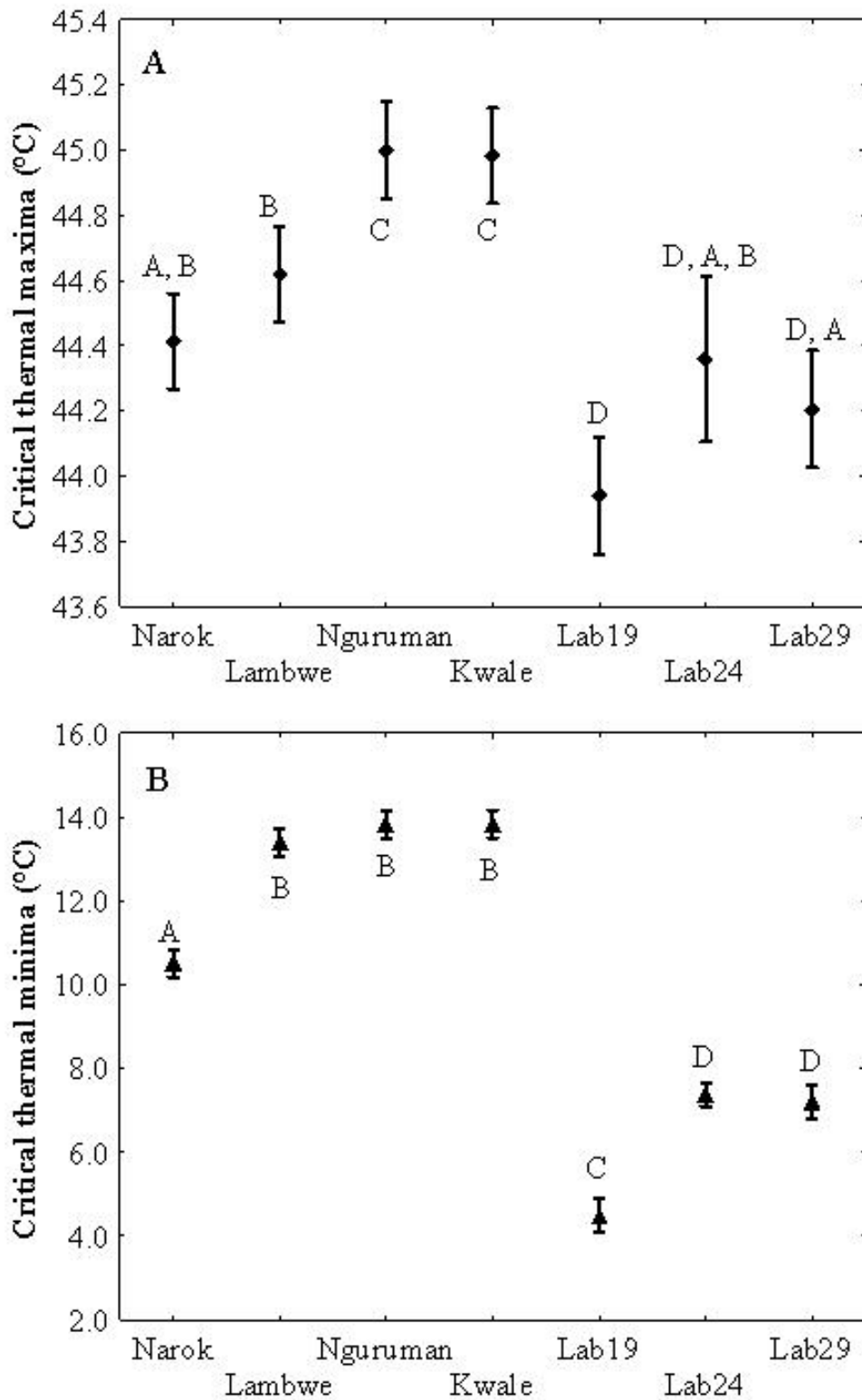


**Figure 3.** Body mass (mean in g ± S.E.) of female *Glossina pallidipes* during the course of acclimation to **A)** 24, **B)** 21, **C)** 19 and **D)** 16 °C. All flies were returned to 25 °C after day nine of acclimation. Although no differences were found in body mass upon completion of the acclimation treatments, there was a significant day \* treatment interaction (see text for statistics).

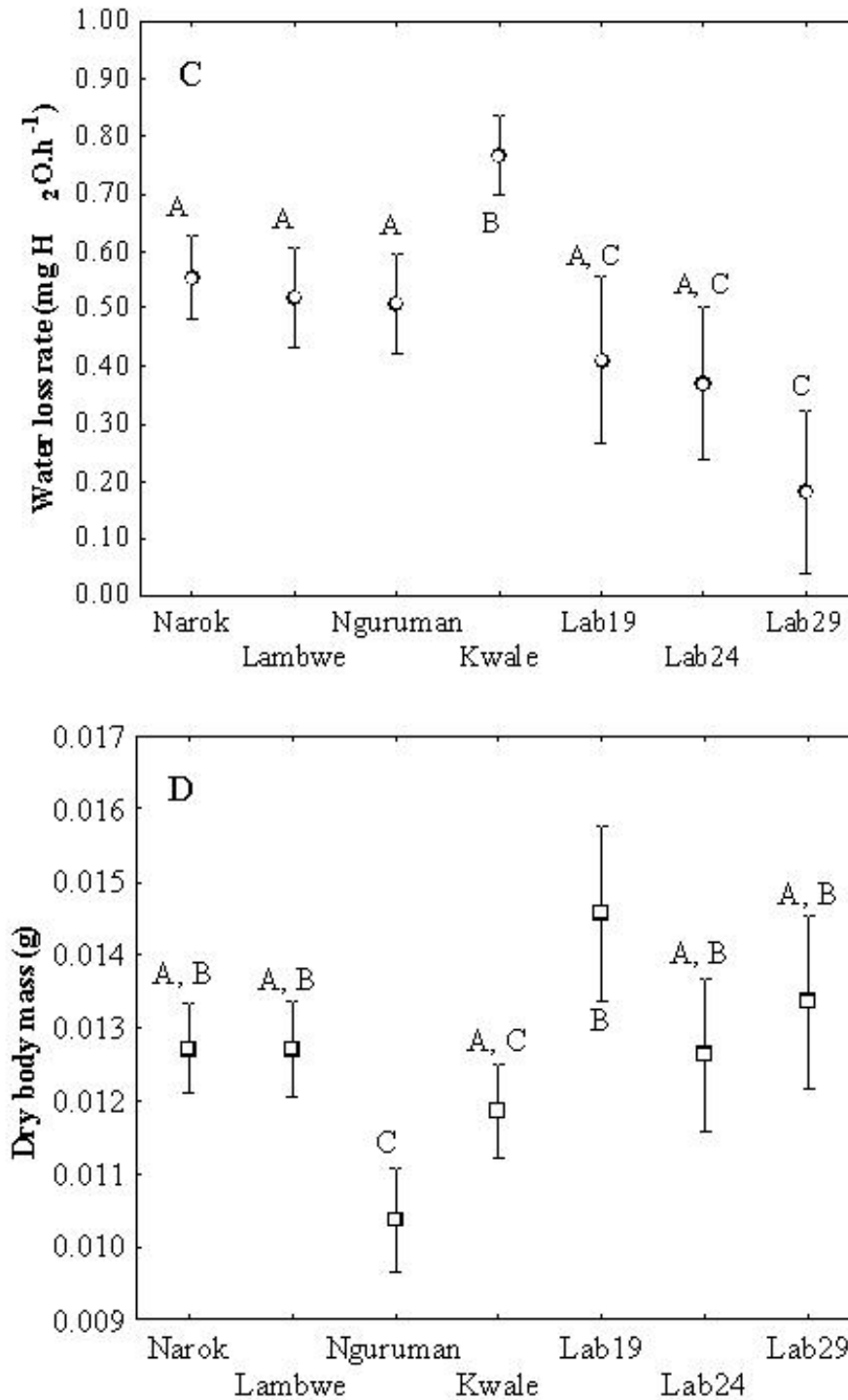
To date, most climate change predictions suggest that tsetse species would show a reduction in the range of the flies owing to increases in temperature (Hulme, 1996). My data support these findings because they show limited plasticity in upper critical temperatures. If upper thermal tolerances are inflexible, then increases in temperature are likely to lead to a reduction in range size as suggested by modelling approaches (Rogers, 2000). What altered rainfall patterns are likely to mean is more difficult to discern for two reasons. First, water loss rates in flies are clearly able to respond both in the laboratory and in the field to changing ambient conditions (Rogers, 1990). Whilst the laboratory response recorded here was to variation in temperature conditions, preliminary data from the field suggest that the strongest predictor of water loss rates is variation in humidity of the site (unpublished data; and see Parkash et al., 2005). Therefore, adult flies might be capable of adjusting water loss rates to changing conditions. However, water balance effects are likely to be most significant for puparia (Hargrove, 2004; Langley, 1977) which were not investigated here, so changing patterns of water availability might have a more substantive effect than the present data suggest. Second, fine-scale predictions of rainfall change are typically not available and only coarse-scale estimates can be made (Watson, 2002). Therefore, it is difficult to be able to predict what the outcome of the interaction between fly desiccation resistance and changing water availability might be. Nonetheless, my results provide further evidence in support of the idea that owing to changes in land use and climate, at least some tsetse may be brought under autonomous control in parts of their natural ranges (Bourn et al., 2001; Rogers & Randolph, 2002).

This work also suggests that long-term rearing of flies might substantially alter the physiological capabilities of the flies. Precisely what the responses of flies might be to other laboratory conditions is not clear, although differences in several aspects of the physiology of the flies develop rapidly once flies are held in captivity (Langley, 1967; Randolph et al., 1990). Moreover, a variety of studies indicates that in arthropods laboratory acclimation can substantially alter physiological performance, and that this change can often be rapid (Sgrò & Partridge, 2000; Hoffmann et al., 2001b; Terblanche et al., 2004b).

In conclusion, this study has shown that limited physiological differentiation between field populations of *G. pallidipes* are due largely or entirely to phenotypic plasticity, that upper critical temperature limits do not readily change, supporting the correlative models of distribution of this species and predictions of the likely response of flies to change, and that there is laboratory evolution in at least some traits in *G. pallidipes*.



**Figure 4A-B.** Traits means ( $\pm$  S.E.), in field and laboratory populations of *Glossina pallidipes*, of **A)** critical thermal maxima (ANOVA,  $F_{6, 161} = 22.38$ ;  $P < 0.0001$ ); **B)** critical thermal minima (ANOVA,  $F_{6, 195} = 453.20$ ;  $P < 0.0001$ ). (The numerals in Lab19, Lab24 and Lab29 refer to the temperature of acclimation in laboratory-reared flies for periods of 9-10 days).



**Figure 4C-D.** Traits means ( $\pm$  S.E.), in field and laboratory populations of *Glossina pallidipes*, of **C)** water loss rate (ANCOVA, covariate, dry body mass;  $F_{6, 126} = 12.378$ ;  $P < 0.0001$ ; least squares adjusted means  $\pm$  95% confidence limits; **D)** body size as dry body mass from all desiccation experiment flies (ANOVA;  $F_{6, 239} = 8.43$ ;  $P < 0.0001$ ; means  $\pm$  95% confidence limits). (The numerals in Lab19, Lab24 and Lab29 refer to the temperature of acclimation in laboratory-reared flies for periods of 9-10 days).

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## References

- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* 267, 739-745.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2001. Revisiting water loss in insects: a large-scale view. *Journal of Insect Physiology* 47, 1377-1388.
- Anderson, A.R., Collinge, J.E., Hoffmann, A.A., Kellett, M., McKechnie, S.W., 2003. Thermal tolerance trade-offs associated with the right arm of chromosome 3 and marked by the *hsr-omega* gene in *Drosophila melanogaster*. *Heredity* 90, 195-202.
- Ayrinhac, A., Debat, V., Gibert, P., Kister, A.G., Legout, H., Moreteau, B. et al., 2004. Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology* 18, 700-706.
- Beniston M, 2002. Climatic change, possible impacts on human health. *Swiss Medical Weekly* 132, 332-337.
- Bourn, D.M., Reid, R.S., Rogers, D.J., Snow, W.F., Wint, G.R.W., 2001. Environmental change and the autonomous control of tsetse and trypanosomiasis in sub-Saharan Africa: Case histories from Ethiopia, Gambia, Kenya, Nigeria and Zimbabwe. Oxford, Environmental Research Group, Oxford Limited <http://ergodd.zoo.ox.ac.uk/ECTCexsum.htm>
- Brady, J., 1988. Circadian ontogeny in the tsetse fly: A permanent major phase change after the first feed. *Journal of Insect Physiology* 34, 743-749.
- Burse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P.C., Baylis, M., 2005. Climate change and the recent emergence of bluetongue in Europe. *Nature Reviews Microbiology* 3, 171-181.
- Bursell, E., 1959. The water balance of tsetse flies. *Transactions of the Royal Entomological Society of London* 111, 205-235.



- Bursell, E., 1960. The effect of temperature on the consumption of fat during puparial development in *Glossina*. *Bulletin of Entomological Research* 51, 583-598.
- Bursell, E., 1961. Post-teneral development of the thoracic musculature in tsetse flies. *Proceedings of the Royal Entomological Society of London A* 36, 69-74.
- Butlin, R.K., Bridle, J.R., Kawata, M., 2003. Genetics and the distribution of species' distributions. In: Blackburn, T.M., Gaston, K.J. (eds), *Macroecology: Concepts and Consequences*. Blackwell Science, Oxford, pp. 274-295.
- Case, T.J., Taper, M.L., 2000. Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. *American Naturalist* 155, 583-605.
- Chen, C.-P., Lee, R.E., Denlinger, D.L., 1990. A comparison of the responses of tropical and temperate flies (Diptera, Sarcophagidae) to cold and heat stress. *Journal of Comparative Physiology B* 160, 543-547.
- Chown, S.L., 2001. Physiological variation in insects, hierarchical levels and implications. *Journal of Insect Physiology* 47, 649-660.
- Chown, S.L., Nicolson, S.W., 2004. *Insect Physiological Ecology. Mechanisms and Patterns*. Oxford University Press, Oxford.
- Chown, S.L., Le Lagadec, M.D., Scholtz, C.H., 1999. Partitioning variance in a physiological trait, desiccation resistance in keratin beetles (Coleoptera, Trogidae). *Functional Ecology* 13, 838-844.
- Davis, A.J., Jenkinson, L.S., Lawton, J.H., Shorrocks, B., Wood, S., 1998. Making mistakes when predicting shifts in species range in response to global warming. *Nature* 391, 783-786.
- Davis, M.B., Shaw, R.G., 2001. Range shifts and adaptive responses to Quaternary climate change. *Science* 292, 673-679.
- Edwards, M., Richardson, A.J., 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature* 430, 881-884.
- Erasmus, B.F.N., van Jaarsveld, A.S., Chown, S.L., Kshatriya, M., Wessels, K.J., 2002. Vulnerability of South African animal taxa to climate change. *Global Change Biology* 8, 679-693.
- Fischer, K., Eenhoorn, E., Bot, A.N.M., Brakefield, P.M., Zwaan, B.J., 2003. Cooler butterflies lay larger eggs: developmental plasticity vs acclimation. *Proceedings of the Royal Society of London B* 270, 2051-2056.
- Forde, S.E., Thompson, J.N., Bohannan, B.J.M., 2004. Adaptation varies through space and time in a coevolving host-parasitoid interaction. *Nature* 431, 841-844.
- Gaston, K.J., 2003. *The Structure and Dynamics of Geographic Ranges*. Oxford University Press, Oxford.
- Gaston, K.J., Chown, S.L., 1999. Elevation and climatic tolerance: a test using dung beetles. *Oikos* 86, 584-590.

- Gibbs, A.G., Matzkin, L.M., 2001. Evolution of water balance in the genus *Drosophila*. *Journal of Experimental Biology* 204, 2331-2338.
- Gibbs, A.G., Chippindale, A.K., Rose, M.R., 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology* 200, 1821-1832.
- Gibert, P., Huey, R.B., Gilchrist, G.W., 2001. Locomotor performance of *Drosophila melanogaster*, interactions among developmental and adult temperatures, age, and geography. *Evolution* 55, 205-209.
- Gilchrist, G.W., Huey, R.B., Partridge, L., 1997. Thermal sensitivity of *Drosophila melanogaster*: evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. *Physiological Zoology* 70, 403-414.
- Githeko, A.K., Lindsay, S.W., Confanonieri, U.E., Patz, J.A., 2000. Climate change and vector-borne diseases: a regional analysis. *Bulletin of the World Health Organisation* 78, 1136-1147.
- Gooding, R.H., Feldmann, U., Robinson, A.S., 1997. Care and maintenance of tsetse colonies. In: Crampton, J.M., Beard, C.B., Louis, C. (eds), *The Molecular Biology of Insect Disease Vectors*. Chapman & Hall, London, pp. 41-55.
- Hadley, N.F. 1994. *Water Relations of Terrestrial Arthropods*. Academic Press, San Diego.
- Hargrove, J.W., 2001. The effect of temperature and saturation deficit on mortality in populations of male *Glossina m. morsitans* (Diptera, Glossinidae) in Zimbabwe and Tanzania. *Bulletin of Entomological Research* 91, 79-86.
- Hargrove, J.W., 2004. Tsetse population dynamics. In: Maudlin, I., Holmes, P.H., Miles, M.A. (eds), *The Trypanosomiases*. CABI Publishing, Wallingford, pp. 113-138.
- Hay, S.I., Rogers, D.J., Randolph, S.E., Stern, D.I., Cox, J., Shanks, G.D. et al., 2002a. Hot topic or hot air? Climate change and malaria resurgence in east African highlands. *Trends in Parasitology* 18, 530-534.
- Hay, S.I., Cox, J., Rogers, D.J., Randolph, S.E., Stern, D.I., Shanks, G.D. et al., 2002b. Climate change, regional warming and malaria resurgence. *Nature* 420, 628.
- Helmuth, B., Kingsolver, J.G., Carrington, E., 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? *Annual Review of Physiology* 67, 177-201.
- Hoffmann, A.A., Blows, M.W., 1994. Species borders, ecological and evolutionary perspectives. *Trends in Ecology and Evolution* 9, 223-227.
- Hoffmann, A.A., Dagher, H., Hercus, M., Berrigan, D., 1997. Comparing different measures of heat resistance in selected lines of *Drosophila melanogaster*. *Journal of Insect Physiology* 43, 393-405.

- Hoffmann, A.A., Hallas, R., Sinclair, C., Mitrovski, P., 2001a. Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions, patterns for desiccation, starvation, cold resistance, and associated traits. *Evolution* 55, 1621-1630.
- Hoffmann, A.A., Hallas, R., Sinclair, C., Partridge, L., 2001b. Rapid loss of stress resistance in *Drosophila melanogaster* under adaptation to laboratory culture. *Evolution* 55, 436-438.
- Hoffmann, A.A., Hallas, R.J., Dean, J.A., Schiffer, M., 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science* 301, 100-102.
- Hoffmann, A.A., Hallas, R., Anderson, A.R., Telonis-Scott, M., 2005a. Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. *Journal of Evolutionary Biology* 18, 804-810.
- Hoffmann, A.A., Shirriffs, J., Scott, M., 2005b. Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Functional Ecology* 19, 222-227.
- Holt, R.D., Lawton, J.H., Gaston, K.J., Blackburn, T.M., 1997. On the relationship between range size and local abundance: back to the basics. *Oikos* 78, 183-190.
- Hulme, M., 1996. Climate change in Southern Africa: An exploration of some potential impacts and implications in the SADC region. Climatic Research Unit, University of East Anglia, Norwich.
- Huntley, B., Green, R.E., Collingham, Y.C., Hill, J.K., Willis, S.G., Bartlein, P.J. et al., 2004. The performance of models relating species geographical distributions to climate is independent of trophic level. *Ecology Letters* 7, 417-426.
- Jeffree, C.E., Jeffree, E.P., 1996. Redistribution of the potential geographical ranges of Mistletoe and Colorado Beetle in Europe in response to the temperature component of climate change. *Functional Ecology* 10, 562-577.
- Kimura, M.T., 2004. Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia* 140, 442-449.
- Kingsolver, J.G., Huey, R.B., 1998. Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist* 38, 545-560.
- Kirkpatrick, M., Barton, N.H., 1997. Evolution of a species' range. *American Naturalist* 150, 1-23.
- Klok, C.J., Chown, S.L., 2003. Resistance to temperature extremes in sub-Antarctic weevils, interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* 78, 401-414.
- Klok, C.J., Sinclair, B.J., Chown, S.L., 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *Journal of Experimental Biology* 207, 2361-2370.

- Kovats, R.S., Campbell-Lendrum, D.H., McMichael, A.J., Woodward, A., Cox, J. St. H., 2001. Early effects of climate change: do they include changes in vector-borne disease? *Philosophical Transactions of the Royal Society of London B* 356, 1057-1068.
- Krafsur, E.S., 2002. Population structure of the tsetse fly *Glossina pallidipes* estimated by allozyme, microsatellite and mitochondrial gene diversities. *Insect Molecular Biology* 11, 37-45.
- Krafsur, E.S., 2003. Tsetse fly population genetics: an indirect approach to dispersal. *Trends in Parasitology* 19, 162-166.
- Krafsur, E.S., Endsley, M.A., 2002. Microsatellite diversities and gene flow in the tsetse fly, *Glossina morsitans s.l.* *Medical and Veterinary Entomology* 16, 292-300
- Krafsur, E.S., Wohlford, D.L. 1999. Breeding structure of *Glossina pallidipes* populations evaluated by mitochondrial variation. *Journal of Heredity* 90, 635-642.
- Krafsur, E.S., Endsley, M.A., Wohlford, D.L., Griffiths, N.T., Allsopp, R., 2001. Genetic differentiation of *Glossina morsitans centralis* populations. *Insect Molecular Biology* 10, 387-395.
- Langley, P.A., 1967. Control of digestion in the tsetse fly, *G. morsitans*: a comparison between field flies and flies reared in captivity. *Journal of Insect Physiology* 13, 477-486.
- Langley, P.A., 1977. Physiology of tsetse flies (*Glossina* spp.)(Diptera, Glossinidae): a review. *Bulletin of Entomological Research* 67, 523-574.
- Langley, P.A., Maudlin, I., Leedham, M.P., 1984. Genetic and behavioural differences between *Glossina pallidipes* from Uganda and Zimbabwe. *Entomologia Experimentalis et Applicata* 35, 55-60.
- Leak, S.G.A., 1999. *Tsetse Biology and Ecology*. CABI Publishing, Wallingford.
- Loeschcke, V., Sorensen, J.G., Kristensen, T.N., 2004. Ecologically relevant stress resistance: from microarrays and quantitative trait loci to candidate genes - A research plan and preliminary results using *Drosophila* as a model organism and climatic and genetic stress as model stresses. *Journal of Bioscience* 29, 503-511.
- Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: history and critique. *Canadian Journal of Zoology* 75, 1561-1574.
- Naidu, S.G., Hattingh, J., 1988. Water balance and osmoregulation in *Physadesmia globosa*, a diurnal tenebrionid beetle from the Namib Desert. *Journal of Insect Physiology* 34, 911-917.
- Opdam, P., Wascher, D., 2004. Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. *Biological Conservation* 117, 285-297.
- Ouma, J.O., Marquez, J.G., Krafsur, E.S., 2005. Macrogeographic population structure of the tsetse fly, *Glossina pallidipes* (Diptera, Glossinidae). *Bulletin of Entomological Research* 95, 437-447.

- Parkash, R., Tyagi, P., Sharma, I., Rajpurohit, S., 2005. Adaptations to environmental stress in altitudinal populations of two *Drosophila* species. *Physiological Entomology* doi: 10.1111/j.1365-3032.2005.00470.x.
- Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37-42.
- Patz, J.A., Graczyk, T.K., Geller, N., Vittor, A.Y., 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* 30, 1-11.
- Patz, J.A., Hulme, M., Rosenzweig, C., Mitchell, T.D., Goldberg, R.A., Githeko, A.K. et al., 2002. Regional warming and malaria resurgence. *Nature* 420, 627-628.
- Pearson, R.G., Dawson, T.P., 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography* 12, 361-371.
- Phelps, R.J., Burrows, P.M., 1969. Prediction of the puparium duration of *Glossina morsitans orientalis* Vanderplank under field conditions. *Journal of Applied Ecology* 6, 323-337.
- Randolph, S.E., Rogers, D.J., 2003. Fragile transmission cycles of tick-borne encephalitis virus may be disrupted by predicted climate change. *Proceedings of the Royal Society of London B* 267, 1741-1744.
- Randolph, S.E., Rogers, D.J., Kiilu, J., 1990. Rapid changes in the reproductive cycle of wild-caught tsetse, *Glossina pallidipes* Austen, when brought into the laboratory. *Insect Science and its Applications* 11, 347-354.
- Robinson, T., Rogers, D., Williams, B., 1997a. Univariate analysis of tsetse habitat in the common fly belt of Southern Africa using climate and remotely sensed vegetation data. *Medical and Veterinary Entomology* 11, 223-234.
- Robinson, T., Rogers, D., Williams, B., 1997b. Mapping tsetse habitat suitability in the common fly belt of Southern Africa using multivariate analysis of climate and remotely sensed vegetation data. *Medical and Veterinary Entomology* 11, 235-245.
- Rogers, D.J., 1990. A general model for tsetse populations. *Insect Science and its Applications* 11, 331-346.
- Rogers, D.J., 2000. Satellites, space, time, and the African trypanosomiasis. *Advances in Parasitology* 47, 129-171.
- Rogers, D.J., Randolph, S.E., 1986. Distribution and abundance of tsetse flies (*Glossina* spp.). *Journal of Animal Ecology* 55, 1007-1025.
- Rogers, D.J., Randolph, S.E., 1991. Mortality rates and population density of tsetse flies correlated with satellite imagery. *Nature* 351, 739-741.

- Rogers, D.J., Williams, B.G., 1994. Tsetse distribution in Africa: seeing the wood *and* the trees. In: Edwards, P.J., May, R.M., Webb, N.R. (eds), Large-scale Ecology and Conservation Biology. Blackwell Science, Oxford, pp. 247-271.
- Rogers, D.J., Randolph, S.E., 2000. The global spread of malaria in a future, warmer world. *Science* 289, 1763-1766.
- Rogers, D.J., Randolph, S.E., 2002. A response to the aim of eradicating tsetse from Africa. *Trends in Parasitology* 18, 534-536.
- Rogers, D.J., Robinson, T.P., 2004. Tsetse distribution. In: Maudlin, I., Holmes, P.H., Miles, M.A. (eds), The Trypanosomiases. CABI Publishing, Wallingford, pp. 139-179.
- Rogers, D.J., Randolph, S.E., Snow, R.W., Hay, S.I., 2002. Satellite imagery in the study and forecast of malaria. *Nature* 415, 710-715.
- Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C., Pounds, J.A., 2003. Fingerprints of global warming on wild animals and plants. *Nature* 421, 57-60.
- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R. et al., 2000. Global biodiversity scenarios for the year 2100. *Science* 287, 1770-1774.
- Samways, M.J., Osborne, R., Hastings, H., Hattingh, V., 1999. Global climate change and accuracy of prediction of species geographical ranges: establishment success of introduced ladybirds (*Coccinellidae*, *Chilocorus* spp.) worldwide. *Journal of Biogeography* 26, 795-812.
- Sgrò, C.M., Blows, M.W., 2004. The genetic covariance among clinal environments after adaptation to an environmental gradient in *Drosophila serrata*. *Genetics* 167, 1281-1291.
- Sgrò, C.M., Partridge, L., 2000. Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *American Naturalist* 156, 341-353.
- Sombroek, W.G., Braun, H.M.H., van der Pouw, B.J.A., 1980. Exploratory soil map and agro-climatic zone map of Kenya. Kenya Soil Survey, Ministry of Agriculture, National Agricultural Laboratories, Nairobi.
- Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. *Science* 301, 65.
- Tatem, A.J., Bayliss, M., Mellor, P.S., Purse, B.V., Capela, R., Pena, I. et al., 2003. Prediction of bluetongue vector distribution in Europe and North Africa using satellite imagery. *Veterinary Microbiology* 97, 13-29.
- Tauber, M.J., Tauber, C.A., Nyrop, J.P., Villani, M.G., 1998. Moisture, a vital but neglected factor in the seasonal ecology of insects, hypotheses and tests of mechanisms. *Environmental Entomology* 27, 523-530.

- Terblanche, J.S., Klok, C.J., Chown, S.L., 2004a. Metabolic rate variation in *Glossina pallidipes* (Diptera, Glossinidae): gender, ageing and repeatability. *Journal of Insect Physiology* 50, 419-428.
- Terblanche, J.S., Klok, C.J., Marais, E., Chown, S.L., 2004b. Metabolic rate in the whip-spider, *Damon annulatipes* (Arachnida, Amblypygi). *Journal of Insect Physiology* 50, 637-645.
- Terblanche, J.S., Sinclair, B.J., Klok, C.J., McFarlane, M.L., Chown, S.L., 2005. The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalconota* (Coleoptera, Chrysomelidae). *Journal of Insect Physiology* 51, 1013-1023.
- Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., et al., 2004. Extinction risk from climate change. *Nature* 427, 145-148.
- Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C. et al., 2002. Ecological responses to recent climate change. *Nature* 416, 389-395.
- Watson, R.T., 2002. *Climate Change 2001: Synthesis Report*. Cambridge University Press, Cambridge.
- Watson, R.T., Zinyowera, M.C., Moss, R.H., Dokken, D.J., 1998. *The Regional Impacts of Climate Change. An Assessment of Vulnerability*. Cambridge University Press, Cambridge.
- Williams, B., Dransfield, R., Brightwell, R., 1992. The control of tsetse flies in relation to fly movement and trapping efficiency. *Journal of Applied Ecology* 29, 163-179.
- Wilson, R.S., Franklin, C.E., 2002. Testing the beneficial acclimation hypothesis. *Trends in Ecology and Evolution* 17, 66-70.
- Wohlford, D.L., Krafur, E.S., Griffiths, N.T., Marquez, J.G., Baker, M.D., 1999. Genetic differentiation of some *Glossina morsitans morsitans* populations. *Medical and Veterinary Entomology* 13, 377-385.
- WHO, 1998. *Control and Surveillance of African Trypanosomiasis. Report of a WHO Expert Committee*. Geneva, World Health Organization Technical Report Series, 1-114.
- WHO, 2004. *Using Climate to Predict Disease Outbreaks*. World Health Organization, Geneva.
- Zeilstra, I., Fischer, K., 2005. Cold tolerance in relation to developmental and adult temperature in a butterfly. *Physiological Entomology* 30, 92-95.



## Chapter 7

### **The relative contributions of developmental plasticity and adult phenotypic flexibility to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera, Glossinidae)\***

*"It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change."*

**Charles Darwin**

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## Introduction

Recently, interest in the nature and magnitude of non-genetic effects on phenotypic variation has increased. Phenotypic plasticity might not only account for much of the variation among populations (Ayrinhac et al., 2004, Hoffmann et al., 2005), but it can also significantly affect the nature of evolutionary responses of populations (Price et al., 2003), and their likely survival especially in the face of rapidly changing modern climates (Helmuth et al., 2005; Somero, 2005). Physiologists have long assumed that acclimation (or acclimatization, both are forms of plasticity) to a particular environment enhances performance in that environment (e.g. Prosser, 1986; Hochachka & Somero, 2002) such that acclimation is beneficial (see Leroi et al., 1994). However, in recent years the generality of this beneficial acclimation hypothesis (BAH) has been questioned because most formal tests of the predictions thereof have been negative (Leroi et al., 1994; Zamudio et al., 1995; Sibly et al., 1997; Woods, 1999; Gibert et al., 2001; Gilchrist & Huey, 2001; Woods & Harrison, 2001; but see Nunney & Cheung, 1997; Thomson et al., 2001). Subsequently, Wilson & Franklin (2002) argued that many of these tests have actually addressed the adaptive significance of developmental plasticity rather than of acclimation. That is, the tests have examined the consequences for the adult phenotype of altered rearing environments, rather than the consequences of environmental variation within a given life-stage, and more specifically the adults. Therefore, they should not be considered tests of the beneficial acclimation hypothesis because acclimation is typically defined as a reversible, facultative response to changes in the adult environment (Spicer & Gaston, 1999; Wilson & Franklin, 2002). Given the wide range of conditions under which adaptive phenotypic plasticity is thought to arise (Scheiner, 1993; Berrigan & Scheiner, 2004), these alternative perspectives on beneficial acclimation have proven controversial.

One outcome of the debate is the realization that few studies have sought to examine the effects of acclimation in the rearing stage (developmental plasticity) relative to those in the adult or within a given life-stage (phenotypic flexibility, see Piersma & Drent, 2003), and the nature of the interactions between them. The most detailed work to date has been that of Fischer et al. (2003) and Zeilstra & Fischer (2005). In the former study, rearing temperature had a substantial effect on the size of eggs laid by *Bicyclus anynana* butterflies. However, these effects were largely reversible after adults were held at different temperatures. Thus, the magnitude of the developmental plasticity and phenotypic flexibility effects was similar. Zeilstra & Fischer (2005) used *Lycaena tityrus* and again found that the effects of developmental plasticity on recovery time from cold shock were largely reversible. They also found

that as long as newly eclosed adults were not exposed to cold shock, adult temperatures (i.e. an adult and a rearing exposure to different temperatures) also affected recovery time, though these times tended to be shorter than those for butterflies exposed to cold shock immediately after eclosion (i.e. a rearing exposure only). Few other studies of this nature have been undertaken (though see Spicer & Gaston, 1999 for discussion of early work; see also Tracy & Walsberg, 2001), and therefore their generality is not certain.

In this study I therefore examine the effects of altering treatment temperatures during pupal development (developmental plasticity), during the adult stage (phenotypic flexibility) and during both stages (combined plasticity) on four traits, *viz.* critical thermal minimum and maximum, water loss rate, metabolic rate in the tsetse fly *Glossina pallidipes* Austen. This species was chosen for several reasons. First, variation in the traits of interest across the Insecta is typically partitioned at family and order levels (Chown et al., 2002). Therefore, investigations of non-lepidopteran species are likely to provide a rapid way of determining how general previous findings are likely to be. Second, temperature and water availability are important correlates of the distribution of *G. pallidipes* and play major roles in influencing its population dynamics directly (Hargrove, 2004; Rogers & Robinson, 2004) and indirectly via metabolic rate (Bursell et al., 1974; Bursell & Taylor, 1980; Terblanche et al., 2004). Investigating the mechanistic bases of the responses of flies to their external environment is therefore a significant link in the causal chain of reasoning from environment to population dynamics. Finally, as a vector of animal disease, *G. pallidipes* has important effects on animal health, and thus indirect effects upon socio-economic development in Africa (Maudlin et al., 2004). Understanding the likely current and future determinants of its abundance and distribution is therefore of considerable significance for ongoing development of the continent (Maudlin et al. 2004).

## **Materials and methods**

### *Laboratory conditions and maintenance*

Twelve to thirteen-day old *Glossina pallidipes* Austen (Diptera, Glossinidae) puparia (hereafter pupae) were received from a laboratory colony maintained at the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, International Atomic Energy Agency, Vienna, Austria. It is unlikely that these experimental flies exhibited inbreeding depression or other deleterious genetic effects of colonization because gene diversities over two mitochondrial loci were within the range of 18 field populations from Ethiopia, East and southern Africa (Krafsur & Wohlford, 1999). It is

these pupae that were subjected to an acclimation treatment, or simply maintained close to the original conditions in which they were held at the Seibersdorf laboratory in the case of controls.

In all cases, on arrival, pupae were immediately placed in Petri dishes ( $n = 50$  per dish) on paper towels which were stored inside two plastic containers with non-airtight lids and transferred to a climate chamber held at  $25\text{ }^{\circ}\text{C}$  (mean  $\pm$  SD:  $24.8 \pm 1.0$ ). Relative humidity (R.H.) inside containers was regulated by means of saturated salt (NaCl) solutions located within each container to give 76 % R.H. (Winston & Bates, 1960). At the first sign of eclosion, pupae were transferred to 10-12 mesh cages (10 cm diameter,  $n < 50$  per cage) and either retained at the original temperature or moved later to a different temperature for an adult acclimation treatment (details below). Cages were stored inside closed, non-airtight plastic containers with relative humidity regulated as above.

The adults were fed using a membrane-tray system (see Gooding et al., 1997) every alternative day (similar to the methods described in Terblanche et al., 2004), and subsequently container locations were randomized within climate chambers. Care was taken to ensure that all treatment groups were handled for the same duration during transfer from the climate chamber to the feeding area, and spent a similar amount of time outside of the climate chambers whilst feeding ( $\sim 25$  min per group). Temperatures during shipment and acclimation were recorded using Thermocron iButtons (Dallas Semiconductors, Dallas, Texas, USA; sampling rate = 15 min; temperature ( $^{\circ}\text{C}$ )  $\pm$  SD during shipment:  $24.2 \pm 2.9$ ).

#### *Acclimation treatments*

Pupae received a six day acclimation in climate chambers set to  $21\text{ }^{\circ}\text{C}$  (mean  $\pm$  SD:  $20.5 \pm 1.0$ ), and  $29\text{ }^{\circ}\text{C}$  ( $28.0 \pm 0.2$ ) starting approximately 12-13 days prior to the expected date of emergence (i.e. just over halfway through the pupal stage). Pupae were also kept at  $25\text{ }^{\circ}\text{C}$ , though handled in the same way as the other groups, and these individuals served as controls (see Sinclair & Chown, 2005) for rationale). After six days of acclimation, all groups were returned to  $25\text{ }^{\circ}\text{C}$  for emergence. This treatment does not constitute a full rearing exposure (as in Fischer et al., 2003), but nonetheless has a marked effect on the pupae (as confirmed in pilot trials). Moreover, it was undertaken because the timecourse of  $CT_{\text{Min}}$  has been examined in adult *G. pallidipes* previously which revealed that a maximum response is typically obtained within five days of exposure, and does not reflect a graded (temperature-dependent) response, but rather demonstrates a distinct temperature threshold (see Terblanche et al., submitted ms). Eclosion commenced in the warmest acclimation group *c.* 7-8 days

before the coolest acclimation group. Thus, depending on the experimental temperature in each pupal acclimation group, a total of 12-19 days passed between the end of the acclimation period and the onset of the experimental assessments. For *G. pallidipes*, ten days at 25 °C is sufficient to completely reverse adult acclimation responses in critical thermal minima after nine days at 19-21 °C (i.e. adult acclimation responses for this trait were reversible) (Terblanche et al., submitted ms). After 30-40% of the flies in an experimental group had begun to eclose, flies were taken through three blood meals, or 'hunger cycles' (~ six days) and used for experimental assessment on approximately the eighth day of the adult stage in a fasted, post-developmental (non-teneral) state. This group was considered the 'developmental plasticity' treatment.

On the day following the experimental assessment, the remaining flies were fed as usual and were then transferred back to the same acclimation treatment received during the pupal stage (e.g. flies which were exposed to 21 °C during the pupal stage were returned to 21 °C) and left to acclimate for a further six days whereupon the same physiological measures were assessed. Preliminary experiments using  $CT_{Min}$  in adult *G. pallidipes* showed that acclimation responses can be fully induced after six days and  $CT_{Min}$  does not change further with longer duration of acclimation (up to 12 days) (Terblanche et al., submitted ms). These flies were labelled the 'combined plasticity' treatment.

In a second experiment, pupae were left at 25 °C ( $24.8 \pm 1.0$ ), and only adult flies were switched to climate chambers set to 21 and 29 °C. The flies were switched to these cabinets on day six after three hunger cycles (as above), and left at these temperatures for six days. Once again, a set of flies held at 25 °C served as the control group. Thereafter, c. 6-7 days elapsed and the traits were then assessed. These flies were regarded the 'adult plasticity' treatment.

### *Experimental assessments*

All experimental assessments were performed on adult flies. In each experiment, care was taken to randomly select flies from as many cages as possible. In cases where the number of flies required was higher than the number of available cages, fly selection was balanced among cages. In addition, all acclimation groups were handled similarly (duration and vigour). Such treatment, in conjunction with frequently randomized cage locations, probably prevented any significant cage by treatment interactions.

### *Critical thermal limits*

An insulated system with eleven double-jacketed isolation chambers was connected to a programmable water bath (LTD 20 with PZ1 programmer, Grant Instruments, Cambridge, UK) which regulated water flow around the chambers. A single fly was placed in each of the ten chambers. A 40 SWG type-T thermocouple was inserted into a control chamber to measure chamber temperatures. The flies were allowed to equilibrate for ten minutes at either 12 or 35 °C before the commencement of the respective minimum and maximum critical thermal limit assessments. Because of their small body mass, the body temperature of flies was considered equivalent to chamber temperature. Moreover, Edney & Barrass (1962) have demonstrated that there is no significant difference between body temperature and ambient temperature across the range of 25-45 °C under moist humidity conditions in *G. morsitans*. After equilibration, the chambers' temperature was decreased or increased at 0.25 °C.min<sup>-1</sup>. Critical thermal minima (CT<sub>Min</sub>) were defined as the loss of coordinated muscle function at decreasing temperatures and critical thermal maxima (CT<sub>Max</sub>) were defined as the onset of muscle spasms at increasing temperatures (Klok & Chown, 1998). Groups of ten flies were assessed at once and the temperature at which these limits were observed was recorded for each individual fly. Preliminary experiments using adult flies found no effect of gender, age or feeding status on critical thermal limits (unpublished data, C.J. Klok and J.S. Terblanche). All observations were performed by a single investigator who was typically unaware of the experimental treatment group.

### *Desiccation rate*

To remove possible confounding effects of sex or pregnancy, only male flies were used in desiccation experiments. Flies (n = 16), individually contained in 5 ml cuvettes, were subjected to desiccation in flowing air (< 2.5 % relative humidity) for ten hours at 25.0 (± 1.0) °C in a climate chamber (Labotech, South Africa). Air flow, produced by an aquarium pump, was directed through a scrubbing column containing silica gel and Drierite as desiccants to remove residual water, and then into a mass flow-controller (MFC) to control the air flow rate. The MFC outlet was connected to a Sable Systems (Las Vegas, NV, USA) MF8 airflow manifold. Each outflow channel of the manifold was further split in two so that two cuvettes, each containing a fly, were attached per manifold channel. The air flow rate through each cuvette, tested with a second MFC, was regulated to 100 ml.min<sup>-1</sup>. Experiments took place during the night (21h00-07h00) because this represents a period of minimal activity in tsetse (Brady, 1988; Kyorku & Brady, 1993). Mass was recorded before and after an

experiment on an electronic microbalance (0.1 mg, Avery Berkel FA 304T, EU) and the difference was assumed to be a result of water loss (acknowledging that some mass loss is due to substrate catabolism but that this is negligible relative to the quantity of water loss over the time scales investigated here (Bursell, 1957)). Flies were dried to constant mass (~50-60 °C for ~72 hrs) and re-weighed to give dry body mass.

### *Metabolic rate*

Metabolic rate was recorded using flow-through respirometry. A calibrated LI-6262 (LiCor; Lincoln, Nebraska) infra-red gas analyzer (IRGA) was connected to a Sable Systems eight channel multiplexer inside a temperature-controlled cabinet (details given in Terblanche et al., 2004). The first seven channels regulated the flow-through respirometry for individual flies and channel eight was used as an empty reference channel for CO<sub>2</sub> and H<sub>2</sub>O baseline measurements. These recordings were performed for fasted, post-development males at 25 °C in each acclimation group. Mass was recorded before and after respirometry recordings as described in the desiccation experiments. Airflow was regulated to 100 ml.min<sup>-1</sup> using a MFC and the outside air was scrubbed through sodalime, silica-gel and Drierite columns to remove water and CO<sub>2</sub>. A Sable Systems AD-1 activity detector was connected to the first cuvette only to compare active and resting gas exchange traces in all flies. Previous studies have shown that in this species activity can be reliably detected from the V̇CO<sub>2</sub> trace without an activity detection system (Terblanche et al. 2004), which was confirmed here. Sable Systems Datacan V software was used to extract and analyze standard (resting) metabolic rate (SMR) data.

### *Statistical analyses*

For all traits, the effect of the treatment (21 or 29 °C) relative to the control (25 °C) was assessed. Significance of these effects was determined by comparing the original trait values between each treatment and the control for each of the different plasticity types. One way analyses of variance were used to compare treatments and the control in the case of CT<sub>Min</sub> and CT<sub>Max</sub>. Because body mass influences both metabolic rate and water loss rate (Addo-Bediako et al., 2001; 2002) analyses of covariance were used for these traits. Two further assessments of the relative contributions of plasticity type and treatment temperature to trait variation were made using nested analyses of variance. In the first analysis, treatment temperature was nested within plasticity type using data for developmental plasticity and phenotypic flexibility only. In the second analysis, all three plasticity types were

assessed, nested within treatment temperature. These two nested analyses of variance present complimentary perspectives on the relative importance of plasticity type and treatment temperature for variation in each of the traits.

## Results

Differences in  $CT_{Max}$  associated with treatment temperature were small irrespective of whether this treatment took place in the pupal or adult stages, and significant in one case of phenotypic flexibility only (Fig. 1). Thus, much of the variance in  $CT_{Max}$  was explained by plasticity type (Table 1). The combined plasticity treatment tended to be most similar to the pupal treatment, and treatment temperature effects were small, resulting in plasticity type retaining its importance in the nested ANOVA including all three plasticity types (Table 2). Differences in  $CT_{Min}$  among treatment temperatures were much more pronounced (Fig. 2). The effect was largest in the case of phenotypic flexibility and combined plasticity for the 21 °C treatment, but in the 29 °C treatment developmental plasticity (and combined plasticity) had the largest effects. Perhaps more significantly, the effects of the two treatment temperatures were opposite in sign. Thus, in both nested ANOVAs treatment temperature accounted for most of the variance (Tables 1 and 2).

Surprisingly little of the variance in desiccation rate could be explained by plasticity type when only developmental plasticity and phenotypic flexibility were considered (Table 1). Treatment temperature in the pupal stage had little effect on water loss rate, and this was true for phenotypic flexibility too, with the exception of a strong decline following adult acclimation at a treatment temperature of 29 °C (Fig. 3). The combined plasticity treatment at 29 °C also resulted in a substantial increase in water loss rate. In consequence, plasticity type explained a larger proportion of the variance when all three plasticity types were included in the nested ANOVA (Table 2).

When only developmental plasticity and phenotypic flexibility were considered, the only significant effect was a response of adult metabolic rate to the 21 °C treatment (Fig. 4). In consequence, treatment temperature explained most of the variance (Table 1) among treatment temperature and plasticity type. This increase in metabolic rate following the 21 °C treatment was also found when both adults and pupae were exposed to acclimation, resulting in a more even partitioning of variance between plasticity type and treatment temperature (Table 2).

**Table 1.** Summary of nested (hierarchical) analyses of variance (ANOVA, Type I) testing for an effect of the plasticity type (developmental vs. adult; Effect: *Plasticity*) and treatment temperature (21, 29 °C; Effect: *Acclimation*) on physiological traits of climatic stress resistance in adult *Glossina pallidipes* (Diptera, Glossinidae). *Plasticity* and *Acclimation* were categorised as random factors. MS = mean squares, DF = degrees of freedom.

Physiological trait	Effect	DF	MS	DF error	MS error	F-ratio	<i>P</i>	% variance explained
Critical Thermal Maxima								
(°C)	<i>Plasticity</i>	1	7.117	2.19	0.322	22.100	< 0.05	66.8
	<i>Acclimation</i>	2	0.352	55.00	0.115	3.048	0.056	4.1
	<i>Error</i>							29.2
Critical Thermal Minima								
(°C)	<i>Plasticity</i>	1	2.408	2.00	35.975	0.067	0.820	0
	<i>Acclimation</i>	2	40.442	56.00	0.240	168.406	< 0.0001	91.8
	<i>Error</i>							8.2
Desiccation Rate <sup>1</sup>								
(mg H <sub>2</sub> O/hr)	<i>Plasticity</i>	1	0.146	2.33	0.080	1.825	0.293	15.5
	<i>Acclimation</i>	2	0.119	43.00	0.016	7.624	< 0.005	30.1
	<i>Error</i>							54.4
Standard Metabolic Rate <sup>2</sup>								
(log <sub>10</sub> µl CO <sub>2</sub> /hr)	<i>Plasticity</i>	1	0.066	2.08	0.087	0.757	0.473	0
	<i>Acclimation</i>	2	0.122	23.00	0.006	21.275	< 0.0001	74.6
	<i>Error</i>							25.4

1 Covarying for dry body mass

2 Covarying for mean experimental body mass

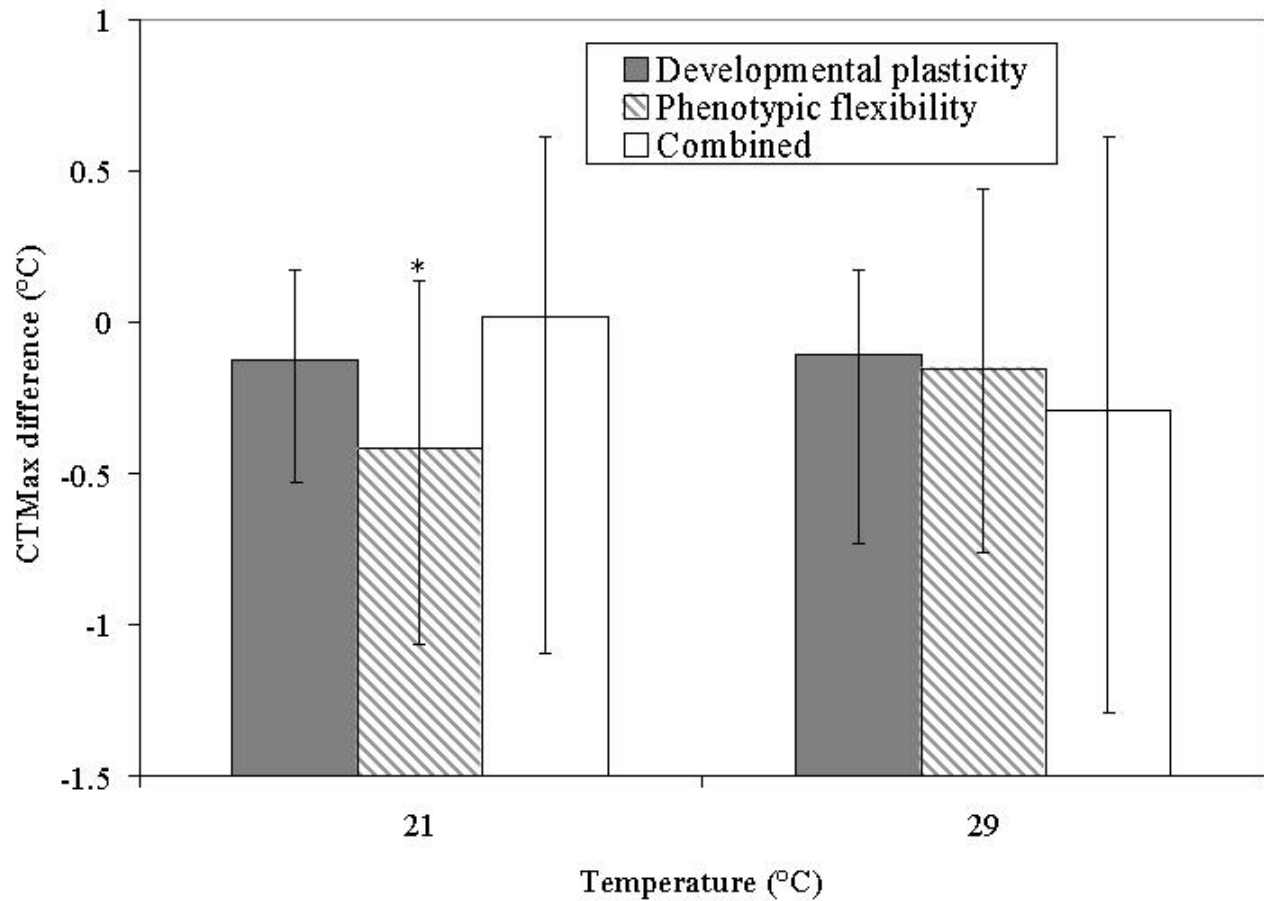


**Table 2.** Summary of nested (hierarchical) analyses of variance (ANOVA, Type I) testing for an effect of treatment temperature (21, 29 °C) (Effect: *Acclimation*) and experimental plasticity treatment (Effect: *Plasticity*) on physiological traits of climatic stress resistance in adult *Glossina pallidipes* (Diptera, Glossinidae). *Acclimation* and *Plasticity* were categorised as random factors. MS = mean squares, DF = degrees of freedom.

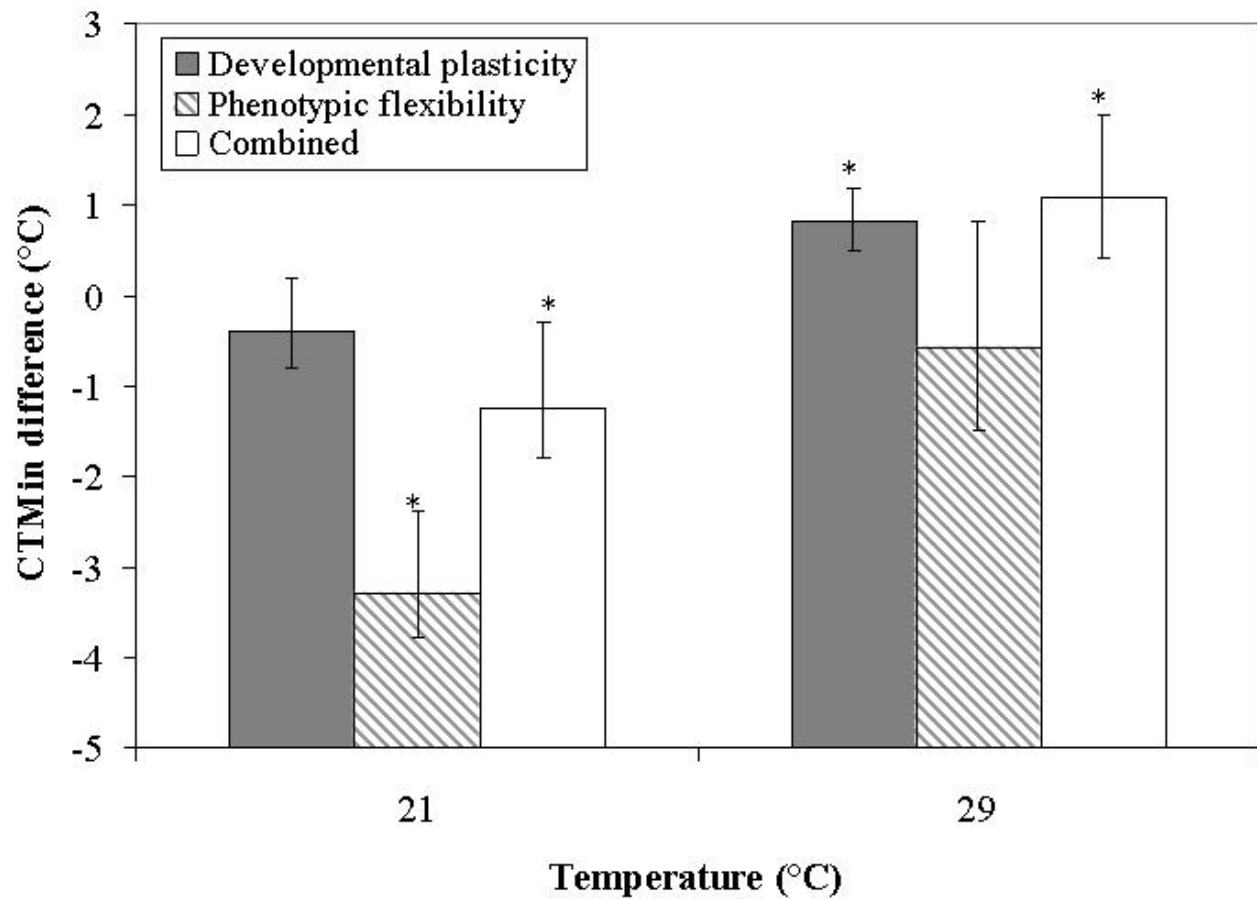
Physiological trait	Effect	DF	MS	DF error	MS error	F-ratio	<i>P</i>	% variance explained
Critical Thermal Maxima								
(°C)	<i>Acclimation</i>	1	0.041	3.89	2.493	0.016	0.904	0
	<i>Plasticity</i>	4	2.077	73.00	0.164	12.665	< 0.0001	48.7
	<i>Error</i>							51.3
Critical Thermal Minima								
(°C)	<i>Acclimation</i>	1	100.352	3.90	3.688	27.213	< 0.01	83.6
	<i>Plasticity</i>	4	3.114	74.00	0.245	12.725	< 0.0001	7.9
	<i>Error</i>							8.5
Desiccation Rate <sup>1</sup>								
(mg H <sub>2</sub> O/hr)	<i>Acclimation</i>	1	0.287	3.89	0.231	1.241	0.329	4.4
	<i>Plasticity</i>	4	0.196	73.00	0.016	12.251	< 0.0001	46.7
	<i>Error</i>							48.9
Standard Metabolic Rate <sup>2</sup>								
(log <sub>10</sub> µl CO <sub>2</sub> /hr)	<i>Acclimation</i>	1	0.211	3.85	0.051	4.137	0.114	36.4
	<i>Plasticity</i>	4	0.046	33.00	0.008	6.045	< 0.001	29.0
	<i>Error</i>							34.6

1 Covarying for dry body mass

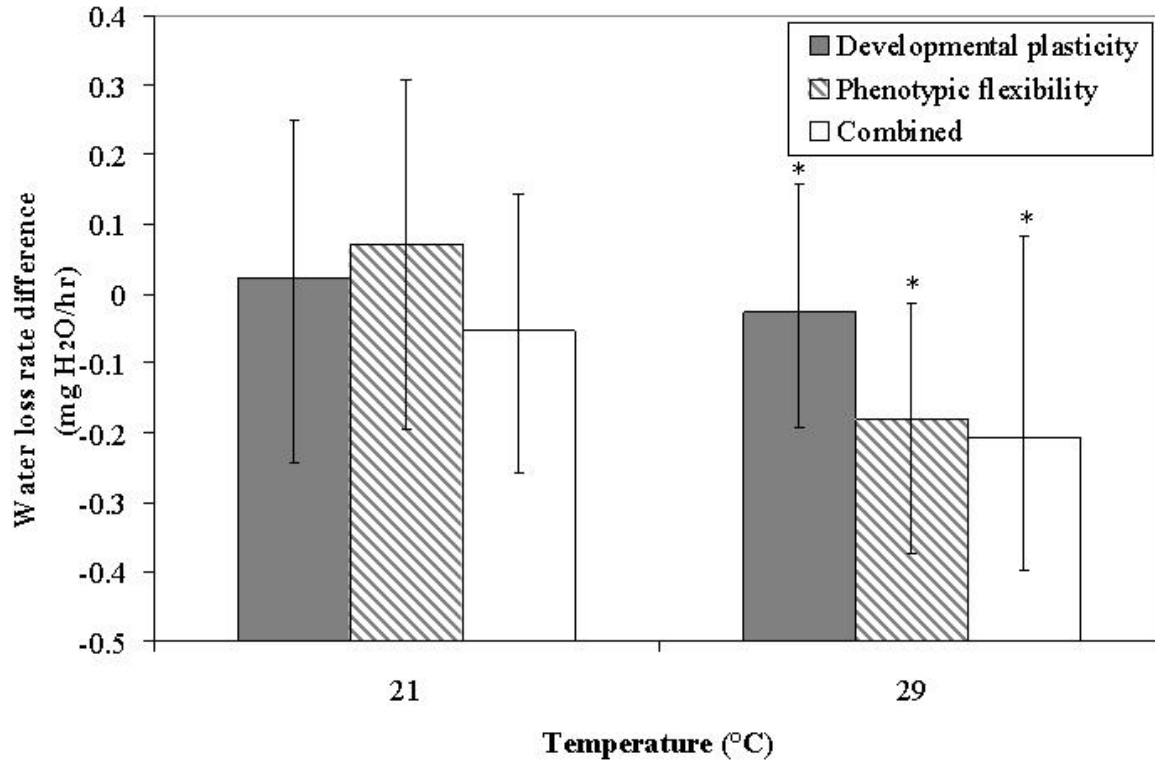
2 Covarying for mean experimental body mass



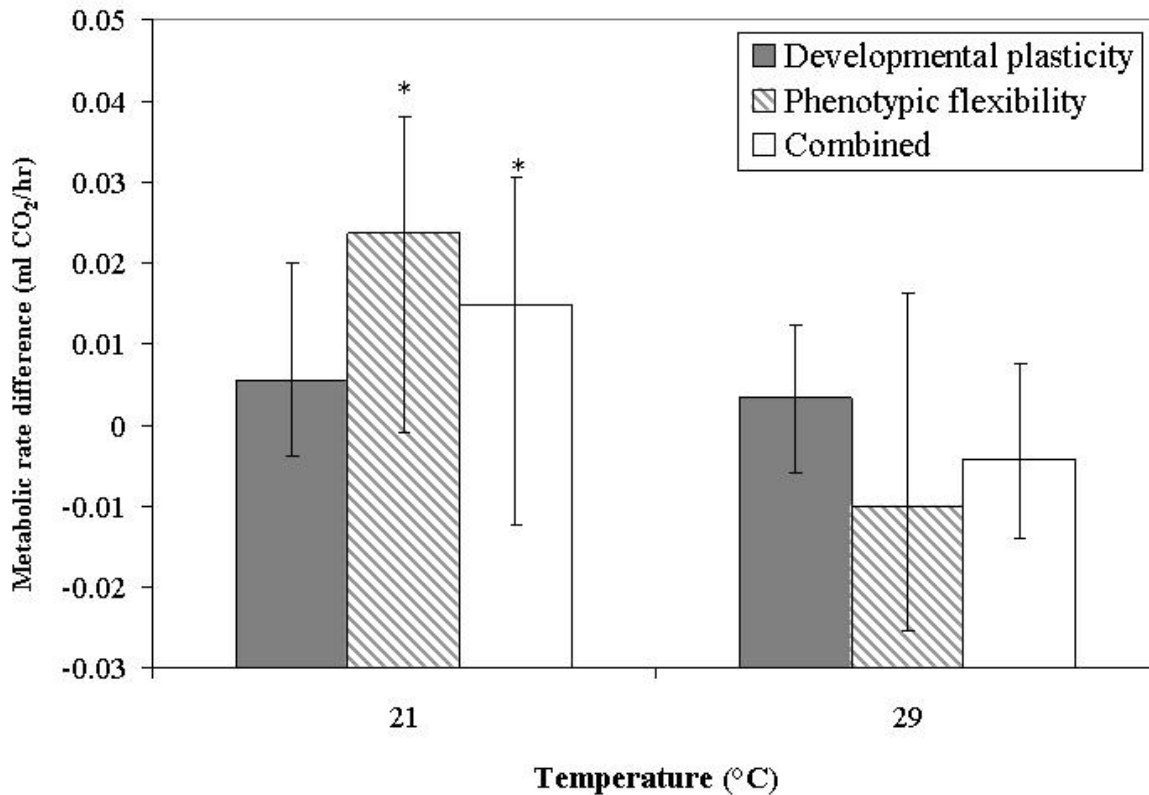
**Figure 1.** Relative change in mean critical thermal maxima for developmental plasticity, adult flexibility and combined plasticity groups within the acclimation temperature treatments expressed as change from the mean of the control (25 °C) group. Asterisks indicate a significant effect of the acclimation relative to the control group by One-way ANOVA ( $P < 0.05$ ). Lower and upper error bars represent minimum and maximum values respectively.



**Figure 2.** Relative change in mean critical thermal minima for developmental plasticity, adult flexibility and combined plasticity groups within the acclimation temperature treatments expressed as change from the mean of the control (25 °C) group. Asterisks indicate a significant effect of the acclimation relative to the control group by One-way ANOVA ( $P < 0.05$ ). Lower and upper error bars represent minimum and maximum values respectively.



**Figure 3.** Relative change in mean water loss rate for developmental plasticity, adult flexibility and combined plasticity groups within the acclimation temperature treatments expressed as change from the mean of the control (25 °C) group. Asterisks indicate a significant effect of the acclimation relative to the control group by GLM (covariate: dry body mass;  $P < 0.05$ ). Lower and upper error bars represent minimum and maximum values respectively.



**Figure 4.** Relative change in mean metabolic rate as indicated by CO<sub>2</sub> production for developmental plasticity, adult flexibility and combined plasticity groups within the acclimation temperature treatments expressed as change from the mean of the control (25 °C) group. Asterisks indicate a significant effect of the acclimation relative to the control group by GLM (covariate: mean experimental body mass;  $P < 0.05$ ). Lower and upper error bars represent minimum and maximum values respectively.

## Discussion

The effects of treatment temperature on the traits investigated in *G. pallidipes* here are largely similar to what has been found in other investigations of insects. In the case of thermal tolerances, acclimation effects were more pronounced for CT<sub>Min</sub> than for CT<sub>Max</sub>, as has been found in several other studies examining acclimation (Klok & Chown, 2003; Slabber & Chown, 2005) or acclimatization (Hoffmann et al., 2005b) effects on thermal limits. Although a more pronounced acclimation effect on lower than on upper limits is not universal (e.g. the converse has been found the caterpillars of the moth

*Embryonopsis halticella*, (Klok & Chown, 1998)), it does seem to be typical of most ectotherms (Kingsolver & Huey, 1998). In insects, greater variation in lower than in upper thermal tolerances is typical not only of acclimation responses, but also of intra- and interspecific geographic variation in thermal limits, and in the responses of these limits to selection (Stanley et al., 1980; Chen et al., 1990; Gilchrist et al., 1997; Hoffmann et al., 1997; Gaston & Chown, 1999; Addo-Bediako et al., 2000; Hercus et al., 2000; Chown, 2001; Kimura, 2004; Terblanche et al., 2005b).

Increases in metabolic rate of the *G. pallidipes* adults with adult exposure to low temperature seems to be typical of this group of tsetse because it has also been found in *Glossina morsitans*. Terblanche et al. (2005a) showed that exposure of adult *G. morsitans* to 29 °C had little effect on metabolic rate relative to adults held at 25 °C, but exposure to 19 °C resulted in a significant increase in metabolic rate across a wide range of test temperatures. Increases of metabolic rate in insects exposed to low temperatures are not uncommon (reviewed in Chown & Gaston, 1999; Addo-Bediako et al., 2002). However, the causes, consequences and likely significance of such whole organismal metabolic upregulation remain controversial and poorly investigated (Chown et al., 2003; Clarke, 2003; Hodkinson, 2003). Why metabolic rate should increase following exposure to a relatively low temperature in tsetse adults is not clear, but might contribute to the absence of this species (and *G. morsitans*) from low temperature areas. Elevated metabolic rates will result in increased use of lipid reserves, lower tolerance of starvation, and increased pressure for foraging, all of which are likely to enhance the chances of mortality (Rajagopal & Bursell, 1966), likely limiting the ability of the flies to survive in low temperature environments (though a lack of pupal development is also important for restricting flies to warmer areas, (for review see Hargrove, 2004; Rogers & Robinson, 2004)).

Perhaps more unusual than the responses of the other traits to acclimation was the change in desiccation rate in response to the temperature treatments. Previous studies have shown strong responses of insect desiccation rate to changes in relative humidity (e.g. Hoffmann, 1990; Gibbs et al., 2003). However, responses of water balance parameters to different temperature regimes are more variable. In some cases treatment temperature has elicited either no response in water balance-related traits (Terblanche et al., 2005b), or a response that is expressed in some traits but not others, such as cuticular hydrocarbon profile changes but no alteration of water loss rates in cactophilic *Drosophila* (Gibbs et al., 1998; see also Cloudsley-Thompson, 1969). In others, pronounced responses in desiccation rate have been found. This is true of *D. melanogaster* from the east coast of Australia, where in several populations in response to summer acclimation temperatures, individuals showed

greater desiccation resistance than flies exposed to constant or winter temperatures (Hoffmann et al., 2005b). Similar declines in desiccation resistance with increasing treatment temperature have also been found for *D. takahashii* and *D. nepalensis* (Parkash et al., 2005). Here, I found that when exposed to 29 °C, either as adults or as pupae, adult *G. pallidipes* had significantly reduced rates of water loss compared to flies held at 21 °C or 25 °C (and similar to results described in Chapter 6). Neither the ultimate, nor proximate causes of this change have been fully investigated. It seems likely that in the former case a reduction in desiccation rate at high temperatures should increase survival given that high temperatures can be associated with low water availability in the habitats occupied by these flies. Indeed, inter-population comparisons of *G. pallidipes* in Kenya have shown that populations from drier areas tend to have the lower water loss rates than those from moister areas (see Chapter 6). How the acclimation or inter-population differences are effected mechanistically has not been well studied, but differences in the amount and composition of cuticular lipids might be significant, as has been found in other species (Gibbs et al., 1991; Rourke, 2000). Preliminary studies in this species have indicated that variation in cuticular hydrocarbon profiles and desiccation rates can occur among populations, however desiccation rates in these flies are not correlated with cuticular lipid mass (i.e. quantity) (R. Jurenka, G. Marquez, J. Odera, J. Terblanche, C. Klok, S. Chown, E. Krafur, unpublished data).

Whilst the extent of the acclimation responses varied among traits, several generalizations can be made about the effects of plasticity type on the traits measured in the adults. Responses to treatment temperature showed an asymmetric effect across all responsive plasticity types, with either the low treatment temperature or the high treatment temperature having a significant effect, but responses to both being found only for  $CT_{Min}$ . In consequence, treatment temperature typically explained less than half of the variance in the measured traits, except in the case of  $CT_{Min}$  where it accounted for more than 80% of the variance. However, the symmetric  $CT_{Min}$  response was found only for the combined plasticity treatment, but in this case appeared to be composed of a response to the 21 °C treatment in the adults, and a response to the 29 °C treatment in the pupae, but not *vice versa*. This finding deserves further exploration. Nonetheless, strong opposing responses to high and low temperature treatments for lower thermal limits have been found in several other studies, though typically these have examined acclimation effects on a single stage only (e.g. Klok & Chown, 2003; Ayrinhac et al., 2004; Hoffmann et al., 2005b; Slabber & Chown, 2005; Terblanche et al., 2005b; Zeilstra & Fischer, 2005). Likewise, asymmetric or threshold effects of treatment temperature have previously been recorded for  $CT_{Max}$

(Klok & Chown, 1998; Hoffmann et al., 2005b; Slabber & Chown, 2005), metabolic rate (Hoffmann, 1985; Terblanche et al., 2005a) and desiccation rate (Rourke, 2000).

By contrast, plasticity type usually accounted for substantial proportions of the variation in the response of the traits to acclimation. Developmental plasticity following the 29 °C temperature treatments was significant and irreversible for  $CT_{Min}$  and desiccation rate, but the pupal treatment was either reversed or had little effect following the 21 °C treatment for these traits and for both temperature treatments in the other traits. The absence of reversibility in  $CT_{Min}$  following exposure to 29 °C is unlike the situation in *Lycaena tityrus*, in which lower thermal limits that change within the pupal stage are typically reversible in the adults (Zeilstra & Fischer, 2005). No other work has examined the effects of developmental plasticity on desiccation rate in insects, but clearly it is significant. Developmental plasticity is known from a wide variety of other traits (e.g. morphological, behavioural, locomotion performance; Atkinson, 1996; Crill et al., 1996; Sheeba et al., 2002; Nijhout, 2003), and generally is assumed to be fixed in the adult stage (e.g. Gibert et al., 2000; and discussed in Wilson & Franklin, 2002). Although early work assumed that such plasticity would be beneficial, most recent studies have shown that this is rarely the case (for discussion see Huey et al., 1999; Wilson & Franklin, 2002). The present design did not enable us to fully explore this hypothesis, though clearly the responses shown by  $CT_{Min}$  and desiccation rate could initially be considered beneficial given that vapour pressure deficit would increase at higher temperatures (for a given quantity of water in the air) (Addo-Bediako et al. 2001), and that a tradeoff between the extent of lower thermal limits and starvation resistance has been found (Hoffmann et al., 2005a).

Phenotypic flexibility (i.e. trait changes following adult acclimation) was most common following low temperature exposures, although for desiccation rate it was only the high temperature exposure that had an effect. Although the time-course of the persistence of changes in physiological tolerances have not yet been investigated, other work on the same species has shown that plasticity in  $CT_{Min}$  is reversible within 10 days (Chapter 6). The generality of these findings for other traits of physiological tolerance in this and other insect species warrants further attention. These findings are in keeping with what has been found in many other studies (reviewed in Spicer & Gaston, 1999; see also Wilson & Franklin, 2002), and confirm the notion that phenotypic flexibility is typically reversible, while developmental plasticity is not (Piersma & Drent, 2003). What was perhaps most significant is that the combined plasticity treatments did not seem to have noticeable additive effects. Rather, the extent of the response following the combined treatments was either quite similar to the pupal or adult



response following exposure, or in the case of the response of critical thermal limits to the low temperatures seemed to have a negative effect on tolerance. What the basis for this negative interaction might be is not clear. However, it is known that basal and induced cold tolerance responses are linked in some species (Chown & Nicolson, 2004).

In conclusion, this study has shown that the stage at which acclimation takes place (i.e. plasticity type) has significant, though often different, effects on several adult traits that are likely to modify environmental effects on populations of *G. pallidipes*. These show that it is not only important to distinguish between developmental plasticity and phenotypic flexibility (Wilson & Franklin, 2002; Piersma & Drent, 2003), but also to consider the direction of the responses and their significance from a life-history perspective (Fischer et al., 2003).

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## References

- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* 267, 739-745.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2001. Revisiting water loss in insects: a large scale view. *Journal of Insect Physiology* 47, 1377-1388.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology* 16, 332-338.
- Atkinson, D., 1996. Ectotherm life-history responses to developmental temperature. In: Johnston, I.A., Bennett, A.F. (eds), *Animals and Temperature. Phenotypic and Evolutionary Adaptation*. Cambridge University Press, Cambridge, pp. 183-204.
- Ayrinhac, A., Debat, V., Gibert, P., Kister, A.-G., Legout, H., Moreteau, B. et al., 2004. Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology* 18, 700-706.

- Berrigan, D., Scheiner, S.M., 2004. Modeling the evolution of phenotypic plasticity. In: De Witt, T.J., Scheiner, S.M. (eds), Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press, New York.
- Brady, J., 1988. Circadian ontogeny in the tsetse fly: A permanent major phase change after the first feed. *Journal of Insect Physiology* 34, 743-749.
- Bursell, E., 1957. Spiracular control of water loss in the tsetse fly. *Proceedings of the Royal Entomological Society of London A* 32, 21-29.
- Bursell, E., Billing, K.C., Hargrove, J.W., McCabe, C.T., Slack, E., 1974. Metabolism of the bloodmeal in tsetse flies. *Acta Tropica* 31, 297-320.
- Bursell, E., Taylor, P., 1980. An energy budget for *Glossina* (Diptera: Glossinidae). *Bulletin of Entomological Research* 70, 187-196.
- Chen, C.-P., Lee Jr, R.E., Denlinger, D.L., 1990. A comparison of the responses of tropical and temperate flies (Diptera: Sarcophagidae) to cold and heat stress. *Journal of Comparative Physiology* 160, 543-547.
- Chown, S.L., 2001. Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* 47, 649-660.
- Chown, S.L., Addo-Bediako, A., Gaston, K.J., 2002. Physiological variation in insects: large-scale patterns and their implications. *Comparative Biochemistry and Physiology B* 131, 587-602.
- Chown, S.L., Addo-Bediako, A., Gaston, K.J., 2003. Physiological diversity: listening to the large-scale signal. *Functional Ecology* 17, 568-572.
- Chown, S.L., Gaston, K.J., 1999. Exploring links between physiology and ecology at macro scales: the role of respiratory metabolism in insects. *Biological Reviews* 74, 87-120.
- Clarke, A., 2003. Costs and consequences of evolutionary temperature adaptation. *Trends in Ecology and Evolution* 18, 573-581.
- Cloudsley-Thompson, J.L., 1969. Acclimation, water and temperature relations of the woodlice *Metoponorthus pruinus* and *Periscyphis jannonei* in the Sudan. *Journal of Zoology* 158, 267-276.
- Crill, W.D., Huey, R.B., Gilchrist, G.W., 1996. Within- and between-generation effects of temperature on the morphology and physiology of *Drosophila melanogaster*. *Evolution* 50, 1205-1218.
- Edney, E.B., Barrass, R., 1962. The body temperature of the tsetse fly, *Glossina morsitans* Westwood (Diptera, Muscidae). *Journal of Insect Physiology* 8, 469-481.

- Gaston, K.J., Chown, S.L., 1999. Elevation and climatic tolerance: a test using dung beetles. *Oikos* 86, 584-590.
- Gibbs, A., Mousseau, T.A., Crowe, J.H., 1991. Genetic and acclimatory variation in biophysical properties of insect cuticle lipids. *Proceedings of the National Academy of Sciences of the USA* 88, 7257-7260.
- Gibbs, A.G., Fukuzato, F., Matzkin, L.M., 2003. Evolution of water conservation mechanisms in *Drosophila*. *Journal of Experimental Biology* 206, 1183-1192.
- Gibbs, A.G., Louie, A.K., Ayala, J.A., 1998. Effects of temperature on cuticular lipids and water balance in a desert *Drosophila*: is thermal acclimation beneficial? *Journal of Experimental Biology* 201, 71-80.
- Gibert, P., Huey, R.B., Gilchrist, G.W., 2001. Locomotor performance of *Drosophila melanogaster*: Interactions among developmental and adult temperatures, age, and geography. *Evolution* 55, 205-209.
- Gibert, P., Moreteau, B., David, J.R., 2000. Developmental constraints on an adaptive plasticity: reaction norms of pigmentation in adult segments of *Drosophila melanogaster*. *Evolution and Development* 2, 249-260.
- Gilchrist, G.W., Huey, R.B., 2001. Parental and developmental temperature effects on the thermal dependence of fitness in *Drosophila melanogaster*. *Evolution* 55, 209-214.
- Gilchrist, G.W., Huey, R.B., Partridge, L., 1997. Thermal sensitivity of *Drosophila melanogaster*: evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. *Physiological Zoology* 70, 403-414.
- Gooding, R.H., Feldmann, U., Robinson, A.S., 1997. Care and maintenance of tsetse colonies. In: Crampton, J.M., Beard, C.B., Louis, C. (eds), *The Molecular Biology of Insect Disease Vectors*. Chapman & Hall, London, pp. 41-55.
- Hargrove, J.W., 2004. Tsetse population dynamics. In: Maudlin, I., Holmes, P.H., Miles, M.A. (eds), *The Trypanosomiases*. CABI Publishing, Wallingford, pp. 113-138.
- Hercus, M.J., Berrigan, D., Blows, M.W., Magiafoglou, A., Hoffmann, A.A., 2000. Resistance to temperature extremes between and within life cycle stages in *Drosophila serrata*, *D. birchii* and their hybrids: intraspecific and interspecific comparisons. *Biological Journal of the Linnean Society* 71, 403-416.

- Hochachka, P.W., Somero, G.N., 2002. Biochemical Adaptation. Mechanism and Process in Physiological Evolution. Oxford University Press, New York.
- Hodkinson, I.D., 2003. Metabolic cold adaptation in arthropods: a smaller-scale perspective. *Functional Ecology* 17, 562-567.
- Hoffmann, A.A., 1990. Acclimation for desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses and genetic variation. *Journal of Insect Physiology* 36, 885-891.
- Hoffmann, A.A., Dagher, H., Hercus, M., Berrigan, D., 1997. Comparing different measures of heat resistance in selected lines of *Drosophila melanogaster*. *Journal of Insect Physiology* 43, 393-405.
- Hoffmann, A.A., Hallas, R., Anderson, A.R., Telonis-Scott, M., 2005a. Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. *Journal of Evolutionary Biology* 18, 804-810.
- Hoffmann, A.A., Shirrifs, J., Scott, M., 2005b. Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Functional Ecology* 19, 222-227.
- Hoffmann, K.H., 1985. Metabolic and enzyme adaptation to temperature. In: Hoffmann, K.H. (ed) *Environmental Physiology and Biochemistry of Insects*. Springer-Verlag, Berlin, pp. 1-32.
- Huey, R.B., Berrigan, D., Gilchrist, G.W., Herron, J.C., 1999. Testing the adaptive significance of acclimation: a strong inference approach. *American Zoologist* 39, 323-336.
- Kimura, M.T., 2004. Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia* 140, 442-449.
- Kingsolver, J.G., Huey, R.B., 1998. Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist* 38, 545-560.
- Klok, C.J., Chown, S.L., 1998. Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-Antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *Journal of Insect Physiology* 44, 615-628.
- Klok, C.J., Chown, S.L., 2003. Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* 78, 401-414.
- Krafsur, E.S., Wohlford, D.L., 1999. Breeding structure of *Glossina pallidipes* populations evaluated by mitochondrial variation. *Journal of Heredity* 90, 635-642.

- Kyorku, C., Brady, J., 1993. A free-running bimodal circadian rhythm in the tsetse fly *Glossina longipennis*. *Journal of Insect Physiology* 40, 63-67.
- Leroi, A.M., Bennett, A.F., Lenski, R.E., 1994. Temperature acclimation and competitive fitness: an experimental test of the Beneficial Acclimation Assumption. *Proceedings of the National Academy of Sciences of the USA* 91, 1917-1921.
- Maudlin, I., Holmes, P.H., Miles, M.A., 2004. *The Trypanosomiases*. CABI Publishing, Wallingford.
- Nijhout, H.F., 2003. Development and evolution of adaptive polyphenisms. *Evolution and Development* 5, 9-18.
- Nunney, L., Cheung, W., 1997. The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: evidence for adaptive plasticity. *Evolution* 51, 1529-1535.
- Parkash, R., Tyagi, P.K., Sharma, I., Rajpurohit, S., 2005. Adaptations to environmental stress in altitudinal populations of two *Drosophila* species. *Physiological Entomology* doi: 10.1111/j.1365-3032.2005.00470.x.
- Piersma, T., Drent, J., 2003. Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution* 18, 228-233.
- Price, T.D., Qvarnström, A., Irwin, D.E., (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London B* 270, 1433-1440.
- Prosser, C.L., 1986. *Adaptational Biology. Molecules to Organisms*. John Wiley & Sons, New York.
- Rajagopal, P.K., Bursell, E., 1966. The respiratory metabolism of resting tsetse flies. *Journal of Insect Physiology* 12, 287-297.
- Rogers, D.J., Robinson, T.P., 2004. Tsetse distribution. In: Maudlin, I., Holmes, P.H., Miles, M.A. (eds), *The Trypanosomiases*. CABI Publishing, Wallingford, pp. 139-179.
- Rourke, B.C., 2000. Geographic and altitudinal variation in water balance and metabolic rate in a California grasshopper, *Melanoplus sanguinipes*. *Journal of Experimental Biology* 203, 2699-2712.
- Scheiner, S.M., 1993. Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* 24, 35-68.
- Sheeba, V., Chandrashekar, M.K., Joshi, A., Sharma, V.K., 2002. Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster*. *Journal of Insect Physiology* 48, 25-32.
- Sibly, R.M., Winokus, L., Smith, R.H., 1997. Interpopulation variation in phenotypic plasticity in the speckled wood butterfly, *Pararge aegeria*. *Oikos* 78, 323-330.

- Sinclair, B.J., Chown, S.L., 2005. Deleterious effects of repeated cold exposure in a freeze-tolerant sub-Antarctic caterpillar. *Journal of Experimental Biology* 208, 869-879.
- Slabber, S., Chown, S.L., 2005. Differential responses of thermal tolerance to acclimation in the sub-Antarctic rove beetle *Halmaeus atriceps*. *Physiological Entomology* 30, 195-204.
- Spicer, J.I., Gaston, K.J., 1999. *Physiological Diversity and its Ecological Implications*. Blackwell Science, Oxford.
- Stanley, S.M., Parsons, P.A., Spence, G.A., Weber, L., 1980. Resistance of species of the *Drosophila melanogaster* subgroup to environmental extremes. *Australian Journal of Zoology* 28, 413-421.
- Terblanche, J.S., Klok, C.J., Chown, S.L., 2004. Metabolic rate variation in *Glossina pallidipes* (Diptera : Glossinidae): gender, ageing and repeatability. *Journal of Insect Physiology* 50, 419-428.
- Terblanche, J.S., Klok, C.J., Chown, S.L., 2005a. Temperature-dependence of metabolic rate in *Glossina morsitans morsitans* (Diptera, Glossinidae) does not vary with gender, age, feeding, pregnancy or acclimation. *Journal of Insect Physiology* 51, 861-870.
- Terblanche, J.S., Sinclair, B.J., Klok, C.J., McFarlane, M.L., Chown, S.L., 2005b. The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalconota* (Coleoptera: Chrysomelidae). *Journal of Insect Physiology* 51, 1013-1023.
- Thomson, L.J., Robinson, M., Hoffmann, A.A., 2001. Field and laboratory evidence for acclimation without costs in an egg parasitoid. *Functional Ecology* 15, 217-221.
- Tracy, R.L., Walsberg, G.E., 2001. Developmental and acclimatory contributions to water loss in a desert rodent: investigating the time-course of adaptive change. *Journal of Comparative Physiology B* 171, 669-679.
- Wilson, R.S., Franklin, C.E., 2002. Testing the beneficial acclimation hypothesis. *Trends in Ecology and Evolution* 17, 66-70.
- Winston, P.W., Bates, D.H., 1960. Saturated solutions for the control of humidity in biological research. *Ecology* 41, 232-237.
- Woods, H.A., 1999. Patterns and mechanisms of growth of fifth-instar *Manduca sexta* caterpillars following exposure to low- or high-protein food during early instars. *Physiological and Biochemical Zoology* 72, 445-454.
- Woods, H.A., Harrison, J.F., 2001. The beneficial acclimation hypothesis versus acclimation of specific traits: physiological change in water-stressed *Manduca sexta* caterpillars. *Physiological and Biochemical Zoology* 74, 32-44.

Zamudio, K.R., Huey, R.B., Crill, W.D., 1995. Bigger isn't always better: body size, temperature and male territorial success in *Drosophila melanogaster*. *Animal Behaviour* 49, 671-677.

Zeilstra, I., Fischer, K., 2005. Cold tolerance in relation to developmental and adult temperature in a butterfly. *Physiological Entomology* 30, 92-95.

## **Chapter 8**

### **Conclusion**

*“When you are face to face with a difficulty, you are up against a discovery”*

**Lord Kelvin**



Physiological variation within and between individuals is a key element underlying adaptive physiological change, and therefore inter-population differentiation (see Chapter 1 and references therein). In consequence, at evolutionary time-scales such variation may even play an important role in speciation (Hoffmann & Parsons, 1997). Physiological variation pervades all levels of hierarchical investigation (Spicer & Gaston, 1999; Chapters 2-7), although in terrestrial arthropods it remains poorly examined at most time-scales and hierarchical levels. Spicer & Gaston (1999) have suggested that the lack of systematic focus on physiological diversity has probably stemmed from the fact that few investigators work on more than one or two hierarchical levels at once (see Figure 1, Chapter 1) and/or that they have failed to consider that variation as something important in itself. Variation is more commonly assumed to be experimental variation and thus treated as a source of error which should be minimized (Spicer & Gaston, 1999). Although this latter opinion is not widely held, as evidenced by several authors who have drawn attention to the evolutionary importance of variation at different hierarchical levels (Bartholomew, 1987; Bennett, 1987; Feder, 1987; Spicer & Gaston, 1999; Chown et al., 2002), the situation is by no means resolved. Indeed, recent reviews of insect physiological patterns have once again highlighted the lack of information on physiological variation (Chown, 2001; Chown et al., 2002; 2003). Emphasis has been placed on understanding such variation, particularly at the within species level, in order to understand ecological processes and potential climate change impacts from a mechanistic viewpoint (e.g. Chown, 2001; Chown et al., 2002; 2003; Clarke, 2003; Stillman, 2003; Helmuth et al., 2005; Somero, 2005; but see Hodkinson, 2003).

In sum, this thesis adds support to the idea that physiological variation within species is pervasive and occurs at temporal scales from days to weeks (Chapters 2, 3 & 5; and see Table 1), in keeping with much of the literature (see e.g. Spicer & Gaston, 1999; Chown & Nicolson, 2004). It has also been demonstrated that considerable variation can occur among populations (Chapter 6), similar to observed physiological variation for several insect and non-insect arthropod species (reviewed in Spicer & Gaston, 1999; Chown & Nicolson, 2004). Nevertheless, exceptions can and do occur, such as the absence of variation in metabolic responses to temperature in *G. morsitans* (slopes of R-T relationships; Chapter 4). Furthermore, it is possible that much of the among-population (and within-species) physiological variation observed is a consequence of physiological flexibility (for example, in the case of lower critical thermal limits and water loss rate in *G. pallidipes*; Chapter 6). My finding that a large proportion of among-population variation in thermal limits and water loss rate tsetse can be accounted for by acclimation responses (Chapter 6) agrees with studies of *Drosophila* (Ayrinhac et al., 2004; Hoffmann et al., 2005; but see Bublly et al., 2002) and sub-Antarctic weevils (Klok & Chown, 2003). The benefits of such plasticity is more difficult to ascertain (Chapter 7; see Woods & Harrison, 2002; Wilson & Franklin, 2002). Flexible

physiological responses are likely pervasive, can develop rapidly and may be reversed equally quickly (Chapter 6; see also Fischer et al., 2003; Zeilstra & Fischer, 2005). The form of plasticity (e.g. developmental plasticity vs. phenotypic flexibility) can have significant, though often different, effects on several adult traits that may mediate environmental effects on populations (Chapter 7). In Chapter 7 it is demonstrated that it is important to distinguish between developmental plasticity and phenotypic flexibility (Wilson & Franklin, 2002; Piersma & Drent, 2003), and also to consider the direction of the responses and their significance from a life-history perspective (Fischer et al., 2003).

Within individuals, the fact that some traits may be substantially less flexible at a given time scale than others (e.g. upper vs. lower critical thermal limits,  $CT_{Max}$  vs.  $CT_{Min}$ ) (Chapter 6 & 7) suggests that the mechanisms underlying these responses differ. Such variation also suggests that both the basal and inducible thermal limits may differ. Limited flexibility of  $CT_{Max}$  relative to  $CT_{Min}$  could indicate a conserved mechanism, and consequently, relatively low trait variation across several highly diverged taxa (see Kingsolver & Huey, 1998 for several non-insect ectotherm examples, and Chown & Nicolson, 2004 for review of insects; Chapter 5 & 6). Additional support for conservation of the underlying mechanisms of thermal limits is contributed by the fact that these parameters appear constrained by the genetic composition of an individual (see Hoffmann et al., 2003). Regardless, variation between thermal limit traits within a population may be a consequence of biochemical (i.e. sub-cellular) limitations (discussed in Klok et al., 2004; Sinclair et al., 2004) or whole-animal (genotypic and/or phenotypic) limitations (discussed in Hoffmann et al., 2003; Anderson et al., 2005; Kellett et al., 2005). The difference in variation between upper and lower temperature tolerance probably reflects fundamental differences in the mechanisms involved. For example,  $CT_{Max}$  is probably more closely linked to cellular level biochemical processes and the associated chemical-bond thresholds (e.g. protein denaturation and/or conformational integrity in membrane composition (Hochachka & Somero, 2002)). Tsetse are no exception to this general pattern, as  $CT_{Max}$  appears highly conserved between field and laboratory populations in *G. pallidipes*, relative to  $CT_{Min}$  (Chapter 6, Fig. 4a, b). By contrast,  $CT_{Min}$  is probably the result of changes that occur at a variety of scales within the insect, from organismal through to sub-cellular adjustments (Klok et al., 2004), and thus may be more pliable and variable in populations (see Addo-Bediako et al., 2000 and discussion in Chown & Nicolson, 2004). However, a fundamental distinction should be made between basal and inducible levels of plasticity. While the absolute magnitude of plasticity available to critical thermal limits may be genetically controlled (e.g. Woods et al., 1999; Hoffmann et al., 2003; Kellett et al., 2005) the mechanisms influencing plasticity of upper and lower limits may be inherently different and are thus able to vary over substantially different magnitudes.

Differences in the magnitude of available plasticity between upper and lower thermal limits have implications for understanding the likely impacts of climate change on species distributions and abundance. For example, when modelling range or abundance changes under future climate scenarios, differences in plasticity (i.e. time course and/or temperature sensitivity) between upper and lower thermal limits could affect the accuracy of predicting vector-host relationships or distributions under different temperature conditions. Typically, plasticity of biological variables is not incorporated into modelling exercises, although the temperature-dependence of biological rate processes (e.g. growth rate) are increasingly considered (e.g. Hoshen & Morse, 2004). Consequently, assumptions of similarly low levels of plasticity in upper and lower temperature limits may therefore over- and underestimate insect responses to temperature variation, respectively. Animals with a high degree of plasticity may show minimal shifts in range or abundance to temperature variation (see discussions in Berteaux et al., 2004; Helmuth et al., 2005), particularly if it is not energetically costly to initiate these plastic responses, and this may differ between upper and lower thermal limits. Naturally, the converse may also be true, in that low levels of plasticity may force animals to respond in alternative ways (e.g. behaviourally) or face extinction as a result of increases in temperature-induced mortality. Indeed, it has been cogently argued that without a mechanistic appreciation for how animals respond to change, the accuracy of predictions of species responses (either physiological or behavioural) to climate change will be compromised (Helmuth et al., 2005). Although climate change effects, either physiological or distributional, have not been well discussed for tsetse, strong relationship between abiotic variables (see Tables 2 & 3) and distribution and abundance suggests high likely responsiveness to climate change.

### **Tsetse Responses to Climate Change**

Recent predictions of anthropogenic climate change have revived interest in the factors influencing insect distribution and abundance, and the potential impact of these on species conservation (e.g. McCarty, 2001; Hill et al., 2002; Thomas et al., 2004). Shifts in geographic distribution and abundance of disease vectors could have serious implications for human and animal health (Cook, 1992; Githeko et al., 2000; Patz et al., 2000; Rogers & Randolph, 2000; Kovats et al., 2001; Beniston, 2002; Lounibos, 2002), particularly in developing countries. Climate change effects on disease transmission have been well explored from theoretical (e.g. Kovats et al., 2001) and empirical (e.g. Purse et al., 2005) perspectives, and are relatively well predicted for vectors of diseases such as malaria (Rogers & Randolph, 2000) and blue-tongue virus (Purse et al., 2005). Mixed support for climate change effects has been obtained. For example, climate change has been strongly associated with increasing risk of disease occurrence in the case of blue-tongue virus (Kuhn et al., 2003; Purse et al., 2005) but not for that of malaria (Randolph & Rogers, 2000). The

lack of evidence for a similar effect of changing climate on trypanosomiasis vectors probably reflects a lack of investigation rather than absence of climate change effects (Kovats et al., 2001). Nonetheless, the balance of accumulated evidence suggests that climate change affects the spread of infectious diseases (e.g. IPCC, 2001; Sutherst, 2004). Resistance to infectious diseases ultimately determines the severity of climate change impact upon human health (Martens & Moser, 2001). The effects of abiotic and biotic factors on vector biology and disease distribution may be complex (Randolph & Rogers, 2000; Purse et al., 2005) but these climate change effects on disease occurrence should in no instance be considered insignificant (Martens & Moser, 2001).

Both the distribution and population dynamics modelling approaches have revealed the importance of climatic variables in determining tsetse abundance and distribution (discussed in Chapters 1 and 6 and reviewed in Rogers & Randolph, 1985; Rogers & Robinson, 2004). It is well established that moisture availability and temperature are important predictors of tsetse distribution at both coarse and fine spatial scales in Africa (Rogers & Robinson, 2004), although this evidence is mostly correlative (e.g. Rogers & Randolph, 1986; 1991; but see Tables 2 & 3). At short and longer time scales, abundance can be positively correlated with temperature and negatively correlated with humidity (Mohamed-Ahmed & Wynholds, 1997; see also Van Etten, 1982; Rogers & Randolph, 1991).

It is also known that tsetse demographics are strongly influenced in all life stages by temperature, and probably to a lesser extent by moisture availability (Hargrove, 2001; 2004), although the functions describing these relationships can differ markedly within and between various life-stages. For example, increasing temperature reduces survival (Hargrove, 2001) and declining water availability may do the same, although variation is found among species (Rogers & Randolph, 1986; Hargrove, 2004). In many cases, however, the mechanisms involved have not been clarified. Indeed, Hargrove (2004) has concluded that in fact “it is unclear why adult mortality increases with temperature.”

The most straightforward way to identify where this lack of clarity lies is by building a simple graphical model (Fig. 1). Initially, consider the information available for tsetse flies. First, development rates have been fairly well described. Increasing temperature generally results in a non-linear decrease in the time to production of larvae and the duration of the pupal stage (see Phelps & Burrows, 1969b; Hargrove, 2004). Second, changes in population size have been also modeled by Hargrove (2004). Generally, increasing temperatures result in a linear increase in mortality (‘loss rates’ in Hargrove, 2004) across most life stages. For growth rates, there is a non-linear ‘a’-shaped relationship with temperature, such that 25 °C indicates an optimum temperature. The most important exception to these trends are the pupae, which show a decline in mortality below 25 °C and an increase above this critical temperature. Therefore, how mortality across

various life-stages causes changes in population size is relatively well established. Third, correlative relationships between abiotic factors and tsetse abundance and distribution have been most clearly demonstrated by Rogers and colleagues (Rogers & Randolph, 1986; 1991; Rogers & Robinson, 2004). At this point, however, it is important to note that the majority of the information is based on work undertaken for only one or two key species, usually *G. pallidipes* and/or *G. morsitans*.

By contrast, relatively little information is available for two key mechanisms, namely, abiotic effects on fecundity and mortality (Fig. 1). Relative to the literature on, for instance, *Drosophila* ( e.g. cold- or heat-induced changes in flight or mating performance (Fasolo & Krebs, 2004; Krebs & Thompson, 2005), environmental effects on adult fecundity in tsetse is relatively poorly explored with the exception of some evidence for heat-induced sterility and general effects of temperature on mating performance (Leegwater-van der Linden, 1984; Mutika et al., 2001) and vector infection rates (Ndegwa et al., 1992). How pupal mortality is affected by temperature has also been relatively poorly examined (Hargrove, 2004). Nevertheless, some information is available. Phelps & Burrows (1969) showed that pupal exposure to 36 °C for 4h per day did not increase mortality, while higher temperature or longer duration resulted in significant increases in mortality. The effects of temperature on teneral mortality have mainly been inferred from size-class elimination studies (e.g. Phelps & Clarke, 1974) or seasonal variation in body size (see Hargrove (2004) for several examples and detailed discussion). Some physiological mechanisms have also been discussed. For example, based on fat content at eclosion it is considered that starvation risk is a real threat to teneral survival (Hargrove, 2004), and that mortality increases at both high and low temperatures (Phelps & Clarke, 1974).

As stated previously, the effects of temperature on adult survival mark a substantial gap in the information scheme (Fig. 1; and see Table 3). Essentially, mark-recapture studies have shown a positive correlation between mortality and temperature, as well as a positive correlation between mortality and saturation deficit. Some models, which are probably biased in terms of the age-classes sampled, identify maximum temperature and mean temperature as being more important than saturation deficit (water availability) (Hargrove, 2004), while other models which sample all life stages (e.g. ovarian age studies) identify saturation deficit as being more important than temperature in determining mortality. As pointed out by Hargrove (2004), if both models are largely correct, this implies that the immature life-stages are most sensitive to moisture availability while the mature life-stages are most sensitive to temperature. In sum, however, the physiological mechanisms underpinning the relationships between abiotic factors and mortality and/or fecundity are poorly established, particularly at temperatures lower than 36 °C (i.e. in the sub-lethal range) (and see Table 2 & 3).

How this study contributes to the scheme in Figure 1 is that it offers potential physiological patterns linking the factors influencing population dynamics in the sub-lethal and lethal temperature ranges. For example, for the lethal temperature range ( $> 40\text{ }^{\circ}\text{C}$ ) this study has shown that limited physiological plasticity in upper critical thermal limits supports the contention that tsetse distribution will likely increase in cooler areas with predicted warming resulting from climate change (Rogers & Packer, 1993), and that species may be separated geographically by as little as  $1\text{ }^{\circ}\text{C}$  (Rogers & Randolph, 1986; Robinson et al., 1997b; a), suggesting that there is little variation among species upper tolerances. On the other hand, highly flexible lower critical thermal limits reflect a likely greater capability that tsetse flies to survive in cooler environments. Thus, when predicting the potential impacts of climate change on tsetse distribution and abundance, it may be necessary to consider the plastic physiological responses to heat and cold separately.

At sub-lethal temperatures, this study yields unusual insight. Here, I suggest an alternative mechanism potentially explaining the sub-lethal mortality-temperature relationship observed in tsetse, while noting that appropriate statistical comparisons and mechanistic exploration is required, and that comparisons of the intercepts would not be possible due to differences in the units of measurement. Consider the slope of the relationship between tsetse mortality and temperature (Fig. 7.4, p. 121; Hargrove, 2004). If metabolic rate-temperature (R-T) relationships had a similar slope, then one might attribute the observed mortality-temperature relationship to this physiological process. Recall that this relationship occurs over the  $25\text{-}35\text{ }^{\circ}\text{C}$  range, temperatures at which no direct effects of temperature on mortality have yet been demonstrated. A mortality R-T across this range may operate via resource acquisition and allocation and are generally predicted by life-history models (see e.g. Reznick et al., 2002; Kozlowski et al., 2004; Yearsley et al., 2005). For example, at higher temperatures flies would metabolize resources more rapidly, require more energy from bloodmeals, and hence take more risks obtaining resources under these temperature conditions (i.e. more feeding events per unit time; see discussion in Yearsley et al., 2005). On the other hand, if metabolic R-T relationships do not fully account for the mortality R-T relationship, one might expect that the desiccation R-T relationship could explain the remainder of the variance, although the degree to which these effects are additive or interactive with temperature would require some evaluation.

There are two main lines of evidence to suggest the possibility of such a mechanism in tsetse flies. First, the slope of the mortality R-T relationship is never exceeded by the slope of the metabolic R-T relationship. Second, the metabolic R-T relationship explains the mortality R-T relationship predictably based on additional physiological information. In *G. m. morsitans*, the mortality R-T relationship has a slope of 0.077 (SE: 0.0045) (derived from the mean of males and females presented in Hargrove (2004)), while the metabolic R-T relationship is 0.035 (SE: 0.003,



Ch. 4). Apparently, the only available relationship of desiccation with temperature is provided by Bursell (1958) for *G. m. morsitans* pupae at a relative humidity of zero for all temperatures, in which the slope of approximately 0.0037 was estimated. However, the desiccation R-T relationships are seldom experimentally determined, probably due to the inherent difficulty of controlling for the non-linear effects of temperature and saturation deficit on relative humidity. In *G. pallidipes*, the slope of the mortality R-T relationship given by Hargrove (2004) is approximately 0.144 (SE: 0.027), while the metabolic R-T slope is 0.029 (SE: 0.003) in field populations of *G. pallidipes* sampled across four geographic locations in Kenya (J.S. Terblanche, unpublished data). If *G. morsitans* is 'dry adapted' (which is how this species has been regarded on several occasions (e.g. Leak, 1999; Hargrove, 2004; Rogers & Robinson, 2004) owing primarily to their increased body water reserves and reduced water loss rates (Bursell, 1959)), then water loss should account for a lower proportion of the mortality R-T relationship slope value than in a more mesic species, such as *G. pallidipes*. Indeed, this could be the case since the slope of the metabolic R-T relationship accounts for a much greater proportion of the overall mortality slope value in *G. m. morsitans* relative to *G. pallidipes* (45 % vs. 19 %) of which the remainder may be due to the desiccation R-T relationship. Obviously, such speculation should be verified with experimental work examining the nature of the desiccation R-T relationship. Nevertheless, it's possible to predict that the combined effects of the metabolic R-T and the desiccation R-T relationships account for the observed mortality R-T relationship. But as yet, such analyses can only be performed in a relatively simplistic manner. Metabolic and desiccation R-T slopes in both cases are likely underestimated in the above approximations due to the exclusion of activity and behaviour (note that activity is correlated with rate of water loss (Bursell, 1959) and activity is also correlated with temperature and humidity (Van Etten, 1982)). Both activity levels and daily behaviour in the wild would need to be accounted for in such an analysis in order to ascertain factors influencing mortality in natural populations.

Clearly, physiological variability of most traits can be expected at several time scales, both within and between generations. Attention, however, needs to be given to the magnitude of variation with reference to the ecological context, especially when making inferences about climate change effects on species responses. For example, although developmental and adult acclimation effects may differ in their magnitude and in some cases direction (Chapter 7), how relevant such variation is to the ecology of the species requires closer scrutiny. Indeed, statistically significant inter-treatment or inter-population differences in  $CT_{Max}$  of the order 0.1-0.3 °C are probably not biologically relevant. Thus, attaining estimates of basal and inducible limits of acclimation responses deserves some consideration in order to predict species responses to climate change, especially over longer time scales.

**Suggested key areas for future research**

The following questions are meant to represent areas of research which may help linking the tsetse distribution and demographics modelling fields with physiological mechanisms causing mortality at sub-lethal temperatures (presented in no particular order). The overarching goal intended for these research foci is the integration of physiological patterns with predicting responses of these vectors to climate change:

1) Several authors have either directly or indirectly implied that physiological variation between species explains local distribution patterns for tsetse (Rogers & Randolph, 1986; Robinson et al., 1997b; a; Rogers & Robinson, 2004). To my knowledge, these theories have not been directly tested. Thus, examining these predictions, specifically, for variation in thermal tolerance and water balance physiology between *Glossina* spp., within a strong phylogenetic framework would provide a worthwhile goal.

2) It is clear that parental conditions may determine offspring performance under various environmental conditions (Mousseau & Fox, 1998), although studies typically assess life-history traits rather than physiological performance. In *Drosophila*, knockdown temperature (and body size) may be influenced by the temperature experienced by the parent (Crill et al., 1996), but the strength of parental effects were weaker than the effects of sex and developmental temperature in this case (Crill et al., 1996; but see Fox et al. (2004) for an exception to this pattern). Cross-generation effects suggest that environmental variation experienced in preceding generations can influence progeny phenotype in a manner that is complex and difficult to predict (Magioglou & Hoffmann, 2003). Regardless, determining the strength of interactions between life-stages via physiological tolerances (e.g. developmental and adult acclimation) or cross-generational effects on physiology of tsetse would provide considerable insight into temperature effects at longer, evolutionarily relevant, time-scales. Determining the degree of reversibility after acquisition of e.g stress resistance at different life-stages would also provide insight into how tsetse may respond to future climate change. Considering the substantial energetic investment associated with larval time *in utero* during tsetse reproduction, parental effects may be more tightly coupled with developmental effects, and thus could be more pronounced in tsetse than in other Dipterans.

3) Research undertaken for several *Drosophila* species has demonstrated that basal and inducible heat tolerance appears phylogenetically constrained, but is usually of a similar magnitude among species (Kellett et al., 2005). Understanding the factors controlling basal and plastic levels of temperature tolerance would provide insight into the mechanisms that should be accounted for when predicting how tsetse may respond to ongoing climate change. Doing

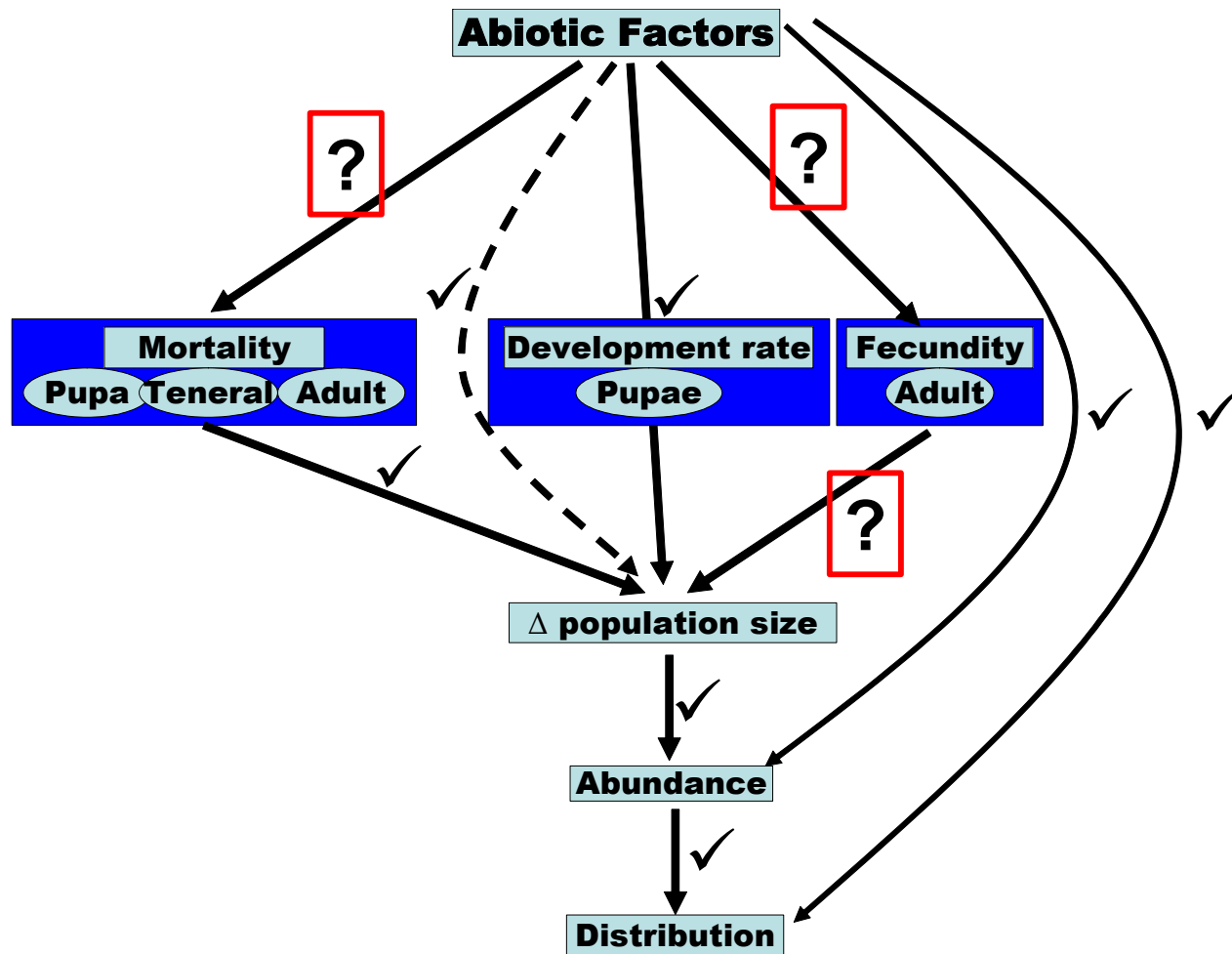


so for a variety of species would help determine whether or not there are important exceptions. Considering the variation in vector-host relationships among tsetse species, single and/or multiple species modelling may be required, and information for several *Glossina* species would thereby make the information more practical for distribution modelling use.

4) For reasons outlined in this chapter (see *Tsetse Responses to Climate Change*), determining the physiological mechanisms accounting for mortality at sub-lethal temperatures (25-35°C) by examining the contribution of metabolic rate and desiccation rate to this relationship in the various key life-stages (pupal, teneral, adult).

5) To further contribute to the aspects of Figure 1 that have not been well explored, understanding environmental factors (e.g. temperature and humidity) influencing fecundity for several tsetse species would be useful. Doing so at sub-lethal and lethal temperatures in a range of tsetse species would be necessary.

In conclusion, the study of variation at the within- and between-individual levels was identified largely by Bartholomew (1987), Bennett (1987), and Feder (1987) (and see discussion in Spicer & Gaston, 1999) as a great challenge for physiologists. This thesis has taken up that challenge and demonstrated the importance of this level of variation in accounting for much population differentiation. Furthermore, such variation may influence laboratory assessments of traits at short time-scales, and contribute to or constrain evolution at longer time-scales. Moreover, incorporating such physiological variation into the prediction of climate change effects will likely improve the accuracy about predictions of how animals will respond to future climate scenarios.



**Figure 1.** Schematic diagram of key population level processes influencing abundance and distribution in tsetse flies (*Glossina* spp.) Tick marks represent lines of causality for which information is available, while interrogation marks represent missing links in the literature.

**Table 1.** Reported differences between laboratory and field-fresh (FF) *Glossina* spp. taken from the modern Anglophone literature.

Species	Trait	Specific measure	Change <sup>1</sup>	Time scale	Reference
<i>G. morsitans</i>	Reproductive success	Abortion	↑	??	(Gaston & Randolph, 1993)
		Mating	↓		(Foster, 1957)
<i>G. pallidipes</i>	Reproductive parameters	Various	↑	2 weeks	(Randolph et al., 1990)*
	Nutritional parameters	Fat content	↓		
		[Haematin]	↑		
<i>G. morsitans</i>	Metabolic rate	Oxygen consumption	↓	??	(Taylor, 1977)
<i>G. morsitans</i>	Digestion rate	Protease activity	↓	??	(Langley, 1967) <sup>o</sup>
		Excretion rates	↓		
<i>G. austeni</i>	Flight musculature	Muscle growth rate	↓	??	(Bursell, 1961)
<i>G. morsitans</i>					(Langley, 1970)
<i>G. morsitans</i>	Activity patterns	Overall mean activity	↓	Years	(Brady & Crump, 1978)

1) relative to field-fresh

\*Lab-maintained, field-collected non-pupal vs. field-fresh

<sup>o</sup> Lab-raised, field-collected pupae vs. field-fresh

**Table 2.** Literature review of studies in which water balance physiology has been conducted on tsetse flies (*Glossina* spp.)

<b>Species</b>	<b>Physiological Trait</b>	<b>Life-stage</b>	<b>Source population</b>	<b>Reference</b>
<i>G. morsitans</i>	Desiccation rate, BWC	Adults	??	(Bursell, 1957)
<i>G. morsitans</i>	Desiccation rate, BWC	Pupae	Laboratory & Field	(Bursell, 1958)
<i>G. swynnertoni</i>	Desiccation rate, BWC	Pupae	Field	(Bursell, 1958)
<i>G. pallidipes</i>				
<i>G. brevipalpis</i>	Desiccation survival, rate, tolerance, BWC	Pupae	Field	(Bursell, 1958)
<i>G. fuscipleuris</i>				
<i>G. longipennis</i>				
<i>G. palpalis</i>				
<i>G. tachinoides</i>				
<i>G. pallidipes</i>				
<i>G. morsitans</i>				
<i>G. m. submorsitans</i>				
<i>G. swynnertoni</i>				
<i>G. austeni</i>				
<i>G. palpalis</i>	Desiccation rate	Pupae	Field	(Bursell, 1958)
<i>G. brevipalpis</i>				
<i>G. fuscipleuris</i>	Desiccation rate, BWC, survival	Adults	Field <sup>1</sup>	(Bursell, 1959)
<i>G. brevipalpis</i>				
<i>G. longipennis</i>				

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*G. palpalis fuscipes*

*G. pallidipes*

*G. morsitans*

*G. swynnertoni*

*G. austeni*

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<i>G. morsitans</i>	Excretory water loss	Adults	Field	(Bursell, 1960b)
<i>G. morsitans</i>	BWC	Adults	Field	(Jackson, 1937)
<i>G. morsitans</i>	BWC, Desiccation rate	Adults	Field	(Jackson, 1932)
<i>G. swynnertoni</i>				
<i>G. palpalis</i>				
<i>G. pallidipes</i>				
<i>G. morsitans</i>	BWC	Adults	Laboratory & Field	(Jackson, 1946)
<i>G. swynnertoni</i>				
<i>G. pallidipes</i>				
<i>G. palpalis</i>	Desiccation survival, water uptake, BWC	Adults	Field	(Mellanby, 1936)

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1 Except for *G. fuscipleuris* and *G. brevipalpis*

**Table 3.** Summary of literature in which thermal limits physiology has been conducted on tsetse flies (*Glossina* spp.).

<b>Species</b>	<b>Physiological trait</b>	<b>Life-stage</b>	<b>Source</b>	<b>Reference</b>
<i>G. m. orientalis</i>	ULT, LLT	Pupae	Laboratory*	(Phelps & Burrows, 1969a)
<i>G. morsitans</i>	ULT, Cold survival, Heat survival	Pupae & Adults	Laboratory & Field	(Potts, 1933)
<i>G. morsitans</i>	ULT, LLT	Pupae	Field	(Jack, 1939) <sup>B</sup>
<i>G. tachinoides</i>	ULT, LLT	Pupae & Adults	Field	(Buxton & Lewis, 1934)
<i>G. m. submorsitans</i>				
<i>G. morsitans</i>	LLT	Pupae	Field	(Bursell, 1960a)
<i>G. morsitans</i>	Survival times, ULT			(Jack, 1942)
<i>G. palpalis</i>	Survival, CTMin/Activity <sup>A</sup>	Adults	Field	(Mellanby, 1936)
<i>G. palpalis</i>	Survival, CTMin/CTMax/Activity <sup>A</sup>	Adults	Field	(Macfie, 1912)

\*colony supplemented with field flies

A) May approximate Critical Thermal Limits

B) cited in (Phelps & Burrows, 1969a)

## References

- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* 267, 739-745.
- Anderson, A.R., Hoffmann, A.A., McKechnie, S.W., 2005. Response to selection for rapid chill-coma recovery in *Drosophila melanogaster*: physiology and life-history traits. *Genetical Research* 85, 15-22.
- Ayrinhac, A., Debat, V., Gibert, P., Kister, A.-G., Legout, H., Moreteau, B. et al., 2004. Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology* 18, 700-706.
- Bartholomew, G.A., 1987. Interspecific comparison as a tool for ecological physiologists. In: Feder, M.E., Bennett, A.F., Burggren, W., Huey, R.B. (eds), *New Directions in Ecological Physiology*. Cambridge University Press, Cambridge, pp. 11-37.
- Beniston, M., 2002. Climate change: possible impacts on human health. *Swiss Medical Weekly* 132, 332-337.
- Bennett, A.F., 1987. Interindividual variability: an underutilized resource. In: Feder, M.E., Bennett, A.F., Burggren, W., Huey, R.B. (eds), *New Directions in Ecological Physiology*. Cambridge University Press, Cambridge, pp. 147-169.
- Berteaux, D., Reale, D., McAdam, A.G., Boutin, S., 2004. Keeping pace with fast climate change: can Arctic life count on evolution? *Integrative and Comparative Biology* 44, 140-151.
- Brady, J., Crump, A.J., 1978. The control of circadian activity rhythms in tsetse flies: environment or physiological clock? *Physiological Entomology* 3, 177-190.
- Bubliy, O.A., Riihimaa, A., Norry, F.M., Loeschke, V., 2002. Variation in resistance and acclimation to low-temperature stress among three geographical strains of *Drosophila melanogaster*. *Journal of Thermal Biology* 27, 337-344.
- Bursell, E., 1957. Spiracular control of water loss in the tsetse fly. *Proceedings of the Royal Entomological Society of London A* 32, 21-29.
- Bursell, E., 1958. The water balance of Tsetse pupae. *Philosophical Transactions of the Royal Society of London B* 241, 179-210.
- Bursell, E., 1959. The water balance of tsetse flies. *Transactions of the Royal Entomological Society of London* 111, 205-235.
- Bursell, E., 1960a. The effect of temperature on the consumption of fat during pupal development in *Glossina*. *Bulletin of Entomological Research* 51, 583-598.
- Bursell, E., 1960b. Loss of water by excretion and defaecation in the tsetse fly. *Journal of Experimental Biology* 37, 689-697.

- Bursell, E., 1961. Post-teneral development of the thoracic musculature in tsetse flies. Proceedings of the Royal Entomological Society of London A 36, 69-74.
- Buxton, P.A., Lewis, D.J., 1934. Climate and tsetse flies: Laboratory studies upon *Glossina submorsitans* and *tachinoides*. Philosophical Transactions of the Royal Society of London B 224, 175-242.
- Chown, S.L., 2001. Physiological variation in insects: hierarchical levels and implications. Journal of Insect Physiology 47, 649-660.
- Chown, S.L., Addo-Bediako, A., Gaston, K.J., 2002. Physiological variation in insects: large-scale patterns and their implications. Comparative Biochemistry and Physiology B 131, 587-602.
- Chown, S.L., Addo-Bediako, A., Gaston, K.J., 2003. Physiological diversity: listening to the large-scale signal. Functional Ecology 17, 568-572.
- Chown, S.L., Nicolson, S.W., 2004. Insect Physiological Ecology. Mechanisms and Patterns. Oxford University Press, Oxford.
- Clarke, A., 2003. Costs and consequences of evolutionary temperature adaptation. Trends in Ecology and Evolution 18, 573-581.
- Cook, G.C., 1992. Effect of global warming on the distribution of parasitic and other infectious diseases: a review. Journal of the Royal Society of Medicine 85, 688-691.
- Crill, W.D., Huey, R.B., Gilchrist, G.W., 1996. Within- and between-generation effects of temperature on the morphology and physiology of *Drosophila melanogaster*. Evolution 50, 1205-1218.
- Fasolo, A.G., Krebs, R.A., 2004. A comparison of behavioural change in *Drosophila* during exposure to thermal stress. Biological Journal of the Linnean Society 83, 197-205.
- Feder, M.E., 1987. The analysis of physiological diversity: the prospects for pattern documentation and general questions in ecological physiology. In: Feder, M.E., Bennett, A.F., Burggren, W., Huey, R.B. (eds), New Directions in Ecological Physiology. Cambridge University Press, Cambridge, pp. 38-75.
- Fischer, K., Eenhorn, E., Bot, A.N.M., Brakefield, P.M., Zwaan, B.J., 2003. Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. Proceedings of the Royal Society of London B 270, 2051-2056.
- Foster, R., 1957. Observations on laboratory colonies of the tsetse flies *Glossina morsitans* Westw. and *Glossina austeni* Newstead. Parasitology 47, 361-374.
- Fox, C.W., Czesak, M.E., Wallin, W.G., 2004. Complex genetic architecture of population differences in adult lifespan of a beetle: nonadditive inheritance, gender differences, body size and a large maternal effect. Journal of Evolutionary Biology 17, 1007-1017.



- Gaston, K.A., Randolph, S.E., 1993. Reproductive under-performance of tsetse flies in the laboratory, related to feeding frequency. *Physiological Entomology* 18, 130-136.
- Githeko, A.K., Lindsay, S.W., Confalonieri, U.E., Patz, J.A., 2000. Climate change and vector-borne diseases: a regional analysis. *Bulletin of the World Health Organisation* 78, 1136-1147.
- Hargrove, J.W., 2001. The effect of temperature and saturation deficit on mortality in populations of male *Glossina m. morsitans* (Diptera: Glossinidae) in Zimbabwe and Tanzania. *Bulletin of Entomological Research* 91, 79-86.
- Hargrove, J.W., 2004. Tsetse population dynamics. In: Maudlin, I., Holmes, P.H., Miles, M.A. (eds), *The Trypanosomiasis*. CABI Publishing, Wallingford, pp. 113-138.
- Helmuth, B., Kingsolver, J.G., Carrington, E., 2005. Biophysics, physiological ecology, and climate change: Does mechanism matter? *Annual Review of Physiology* 67, 177-201.
- Hill, J.K., Thomas, C.D., Fox, R., Telfer, M.G., Willis, S.G., Asher, J. et al., 2002. Responses of butterflies to twentieth century climate warming: implications for future ranges. *Proceedings of the Royal Society of London B* 269, 2163-2171.
- Hochachka, P.W., Somero, G.N., 2002. *Biochemical Adaptation. Mechanism and Process in Physiological Evolution*. Oxford University Press, New York.
- Hodkinson, I.D., 2003. Metabolic cold adaptation in arthropods: a smaller-scale perspective. *Functional Ecology* 17, 562-567.
- Hoffmann, A.A., Parsons, P.A., 1997. *Extreme Environmental Change and Evolution*. Cambridge University Press, Cambridge.
- Hoffmann, A.A., Shirrifs, J., Scott, M., 2005. Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Functional Ecology* 19, 222-227.
- Hoffmann, A.A., Sorensen, J.G., Loeschke, V., 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology* 28, 175-216.
- Hoshen, M.B., Morse, A. P., 2004. A weather-driven model of malaria transmission. *Malaria Journal* 3, 32.
- IPCC, 2001. *Climate Change 2001: Synthesis Report. Summary for policymakers. An assessment of the Intergovernmental Panel on Climate Change*. Intergovernmental Panel on Climate Change, Geneva, Switzerland.
- Jack, R.W., 1939. Studies in the physiology and behaviour of *Glossina morsitans* Westw. *Memoirs of the Department of Agriculture of South Rhodesia* 1, 203.

- Jack, R.W., 1942. The life economy of a Tsetse fly. Proceedings of the Rhodesia Scientific Association 34, 2-15.
- Jackson, C.H.N., 1932. Comparative studies of the habitat requirements of tsetse fly species. Journal of Animal Ecology 14, 46-51.
- Jackson, C.H.N., 1937. Water and fat content of Tsetse flies. Nature 140, 674-675.
- Jackson, C.H.N., 1946. An artificially isolated generation of tsetse-flies (Diptera). Bulletin of Entomological Research 32, 291-299.
- Kellett, M., Hoffmann, A.A., McKechnie, S.W., 2005. Hardening capacity in the *Drosophila melanogaster* species group is constrained by basal thermotolerance. Functional Ecology 19, 853-858.
- Kingsolver, J.G., Huey, R.B., 1998. Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. American Zoologist 38, 545-560.
- Klok, C.J., Chown, S.L., 2003. Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. Biological Journal of the Linnean Society 78, 401-414.
- Klok, C.J., Sinclair, B.J., Chown, S.L., 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. Journal of Experimental Biology 207, 2361-2370.
- Kovats, R.S., Campbell-Lendrum, D.H., McMichael, A.J., Woodward, A., Cox, J.S., 2001. Early effects of climate change: do they include changes in vector-borne disease? Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 356, 1057-1068.
- Kozłowski, J., Czarnoleski, M., Danko, M., 2004. Can optimal resource allocation models explain why ectotherms grow larger in cold? Integrative and Comparative Biology 44, 480-493.
- Krebs, R.A., Thompson, K.A., 2005. A genetic analysis of variation for the ability to fly after exposure to thermal stress in *Drosophila mojavensis*. Journal of Thermal Biology 30, 335-342.
- Kuhn, K.G., Campbell-Lendrum, D.H., Armstrong, B., Davies, C.R., 2003. Malaria in Britain: Past, present, and future. Proceedings of the National Academy of Sciences of the USA 100, 9997-10001.
- Langley, P.A., 1967. Control of digestion in the tsetse fly, *G. morsitans*: a comparison between field flies and flies reared in captivity. Journal of Insect Physiology 13, 477-486.
- Langley, P.A., 1970. Post-teneral development of thoracic musculature in the tsetse-flies *Glossina austeni* and *G. morsitans*. Entomologia Experimentalis et Applicata 13, 133-140.
- Leak, S.G.A., 1999. Tsetse Biology and Ecology. Their Role in the Epidemiology and Control of Trypanosomosis. CABI Publishing, New York.

- Leegwater-van der Linden, M.E., 1984. Temperature and fertility in *Glossina pallidipes*. Tropical and Geographical Medicine. 36, 391.
- Lounibos, L.P., 2002. Invasions by insect vectors of disease. Annual Review of Entomology 47, 233-266.
- Macfie, J.W.S., 1912. Experiments and observations upon *Glossina palpalis*. Bulletin of Entomological Research 3, 61-72.
- Magioglou, A., Hoffman, A.A., 2003. Cross-generation effects due to cold-exposure in *Drosophila serrata*. Functional Ecology 17, 664-672.
- Martens, P., Moser, S.C., 2001. Health impacts of climate change. Science 292, 1065-1066.
- McCarty, J.P., 2001. Ecological consequences of recent climate change. Conservation Biology 15, 320-331.
- Mellanby, K., 1936. Experimental work with the tsetse fly *Glossina palpalis* in Uganda. Bulletin of Entomological Research 27, 611-633.
- Mohamed-Ahmed, M.M., Wynholds, Y., 1997. Effects of vegetation and weather on trap catches of *Glossina fuscipes fuscipes* near Lake Victoria, Kenya. Entomologia Experimentalis et Applicata 85, 231-236.
- Mousseau, T.A., Fox, C.W., 1998. The adaptive significance of maternal effects. Trends in Ecology and Evolution 13, 403-407.
- Mutika, G.N., Opiyo, E., Robinson, A.S., 2001. Assessing mating performance of male *Glossina pallidipes* (Diptera: Glossinidae) using a walk-in field cage. Bulletin of Entomological Research 91, 281-287.
- Ndegwa, P.N., Irungu, L.W., Moloo, S.K., 1992. Effect of puparia incubation temperature: increased infection rates of *Trypanosoma congolense* in *Glossina morsitans centralis*, *G. fuscipes fuscipes* and *G. brevipalpis*. Medical and Veterinary Entomology 6, 127-130.
- Patz, J.A., Graczyk, T.K., Geller, N., Vittor, A.Y., 2000. Effects of environmental change on emerging parasitic diseases. International Journal for Parasitology 30, 1395-1405.
- Phelps, R.J., Burrows, P.M., 1969a. Lethal temperature for puparia of *Glossina morsitans orientalis*. Entomologia Experimentalis et Applicata 22, 23-32.
- Phelps, R.J., Burrows, P.M., 1969b. Prediction of the pupal duration of *Glossina morsitans orientalis* under field conditions. Journal of Applied Ecology 6, 323-337.
- Phelps, R.J., Clarke, G.P.Y., 1974. Seasonal elimination of some classes in males of *Glossina morsitans morsitans* (Diptera: Glossinidae). Bulletin of Entomological Research 64, 313-324.
- Piersma, T., Drent, J., 2003. Phenotypic flexibility and the evolution of organismal design. Trends in Ecology and Evolution 18, 228-233.

- Potts, W.H., 1933. Observations on *Glossina morsitans*, Westw., in East Africa. Bulletin of Entomological Research 24, 293-300.
- Purse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P.C., Baylis, M., 2005. Climate change and the recent emergence of bluetongue in Europe. Nature Reviews Microbiology 3, 171-181.
- Randolph, S.E., Rogers, D.J., 2000. Fragile transmission cycles of tick-borne encephalitis virus may be disrupted by predicted climate change. Proceedings of the Royal Society of London B 267, 1741-1744.
- Randolph, S.E., Rogers, D.J., Kiilu, J., 1990. Rapid changes in the reproductive cycle of wild-caught tsetse, *Glossina pallidipes* Austen, when brought into the laboratory. Insect Science and its Applications 11, 347-354.
- Reznick, D., Bryant, M.J., Bashey, F., 2002. r- and K-selection revisited: The role of population regulation in life-history evolution. Ecology 83, 1509-1520.
- Robinson, T., Rogers, D., Williams, B., 1997a. Mapping tsetse habitat suitability in the common fly belt of Southern Africa using multivariate analysis of climate and remotely sensed vegetation data. Medical and Veterinary Entomology 11, 235-245.
- Robinson, T., Rogers, D., Williams, B., 1997b. Univariate analysis of tsetse habitat in the common fly belt of Southern Africa using climate and remotely sensed vegetation data. Medical and Veterinary Entomology 11, 223-234.
- Rogers, D.J., Packer, M.J., 1993. Vector-borne diseases, models, and global change. Lancet 342, 1282-1284.
- Rogers, D.J., Randolph, S.E., 1985. Population ecology of tsetse. Annual Review of Entomology 30, 197-216.
- Rogers, D.J., Randolph, S.E., 1986. Distribution and abundance of tsetse flies (*Glossina* Spp.). Journal of Animal Ecology 55, 1007-1025.
- Rogers, D.J., Randolph, S.E., 1991. Mortality rates and population density of tsetse flies correlated with satellite imagery. Nature 351, 739-741.
- Rogers, D.J., Randolph, S.E., 2000. The global spread of malaria in a future, warmer world. Science 289, 1763-1766.
- Rogers, D.J., Robinson, T.P., 2004. Tsetse distribution. In: Maudlin, I., Holmes, P.H., Miles, M.A. (eds), The Trypanosomiasis. CABI Publishing, Wallingford, pp. 139-180.
- Sinclair, B.J., Klok, C.J., Chown, S.L., 2004. Metabolism of the sub-Antarctic caterpillar *Pringleophaga marioni* during cooling, freezing and thawing. Journal of Experimental Biology 207, 1287-1294.

- Somero, G.N., 2005. Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Frontiers in Zoology* 2.
- Spicer, J.I., Gaston, K.J., 1999. *Physiological Diversity and its Ecological Implications*. Blackwell Science, Oxford.
- Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. *Science* 301, 65-65.
- Sutherst, R.W., 2004. Global change and human vulnerability to vector-borne diseases. *Clinical Microbiology Reviews* 17, 136-173.
- Taylor, P., 1977. The respiratory metabolism of the tsetse (Diptera: Glossinidae) in relation to temperature, blood-meal size and the pregnancy cycle. *Physiological Entomology* 2, 317-322.
- Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C. et al., 2004. Extinction risk from climate change. *Nature* 427, 145-148.
- Van Etten, J., 1982. Comparative studies on the diurnal activity pattern in two field and laboratory populations of *Glossina pallidipes*. *Entomologia Experimentalis et Applicata* 32, 38-45.
- Wilson, R.S., Franklin, C.E., 2002. Testing the beneficial acclimation hypothesis. *Trends in Ecology and Evolution* 17, 66-70.
- Woods, H.A., Harrison, J.F., 2002. Interpreting rejections of the beneficial acclimation hypothesis: when is physiological plasticity adaptive? *Evolution* 56, 1863-1866.
- Woods, R.E., Sgrò, C.M., Hercus, M.J., Hoffmann, A.A., 1999. The association between fluctuating asymmetry, trait variability, trait heritability, and stress: a multiply replicated experiment on combined stresses in *Drosophila melanogaster*. *Evolution* 53, 493-505.
- Yearsley, J.M., Kyriazakis, A., Gordon, I.J., Johnston, S.L., Speakman, J.R., Tolcamp, B.J. et al., 2005. A life history model of somatic damage associated with resource acquisition: damage protection or prevention? *Journal of Theoretical Biology* 235, 305-317.
- Zeilstra, I., Fischer, K., 2005. Cold tolerance in relation to developmental and adult temperature in a butterfly. *Physiological Entomology* 30, 92-95.

## **Appendix 1**



## Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica

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### Abstract

Daily changes in microclimate temperature and supercooling point (SCP) of Collembola were measured during summer at Cape Hallett, North Victoria Land, Antarctica. *Isotoma klovstadi* and *Cryptopygus cisantarcticus* (Isotomidae) showed bimodal SCP distributions, predominantly in the high group during the day and in the low group during the night. There were no concurrent diurnal changes in water content or haemolymph osmolality. By contrast, *Friesea grisea* (Neanuridae) had a unimodal distribution of SCPs that was invariant between daytime and nighttime. *Isotoma klovstadi* collected foraging on moss had uniformly high SCPs, which shifted towards the low group when the animals were starved for 2–8 h. When *I. klovstadi* was acclimated for five days with lichen or algae, SCPs were higher than if they were supplied with moss, while those that were starved (with free water or 100% relative humidity) displayed a trimodal SCP distribution. A variety of pre-treatments, including cold, heat, desiccation and slow cooling were ineffective at inducing SCP shifts in *C. cisantarcticus* or *I. klovstadi*. It is postulated that behavioural avoidance of low temperatures by vertical migration may be key in *I. klovstadi*'s short-term survival of nighttime temperatures. These data suggest that the full range of thermal responses of Antarctic Collembola is yet to be elucidated.

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### 1. Introduction

Cold tolerance is generally essential if arthropods inhabiting seasonally cold environments are to survive the winter. Indeed, much attention has been focused on wintering biology in these environments (Cannon and Block, 1988; Lee, 1991; Sømme, 1999; Danks, 2000; Sinclair et al., 2003), and the physiological processes that mediate seasonal changes in cold tolerance (Cannon and Block, 1988; Coulson et al., 1995b; Convey, 1996; Sinclair and Sjørnsen, 2001). However, attention has recently been increasingly focused on physiological changes over other periods, and it is now apparent that

physiological changes may be manifest at three broad time scales: (1) evolutionary time scales, leading to inter-specific and inter-population differences in cold tolerance abilities; for example, different populations of the gall fly *Eurosta solidaginis* (Baust and Lee, 1981), various *Hemideina* species (Sinclair et al., 1999), and insects in general (Addo-Bediako et al., 2000); (2) seasonal time scales, including summer–winter transitions (numerous examples in insects and other arthropods, see Bale (2002) and Sømme (1999) for recent reviews); and (3) short-term time scales, allowing arthropods to track changes in habitat temperature, for example on a daily basis (the rapid cold hardening (RCH) response, see Lee et al., 1987 and Kelty and Lee, 1999 for examples, and Bale, 2002, for review).

Rapid cold hardening responses in the strict sense refer only to changes in chill tolerance after a prior exposure to a sub-lethal low temperature (Lee et al.,

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1987), but as the dynamic nature of arthropod responses to temperature have become more apparent, the term has also been applied to changes in cold tolerance in response to other (non-cold) stresses (e.g. Coulson and Bale, 1991; Tammariello et al., 1999; Sinclair and Chown, 2003), to changes in freezing survival (Sinclair and Chown, 2003), and to shifts in supercooling point distribution (Worland and Convey, 2001). The presence of these dynamic responses in almost all groups that have been studied must lead to a re-evaluation of the way that an organism's response to its environment at any time scale is conceptualised. On a long-term scale, RCH means that predictions of responses to climate change based on static investigations of environmental tolerances must be made more flexible. This includes almost all published accounts of this nature, e.g. Bale (1994), Coulson et al. (1995a) and Sinclair (2001). On a medium-term scale, it is important to recognize that the physiology of overwintering insects during transitional periods of autumn, spring and, in terrestrial Antarctic habitats, summer, may be governed by rules different to those followed during the winter proper (Davey et al., 1992; Sinclair and Sjørnsen, 2001; Worland and Convey, 2001). Finally, on a short-term scale, it is important to assess the frequency, likelihood and responses to stresses that may occur on a daily basis (Sinclair et al., 2003).

Alongside mites, Collembola are one of two dominant groups of arthropods inhabiting ice-free areas on the Antarctic continent (Gressitt, 1967; Wise, 1967), and have received considerable attention, both as physiological models (Cannon and Block, 1988) and because of their role in the ecosystem (Janetschek, 1967; Block, 1982). Antarctic Collembola employ a cold tolerance strategy of freeze avoidance. Conveniently, the temperature at which they freeze (the supercooling point, SCP) is a valid measure of their lower lethal temperature, enabling SCP distributions to be used to examine population cold tolerance (Block et al., 1978; Sømme, 1981; Cannon, 1983; Cannon and Block, 1988; Sinclair and Sjørnsen, 2001; Worland and Convey, 2001), although this is not globally the case for Collembola (Holmstrup and Sømme, 1998; Worland et al., 1998; Hanc and Nedved, 1999). Supercooling points of Antarctic Collembola are often bimodal, a function of the nucleating effects of gut material (Sømme, 1981; Cannon and Block, 1988; Worland and Lukešová, 2000). This bimodal distribution may be modified through diet and gut clearance (Sømme, 1978; Cannon et al., 1985; Cannon, 1986; Cannon and Block, 1988; Worland and Lukešová, 2000), while the absolute value of SCP within the low and high groups can be conditional on hydration state (Block, 2002), carbohydrates and thermal hysteresis proteins (Cannon and Block, 1988; Sinclair and Sjørnsen, 2001). Although there appears to be a considerable body of literature on cold tolerance of Antarctic Collembola (Sømme, 1995; Hopkin, 1997), in reality, this research

is focused on Isotomidae (e.g. Sømme, 1986; Cannon and Block, 1988), and has been overwhelmingly located in the Maritime Antarctic (Block and Worland, 2001; Worland and Convey, 2001), in spite of the wide distribution of Collembola elsewhere on the continent (Gressitt, 1967). However, cold tolerance of continental Antarctic species has been addressed explicitly by Sømme (1978, 1986) and Sinclair and Sjørnsen (2001), while Pryor (1962), Janetschek (1970) and Fitzsimons (1971) address aspects of cold tolerance by Collembola in these habitats.

Although changes in SCP have been largely regarded as a seasonal phenomenon, Collembola are small and ectothermic, and their body temperatures consequently approximate those of their microhabitat. Numerous studies of Antarctic microclimate have shown variation in temperature at both seasonal and daily scales (Pryor, 1962; Janetschek, 1967; Davey et al., 1992; Marshall et al., 1995; Sinclair and Sjørnsen, 2001; Worland and Convey, 2001; Moorhead et al., 2002), and it is clear that Collembola might therefore be thermally challenged on a diurnal, as well as seasonal, timescale. Rapid cold hardening has been shown to occur at ecologically relevant timescales and cooling rates (Kelty and Lee, 1999), and Worland and Convey (2001) have shown on the Antarctic peninsula that SCPs, of the isotomid collembolan *Cryptopygus antarcticus* and two species of mites, closely track thermal changes in the microhabitat on a daily basis, and that these changes may be induced by pre-cooling, and slow cooling.

This paper investigates diurnal variation in SCP in three species of Antarctic Collembola occupying a small, ice-free area at Cape Hallett, North Victoria Land, Antarctica. The aims were to investigate the extent of diurnal variation in SCP by comparing nighttime and daytime SCP distributions in three species of Collembola, and to examine the cues inducing these changes by applying a variety of laboratory pre-treatments standard in RCH studies.

## 2. Methods

### 2.1. Study site and animals

The Cape Hallett ice-free area (Antarctic Specially Protected Area no. 106, <http://www.era.gs/resources/apa/>) is a small (ca. 72 Ha) ice-free area in North Victoria Land, Antarctica (72°19'S, 170°13'E). It consists of basalt screes and moraines, and approximately half of the ice-free area is occupied by an Adelie Penguin (*Pygoscelis adeliae*) colony, and consequently does not have any terrestrial plant or invertebrate community (M.B. Scott and B.J. Sinclair, unpublished). The area not occupied by penguins includes an undulating moraine-flat a few metres above sea level with moss and algal



communities, ephemeral meltwater ponds, streams and low-lying winter snowbanks, and a substantial scree slope with abundant lichen and occasional moss and algal communities (more abundant near the bottom of the slope). The scree slope rises steeply to approximately 300 m asl, and the lower portions of the scree slope and the algal flats are sparsely occupied by a Skua (*Catharacta maccormicki*) colony.

The site faces northwest, and on fine days receives direct sunlight from approximately 09:00 h until approximately 22:00 h (New Zealand Daylight Savings Time), during which the dark surfaces of the rocks can heat up considerably, while outside these hours the sun is low in the sky, behind the main ridge of the peninsula, and the site is shaded, resulting in a strong diurnal temperature cycle. Both daytime and nighttime temperatures depend on cloud cover and snow (C.J. Klok et al., personal observations).

There are three species of Collembola at Cape Hallett (Wise and Shoup, 1967): *Friesea grisea* (Schäffer) (Neanuridae), which is sparsely distributed through the entire area, with foci at the edges of moss banks, where we collected them. *Cryptopygus cisantarcticus* Wise (Isotomidae), which is found largely in moist algal flats, and *Isotoma klovstadi* Carpenter (Isotomidae), which is extremely abundant in vegetated areas on the scree slope (Pryor, 1962; Wise and Shoup, 1967).

All work was conducted from a tent-based field laboratory at Cape Hallett between November 9 2002 and January 27 2003, with the majority of work for the present study conducted from mid-December to January. Field generators, which typically ran for only 14 h per day, provided power. Consequently, the scope of the work, particularly regarding acclimation treatments, was limited by logistic capabilities.

## 2.2. Measurement of microclimate temperatures

Microclimate temperatures were measured every 10 min throughout the summer in triplicate beneath rocks representative of those that were turned to collect each species. In all cases, the stones were level with one another, on a similar substrate, and less than 1 m apart. For *C. cisantarcticus*, we chose rocks in an area of moist algal flat, and the rocks ranged in area from 67.3 to 127.3 cm<sup>2</sup>, and in thickness from 34 to 76 mm. *Friesea grisea* stones ranged from 33.3 to 382.2 cm<sup>2</sup> (reflecting the broad range of sizes present in that part of the habitat) and 23 to 57 mm thick, and were at the edge of a moss band in a small drainage line. *Isotoma klovstadi* stones were elevated approximately 10 m above the moss/algal flats on the scree slope, and ranged in area from 25.9 to 150.7 cm<sup>2</sup>, and in thickness from 12–74 mm. Temperatures were logged at the *F. grisea* and *C. antarcticus* sites by calibrated iButton Thermochron loggers (accurate to  $\pm 1.0$  °C, Dallas Semiconductors, Dallas, TX,

USA), and at the *I. klovstadi* site with a combination of an iButton and two channels of a Hobo H-8 logger with TMCx-HA thermistor probes (Onset Computer Corporation, Pocasset, MA, USA). Mean and standard deviation of temperatures under the three stones were calculated for each site.

## 2.3. Supercooling point determination

Collembola were collected with an aspirator from beneath rocks into dry plastic containers and returned to the laboratory tent within 5 min. Two or three springtails were carefully affixed to a 40-gauge Copper-Constantan (Type T) thermocouple with silicone grease, connected to an 8 channel Grant Squirrel SQ800 data logger (Grant Instruments, Cambridge, UK) interfaced to a PC. Temperature measurements were made continuously and averaged at 4 s intervals. This setup allowed the simultaneous measurement of 16–24 SCPs, making our methods comparable to the DSC technique employed by Worland and Convey (2001). Thermocouples and springtails were placed in 1.5 ml Eppendorf tubes in an aluminium block cooled by an electronically-controlled Peltier module setup which in turn used circulating ice water from a bucket as a heat sink. The Peltier system was developed specifically for the project, and is similar in principle to systems described elsewhere (Wharton and Rowland, 1984; Worland, 2000; Sinclair and Sjurson, 2001). Springtails were cooled at 0.5 °C/min from 0 to –36 °C (preliminary experiments indicated that SCP was not affected by varying the cooling rate between 0.8 and 0.05 °C/min, so this rate was used for all experiments), and SCPs were derived from the data file as the temperature recorded immediately prior to the upward inflection of temperature caused by the release of the latent heat of crystallization. Preliminary experiments exposing groups of animals from all three species to successively lower temperatures indicated that distributions of lower lethal temperatures (LLTs) closely matched SCP distributions measured concurrently (C.J. Klok and B.J. Sinclair, unpublished data). Thus, it was assumed that the SCP is a valid measure of LLT.

## 2.4. Diurnal variation in SCP

Diurnal variation in SCP was measured for each species by measuring the SCP of a group of individuals collected during the ‘day’ (when the study sites were receiving full sun, and always between 11:00 h and 16:30 h, when microclimate temperatures were around their peak), and animals collected at ‘night’ (when the study area was in shadow and microclimate temperatures were low; between 23:30 and 04:30 h). SCPs were measured for each species (in randomized order) on two nights and days (consecutive unless interrupted by poor weather) in mid- (12 & 13) and late- (21–24) December 2002.

### 2.5. Diet effects on SCP of *Isotoma klovstadi*

To investigate the effects of diet on SCP in *Isotoma klovstadi*, *I. klovstadi* were collected from beneath stones in the field during the 'day' (see above for definition) and acclimated for five days at 5 °C in a Sable Systems PTC-001 Peltier-effect Temperature cabinet (Sable Systems Inc. Henderson, Nevada, USA) with 24 h light provided by a battery powered white Light-emitting Diode lamp (Black Diamond Ion Headlamp, Black Diamond Equipment Ltd, Salt Lake City, UT, USA). Because the Peltier cabinet was dependent on generator power, it was switched off during periods of poor weather and often at night (between 22:00 and 08:00 h). The internal temperature was prevented from dropping below -2 °C by the addition of a hot water bottle, and all treatments were run concurrently (and positions within the cabinet randomized) to reduce the likelihood of an effect due to the action of the cabinet. Collembola were placed into constant-humidity acclimation chambers (which consisted of mesh-lidded containers within larger airtight jars that contained distilled water to provide 100% RH). Six feeding treatments were used, and wherever possible, these were duplicated: (1) starved—Collembola in 100% RH, but with no access to food or free water. (2) Liquid water—Collembola with no food, but access to liquid water in the form of wet filter paper. (3) Algae1—Collembola provided with moist *Prasiola crispa* algae collected from the algal flats away from the scree slope. (4) Algae2—Collembola collected while feeding from the surface of algae (possibly also *P. crispa*) on the scree slope, and provided with the same algae. (5) Lichen—Collembola provided with pieces of *Caloplaca* sp., a common orange lichen from the scree slope. (6) Moss—Collembola provided with living Moss (*Bryum* sp.) collected from the moss flats near the base of the scree slope. This moss was free of rotting material or visible epiphytic algae. After 5 days, living Collembola (in excess of 80% of individuals originally allocated to each chamber, with drowning in water droplets the main cause of mortality) were used for SCP determination as described above.

The time scale over which starvation (and consequent gut-clearing) could affect the SCP in *Isotoma klovstadi* was investigated. For these experiments, actively feeding *I. klovstadi* was collected from the surface of vegetation at ca. 11:00 h and SCPs were determined on field-fresh individuals. Other individuals were placed in groups (ca. 50 animals) in acclimation chambers at 100% RH and 5 °C with 24 h light, but with no access to food or liquid water, and SCPs determined after 1, 2, 4 and 8 h.

### 2.6. Rapid cold hardening

An attempt was made to induce the observed diurnal SCP changes in *C. cisantarcticus* and *I. klovstadi* by pre-

treating groups of animals with a variety of temperature and environmental cues. Groups of ca. 30 animals were collected from the edges of stones near vegetation (*C. cisantarcticus*), or from actively feeding groups on the surface of vegetation (*I. klovstadi*), and exposed to 2 h pre-treatments (unless otherwise stated, in 1.5 ml Eppendorf tubes with a small piece of moist filter paper). These treatments were (1) Control—maintained at 5 °C for 2 h. (2) Temperature treatments—groups kept at 28, 0, -2 (*C. cisantarcticus* only), -5 or -9 °C (*I. klovstadi* only) for 2 h. (3) -5 °C with delay (*I. klovstadi* only)—a 2 h pre-treatment at -5 °C, followed by 2 h recovery at +5 °C (see Sinclair and Chown, 2003 for rationale). (4) Slow cooling—groups of springtails for which SCP was determined at 0.1 °C/min (*I. klovstadi* only) and which were pre-treated with a slow cooling regime (1 °C/h from +5 to -5 °C, as used by Worland and Convey, 2001). (5) Desiccation (*I. klovstadi* only)—Collembola exposed to a 2 h desiccation stress (an acclimation chamber with silica gel in place of distilled water, giving <5% RH). After each pre-treatment, SCP determination was carried out as above. In all cases, a concurrent field fresh (FF) SCP determination was made. The variety in treatments for the different species reflects the availability of material, and the constraints of the field site.

### 2.7. Analysis and interpretation of SCP distributions

Supercooling points are not easily interpreted with conventional statistics, because they are often bimodal (Spicer and Gaston, 1999). Several different analytical approaches were used in an attempt to retain the variation in the data, and in some cases, statistical analysis is eschewed in favour of common-sense interpretation of observed patterns. *Friesea grisea* did not show bimodal SCPs, but both *C. cisantarcticus* and *I. klovstadi* did. Although techniques do exist to determine breakpoints in bimodal distributions (e.g. Aldrich, 1987), it was simplest to decide on a high group-low group divider by collating all SCP data and visually assessing a histogram for an obvious break (e.g. Block and Sømme, 1982; Sømme and Block, 1982). The high-low group division was thus set at -16 °C for *C. cisantarcticus*, and at -18 °C for *I. klovstadi*. Median SCPs for high and low groups were compared using Kruskal-Wallis Rank ANOVA with multiple comparisons (Statistica 6.0, Statsoft, Oklahoma, USA), while non-independence of high- or low- group SCP frequencies among treatments were compared using chi-square contingency tables.

### 2.8. Water content and haemolymph osmolality determination

Water content of *Isotoma klovstadi* was measured gravimetrically for groups of 20 animals at midday and midnight (2–6 December, 2002), and also for each treat-

ment of the rapid cold hardening experiments. Collembola were placed in a pre-weighed Eppendorf vial, weighed ( $\pm 0.5 \mu\text{g}$ ) using a Mettler-Toledo UMX-2 microbalance before being dried for 2 h at ca.  $50^\circ\text{C}$  (maintained by judicious use of a cast aluminium pot with lid and a Primus cooker), and stored for 24 h over silica gel at ca.  $5^\circ\text{C}$  before dry mass was determined with the microbalance.

Haemolymph osmolality was determined for individual *I. klovstadi* individuals in the RCH experiment using a calibrated Clifton Nanoliter Osmometer (see Sinclair and Sjørnsen, 2001; Sinclair and Chown, 2002 for methods).

### 3. Results

#### 3.1. Diurnal variation in SCP

Supercooling points of both *Isotoma klovstadi* and *Cryptopygus cisantarcticus* were bimodally distributed, with a pronounced diurnal pattern of SCP (Figs. 1 and 2). By contrast, although right-skewed, SCPs of *Friesea grisea* were clearly unimodal, and did not show any discernible diurnal variation (Fig. 3). Mean SCPs for all species and times are summarised in Table 1. There was a heavy snow and wind storm on the 22nd of December, resulting in increased insulation, reduced amplitude of the temperature cycle, and the causative cloud continued

this pattern for several days. Thus, the temperature differences between day and night were reduced in the late December period compared to mid-December.

*Isotoma klovstadi* collected in mid-December displayed a significant change in SCP high group:low group ratio (HG:LG) from 14:17 during the night to 34:2 during the day (Fig. 1b,  $\chi^2 = 19.9$ ,  $\text{df} = 1$ ,  $p < 0.001$ ). In late December, daytime measurements included animals collected foraging from vegetation as well as from under stones, with the latter showing a clear bimodal distribution, whereas SCPs of foraging animals unimodal, with high SCPs. Nighttime SCP frequencies were strongly skewed towards the low group. A chi-square test of independence showed that the frequencies of SCPs were not distributed independently between day and night and the two habitats ( $\chi^2 = 59.6$ ,  $\text{df} = 2$ ,  $p < 0.001$ ).

*Cryptopygus cisantarcticus* also showed a strong diurnal change in distribution of SCPs both at mid December and late December (Fig. 2), and this difference was again not independent of time of day in mid December ( $\chi^2 = 6.6$ ,  $\text{df} = 1$ ,  $p < 0.025$ ) but in late December, there was no significant difference between day and night frequencies ( $\chi^2 = 2.0$ ,  $\text{df} = 1$ ,  $p > 0.1$ ).

*Friesea grisea* did not show any change in SCP distributions when day and night were compared (Fig. 3, Table 1), and the very slight differences in mean SCP between night and day were both within 1 standard error of the mean, and not consistent between the two sampling points.

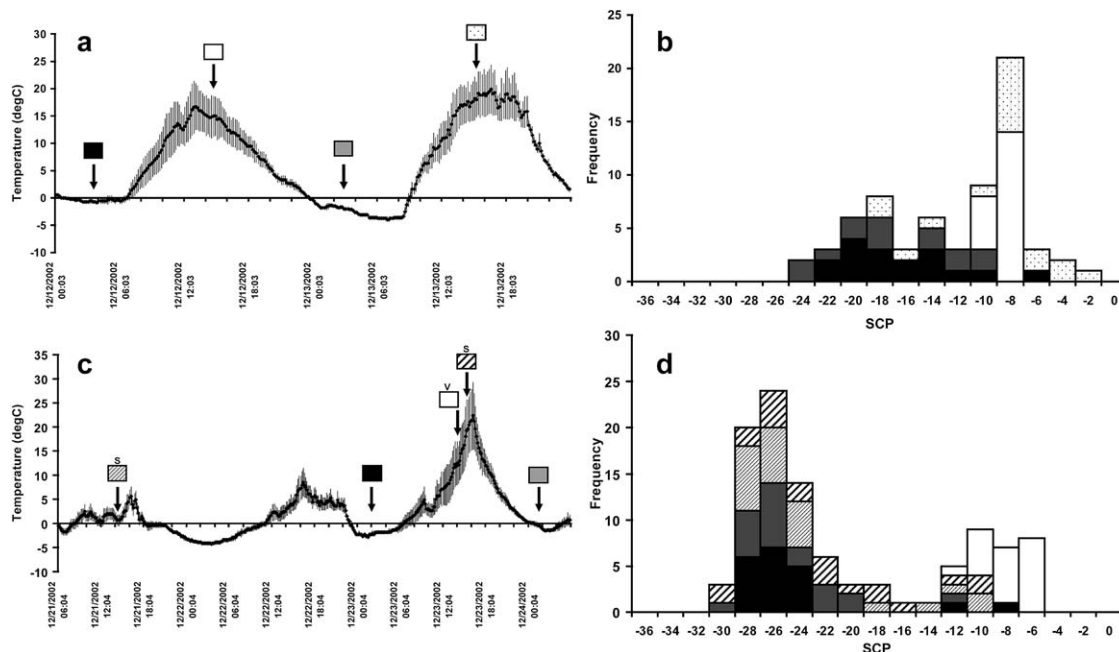


Fig. 1. Diurnal variation in microclimate temperature (mean  $\pm$  s.d. of three data loggers) and supercooling point of *Isotoma klovstadi* from Cape Hallett. Mid-December: (a) microclimate temperature, (b) SCP distribution. Late-December: (c) microclimate temperature, (d) SCP distribution. Arrows and blocks indicate points in microclimate record where Collembola were collected for SCP determination, and correspond to patterns in the histogram. s, animals collected from stones; v, animals collected while foraging on vegetation. See Table 1 for a statistical summary of the SCP values and HG:LG ratios.

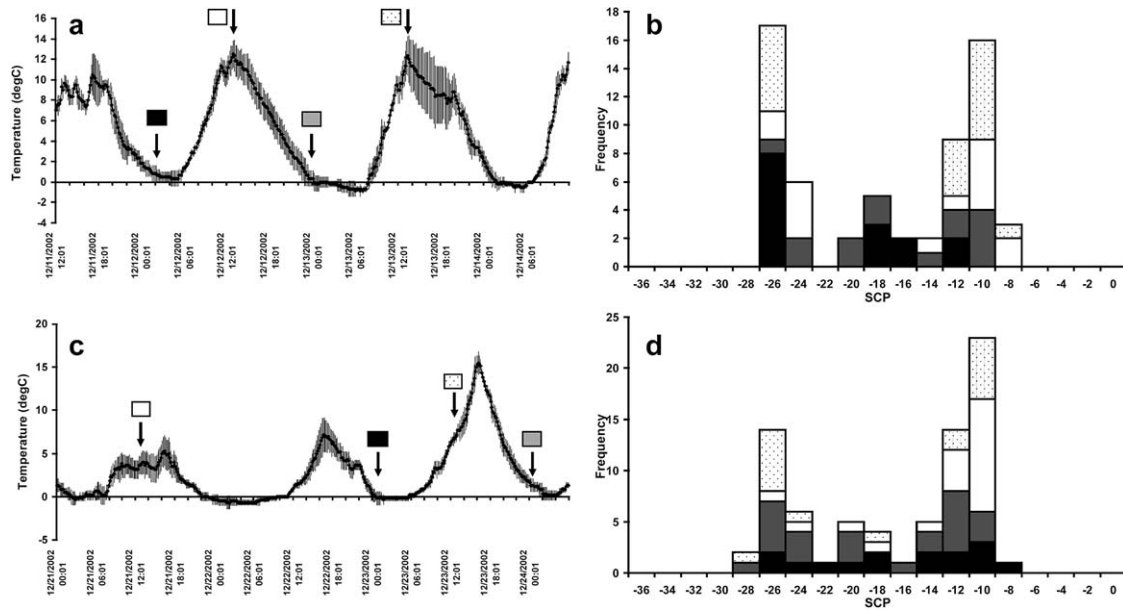


Fig. 2. Diurnal variation in microclimate temperature (mean  $\pm$  s.d. of three data loggers) and supercooling point of *Cryptopygus cisantarcticus* from Cape Hallett. Mid-December: (a) microclimate temperature, (b) SCP distribution. Late-December: (c) microclimate temperature, (d) SCP distribution. Arrows and blocks indicate points in microclimate record where Collembola were collected for SCP determination, and correspond to patterns in the histogram. See Table 1 for a statistical summary of the SCP values and H:L group ratios.

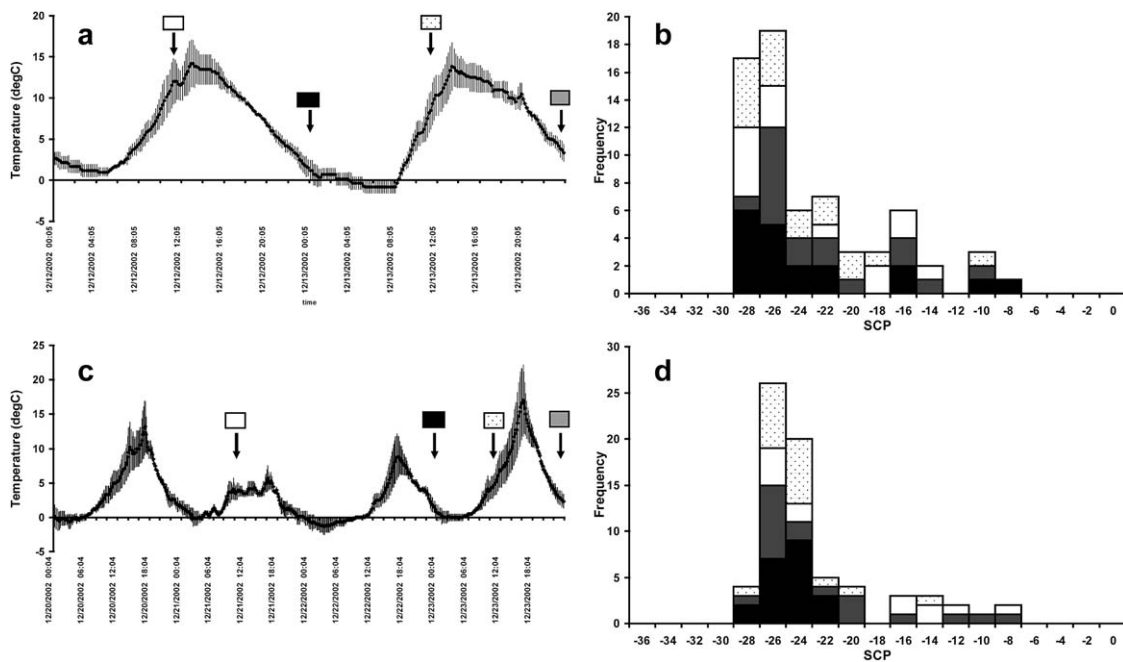


Fig. 3. Diurnal variation in microclimate temperature (mean  $\pm$  s.d. of three data loggers) and supercooling point of *Friesia grisea* from Cape Hallett. Mid-December: (a) microclimate temperature, (b) SCP distribution. Late-December: (c) microclimate temperature, (d) SCP distribution. Arrows and blocks indicate points in microclimate record where Collembola were collected for SCP determination, and correspond to patterns in the histogram. See Table 1 for a summary of the SCP values and HG:LG ratios.

### 3.2. Diet effects on SCP

*Isotoma klovstadi* individuals collected while foraging on moss and algal surfaces had unimodal distributions of SCP (data not shown), and their mean SCPs did not differ significantly (moss:  $-8.5 \pm 0.2$  °C  $n = 46$ , algae:

$-8.2 \pm 0.1$  °C  $n = 34$ ;  $t_{78} = 1.28$ ,  $p = 0.2$ ). When acclimated with different food sources, there were significant differences in median SCP between the groups. Animals held in high humidity (Fig. 4d), with free water (Fig. 4a) and with algae (Fig. 4b) showed an essentially trimodal distribution of SCPs, while those held with



Table 1

Summary of supercooling point distributions and means for three species of Collembola at Cape Hallett. Low group included those individuals with SCP < -18 °C (*I. klovstadi*) or < -16 °C (*C. cisantarcticus*)

	Time of day	HG:LG ratio	High mean ± s.e. (°C)	Low mean ± s.e. (°C)
<i>Isotoma klovstadi</i>				
Mid-December	Day	34:2	-9.0 ± 0.4	-18.7
	Night	14:17	-13.6 ± 0.7	-20.7 ± 0.5
Late-December	Day (from algae)	20:0	-9.0 ± 0.4	
	Day (from stones)	8:35	-12.4 ± 0.8	-26.1 ± 0.5
	Night	3:38	-11.1 ± 1.6	-26.5 ± 0.4
<i>Cryptopygus cisantarcticus</i>				
Mid-December	Day	21:12	-11.1 ± 0.3	-26.5 ± 0.3
	Night	9:20	-12.0 ± 0.5	-23.0 ± 0.9
Late-December	Day	24:13	-11.5 ± 0.2	-25.1 ± 0.9
	Night	19:20	-12.4 ± 0.4	-23.9 ± 0.8
<i>Friesea grisea</i> <sup>a</sup>				
Mid-December	Day	<i>n</i> = 31		-24.4 ± 0.9
	Night	<i>n</i> = 36		-23.6 ± 0.9
Late-December	Day	<i>n</i> = 30		-23.2 ± 1.0
	Night	<i>n</i> = 40		-24.1 ± 0.7

<sup>a</sup> *Friesea grisea* did not display a bimodal SCP distribution, so only a single mean and standard error is presented.

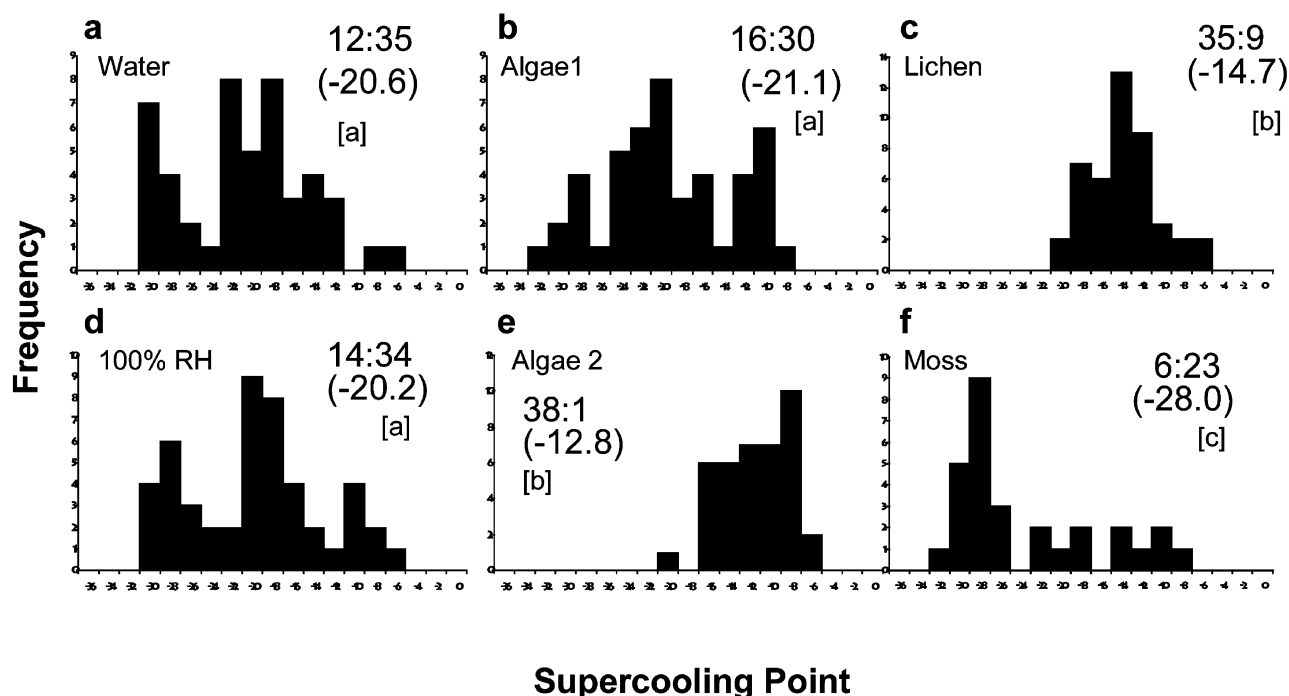


Fig. 4. Supercooling point distributions of *Isotoma klovstadi* in response to 5 days acclimation under different feeding conditions. High group:low group ratios of SCPs are shown (SCPs below -18 °C were included in the low group), and medians are in parentheses. There were significant differences in median SCP between the groups (Kruskal–Wallis  $H(df = 5, n = 253) = 80.7, p < 0.001$ ), treatments with different letters [in square brackets] have medians significantly different from one another (multiple range test results,  $p < 0.05$ ).

algae (which had been collected while foraging, Fig. 4e) and lichen (Fig. 4c) showed a unimodal distribution, although in the case of lichen, this seems to have involved a downward shift in SCPs. Supercooling points of those animals acclimated with moss showed a strong

shift towards the low group (Fig. 4f). *Isotoma klovstadi* collected while foraging showed a shift in SCP with starvation, with more than 20% of individuals in the low group after 2 h, and more than 30% after 8 h (Fig. 5). In addition, the mean high group SCP had decreased from

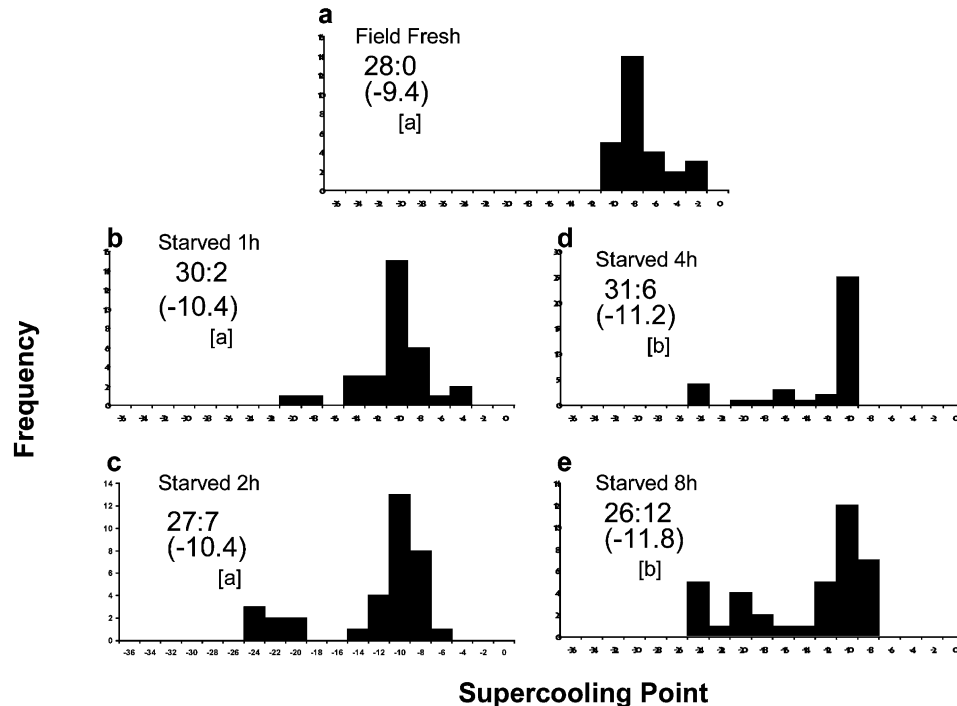


Fig. 5. Supercooling point distributions of *Isotoma klovstadi* starved for 1–8 h and field-fresh. High group:low group ratios of SCPs are shown (SCPs below  $-18$  °C were included in the low group), and medians are in parentheses. There were significant differences in median SCP between the groups (Kruskal–Wallis  $H(df = 4, n = 169) = 51.9, p < 0.001$ ), treatments with different letters [in square brackets] have medians significantly different from one another (multiple range test results,  $p < 0.05$ ).

$-8.2 \pm 0.4$  when field fresh to  $-10.5 \pm 0.4$  °C after 2 h of starvation, and the medians of those starved for 4 and 8 h were significantly lower than the field fresh group (Fig. 5).

### 3.3. Rapid cold hardening effects on SCP

Exposure of *I. klovstadi* to RCH treatments did not result in any substantial increase in the frequency of low group SCPs (Fig. 6). Control animals (simply kept for 2 h at 5 °C), as well as animals exposed to 28 °C and desiccation stress all had median SCPs slightly lower than that of field fresh individuals (Kruskal–Wallis  $H(df = 9, n = 220) = 32.98, p = 0.0001$ , multiple range tests  $p < 0.02$ ), suggesting that there may be a starvation effect at higher temperatures (see also Fig. 5), and both the slow cooling treatments (0.1 °C/min and 1 °C/h) and desiccation groups had medians significantly different from the control (Kruskal–Wallis test above, multiple range test,  $p < 0.02$ ). However, the 0.1 °C/min and 1 °C/h medians showed shifts in opposite directions (Fig. 6), suggesting that there is no overarching directional response.

Although data for this species is less comprehensive, *Cryptopygus cisantarcticus* also showed no clear response to the RCH treatments (Fig. 7). In mid December, the 1 °C/h treatment actually resulted in a higher median SCP than field fresh individuals (although

HG:LG ratio remained roughly constant) (Kruskal–Wallis  $H(df = 6, n = 194) = 20.43, p = 0.0023$ ), and in January, the control groups had a much higher frequency of low group SCP and also a lower median (Fig. 7).

### 3.4. Water content and haemolymph osmolality

There was no significant difference between the water content of *Isotoma klovstadi* collected during the day ( $2.43 \pm 0.16$  g water/g dry mass) and night ( $2.23 \pm 0.21$  g water/g dry mass) (ANCOVA:  $F_{1,21} = 0.133, p = 0.72$ ).

Of the rapid cold hardening treatments on *I. klovstadi*, desiccation resulted in water content that was significantly lower than the control and 28 °C treatments (Table 2), while haemolymph osmolality in this species was significantly elevated as a result of the desiccation treatment (Table 2).

## 4. Discussion

Terrestrial Antarctic habitats are poorly thermally buffered and, in spite of 24 h daylight, show strong diurnal cycles in temperature (Worland and Convey, 2001). Thus, it is essential for the small ectotherms that inhabit these habitats to be able to survive not only the long cold winter, but also the daily temperature fluctuations

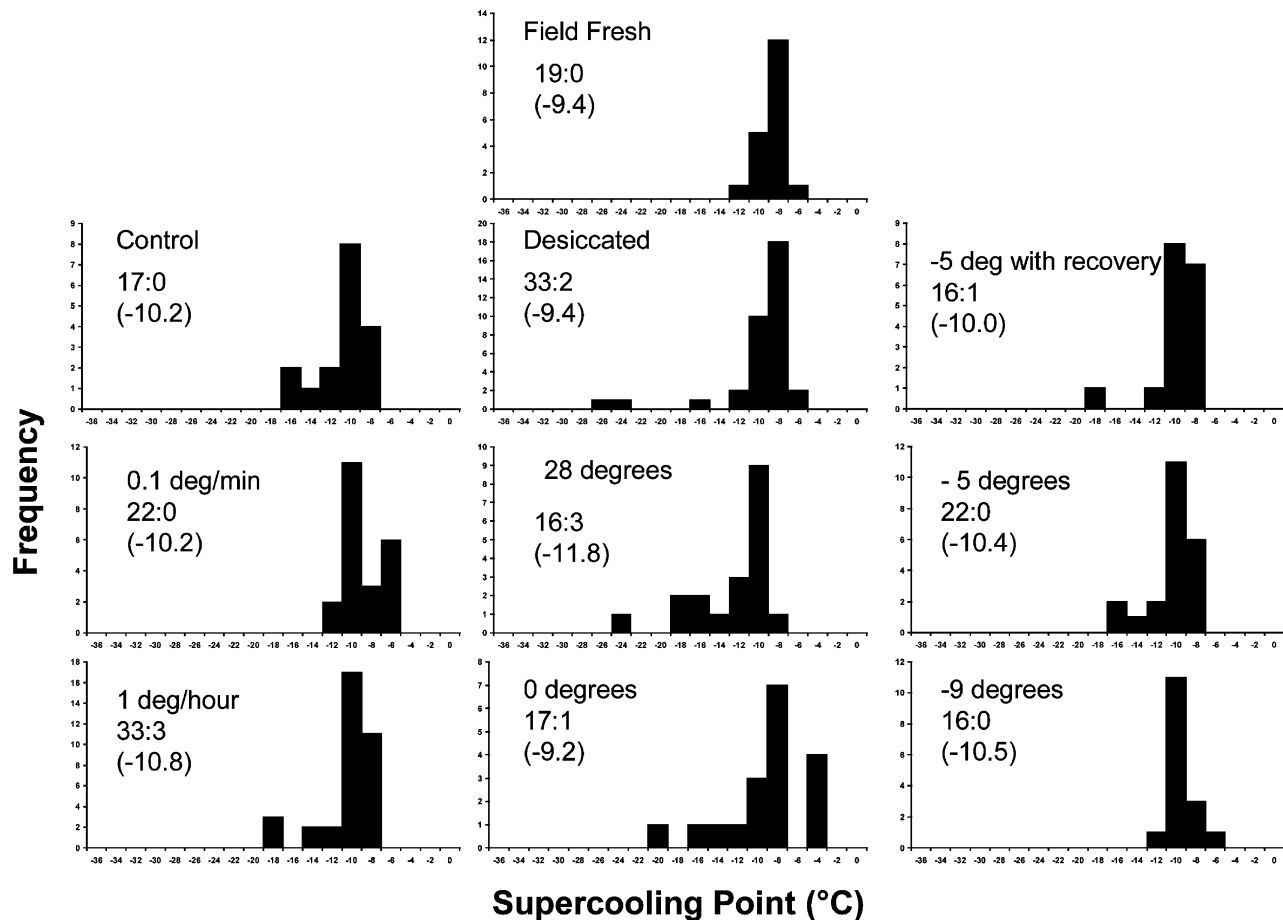


Fig. 6. Supercooling point distributions of *Isotoma klovstadi* in response to rapid cold hardening treatments. High group:low group ratios (low group includes SCPs below  $-18^{\circ}\text{C}$ ) and median SCP (in parentheses) are shown.

during the summer, while maintaining growth and reproduction. The strategies to achieve this are only beginning to be explored, but include cryoprotective dehydration in nematodes (Wharton et al., 2003), behavioural avoidance (Leather et al., 1993), and rapid cold hardening (Kelty and Lee, 2001; Worland and Convey, 2001). Here, we present results for three species of Collembola in a continental Antarctic habitat, which show three somewhat different strategies, none of which matches the rapid supercooling point change described for *Cryptopygus antarcticus* by Worland and Convey (2001).

#### 4.1. Diurnal cycles in supercooling point

*Isotoma klovstadi* has a diurnal cycle in SCP distribution, with a pronounced shift to the low group at night. The intensity of this shift appears in part to be mediated by the amplitude of the current temperature cycle (the extent of the shift was greater in mid than late December), however, diet also plays a role. If only foraging animals (presumably with material in the gut) are selected, then SCP is unimodal and high, and this effect begins to diminish after only 2 h of starvation (Fig. 5).

Nevertheless, total starvation, and a variety of food sources had only a partial effect on SCP distributions (Fig. 4), suggesting that diet and starvation alone are not adequate to explain the diurnal shift in SCP distribution in this species. However, if animals are collected from beneath stones, even during the day, there is a substantial low group present (Fig. 1), suggesting that even during the day a considerable portion of the population are not feeding.

Rapid supercooling point change in Antarctic Collembola was first described for *Cryptopygus antarcticus* on the Antarctic peninsula (Worland et al., 2000; Worland and Convey, 2001). Here, we find a significant diurnal shift in SCP distribution for its congener *C. cisantarcticus* from a habitat on the opposite side of the continent. The lack of difference between night and day SCP in late December, following a period of bad weather (Fig. 2), compared to mid December (when the weather was clear and sunny) is suggestive of a strong relationship between SCP distribution and immediate environmental conditions. However, as for *I. klovstadi*, there was always a significant low group present, during both day and night, which also suggests some degree of bet-hedg-

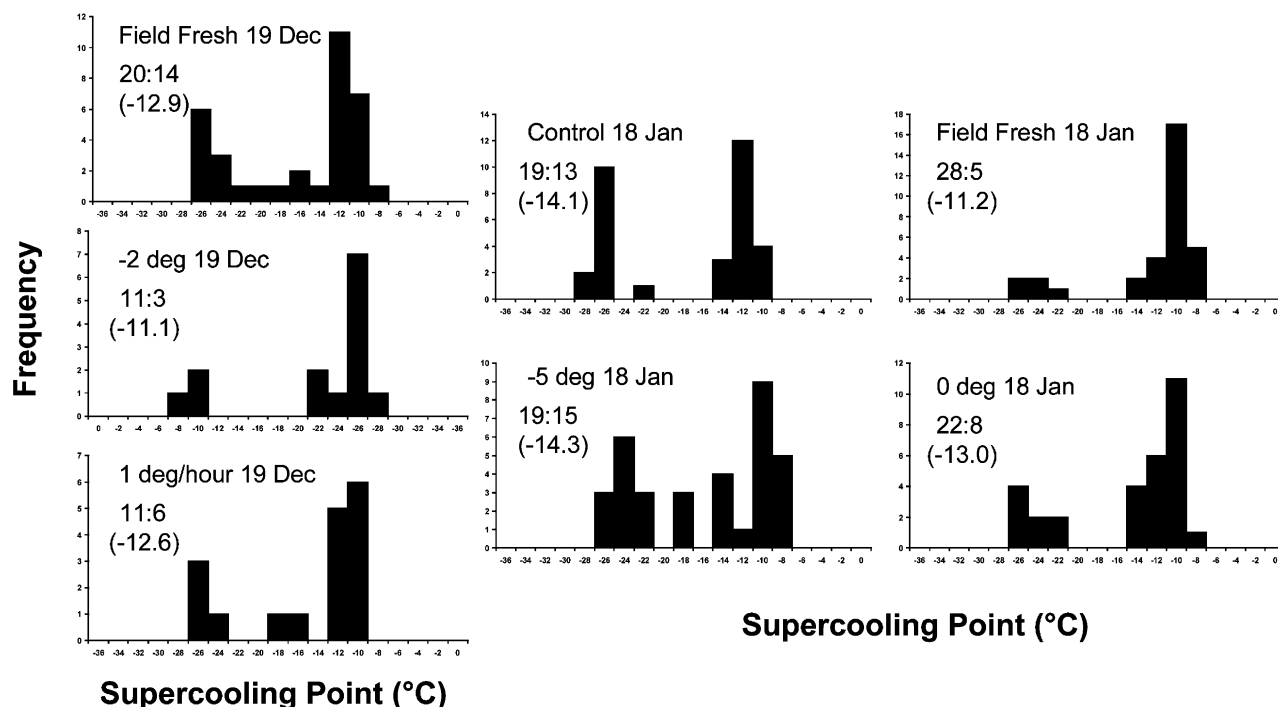


Fig. 7. Supercooling point distributions of *Cryptopygus cisantarcticus* in response to rapid cold hardening treatments. High group:low group ratios (low group includes SCPs below  $-16^{\circ}\text{C}$ ) and median SCP (in parentheses) are shown.

Table 2

Water content and haemolymph osmolality of *Isotoma klovstadi* in response to rapid cold hardening treatments. See methods for full description of treatments. Differences from control treatments at  $p < 0.05$  (\*) and  $p < 0.001$  (\*\*) (by ANCOVA, in the case of water content) are indicated. In addition, water contents of animals exposed to desiccation and  $28^{\circ}\text{C}$  were significantly different ( $p < 0.02$ )

Treatment	Mean $\pm$ s.e. (n) water content (g $\text{H}_2\text{O}$ /g dry mass)	Mean $\pm$ s.e. (n) haemolymph osmolality (mOsm)
Field fresh	$2.00 \pm 0.03$ (3) <sup>a</sup>	$666 \pm 7$ (10) <sup>b</sup>
Control	$2.26 \pm 0.08$ (3)	$708 \pm 10$ (5)
1 $^{\circ}\text{C}/\text{h}$		$688 \pm 15$ (5)
0 $^{\circ}\text{C}$	$1.64 \pm 0.04$ (3)	$714 \pm 13$ (10)
-5 $^{\circ}\text{C}$	$1.48 \pm 0.10$ (3)	$741 \pm 15$ (6)
-5 $^{\circ}\text{C}$ with 2 h recovery	$1.79 \pm 0.12$ (3)	$728 \pm 15$ (5)
-9 $^{\circ}\text{C}$	$1.65 \pm 0.14$ (3)	$698 \pm 10$ (5)
Desiccation	$0.80 \pm 0.20$ (6)*	$998 \pm 30$ (10)**
28 $^{\circ}\text{C}$	$1.67 \pm 0.04$ (3)	$699 \pm 6$ (5)

<sup>a</sup> From samples collected 21 December 2002.

<sup>b</sup> From samples collected 23 December 2002.

ing, particularly if SCP distribution is indeed largely driven by feeding status.

*Friesea grisea* is a near-circumantarctic species, and also occurs in sympatry with *C. antarcticus* on the Antarctic peninsula (Wise, 1967; Convey et al., 1999). At Cape Hallett, we found no evidence of a difference in SCP distribution between night and day (Fig. 3) on either

sampling date. This seems to imply that *F. grisea* either has a nucleator-free diet (other species in this genus are carnivorous (Hopkin, 1997), which may result in a low-nucleator diet), or that it has some very strong mechanism to avoid ice nucleation by the gut material. However, another possibility is simply that the feeding population of *F. grisea* is not to be found under stones, but is, instead, in the soil. There appears to be a negative trend between the number of *F. grisea* found on stones and the number found in soil at a site, suggesting that this may indeed be the case (more animals on stones mean that less of the population is in the soil feeding), but a correlation of number of individuals on stones and from soil within a  $25 \times 25$  cm plot was not statistically significant ( $r = -0.25$ ,  $p \gg 0.05$ ,  $n = 16$  sites, M.B. Scott and B.J. Sinclair, unpublished data).

#### 4.2. Diet effects on SCP of *Isotoma klovstadi*

Most of our evidence points to *I. klovstadi* being primarily an algal consumer (see also Pryor, 1962), since SCP remains highest when acclimated with algae or lichen (Fig. 4), and although *I. klovstadi* is often found on the surface of moss, when placed with clean, living moss, there is little evidence that feeding occurs. However, the difference between Algae1 (animals collected and acclimated with algae from algal flats—*C. cisantarcticus* habitat where *I. klovstadi* is not found), and Algae2 (animals collected from feeding on algae and fed same algae) (Fig. 4) suggests that (1) there is some dietary



preference or difference between the nucleator content of algae from the two sites and/or (2) that animals that are non-feeding take more than 5 days to change their status. A second unexpected observation is the trimodal SCP distribution that appears with acclimation in three of the treatments (Fig. 4a,b,d). Trimodal SCP distributions were not observed at any point in field fresh samples, and the position of the middle mode (by visual assessment), is consistent between water (ca.  $-19^{\circ}\text{C}$ ), 100% RH (ca.  $-20^{\circ}\text{C}$ ), and the Algae1 group (ca.  $-20^{\circ}\text{C}$ ). This middle peak may be formed by a transition between high and low (which implies that it is a continuum, not a bimodality), or by some other biochemical state induced by lack of nutrition in these individuals.

In addition, although Fig. 5 shows that a substantial proportion of individuals (20%) shift to the low group after 2 h of starvation, after 8 h this proportion is scarcely higher, and Fig. 4d shows that after 5 days of starvation there is still a definite high SCP group; although we cannot preclude the possibility that these animals were eating faecal pellets, this has not been reported in Antarctic Collembola (Hopkin, 1997). This suggests that although starvation does have an effect on SCP, it is not sufficient to account for wholesale changes in SCP frequencies.

#### 4.3. Induction of SCP change—rapid cold hardening?

We found no evidence of an inducible rapid cold hardening response in either *Cryptopygus cisantarcticus* or *Isotoma klovstadi*. In both species, control (starved for 2 h at  $5^{\circ}\text{C}$ ) animals had slightly lower SCPs than field fresh, suggesting that starvation can play a role in SCP modification, and slowly cooled *C. cisantarcticus* also displayed modified SCP distributions, although this was not in a consistent direction. Unsurprisingly, desiccation (which was shown to increase haemolymph osmolality substantially) did result in a lowering of the median SCP in *I. klovstadi*, however, so did the  $28^{\circ}\text{C}$  treatment (in which we prevented water loss by the addition of moist filter paper). Since the  $28^{\circ}\text{C}$  treatment is close to the critical thermal maximum of this species (ca.  $33^{\circ}\text{C}$ , C.J. Klok, unpublished data), it is likely that some sort of stress response is being enacted. However, heat shock proteins, which have been implicated in cross-tolerance in several other species (Bayley et al., 2001, but see Kelty and Lee, 2001; Tammariello et al., 1999), and presumed important in heat effects on freeze tolerance in the sub-Antarctic caterpillar *Pringleophaga marioni* (Sinclair and Chown, 2003) are unlikely to make a contribution to SCP modification. Investigations into the heat shock response in insects have focused largely on heat shock proteins (Denlinger et al., 1991; Denlinger and Yocum, 1998), although carbohydrates (which may have an effect on SCP) have been implicated in some species (Wolfe et al., 1998; Salvucci, 2000).

#### 4.4. Responses to diurnal temperature variation in Antarctic Collembola

Diurnal variation in temperature is a feature of all Antarctic Terrestrial habitats for which microclimate temperature data are available (Davey et al., 1992; Marshall et al., 1995; Sinclair and Sjørnsen, 2001; Worland and Convey, 2001; Doran et al., 2002), requiring some form of adaptive response in cold tolerance. To date, the only mechanism proposed for freeze-avoiding arthropods has been the rapid supercooling point changes observed by Worland and Convey (2001). Here, it is shown that although two of the three species of Collembola at Cape Hallett display a diurnal pattern in supercooling points, this is not inducible by a variety of temperature (and other environmental stress) treatments, although in *I. klovstadi* there is some evidence that the response, in the field at least, may be mediated by temperature. By contrast, *Friesea grisea* does not apparently show any diurnal cycle in SCP at Cape Hallett.

In addition to physiological responses, behaviour can also be important in avoiding cold (Leather et al., 1993). At Cape Hallett, the algal/moss flats where *F. grisea* and *C. cisantarcticus* are predominantly found are on permafrost-underlain, sorted and compacted glacial tills offering minimal opportunity for vertical migration by Collembola. However, the loose scree slopes where *I. klovstadi* is abundant offer much more of a three dimensional structure, and the opportunity to migrate tens of centimeters below the surface, which is likely to provide significant thermal buffering (Ružicka et al., 1995) describe cold air drainage in scree microhabitats, but in this case, the blocks are too small to allow airflow through the slope). We observed that throughout the duration of our field work, *I. klovstadi* were more easily collected in large numbers during the day than at night, and that during the night, stones would have to be moved to allow access to a depth of 30 cm or more to collect animals (B.J. Sinclair, J.S. Terblanche, M.B. Scott and C.J. Klok, unpublished observations). In addition, *I. klovstadi* have a very low critical thermal minimum: the animals enter chill coma at around  $-12^{\circ}\text{C}$  (C.J. Klok, unpublished data, see also a brief mention by Pryor, 1962), and it is proposed that the main protective mechanism of *I. klovstadi* to potentially lethal diurnal temperature minima is in fact behavioural, and that individuals migrate downwards in the scree in response to decreasing temperatures. Although temperatures are likely to be buffered under stones in habitats that *C. cisantarcticus* occupies, it is less clear whether there is a diurnal behavioural cycle, and whether this would be adequate to provide significant thermal buffering.

Thus, it is proposed that Antarctic Collembola may utilise three different mechanisms to survive diurnal temperature minima: rapid supercooling point modification (*C. antarcticus*, described by Worland and Con-

vey, 2001) behavioural avoidance (*I. klovstadi*, this paper) and perennially low SCPs (*F. grisea*, this paper, and possibly also *Gomphiocephalus hodgsoni* (Hypogastruridae), see Sinclair and Sjørnsen (2001), and *Cryptopygus sverdrupi* see Sømme, 1986). In addition, both *I. klovstadi* and *C. cisantarcticus* displayed diurnal changes in SCP distributions, but we could not induce these changes in the laboratory. The rapid supercooling point modification described by Worland and Convey (2001), whilst present in other Antarctic Collembola, might not always be a direct response to diurnal microclimate variation, but might rather be correlated with this variation in a fashion that is not directly causal. It is apparent that, in spite of nearly three decades' effort, the range of responses to temperature in Antarctic Collembola has not been fully explored, and especially not for continental species.

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### References

- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* 267, 739–745.
- Aldrich, J.C., 1987. Graphical analyses of bimodal frequency distributions with an example using *Cancer pagurus*. *Journal of Zoology, London* 211, 307–319.
- Bale, J.S., 1994. Indices of insect cold tolerance in an era of climate change. In: Leather, S.R., Watt, A.D., Mills, N.J., Walters, K.F.A. (Eds.), *Individuals, Populations and Patterns in Ecology*. Intercept, Hampshire, pp. 45–60.
- Bale, J.S., 2002. Insects and low temperatures: from molecular biology to distributions and abundance. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 357, 849–861.
- Baust, J.G., Lee, R.E. Jr., 1981. Divergent mechanisms of frost-hardiness in two populations of the gall fly, *Eurosta solidaginis*. *Journal of Insect Physiology* 27, 485–490.
- Bayley, M., Petersen, S.O., Knigge, T., Kohler, H.R., Holmstrup, M., 2001. Drought acclimation confers cold tolerance in the soil collembolan *Folsomia candida*. *Journal of Insect Physiology* 47, 1197–1204.
- Block, W., 1982. The Signy Island terrestrial reference sites: XIV. Population studies on the Collembola. *British Antarctic Survey Bulletin* 55, 33–49.
- Block, W., 2002. Interactions of water, ice nucleators and desiccation in invertebrate cold survival. *European Journal of Entomology* 99, 259–266.
- Block, W., Sømme, L., 1982. Cold hardiness of terrestrial mites at Signy Island, Maritime Antarctic. *Oikos* 38, 157–167.
- Block, W., Worland, M.R., 2001. Experimental studies of ice nucleation in an Antarctic springtail (Collembola, Isotomidae). *Cryobiology* 42, 170–181.
- Block, W., Young, S.R., Conradi-Larsen, E.M., Sømme, L., 1978. Cold tolerance of two Antarctic terrestrial arthropods. *Experientia* 34, 1166–1167.
- Cannon, R.J., 1986. Diet and acclimation effects on the cold tolerance and survival of an Antarctic springtail. *British Antarctic Survey Bulletin* 71, 19–30.
- Cannon, R.J.C., 1983. Experimental studies on supercooling in two Antarctic micro-arthropods. *Journal of Insect Physiology* 29, 617–624.
- Cannon, R.J.C., Block, W., 1988. Cold tolerance of microarthropods. *Biological Reviews* 63, 23–77.
- Cannon, R.J.C., Block, W., Collett, G.D., 1985. Loss of supercooling ability in *Cryptopygus antarcticus* (Collembola: Isotomidae) associated with water uptake. *Cryo-Letters* 6, 73–80.
- Convey, P., 1996. Overwintering strategies of terrestrial invertebrates in Antarctica—the significance of flexibility in extremely seasonal environments. *European Journal of Entomology* 93, 489–505.
- Convey, P., Greenslade, P., Arnold, R.J., Block, W., 1999. Collembola of sub-Antarctic South Georgia. *Polar Biology* 22, 1–6.
- Coulson, S.J., Bale, J.S., 1991. Anoxia induces rapid cold hardening in the housefly *Musca domestica* (Diptera, Muscidae). *Journal of Insect Physiology* 37, 497–501.
- Coulson, S.J., Hodkinson, I.D., Block, W., Webb, N.R., Worland, M.R., 1995a. Low summer temperatures: a potential mortality factor for high arctic soil microarthropods? *Journal of Insect Physiology* 41, 783–792.
- Coulson, S.J., Hodkinson, I.D., Strathdee, A.T., Block, W., Webb, N.R., Bale, J.S., Worland, M.R., 1995b. Thermal environments of Arctic soil organisms during winter. *Arctic and Alpine Research* 27, 364–370.
- Danks, H.V., 2000. Insect cold hardiness: a Canadian perspective. *Cryo-Letters* 21, 297–308.
- Davey, M.C., Pickup, J., Block, W., 1992. Temperature variation and its biological significance in fellfield habitats on a maritime Antarctic island. *Antarctic Science* 4, 383–388.
- Denlinger, D.L., Joplin, K.H., Chen, C.-P., Lee, R.E. Jr., 1991. Cold shock and heat shock. In: Lee, R.E. Jr., Denlinger, D.L. (Eds.), *Insects at Low Temperature*. Chapman and Hall, New York, pp. 131–148.
- Denlinger, D.L., Yocum, G.D., 1998. Physiology of heat sensitivity. In: Hallam, G.J., Deninger, D.L. (Eds.), *Temperature Sensitivity in Insects and Application in Integrated Pest Management*. Westview Press, Boulder, pp. 7–53.
- Doran, P.T., Priscu, J.C., Lyons, W.B., Walsh, J.E., Fountain, A.G., McKnight, D.M., Moorhead, D.L., Virginia, R.A., Wall, D.H., Clow, G.D., Fritsen, C.H., McKay, C.P., Parsons, A.N., 2002. Antarctic climate cooling and terrestrial ecosystem response. *Nature* 415, 517–520.
- Fitzsimons, J.M., 1971. Temperature and three species of Antarctic arthropods. *Pacific Insects Monographs* 25, 127–135.
- Gressitt, J.L., 1967. Introduction. In: Gressitt, J.L. (Ed.), *Entomology of Antarctica*. American Geophysical Union, Washington, DC, pp. 1–33.
- Hanc, Z., Nedved, O., 1999. Chill injury at alternating temperatures in

- Orchesella cincta* (Collembola: Entomobryidae) and *Pyrrhochoris apterus* (Heteroptera: Pyrrhocoridae). *European Journal of Entomology* 96, 165–168.
- Holmstrup, M., Sømme, L., 1998. Dehydration and cold hardiness in the Arctic collembolan *Onychiurus arcticus* Tullberg 1876. *Journal of Comparative Physiology B* 168, 197–203.
- Hopkin, S.P., 1997. *Biology of the Springtails* (Insecta: Collembola). Oxford University Press, Oxford.
- Janetschek, H., 1967. Arthropod ecology of South Victoria Land. In: Gressitt, J.L. (Ed.), *Entomology of Antarctica*. American Geophysical Union, Washington, DC, pp. 205–293.
- Janetschek, H., 1970. Environments and ecology of terrestrial arthropods in the high Antarctic. In: Holdgate, M.W. (Ed.), *Antarctic Ecology*, vol. 2. Academic Press, London, pp. 871–885.
- Kelty, J.D., Lee, R.E. Jr., 1999. Induction of rapid cold hardening by ecologically relevant cooling rates in *Drosophila melanogaster*. *Journal of Insect Physiology* 45, 719–726.
- Kelty, J.D., Lee, R.E. Jr., 2001. Rapid cold-hardening of *Drosophila melanogaster* (Diptera: Drosophilidae) during ecologically based thermoperiodic cycles. *Journal of Experimental Biology* 204, 1659–1666.
- Leather, S.R., Walters, K.F.A., Bale, J.S., 1993. *The Ecology of Insect Overwintering*. Cambridge University Press, Cambridge.
- Lee, R.E. Jr., 1991. Principles of insect low temperature tolerance. In: Lee, R.E. Jr., Denlinger, D.L. (Eds.), *Insects at Low Temperature*. Chapman and Hall, New York, pp. 17–46.
- Lee, R.E. Jr., Chen, C.-P., Denlinger, D.L., 1987. A rapid cold-hardening process in insects. *Science* 238, 1415–1417.
- Marshall, D.J., Newton, I.P., Crafford, J.E., 1995. Habitat temperature and potential locomotor activity of the continental Antarctic mite, *Maudheimia petronia* Wallwork (Acari: Oribatei). *Polar Biology* 15, 41–46.
- Moorhead, D.L., Wall, D.H., Virginia, R.A., Parsons, A.N., 2002. Distribution and life-cycle of *Scottinema lindsayae* (Nematoda) in Antarctic soils: a modeling analysis of temperature responses. *Polar Biology* 25, 118–125.
- Pryor, M.E., 1962. Some environmental features of Hallett Station, Antarctica, with special reference to soil arthropods. *Pacific Insects* 4, 681–728.
- Ružicka, V., Hajer, J., Zacharda, M., 1995. Arachnid population patterns in underground cavities of a stony debris field (Araneae, Opiliones, Pseudoscorpionidea, Acari: Prostigmata, Rhagidiidae). *Pedobiologia* 39, 42–51.
- Salvucci, M.E., 2000. Sorbitol accumulation in whiteflies: evidence for a role in protecting proteins during heat stress. *Journal of Thermal Biology* 25, 353–361.
- Sinclair, B.J., 2001. Field ecology of freeze tolerance: interannual variation in cooling rates, freeze–thaw and thermal stress in the microhabitat of the alpine cockroach *Celatoblatta quinque maculata*. *Oikos* 93, 286–293.
- Sinclair, B.J., Chown, S.L., 2002. Haemolymph osmolality and thermal hysteresis activity in 17 species of arthropods from sub-Antarctic Marion Island. *Polar Biology* 25, 928–933.
- Sinclair, B.J., Chown, S.L., 2003. Rapid responses to high temperature and desiccation but not to low temperature in the freeze tolerant sub-Antarctic caterpillar *Pringleophaga marioni* (Lepidoptera, Tineidae). *Journal of Insect Physiology* 49, 45–52.
- Sinclair, B.J., Sjørnsen, H., 2001. Cold tolerance of the Antarctic springtail *Gomphiocephalus hodgsoni* (Collembola, Hypogastruridae). *Antarctic Science* 13, 271–279.
- Sinclair, B.J., Worland, M.R., Wharton, D.A., 1999. Ice nucleation and freezing tolerance in New Zealand alpine and lowland weta, *Hemideina* spp. (Orthoptera; Stenopelmatidae). *Physiological Entomology* 24, 56–63.
- Sinclair, B.J., Vernon, P., Klok, C.J., Chown, S.L., 2003. Insects at low temperatures: an ecological perspective. *Trends in Ecology and Evolution* 18, 257–262.
- Sømme, L., 1978. Cold-hardiness of *Cryptopygus antarcticus* (Collembola) from Bouvetøya. *Oikos* 31, 94–97.
- Sømme, L., 1981. Cold tolerance of Alpine, Arctic and Antarctic Collembola and Mites. *Cryobiology* 18, 212–220.
- Sømme, L., 1986. Ecology of *Cryptopygus sverdrupi* (Insecta: Collembola) from Dronning Maud Land, Antarctica. *Polar Biology* 6, 179–184.
- Sømme, L., 1995. *Invertebrates in Hot and Cold Arid Environments*. Springer-Verlag, Berlin.
- Sømme, L., 1999. The physiology of cold hardiness in terrestrial arthropods. *European Journal of Entomology* 96, 1–10.
- Sømme, L., Block, W., 1982. Cold hardiness of Collembola at Signy Island, maritime Antarctic. *Oikos* 38, 168–176.
- Spicer, J.I., Gaston, K.J., 1999. *Physiological Diversity and its Ecological Implications*. Blackwell, Oxford.
- Tammariello, S.P., Rinehart, J.P., Denlinger, D.L., 1999. Desiccation elicits heat shock protein transcription in the flesh fly, *Sarcophaga crassipalpis*, but does not enhance tolerance to high or low temperatures. *Journal of Insect Physiology* 45, 933–938.
- Wharton, D.A., Rowland, J.J., 1984. A thermoelectric cooling stage for the measurement of the supercooling points of microscopic organisms. *Journal of Microscopy* 134, 299–305.
- Wharton, D.A., Goodall, G., Marshall, C.J., 2003. Freezing survival and cryoprotective dehydration as cold tolerance mechanisms in the Antarctic nematode *Panagrolaimus davidi*. *Journal of Experimental Biology* 206, 215–221.
- Wise, K.A.J., 1967. Collembola (Springtails). In: Gressitt, J.L. (Ed.), *Entomology of Antarctica*. American Geophysical Union, Washington, DC, pp. 123–148.
- Wise, K.A.J., Shoup, J., 1967. Distribution of Collembola at Cape Hallett. In: Gressitt, J.L. (Ed.), *Entomology of Antarctica*. American Geophysical Union, Washington, DC, pp. 325–330.
- Wolfe, G.R., Hendrix, D.L., Salvucci, M.E., 1998. A thermoprotective role for sorbitol in the silverleaf whitefly, *Bemisia argentifolii*. *Journal of Insect Physiology* 44, 597–603.
- Worland, M.R., 2000. Temperature gradients in intact/undisturbed soil cores—an experimental system for use in freeze–thaw studies. *Cryo-Letters* 21, 71–72.
- Worland, M.R., Convey, P., 2001. Rapid cold hardening in Antarctic microarthropods. *Functional Ecology* 15, 515–524.
- Worland, M.R., Lukešová, A., 2000. The effect of feeding on specific soil algae on the cold-hardiness of two Antarctic micro-arthropods (*Alaskozetes antarcticus* and *Cryptopygus antarcticus*). *Polar Biology* 23, 766–774.
- Worland, M.R., Grubor-Lajsic, G., Montiel, P.O., 1998. Partial desiccation induced by sub-zero temperatures as a component of the survival strategy of the Arctic collembolan *Onychiurus arcticus* (Tullberg). *Journal of Insect Physiology* 44, 211–219.
- Worland, M.R., Convey, P., Lukešová, A., 2000. Rapid cold hardening: a gut feeling. *Cryo-Letters* 21, 315–324.

## **Appendix 2**

## Insect gas exchange patterns: a phylogenetic perspective

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### Summary

Most investigations of insect gas exchange patterns and the hypotheses proposed to account for their evolution have been based either on small-scale, manipulative experiments, or comparisons of a few closely related species. Despite their potential utility, no explicit, phylogeny-based, broad-scale comparative studies of the evolution of gas exchange in insects have been undertaken. This may be due partly to the preponderance of information for the endopterygotes, and its scarcity for the apterygotes and exopterygotes. Here we undertake such a broad-scale study. Information on gas exchange patterns for the large majority of insects examined to date (eight orders, 99 species) is compiled, and new information on 19 exemplar species from a further ten orders, not previously represented in the literature (Archaeognatha, Zygentoma, Ephemeroptera, Odonata, Mantodea, Mantophasmatodea,

Phasmatodea, Dermaptera, Neuroptera, Trichoptera), is provided. These data are then used in a formal, phylogeny-based parsimony analysis of the evolution of gas exchange patterns at the order level. Cyclic gas exchange is likely to be the ancestral gas exchange pattern at rest (recognizing that active individuals typically show continuous gas exchange), and discontinuous gas exchange probably originated independently a minimum of five times in the Insecta.

Supplementary material available online at  
<http://jeb.biologists.org/cgi/content/full/208/23/4495/DC1>

Key words: adaptation, discontinuous gas exchange, periodic breathing, phylogeny.

### Introduction

Discontinuous gas exchange cycles are one of the most striking gas exchange patterns shown by resting or quiescent insects. They were originally described in lepidopteran pupae (by Heller, 1930; cf. Buck, 1962) and the adults of several species (Punt et al., 1957; Wilkins, 1960). However, it was the extensive investigation of discontinuous gas exchange cycles in diapausing saturniid pupae by Schneiderman and his colleagues that resulted in the first comprehensive description of this pattern of gas exchange, and elucidation of the mechanisms underlying it (e.g. Schneiderman, 1960; Levy and Schneiderman, 1966a,b; Schneiderman and Schechter, 1966). This work, as well as later studies on adult cockroaches, ants and beetles, provided strong support for the idea that discontinuous gas exchange cycles (DGCs) evolved to reduce respiratory water loss (Kestler, 1985; Lighton, 1994; Chown, 2002).

Subsequent investigations cast doubt on this hypothesis, and six competing explanations have now been formulated to account for the evolution of DGCs (reviewed in Chown et al., 2005). A prominent feature of virtually all tests of the competing hypotheses is that they have been based either on small-scale, manipulative experiments (Lighton and Berrigan, 1995; Chown and Holter, 2000), or comparative investigations

of a few closely related species (e.g. Lighton, 1991a; Duncan et al., 2002; Chown and Davis, 2003). These approaches have provided many valuable insights into the evolution of insect gas exchange patterns, and especially the mechanisms underlying them. However, broader comparative analyses can also be informative. Indeed, Huey and Kingsolver (1993) have cogently argued that a combined approach involving mechanistic investigations, laboratory selection and comparative methods is essential if an integrated understanding of the evolution of physiological traits, and their broader ecological implications, is to be achieved (see also Kingsolver and Huey, 1998; Feder and Mitchell-Olds, 2003). Moreover, comparative analyses undertaken in a phylogenetic context can provide useful information on the history of a given trait, including its origin, whether or not it should be considered adaptive (in the strict sense, such that natural selection is responsible for its origin and maintenance; Coddington, 1988; Baum and Larson, 1991), and the likelihood of repeated and/or convergent evolution (Brooks and McLennan, 1991). In the context of gas exchange patterns, the value of such a phylogeny-based comparative approach has already been established by Klok et al. (2002), who demonstrated that discontinuous gas exchange probably arose



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independently at least four times in the Arthropoda. Nonetheless, no phylogeny-based comparative analysis of the occurrence of gas exchange patterns, and particularly discontinuous gas exchange, in insects (which form a monophyletic unit; Giribet et al., 2001) has been undertaken.

This situation at first appears remarkable, given that published investigations of gas exchange patterns are available for 99 insect species, and it is known that these patterns vary considerably among, and sometimes within, species at rest: from continuous, to cyclic, to discontinuous (Lighton, 1998; Marais and Chown, 2003; Gibbs and Johnson, 2004). However, on closer inspection it is clear that there are probably several reasons why no phylogeny-based analysis has been undertaken, amongst which two are perhaps most significant. First, there is probably a file-drawer problem (Csada et al., 1996), such that in instances where species do not show discontinuous gas exchange the data are not published, thus biasing the literature in favour of reports of discontinuous gas exchange cycles (Lighton, 1998; Chown, 2001). Second, and possibly as a consequence of the file-drawer problem, the taxa for which gas exchange patterns are available is highly skewed towards the holometabolous insects. Thus, of the approx. 100 insect species for which information on gas exchange patterns is presently available, 83 are holometabolous, and of these, 44 are Coleoptera. By contrast, the Exopterygota is comparatively under-represented in the literature, with published information available for six cockroach species, six termite species, and five species of Orthoptera. No Apterygotes (*Zygentoma* and *Archaeognatha*) have been investigated.

In this paper, we address some of the above problems and provide the first, phylogeny-based comparative analysis of the distribution of insect gas exchange patterns at the order level. Whilst we cannot resolve the file-drawer issue, we comprehensively review patterns documented by the existing literature, provide information on several exemplar taxa representing orders of insects that have not previously been investigated (*Archaeognatha*, *Zygentoma*, *Ephemeroptera*, *Odonata*, *Mantodea*, *Mantophasmatodea*, *Phasmatodea*, *Dermaptera*, *Neuroptera* and *Trichoptera*), and add to the data on little studied groups (*Blattodea*, *Orthoptera*, *Hemiptera* and *Diptera*). In undertaking this work we realise that a comprehensive comparative analysis of insect gas exchange patterns is still some way off. The 118 species included here represent less than 0.003% of the estimated global insect fauna (May, 2000). However, because much of the variation in insect physiological traits is partitioned at higher taxonomic levels (Chown et al., 2002), we begin by using exemplar taxa at the Order level. Thus, our aim is to provide a working phylogenetic framework within which the evolution of gas exchange patterns in insects can be discussed. To date, such a framework has been missing.

## Materials and methods

### *Data from the literature*

The literature concerning insect gas exchange patterns was

reviewed as far back as 1950, and all studies in the Anglophone literature reporting gas exchange patterns were included. Where authors provided figures of the gas exchange patterns of the species they studied, these were used for assessments of the type of gas exchange pattern (either DGC, cyclic or continuous). DGCs were identified on the basis of the presence of C and F periods (Lighton, 1996; Chown et al., in press) in the figures presented by the authors. For the other patterns our assessments were based on the protocol described in the next section. In those instances where figures were not available, the authors' view on the type of pattern was accepted as correct, although confidence in the pattern assessment was marked lower (confidence in the data was ranked either as high, medium or low, which reflects our access to original data, rather than the abilities of the original authors). These assessments were made independently by E.M. and C.J.K., and in instances of disagreement a consensus was reached following discussion or analysis. The data were then tabulated. In two instances original data from published (Shelton and Appel, 2000) and unpublished works (B. A. Klein, K. M. F. Larsen and A. G. Gibbs) were obtained to verify these assessments. Each species was also scored for whether it is winged or wingless, from a mesic or xeric habitat, or expected to have a subterranean lifestyle, based on comments provided by the authors in the original works, and/or information on the species or higher taxon available elsewhere in the literature.

### *Experimental investigations*

The additional species collected for investigation were chosen based on Order-level deficiencies in the literature on gas exchange patterns. Adult individuals of 19 species representing the *Archaeognatha* (1 sp.), *Zygentoma* (3 spp.), *Ephemeroptera* (1 sp.), *Odonata* (2 spp.), *Blattodea* (1 sp.), *Mantodea* (1 sp.), *Mantophasmatodea* (1 sp.), *Phasmatodea* (1 sp.), *Orthoptera* (1 sp.), *Dermaptera* (1 sp.), *Hemiptera* (2 spp.), *Neuroptera* (1 sp.), *Diptera* (1 sp.), *Trichoptera* (1 sp.) and *Lepidoptera* (1 sp.) were collected from several localities in South Africa (Table 1) and returned to the laboratory within 1 week of collection. Most experiments started within 12 h of the arrival of the insects at the laboratory because little is known about how long they survive in captivity. Insects were held in an incubator at 22±1°C (12 h:12 h L:D photoperiod), with access to water but not to food (with the exception of the hemipterans, mantophasmatodeans, cockroaches and the stick insects, where food was provided, but where a period of starvation preceded respirometry), before their gas exchange patterns were examined. Assessments were made in dry air for technical reasons and because under these conditions discontinuous gas exchange would seem most likely as a means to conserve water (Duncan et al., 2002). Each individual was weighed using an analytical balance (0.1 mg resolution; Mettler Toledo AX504, Columbus, OH, USA), and placed into a cuvette kept at 20±0.2°C, using either a water bath (Grant LTD20, Cambridge, UK) or a temperature-controlled cabinet (Labcon, Johannesburg, South Africa). This slightly lower temperature was selected because it improved quiescence and

Table 1. *Species examined for gas exchange patterns in this study*

	Locality	Response time, lag time (s)
<b>Archaeognatha</b>		
Meinertellidae, sp.	Helderberg Nature Reserve, Somerset West, South Africa (34°02.579'S, 18°52.472'E)	6, 120
<b>Zygentoma</b>		
Lepismatidae		
Lepismatidae sp. 1	Sutherland, South Africa (32°34.105'S, 20°57.747'E)	6, 120
Lepismatidae sp. 2	Cederberg, South Africa (31°51.611'S, 18°55.122'E)	6, 120
<i>Ctenolepisma longicaudata</i> (Echerich, 1905)	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	6, 120
<b>Ephemeroptera</b>		
Heptageniidae sp.	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	9, 210
<b>Odonata</b>		
Coenagrionidae		
<i>Ischnura senegalensis</i> (Rambur, 1842)	Jonkershoek, Stellenbosch, South Africa (33°57.814'S, 18°55.514'E)	7, 130
Libellulidae		
<i>Trithemis arteriosa</i> (Burmeister, 1839)	Jonkershoek, Stellenbosch, South Africa (33°57.814'S, 18°55.514'E)	18, 270
<b>Blattodea</b>		
Blaberidae, sp.	Cederberg, South Africa (31°51.611'S, 18°55.122'E)	4, 90
<b>Mantodea</b>		
Mantidae		
<i>Sphodromantis gastrica</i> Stål	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	7, 130
<b>Mantophasmatodea</b>		
Austrophasmatidae		
<i>Karoophasma biedouwensis</i> (Klass et al., 2003)	Cederberg, South Africa (32°05'S, 19°15'E)	9, 210
<b>Phasmatodea</b>		
Phasmatidae		
<i>Extatosoma tiaratum</i> (Macleay, 1826)	Butterfly World, Klapmuts, South Africa, but originally from Australia	7, 130
<b>Orthoptera</b>		
Pneumoridae		
<i>Bullacris intermedia</i> (Péringuey, 1916)	Zuurberg, South Africa (33°48'S, 25°14'E)	7, 130
<b>Dermaptera</b>		
Labiduridae		
<i>Euborellia annulipes</i> (Lucas, 1847)	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	6, 120
<b>Hemiptera</b>		
Coreidae sp.	Nigel, South Africa (26°25.422'S, 28°28.349'E)	6, 120
Lygaeidae sp.	Somerset West, South Africa (34°03.806'S, 18°49.473'E)	6, 120
<b>Neuroptera</b>		
Chrysopidae		
<i>Chrysoperla</i> sp.	Somerset West, South Africa (34°03.806'S, 18°49.473'E)	9, 210
<b>Diptera</b>		
Glossinidae		
<i>Glossina morsitans</i> Westwood	FAO/IAEA, Vienna, Austria (Laboratory colony)	6, 120
<b>Trichoptera</b>		
Leptoceridae		
<i>Leptocerina</i> sp.	Olifants River, Citrusdal (32°35'S, 18°40'E)	9, 210
<b>Lepidoptera</b>		
Plutellidae		
<i>Plutella xylostella</i> (Linnaeus, 1758)	Somerset West, South Africa (34°03.806'S, 18°49.473'E)	9, 210

Localities are provided, and species names where these are available. However, the taxonomic impediment in South Africa means that the latter has not always been possible. Response and lag times refer to delay in first detection of CO<sub>2</sub>, and time to zero baseline, respectively, of each of the designs used to examine gas exchange patterns. With one exception these times are well within those calculated from designs typically described in the literature for gas exchange analyses in insects.

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might have also induced discontinuous gas exchange. Previous work (Chown, 2001; Marais and Chown, 2003) indicated that gas exchange patterns, whilst repeatable, can be variable within individuals and species. In consequence, conditions favourable to the induction of discontinuous gas exchange were used, and particularly temperatures that are typically lower than mean summer microclimate temperatures in the region (which range from 24°C at sea level, to 22°C at the highest inland site of collection, with absolute maxima ranging from 50°C at the sea level site to 53°C at the high altitude site; see also Botes et al., in press).

Air, scrubbed of CO<sub>2</sub> (using soda lime) and water (using silica gel and then Drierite®, Xenia, OH, USA) was passed through the cuvette (see Table 1 for response times, regulated using a Sidetrak Mass Flow Controller, Monterey, USA) and into a calibrated infrared gas analyzer (Li-Cor Li7000 or Li-Cor Li6262; Lincoln, NE, USA) to measure CO<sub>2</sub> production. Flow rates and cuvette sizes varied according to the species and in a manner such that washout was unlikely to be significant (see Results, and Lighton, 1991b). A Sable Systems (Las Vegas, NV, USA) AD-1 activity detector was used to detect any movement of the individual in the cuvette during the experiment, and the output of the detector was fed into the auxiliary channel of the Li7000 or Li6262. The AD-1 registers activity as a value between -5 and +5 V, where little deviation from the mean indicates that the animal is inactive, and a large deviation indicates high levels of activity (for detail see [www.sablesys.com/ad1.html](http://www.sablesys.com/ad1.html)). Each experimental assessment lasted for approximately 2 h, which is typically sufficient to detect variation in gas exchange traces (Chown, 2001) without dehydrating animals to such an extent that the gas exchange pattern might switch to continuous, owing to dehydration, as has been found in some species (Quinlan and Hadley, 1993; Chappell and Rogowitz, 2000). The data file generated by the Li7000 software was exported, *via* Microsoft Excel, to DATACAN V (Sable Systems,), whilst the data stream from the Li6262 was captured directly using Sable Systems hardware and software. DATACAN V was used for initial analysis of the respirometry data (corrected to standard temperature and pressure) for periods of inactivity only.

Traces of rates of CO<sub>2</sub> production ( $\dot{V}_{CO_2}$ ) were categorized as continuous, cyclic or discontinuous gas exchange by inspection. The DGCs were readily identified based on the presence of C-periods and F-periods. However, identification of gas exchange patterns in the absence of the C- and F-periods is less straightforward. Several statistical approaches were explored for distinguishing continuous from cycling patterns objectively. These included spectral analysis and the modification thereof that has been used to identify population cycles (Cohen et al., 1998). Unfortunately, these methods typically did not allow continuous and cyclic gas exchange to be distinguished, most notably because even continuous gas exchange has some periodicity. The variance approach adopted by Williams et al. (1997) is also unsuitable because it does not take temporal autocorrelation into account. In consequence, any comparison of variances between species would be

confounded. Nonetheless, it is essential that some objective criterion has to be developed to allow traces to be classified or distinguished in a repeatable manner.

Therefore, we developed a simple, alternative convention based on the principle of the presence of regular bursts. We assumed that when a line is drawn through the centre of the  $\dot{V}_{CO_2}$  trace a cyclic trace should have fewer data points above this line than below it. By contrast, more continuous traces should show the converse. We applied this convention to the traces we recorded by adhering to the following steps. First, subtract the minimum point of the time series that has to be analysed from all the data points. Second, shift the data series down by 50% to ensure that the zero line passes through the centre of the trace. Third, calculate the percentage of data points that lie above the zero line. We adopted a conservative approach here and assumed that if the percentage of data points above the zero line was <30% the trace was cyclic, whilst if the percentage was >30% the trace was more likely to be continuous (Fig. 1). This technique is sensitive to traces that show drift and/or outliers (e.g. electronic glitches, baseline measurements) in the time series that result in artificial minima or maxima. However, such errors can routinely be corrected using modern analytical software. Although it might be argued

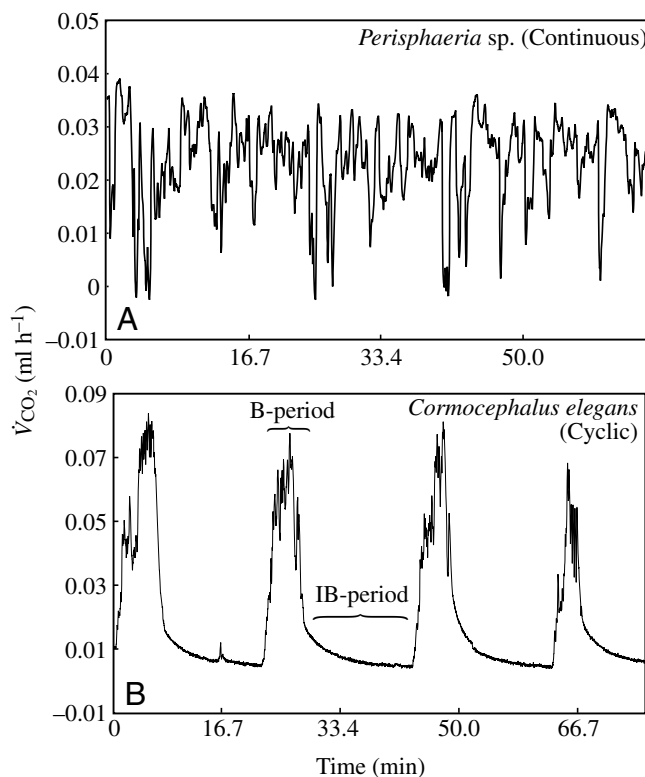


Fig. 1. Continuous gas exchange patterns (A; from a cockroach) are defined here as those in which the fluctuations between high and low  $\dot{V}_{CO_2}$  release are not regular, while cyclic patterns (B; from a centipede) are defined as those which show bursts at more regular intervals. The cyclic patterns are characterised by interburst periods (IB) and burst periods (B).



that an alternative set of criteria should be used, the approach we adopted makes any decision on cyclic *vs* continuous traces explicit, repeatable, and more objective than simple inspection. Here, once this method had been applied to the traces, summary statistics for the data were calculated, based on the approach to cyclic and discontinuous gas exchange patterns adopted by Marais and Chown (2003).

### *Analyses*

Based on the data from the literature and the data generated in this study we assigned gas exchange patterns (continuous, cyclic and discontinuous) to all of the insect orders that have been investigated to date, and these were plotted onto the phylogeny of the orders provided by Gullan and Cranston (2005). In those orders where species showed different gas exchange patterns, or where a single species showed more than one pattern, all gas exchange patterns were listed. A formal parsimony analysis (see Brooks and McLennan, 1991) was undertaken and used to assess the likely evolution of gas exchange patterns [see Scholtz and Chown (1995) for use of this approach to investigate the evolution of scarabaeoid diets]. In cases where both unknown patterns (orders not yet investigated) and known patterns were present on shallower nodes, preference was given to the known patterns at the deeper nodes. It should be noted that although the tree provided by Gullan and Cranston (2005) indicates some controversy in interpretation of the branching patterns, it was not presented with likelihood values for these alternatives. Therefore, a single parsimony analysis for one character (gas exchange pattern, with three states) based on the given tree was undertaken. Adopting the same approach with the consensus phylogeny presented by Grimaldi and Engel (2005) did not change our conclusions.

### **Results**

Reliable assessments of gas exchange patterns could be made for 99 species for which published data are available (and in one case we used unpublished information; see supplementary material, Appendix 1). Of these species, 59 typically showed discontinuous gas exchange cycles, 35 showed cyclic gas exchange, and 31 continuous gas exchange. Four species showed all three patterns, 19 species showed two of them, and in 76 species the work in which the outcome was reported suggested that the focal species showed only a single pattern. Indeed, in many published investigations the authors discussed the most common gas exchange pattern without providing explicit information on whether other gas exchange patterns were also evident in the species being examined. Thus, our characterization reflects the reporting convention of the previous literature, and probably under-represents multiple gas exchange patterns (especially the frequency of continuous gas exchange). It is now more common to find reporting of all gas exchange patterns (e.g. Gibbs and Johnson, 2004).

Our own investigations added 19 species to the list of those that have been investigated, and importantly most of these

were Exopterygotes or Apterygotes: groups that have enjoyed little attention to date (Fig. 2). In a few instances, sample sizes were low, but sufficient to indicate which kinds of gas exchange patterns were present. In these instances repeated measures of the individuals at hand were also undertaken (for rationale, see McNab, 2003). Likewise, although the response-time of one of our designs was slow (280 ml cuvette with 150 ml flow rate, to accommodate dragonflies), in none of the cases did Z-transformations (Bartholomew et al., 1981), using empirically derived response-time information from the experimental set-ups (Table 1), suggest that evidence for a lack of spiracle closure was a consequence of the experimental design. Moreover, the empirically derived times were well within those that would be typical of the majority of published studies, based on the cuvette sizes and flow rates reported in those studies (e.g. Lighton, 1990; Harrison et al., 1991; Duncan and Lighton, 1997).

In virtually all of the species, substantial among-individual variation in gas exchange pattern at rest was common, such that several individuals showed continuous gas exchange while a few showed cyclic or discontinuous patterns, or *vice versa* (Table 2). In most cases, cyclic gas exchange patterns were clearly identifiable as such, as were discontinuous gas exchange cycles (Fig. 2A–T). Thus, on the basis of these data, and on that available in the literature, gas exchange patterns were assigned to 18 of the 30 orders of insects (Fig. 3). Within all of the orders examined some species show continuous gas exchange, many show cyclic gas exchange, and only five orders contain species that show clear evidence of discontinuous gas exchange cycles. Moreover, within the latter orders where more than one or two species have been investigated substantial variation between species is typical (supplementary material, Appendix 1; Table 2). Nonetheless, the parsimony analysis demonstrated that, at the order level, continuous and cyclic gas exchange are basal, and that discontinuous gas exchange has evolved independently at least five times: in the Blattodea, Orthoptera, Coleoptera, Lepidoptera, and Hymenoptera.

### **Discussion**

Gas exchange patterns have now been reported for 118 species from 18 insect orders. Although this represents an increase of nearly 125% in the number of orders for which data are available, much remains to be done, and in this context data on exemplars from the 12 outstanding orders (see Fig. 3) would be a useful step forward. The question nonetheless remains of what can be learned from the comparative data that are at hand, especially in the context of the adaptive hypotheses that have been proposed to account for the evolution of discontinuous gas exchange cycles (Chown et al., in press). Because only a few species have been examined in most orders, little certainty can be attached to the absence of a particular pattern in a given order. Thus, although it now appears that DGCs have evolved independently five times in the insects at the order level, this situation may change as additional species are investigated in

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 Table 2. *CO<sub>2</sub> emission volumes, period durations, emission rates, total CO<sub>2</sub> emission rates and masses for each of the species investigated in this study*

	Mean	Standard deviation	<i>N</i>		Mean	Standard deviation	<i>N</i>
<b>Archaeognatha</b>				<i>Interburst</i>			
<b>Meinertellidae sp.</b> (Fig. 2A)				Emission volume (μl)	3.309	2.881	5
Mass (mg)	12.75	5.18	4	Duration (s)	1160.84	908.49	5
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	$4.036 \times 10^{-3}$	$0.713 \times 10^{-3}$	4	Emission rate (μl min <sup>-1</sup> )	0.302	0.422	5
<b>Zygentoma</b>				<i>Burst</i>			
<b>Lepismatidae sp. 1</b> (Fig. 2B)				Emission volume (μl)	1.215	1.106	5
Mass (mg)	23.04	1.99	5	Duration (s)	218.83	104.455	5
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	$3.89 \times 10^{-3}$	$0.884 \times 10^{-3}$	5	Emission rate (μl min <sup>-1</sup> )	0.291	0.157	5
Frequency (mHz)	$3.701 \times 10^3$	$0.521 \times 10^3$	5	<b>Libellulidae</b>			
<i>Interburst</i>				<b><i>Trithemis arteriosa</i></b> (Fig. 2G)			
Emission volume (μl)	0.179	0.078	5	Mass (mg)	103.06	37.88	8
Duration (s)	218.43	45.87	5	$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	$3.400 \times 10^{-2}$	$0.828 \times 10^{-2}$	8
Emission rate (μl min <sup>-1</sup> )	0.046	0.012	5	Frequency (mHz)	4.969		2
<i>Burst</i>				<i>Interburst</i>			
Emission volume (μl)	0.222	0.073	5	Emission volume (μl)	0.702		2
Duration (s)	106.60	9.050	5	Duration (s)	80.0		2
Emission rate (μl min <sup>-1</sup> )	0.120	0.032	5	Emission rate (μl min <sup>-1</sup> )	0.495		2
<b>Lepismatidae sp. 2</b> (Fig. 2C)				<i>Burst</i>			
Mass (mg)	17.18	0.72	6	Emission volume (μl)	2.344		2
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	$4.450 \times 10^{-3}$	$1.130 \times 10^{-3}$	6	Duration (s)	205.0		2
Frequency (Hz)	5.140	1.240	6	Emission rate (μl min <sup>-1</sup> )	0.690		2
<i>Interburst</i>				<b>Blattodea</b>			
Emission volume (μl)	0.143	0.070	6	<b>Blaberidae sp.</b> (Fig. 2H)			
Duration (s)	172.0	35.84	6	Mass (mg)	$2.54 \times 10^3$	$0.94 \times 10^3$	6
Emission rate (μl min <sup>-1</sup> )	0.052	0.017	6	$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	$9.100 \times 10^{-2}$	$0.004 \times 10^{-2}$	6
<i>Burst</i>				<i>DGC</i>			
Emission volume (μl)	0.166	0.041	6	Frequency (mHz)	1.579		2
Duration (s)	108.74	13.34	6	<i>C-period</i>			
Emission rate (μl min <sup>-1</sup> )	0.102	0.026	6	Emission volume (μl)	0.306		2
<b>Lepismatidae</b>				Duration (s)	144.5		2
<b><i>Ctenolepisma longicaudata</i></b> (Fig. 2D)				Emission rate (μl min <sup>-1</sup> )	0.130		2
Mass (mg)	26.64	5.46	9	<i>F-period</i>			
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	$2.430 \times 10^{-3}$	$0.492 \times 10^{-3}$	9	Emission volume (μl)	0.800		2
Frequency (mHz)	5.627	3.501	7	Duration (s)	152.40		2
<i>Interburst</i>				Emission rate (μl min <sup>-1</sup> )	0.336		2
Emission volume (μl)	0.046	0.032	7	<i>O-period</i>			
Duration (s)	90.86	63.73	7	Emission volume (μl)	11.71		2
Emission rate (μl min <sup>-1</sup> )	0.034	0.040	7	Duration (s)	261.5		2
<i>Burst</i>				Emission rate (μl min <sup>-1</sup> )	2.686		2
Emission volume (μl)	0.137	0.068	7	<i>Interburst-burst pattern</i>			
Duration (s)	129.71	30.30	7	Frequency (mHz)	5.319	6.081	3
Emission rate (μl min <sup>-1</sup> )	0.063	0.021	7	<i>Interburst</i>			
<b>Ephemeroptera</b>				Emission volume (μl)	1.237	1.579	3
<b>Heptageniidae sp.</b> (Fig. 2E)				Duration (s)	113.86	71.01	3
Mass (mg)	5.61	5.98	12	Emission rate (μl min <sup>-1</sup> )	0.530	0.578	3
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	$1.425 \times 10^{-3}$	$1.143 \times 10^{-3}$	12	<i>Burst</i>			
<b>Odonata</b>				Emission volume (μl)	14.55	12.82	3
<b>Coenagrionidae</b>				Duration (s)	340.86	358.38	3
<b><i>Ischnura senegalensis</i></b> (Fig. 2F)				Emission rate (μl min <sup>-1</sup> )	2.733	1.705	3
Mass (mg)	21.16	4.58	10	<b>Mantodea</b>			
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	$1.265 \times 10^{-2}$	$0.658 \times 10^{-2}$	10	<b>Mantidae</b>			
Frequency (mHz)	0.629	0.331	5	<b><i>Sphodromantis gastrica</i></b> (Fig. 2I)			
				Mass (mg)	335.7	276.7	7

Table continued on next page.

Table 2. Continued

	Mean	Standard deviation	N		Mean	Standard deviation	N
<i>Sphodromantis gastrica</i> (continued)				<b>Dermaptera</b>			
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	6.440×10 <sup>-2</sup>	0.005×10 <sup>-2</sup>	7	<b>Labiduridae</b>			
Frequency (mHz)	5.648	2.519	4	<i>Euborellia annulipes</i> (Fig. 2N)			
<i>Interburst</i>				Mass (mg)	31.76	26.50	12
Emission volume (μl)	0.451	0.810	4	$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	8.727×10 <sup>-3</sup>	8.004×10 <sup>-3</sup>	12
Duration (s)	66.65	82.31	4	Frequency (mHz)	1.044×10 <sup>3</sup>		2
Emission rate (μl min <sup>-1</sup> )	0.531	0.886	4	<i>Interburst</i>			
<i>Burst</i>				Emission volume (μl)	1.398	0.974	3
Emission volume (μl)	3.683	4.027	4	Duration (s)	533.88	341.90	3
Duration (s)	214.35	119.81	4	Emission rate (μl min <sup>-1</sup> )	0.172	0.131	3
Emission rate (μl min <sup>-1</sup> )	0.806	0.515	4	<i>Burst</i>			
<b>Mantophasmatodea</b>				Emission volume (μl)	0.621	0.571	3
<b>Austrophasmatidae</b>				Duration (s)	306.50	136.13	3
<i>Karoophasma biedouwensis</i> (Fig. 2J)				Emission rate (μl min <sup>-1</sup> )	0.107	0.065	3
Mass (mg)	98.62	83.50	14	<b>Hemiptera</b>			
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	2.396×10 <sup>-2</sup>	1.487×10 <sup>-2</sup>	14	<b>Coreidae sp.</b> (Fig. 2O)			
Frequency (mHz)	3.450	1.01	14	Mass (mg)	556.1	63.64	4
<i>Interburst</i>				$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	0.164	0.073	4
Emission volume (μl)	0.416	0.239	11	Frequency (mHz)	4.208	2.048	4
Duration (s)	120	69	11	<i>Interburst</i>			
Emission rate (μl min <sup>-1</sup> )	0.216	0.010	11	Emission volume (μl)	2.88	3.137	3
<i>Burst</i>				Duration (s)	134.15	120.44	3
Emission volume (μl)	1.254	0.566	11	Emission rate (μl min <sup>-1</sup> )	1.486	0.691	3
Duration (s)	192	63	11	<i>Burst</i>			
Emission rate (μl min <sup>-1</sup> )	0.394	0.144	11	Emission volume (μl)	12.03	2.164	3
<b>Phasmatodea</b>				Duration (s)	172.92	39.28	3
<b>Phamatidae</b>				Emission rate (μl min <sup>-1</sup> )	4.233	0.522	3
<i>Extatosoma tiaratum</i> (Fig. 2K)				<b>Lygaeidae sp.</b> (Fig. 2P)			
Mass (mg)	0.59×10 <sup>3</sup>	0.42×10 <sup>3</sup>	10	Mass (mg)	19.66	3.94	9
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	0.218×10 <sup>-3</sup>	0.066×10 <sup>-3</sup>	10	$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	1.103×10 <sup>-2</sup>	1.873×10 <sup>-2</sup>	9
Frequency (mHz)	7.650	0.022	10	<b>Trichoptera</b>			
<i>Interburst</i>				<b>Leptoceridae</b>			
Emission volume (μl)	9.59		1	<i>Leptocerina</i> sp. (Fig. 2S)			
Duration (s)	39.75		1	Mass (mg)	5.20		2
Emission rate (μl min <sup>-1</sup> )	1.41		1	$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	3.532×10 <sup>-3</sup>		2
<i>Burst</i>				Frequency (mHz)	10.21		2
Emission volume (μl)	13.95		1	<i>Interburst</i>			
Duration (s)	80.75		1	Emission volume (μl)	0.054		2
Emission rate (μl min <sup>-1</sup> )	1.14		1	Duration (s)	51.5		2
<b>Orthoptera</b>				Emission rate (μl min <sup>-1</sup> )	0.045		2
<b>Pneumoridae</b>				<i>Burst</i>			
<i>Bullacris intermedia</i> (Fig. 2M)				Emission volume (μl)	0.141		2
Mass (mg)	1.69×10 <sup>3</sup>	1.19×10 <sup>3</sup>	5	Duration (s)	83.5		2
$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	0.297	0.135	5	Emission rate (μl min <sup>-1</sup> )	0.085		2
<i>Interburst</i>				<b>Lepidoptera</b>			
Emission volume (μl)	17.71		1	<b>Plutellidae</b>			
Duration (s)	160.5		1	<i>Plutella xylostella</i> (Fig. 2T)			
Emission rate (μl min <sup>-1</sup> )	6.643		1	Mass (mg)	3.52	0.93	13
<i>Burst</i>				$\dot{V}_{CO_2}$ (ml CO <sub>2</sub> h <sup>-1</sup> )	3.751×10 <sup>-3</sup>	0.578×10 <sup>-3</sup>	13
Emission volume (μl)	1.40		1				
Duration (s)	90.75		1				
Emission rate (μl min <sup>-1</sup> )	0.926		1				

Note that differences in sample size between mass data and frequency data provide an indication of the frequency of cyclic or DGC patterns relative to continuous gas exchange, thus providing a measure of within-species variation.  $\dot{V}_{CO_2}$ , rate of CO<sub>2</sub> production.

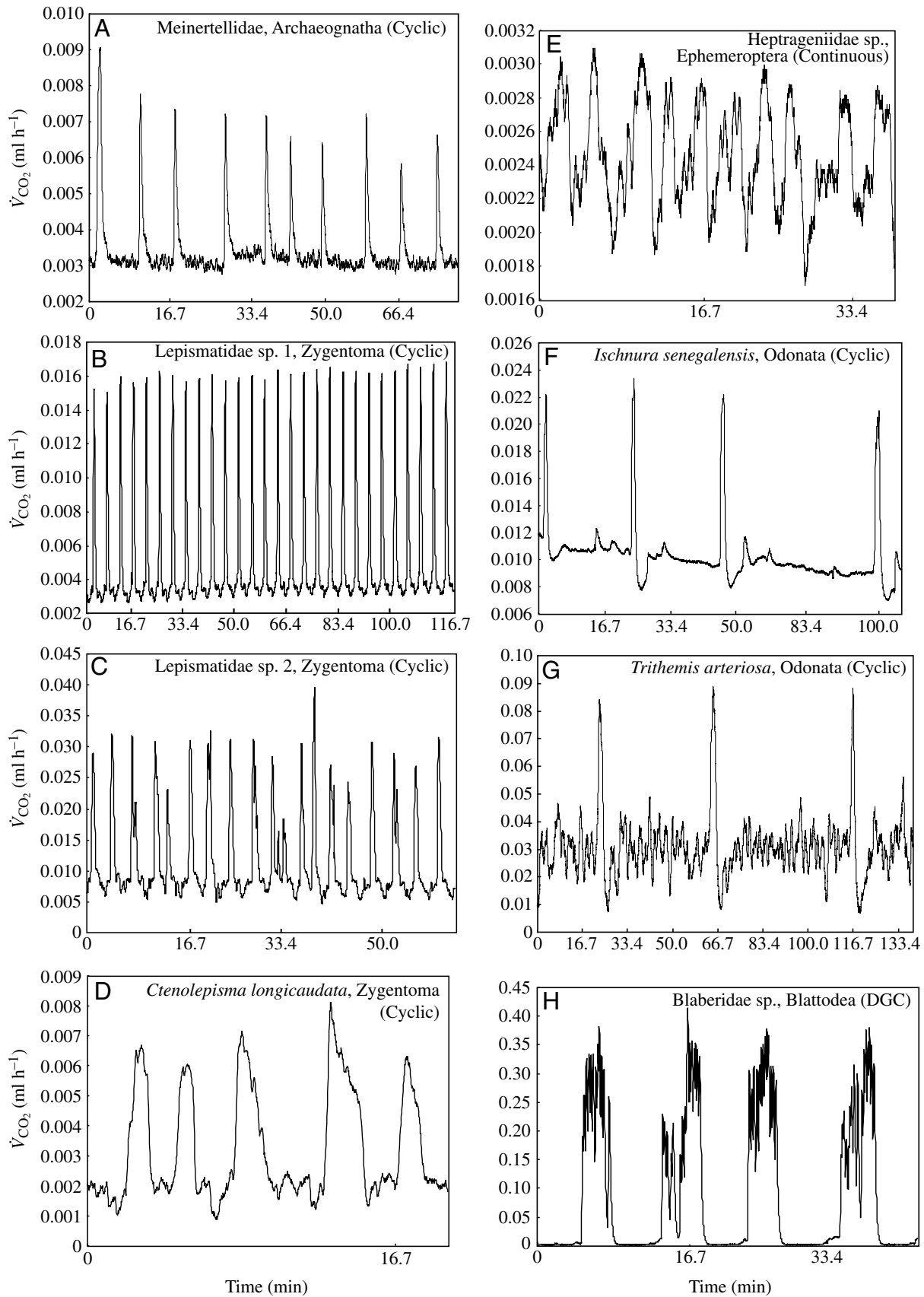


Fig. 2A-H

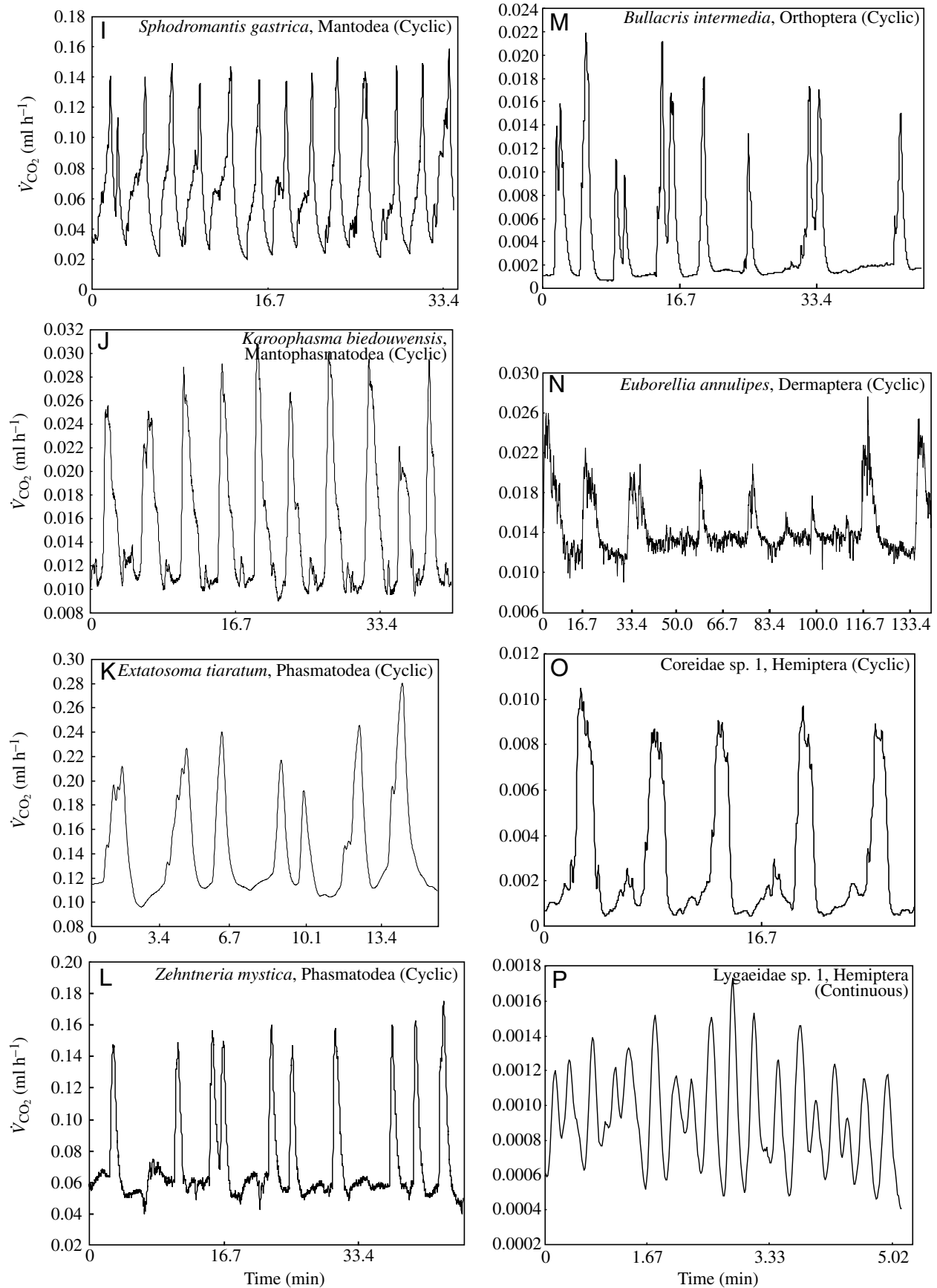


Fig. 2I-P

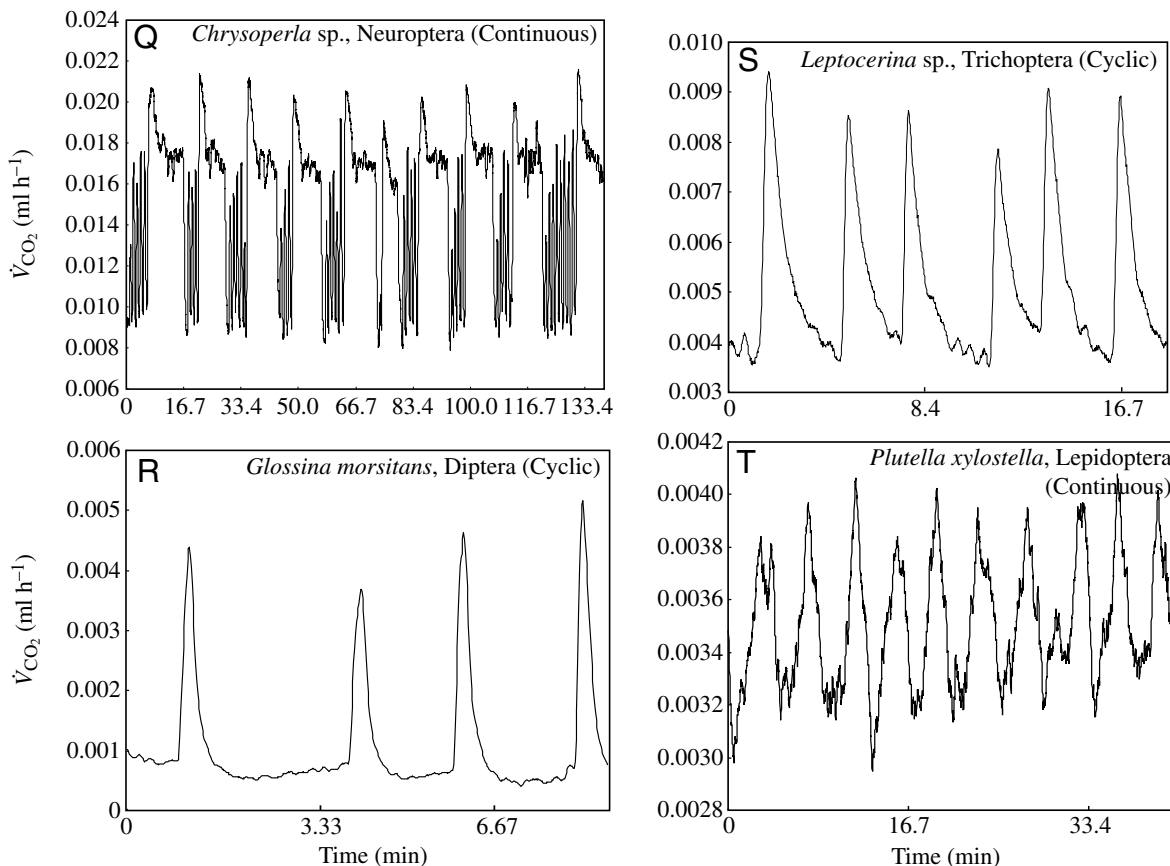


Fig. 2. (A–T) Representative continuous, cyclic or discontinuous gas exchange patterns for all of the species and orders investigated in this study. *Zehntneria mystica* (Phasmatodea, Phasmatidae) (L) is not discussed in the text. However, a single specimen showed a clear cyclic trace, therefore the graph is included. The other phasmid, *Extatosoma tiaratum* (K), is a leaf-mimicking species that shows a swaying response to the slightest air flow, which explains the limited evidence for cyclic gas exchange in this species.

each of the orders and as species from previously unstudied orders are examined. Even so, the independent evolution of DGCs in five insect orders is not unlike the situation amongst the Arthropoda as a whole, where discontinuous gas exchange cycles appear to have evolved independently at least four times, with the Pseudoscorpiones and Solifugae possibly inheriting DGCs from a common ancestor, and in one instance with a control system different to that of the insects (Klok et al., 2002; Lighton and Joos, 2002). Such independent evolution of DGCs, and the basal nature of both continuous and cyclic gas exchange, suggest that discontinuous gas exchange might well be adaptive, rather than an ancestral feature of the insects. The presence in particular families of some species with the trait and others without it (supplementary material, Appendix 1; Table 2) provides additional evidence for this idea. Perhaps more importantly, this independent evolution indicates that there is no *a priori* reason why control of discontinuous gas exchange cycles should be similar in the different orders of insects (see also Lighton and Joos, 2002), and that considerable care must be taken in applying the outcomes of the careful and informative analyses of model preparations (such as Lepidopteran pupae, cockroaches and grasshoppers e.g. Kestler, 1985; Harrison, 1997; Levy and Schneiderman,

1966a,b) more broadly. This caveat has been raised previously by work showing substantial differences between insect species (Harrison, 1997; Lighton, 1998; Davis et al., 1999), and the present study lends further support to it.

Whilst the comparative analysis does indicate which gas exchange characteristics are basal, it does not clearly resolve the reason(s) for the origin and/or maintenance of discontinuous gas exchange. Nonetheless, examination of the data (supplementary material, Appendix 1; Table 2) reveals several interesting patterns to the presence and absence of DGCs. There is no clear pattern of association between DGCs and subterranean vs non-subterranean lifestyles ( $\chi^2=1.08$ ,  $P=0.30$ ). Likewise, both winged and wingless species show DGCs ( $\chi^2=0.17$ ,  $P=0.68$ ), despite their apparently different oxygen demands (Reinhold, 1999; Addo-Bediako et al., 2002), which should mean greater threat of oxidative damage (see Hetz and Bradley, 2005) in flying species at rest because of their highly developed tracheal system that should mean enhanced oxygen access to tissues (Chapman, 1998). However, associations between DGCs and xeric environments ( $\chi^2=9.26$ ,  $P=0.002$ ), as might be predicted from the hygric hypothesis (Lighton, 1998; Chown and Nicolson, 2004), were found. These preliminary analyses broadly suggest that cyclic and



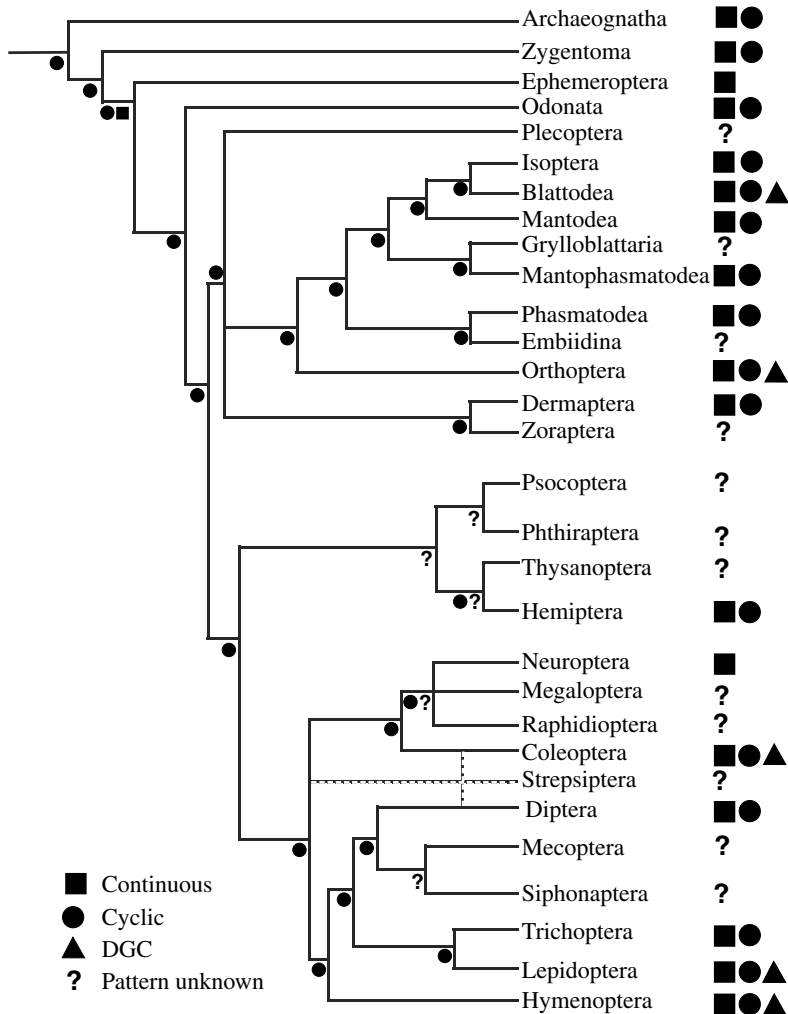


Fig. 3. Phylogeny of insects (redrawn from Gullan and Cranston, 2005) indicating the gas exchange patterns that are shown by species within each of the orders. Symbols for the gas exchange patterns are as follows: squares, continuous gas exchange; circles, cyclic gas exchange; triangles, discontinuous gas exchange. The parsimony analysis is also indicated on the phylogeny and shows that the cyclic pattern is the ancestral condition at rest. Discontinuous gas exchange cycles (DGCs) have been observed in only 5 of the 30 orders of insects, while continuous and cyclic patterns have been observed in all of the orders examined.

continuous gas exchange at rest are more likely in mesic than in xeric environments, than are DGCs, but that DGCs can evolve in both kinds of environments. However, the data have a strong phylogenetic signal such that DGCs are restricted to a few families. If these analyses are repeated within orders (Coleoptera, Hymenoptera, Lepidoptera, Orthoptera) or families (Tenebrionidae, Scarabaeidae, Formicidae) that have sufficiently large sample sizes, in what is essentially then a phylogenetically nested approach to examining these associations (see Harvey and Pagel, 1991), none of the associations are significant ( $\chi^2$ ,  $P > 0.07$  in all cases). Thus, the overall result does not appear to be strongly biased by a single taxon. However, such an approach does not adequately exclude phylogenetic signal (Garland et al., 2005), and does not make

full use of the potentially available environmental data. To this end, conversion of the environmental data to a continuous, rather than categorical form, a phylogeny at the species level for the 118 species that have been studied, and a phylogenetic generalized least-squares analysis (Grafen, 1989) is required. Such work is now underway (C. L. White, S. L. Chown and others, unpublished data).

In the few studies where a tally has been kept of individuals showing DGCs vs other gas exchange patterns (e.g. Gibbs and Johnson, 2004), one of the predictions of the emergent property hypothesis also seems to be supported. That is, DGCs should emerge whenever the gas exchange system has little demand placed on it, but that this might vary given initial conditions (Chown and Holter, 2000). Where there is variation in gas exchange patterns (see also Table 2), considerable variation among individuals in gas exchange pattern has been found. Therefore, DGCs might be an emergent property of the interacting CO<sub>2</sub> and O<sub>2</sub> setpoints, although it is not clear why DGCs emerge in only a few orders. In consequence, there is good reason to undertake modelling work of interactions between the CO<sub>2</sub> and O<sub>2</sub> setpoints, in the context of knowledge of gas exchange regulation (reviewed in Chown and Nicolson, 2004), as well as to examine and, just as importantly, to report variation in gas exchange patterns within and between individuals (for additional discussion, see Lighton, 1998; Chown, 2001).

Another striking outcome of this phylogeny-based analysis of insect gas exchange patterns is that, despite a wide range of studies, undertaken over many years, nothing remains known of gas exchange in 12 of the 30 insect orders, and that of the remaining orders, only the Coleoptera and Hymenoptera have had investigations undertaken on more than ten species. The same is true of the Arthropoda as a whole, where gas exchange investigations of terrestrial groups are restricted to only a handful of species (see e.g. Lighton et al., 1993; Lighton and Fielden, 1996; Lighton, 2002; Lighton and Joos, 2002; Klok et al., 2002; Terblanche et al., 2004). This bias in the data does not mean that a clear understanding of the mechanisms underlying gas exchange, and particularly discontinuous gas exchange, is not emerging (reviewed by in Lighton, 1996, 1998; Chown and Nicolson, 2004). However, it does suggest that investigations of the reasons for the origin and maintenance of particular forms of gas exchange, in other words their likely adaptive value, will be constrained, at least from a comparative perspective, by the absence of appropriate information. To some extent this is true also of comparisons at the species level where, to date, not a single comparative analysis, in the strict phylogenetically independent sense (see Harvey and Pagel, 1991), or using a parsimony style approach

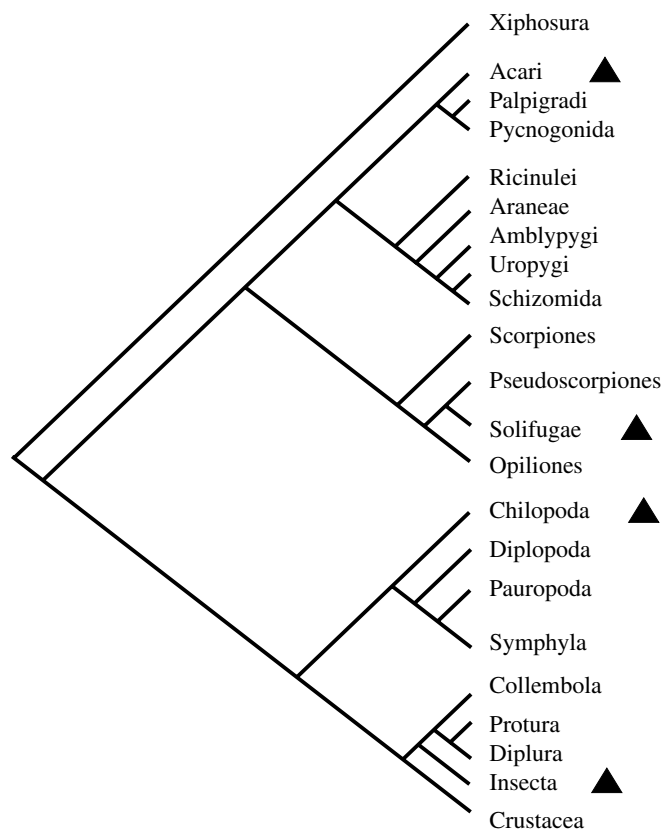


Fig. 4. The distribution of discontinuous gas exchange cycles (DGCs) across the arthropod phylogeny (redrawn from Chown and Nicolson, 2004).

(see Brooks and McLennan, 1991), has been undertaken for a multi-species monophyletic unit. In addition, laboratory selection experiments investigating the response of gas exchange patterns to different conditions have been restricted to a few *Drosophila* species and then only under conditions of starvation and desiccation (Gibbs et al., 1997; Williams et al., 1997, 2004).

Thus, of the approaches that Huey and Kingsolver (1993; see also Kingsolver and Huey, 1998) suggested are essential for providing an integrated understanding of the evolution of physiological traits, insect gas exchange physiology has been successful at the mechanistic investigations (reviewed by Harrison, 1997; Lighton, 1996, 1998; Chown and Nicolson, 2004). However, it has so far performed relatively poorly when it comes to laboratory selection and comparative approaches. Remedying the situation does not mean undertaking 4–8 million studies to assess gas exchange patterns in all of the insects (May, 2000). Rather, there should be a concerted approach to identify gas exchange patterns in a variety of species from the major orders, given that variation in many insect physiological traits is partitioned at the order and family levels (Chown et al., 2002). This work could be combined with smaller scale comparative investigations, laboratory selection experiments (for an excellent example of this combined approach, see Gibbs et al., 1997), and the kinds of mechanistic

studies for which the field is best known, and which have revealed striking differences amongst regulatory systems in the arthropods (Lighton and Joos, 2002).

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## References

- Addo-Bediako, A., Chown, S. L. and Gaston, K. J.** (2002). Metabolic cold adaptation in insects: a large-scale perspective. *Funct. Ecol.* **16**, 332–338.
- Bartholomew, G. A., Vleck, D. and Vleck, C. M.** (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J. Exp. Biol.* **90**, 17–32.
- Baum, D. A. and Larson, A.** (1991). Adaptation reviewed: a phylogenetic methodology for studying character macroevolution. *Syst. Zool.* **40**, 1–18.
- Botes, A., McGeoch, M. A., Robertson, H. G., van Niekerk, A., Davids, H. P. and Chown, S. L.** (in press). Ants, altitude and change in the northern Cape Floristic Region. *J. Biogeog.*
- Brooks, D. R. and McLennan, D. A.** (1991). *Phylogeny, Ecology and Behaviour: A Research Program in Comparative Biology*. Chicago: Chicago University Press.
- Buck, J.** (1962). Some physical aspects of insect respiration. *Annu. Rev. Entomol.* **7**, 27–56.
- Chapman, R. F.** (1998). *The Insects: Structure and Function*, 4th edn. Cambridge: Cambridge University Press.
- Chappell, M. A. and Rogowitz, G. L.** (2000). Mass, temperature and metabolic effects on discontinuous gas exchange cycles in *Eucalyptus*-boring beetles (Coleoptera: Cerambycidae). *J. Exp. Biol.* **203**, 3809–3820.
- Chown, S. L.** (2001). Physiological variation in insects: hierarchical levels and implications. *J. Insect Physiol.* **47**, 649–660.
- Chown, S. L.** (2002). Respiratory water loss in insects. *Comp. Biochem. Physiol.* **133A**, 791–804.
- Chown, S. L. and Davis, A. L. V.** (2003). Discontinuous gas exchange and the significance of respiratory water loss in scarabaeine beetles. *J. Exp. Biol.* **206**, 3547–3556.
- Chown, S. L. and Holter, P.** (2000). Discontinuous gas exchange cycles in *Aphodius fossor* (Scarabaeidae): a test of hypotheses concerning origins and mechanisms. *J. Exp. Biol.* **203**, 397–403.
- Chown, S. L. and Nicolson, S. W.** (2004). *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford: Oxford University Press.
- Chown, S. L., Addo-Bediako, A. and Gaston, K. J.** (2002). Physiological variation in insects: large-scale patterns and their implications. *Comp. Biochem. Physiol.* **131B**, 587–602.
- Chown, S. L., Gibbs, A. G., Hetz, S. K., Klok, C. J., Lighton, J. R. B. and Marais, E.** (in press). Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. *Physiol. Biochem. Zool.*
- Coddington, J. A.** (1988). Cladistic tests of adaptational hypotheses. *Cladistics* **4**, 3–22.
- Cohen, A. E., Gonzalez, A., Lawton, J. H., Petchey, O. L., Wildman, D. and Cohen, J. E.** (1998). A novel experimental apparatus to study the impact of white noise and 1/f noise on animal populations. *Proc. R. Soc. Lond. B* **265**, 11–15.
- Csada, R. D., James, P. C. and Espie, R. H. M.** (1996). The ‘file drawer



- problem' of non-significant results: does it apply to biological research? *Oikos* **76**, 591-593.
- Davis, A. L. V., Chown, S. L. and Scholtz, C. H.** (1999). Discontinuous gas-exchange cycles in *Scarabaeus* dung beetles (Coleoptera: Scarabaeidae): Mass-scaling and temperature dependence. *Physiol. Biochem. Zool.* **72**, 555-565.
- Duncan, F. D. and Lighton, J. R. B.** (1997). Discontinuous ventilation and energetics of locomotion in the desert-dwelling female mutillid wasp, *Dasyutilla gloriosa*. *Physiol. Entomol.* **22**, 310-315.
- Duncan, F. D., Krasnov, B. and McMaster, M.** (2002). Metabolic rate and respiratory gas-exchange patterns in tenebrionid beetles from the Negev Highlands, Israel. *J. Exp. Biol.* **205**, 791-798.
- Feder, M. E. and Mitchell-Olds, T.** (2003). Evolutionary and ecological functional genomics. *Nat. Rev. Genet.* **4**, 649-655.
- Garland, T., Jr, Bennett, A. F. and Rezende, E. L.** (2005). Phylogenetic approaches in comparative physiology. *J. Exp. Biol.* **208**, 3015-3035.
- Gibbs, A. G. and Johnson, R. A.** (2004). The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. *J. Exp. Biol.* **207**, 3477-3482.
- Gibbs, A. G., Chippindale, A. K. and Rose, M. R.** (1997). Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J. Exp. Biol.* **200**, 1821-1832.
- Giribet, G., Edgecombe, G. D. and Wheeler, W. C.** (2001). Arthropod phylogeny based on eight molecular loci and morphology. *Nature* **413**, 157-161.
- Grafen, A.** (1989). The phylogenetic regression. *Phil. Trans. R. Soc. Lond. B* **326**, 119-157.
- Grimaldi, D. and Engel, M. S.** (2005). *Evolution of the Insects*. Cambridge: Cambridge University Press.
- Gullan, P. J. and Cranston, P. S.** (2005). *The Insects: An Outline of Entomology*. United Kingdom: Blackwell Publishing.
- Harrison, J. F.** (1997). Ventilatory mechanism and control in grasshoppers. *Am. Zool.* **37**, 73-81.
- Harrison, J. F., Philips, J. E. and Gleeson, T. T.** (1991). Activity physiology of the two-striped grasshopper, *Melanoplus bivittatus*: gas exchange, hemolymph acid-base status, lactate production, and the effect of temperature. *Physiol. Zool.* **64**, 451-472.
- Harvey, P. H. and Pagel, M. D.** (1991). *The Comparative Method in Evolutionary Biology*. Oxford: Oxford University Press.
- Heller, J.** (1930). Sauerstoffverbrauch der schmetterlingspuppen in abhängigkeit von der temperatur. *Z. Vergleich. Physiol.* **11**, 448-460.
- Hetz, S. K. and Bradley, T. J.** (2005). Insects breathe discontinuously to avoid oxygen toxicity. *Nature* **433**, 516-519.
- Huey, R. B. and Kingsolver, J. G.** (1993). Evolution of resistance to high temperature in ectotherms. *Am. Nat.* **142**, S21-S46.
- Kestler, P.** (1985). Respiration and respiratory water loss. In *Environmental Physiology and Biochemistry of Insects* (ed. K. H. Hoffmann), pp. 137-183. Berlin: Springer.
- Kingsolver, J. G. and Huey, R. B.** (1998). Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *Am. Zool.* **38**, 545-560.
- Klok, C. J., Mercer, R. D. and Chown, S. L.** (2002). Discontinuous gas exchange in centipedes and its convergent evolution in tracheated arthropods. *J. Exp. Biol.* **205**, 1031-1036.
- Levy, R. I. and Schneiderman, H. A.** (1966a). Discontinuous respiration in insects - II. The direct measurement and significance of changes in tracheal gas composition during the respiratory cycle of silkworm pupae. *J. Insect Physiol.* **12**, 83-104.
- Levy, R. I. and Schneiderman, H. A.** (1966b). Discontinuous respiration in insects - IV. Changes in intratracheal pressure during the respiratory cycle of silkworm pupae. *J. Insect Physiol.* **12**, 465-492.
- Lighton, J. R. B.** (1990). Slow discontinuous ventilation in the Namib dunesea ant *Camponotus detritus* (Hymenoptera, Formicidae). *J. Exp. Biol.* **151**, 71-82.
- Lighton, J. R. B.** (1991a). Ventilation in Namib desert tenebrionid beetles: mass scaling and evidence of a novel quantized flutter-phase. *J. Exp. Biol.* **159**, 249-268.
- Lighton, J. R. B.** (1991b). Insects: measurements. In *Concise Encyclopedia on Biological and Biomedical Measurement Systems* (ed. P. A. Payne), pp. 201-20. Oxford: Pergamon Press.
- Lighton, J. R. B.** (1994). Discontinuous ventilation in terrestrial insects. *Physiol. Zool.* **67**, 142-162.
- Lighton, J. R. B.** (1996). Discontinuous gas exchange in insects. *Annu. Rev. Entomol.* **41**, 309-324.
- Lighton, J. R. B.** (1998). Notes from the underground: towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods. *Am. Zool.* **38**, 483-491.
- Lighton, J. R. B.** (2002). Lack of discontinuous gas exchange in a tracheate arthropod, *Leiobunum townsendi* (Arachnida, Opiliones). *Physiol. Entomol.* **27**, 170-174.
- Lighton, J. R. B. and Berrigan, D.** (1995). Questioning paradigms: caste-specific ventilation in harvester ants, *Messor pergandei* and *M. julianus* (Hymenoptera: Formicidae). *J. Exp. Biol.* **198**, 521-530.
- Lighton, J. R. B. and Fielden, L. J.** (1996). Gas exchange in wind spiders (Arachnida, Solpugidae): Independent evolution of convergent control strategies in solpugids and insects. *J. Insect Physiol.* **42**, 347-357.
- Lighton, J. R. B. and Joos, B.** (2002). Discontinuous gas exchange in the pseudoscorpion *Garypus californicus* is regulated by hypoxia, not hypercapnia. *Physiol. Biochem. Zool.* **75**, 345-349.
- Lighton, J. R. B., Fielden, L. J. and Rechav, Y.** (1993). Discontinuous ventilation in a non-insect, the tick *Amblyomma marmoreum* (Acari, Ixodidae): characterization and metabolic modulation. *J. Exp. Biol.* **180**, 229-245.
- Marais, E. and Chown, S. L.** (2003). Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. *J. Exp. Biol.* **206**, 4565-4574.
- May, R. M.** (2000). *The Dimensions of Life on Earth in Nature and Human Society: The Quest for a Sustainable World* (ed. P. H. Raven and T. Williams), pp. 30-45. Washington, DC: National Academy Press.
- McNab, B. K.** (2003). Sample size and the estimation of physiological parameters in the field. *Funct. Ecol.* **17**, 82-86.
- Punt, A., Parser, W. J. and Kuchlein, J.** (1995). Oxygen uptake in insects with cyclic CO<sub>2</sub> release. *Biol. Bull.* **112**, 108-119.
- Quinlan, M. C. and Hadley, N. F.** (1993). Gas exchange, ventilatory patterns, and water loss in two lubber grasshoppers: quantifying cuticular and respiratory transpiration. *Physiol. Zool.* **66**, 628-642.
- Reinhold, K.** (1999). Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Funct. Ecol.* **13**, 217-224.
- Schneiderman, H. A.** (1960). Discontinuous respiration in insects: role of the spiracles. *Biol. Bull.* **119**, 494-528.
- Schneiderman, H. A. and Schechter, A. N.** (1966). Discontinuous respiration in insects - V. Pressure and volume changes in the tracheal system of silkworm pupae. *J. Insect Physiol.* **12**, 1143-1170.
- Scholtz, C. H. and Chown, S. L.** (1995). The evolution of habitat use and diet in the Scarabaeoidea: a phylogenetic approach. In *Biology, Phylogeny, and Classification of Coleoptera. Paper celebrating the 80<sup>th</sup> birthday of Roy A. Crowson* (ed. J. Pakaluk and S. A. Slipinski), pp. 355-374. Warszawa: Muzeum i Instytut Zoologii PAN.
- Shelton, T. G. and Appel, A. G.** (2000). Cyclic carbon dioxide release in the dampwood termite, *Zootermopsis nevadensis* (Hagen). *Comp. Biochem. Physiol.* **126A**, 539-545.
- Terblanche, J. S., Klok, C. J., Marais, E. and Chown, S. L.** (2004). Metabolic rate in the whip spider, *Damon annulatipes* (Arachnida: Amblypygi). *J. Insect Physiol.* **50**, 637-645.
- Wilkins, M. B.** (1960). A temperature-dependent endogenous rhythm in the rate of carbon dioxide output of *Periplaneta americana*. *Nature* **185**, 481-482.
- Williams, A. E., Rose, M. R. and Bradley, T. J.** (1997). CO<sub>2</sub> release patterns in *Drosophila melanogaster*: The effect of selection for desiccation resistance. *J. Exp. Biol.* **200**, 615-624.
- Williams, A. E., Rose, M. R. and Bradley, T. J.** (2004). The respiratory pattern in *Drosophila melanogaster* selected for desiccation resistance is not associated with the observed evolution of decreased locomotor activity. *Physiol. Biochem. Zool.* **77**, 10-17.

## **Appendix 3**



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## Environmental physiology of three species of Collembola at Cape Hallett, North Victoria Land, Antarctica

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### Abstract

The environmental physiology of three species of Collembola: *Cryptopygus cisantarcticus*, *Isotoma klovstadi* (Isotomidae) and *Friesea grisea* (Neanuridae) was investigated from November 2002 to February 2003 at Cape Hallett, North Victoria Land, Antarctica. All three species were freeze avoiding, and while supercooling points were variable on seasonal and daily scales in *I. klovstadi* and *C. cisantarcticus*, they remained largely static in *F. grisea*. LT50 (temperature where 50% of animals are killed by cold) was  $-13.6$ ,  $-19.1$  and  $-19.8$  °C for *C. cisantarcticus*, *I. klovstadi* and *F. grisea*, respectively. Upper lethal temperature was 34, 34 and 38 °C for *C. cisantarcticus*, *I. klovstadi* and *F. grisea*. Critical thermal minimum onset (the temperature where individuals entered chill coma) was ca.  $-7$ ,  $-12$  and  $-8$  °C for *C. cisantarcticus*, *I. klovstadi* and *F. grisea*, and 25% of *I. klovstadi* individuals froze without entering chill coma. Critical thermal maximum (the onset of spasms at high temperature) was 30, 33 and 34 °C for *C. cisantarcticus*, *I. klovstadi* and *F. grisea*. Haemolymph osmolality was approximately 720 mOsm for *C. cisantarcticus* and 680 mOsm for *I. klovstadi*, and both species showed a moderate degree of thermal hysteresis, which persisted through the season. Desiccation resistance was measured as survival above silica gel, and the species survived in the rank order of *C. cisantarcticus* << *I. klovstadi* = *F. grisea*. Desiccation resulted in an increase in haemolymph osmolality in *I. klovstadi*, and water was quickly regained by desiccation-stressed individuals that had access to liquid water, but not by individuals placed in high humidity, indicating that this species is unable to absorb atmospheric water vapour. SDS-PAGE did not suggest any strong patterns in protein synthesis either seasonally or in response to temperature or desiccation stress. Microclimate temperatures were measured at sites representative of collection sites for the three species. Microclimate temperatures were highly variable on a diurnal and weekly scale (the latter relating to weather patterns), but showed little overall variation across the summer season. Potentially lethal high and low temperatures were recorded at several sites, and it is suggested that these temperature extremes account for the observed restriction of the less-tolerant *C. cisantarcticus* at Cape Hallett. Together, these data significantly increase the current knowledge of the environmental physiology of Antarctic Collembola.

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**Keywords:** Cold tolerance; Supercooling point; Critical thermal limits; Desiccation; Osmoregulation; Osmolality; Stress protein; Microclimate

### 1. Introduction

The organisms that live in terrestrial Antarctic habitats must tolerate the temperature extremes, aridity, short growing season and long months of complete darkness that characterise Antarctica (Block, 1994). Given current climate change (IPCC, 2001; Doran et al., 2002; Parmesan

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and Yohe, 2003), understanding the physiological tolerances of Antarctic species, and the extent to which these tolerances may influence community dynamics and determine distribution, is an important aspect of managing and conserving Antarctic biodiversity (Convey et al., 2002, 2003; Peck, 2002; Walther et al., 2002; Peck et al., 2004). In terms of species diversity, distribution and abundance, Collembola (springtails) are one of the dominant members of the terrestrial Antarctic fauna. Endemism in the Antarctic springtail fauna is high, and the group is found in habitats extending almost as far south as ice-free land permits (Wise and Gressitt, 1965; Block, 1994). In consequence, research on the ecology and physiology of Antarctic Collembola has a long history (e.g. Ewing, 1922; Gressitt, 1967; Janetschek, 1967; Tillbrook, 1977; Cannon and Block, 1988; Sinclair and Sjørnsen, 2001a; Worland and Convey, 2001).

All Antarctic Collembola that have been studied are freeze avoiding, surviving sub-zero temperatures by maintaining their body fluids in a liquid state, and are killed upon freezing at a temperature referred to as the supercooling point (SCP) (Cannon and Block, 1988; Sinclair et al., 2003b). Consequently SCP frequency distributions are a valid and useful way to assess the population distribution of cold tolerance in these species (Cannon and Block, 1988; Worland and Convey, 2001). Bimodal SCP distributions have been commonly described, and seem to be a consequence of ice nucleators from food in the gut (Cannon and Block, 1988). The higher (less cold-hardy) mode of the distribution is thought to be made up largely of active, foraging individuals, while the low (more cold-hardy) group are thought to be non-feeding or moulting individuals (Worland, 2005), with a general shift to the low group also observed in preparation for winter (Cannon and Block, 1988). Recently, rapid shifts between these groups in response to diurnal temperature cycles have been demonstrated in species from the maritime (Worland and Convey, 2001) and continental (Sinclair et al., 2003b) Antarctic. However, bimodal SCP distributions are absent in *Friesea grisea* (Neanuridae) at Cape Hallett (Sinclair et al., 2003b) and possibly also in *Gomphiocephalus hodgsoni* (Hypogastruridae) at Cape Bird (Sinclair and Sjørnsen, 2001a), suggesting that bimodality may not be the rule in non-isotomid species. Thermal hysteresis proteins, which stabilise the body fluids of supercooled insects against homogeneous ice nucleation and prevent inoculative freezing (Zachariassen and Husby, 1982; Zachariassen and Kristiansen, 2000) are present in several alpine Collembola (Zettel, 1984) and have been detected in *G. hodgsoni* (Sinclair and Sjørnsen, 2001a), but are not present in *Cryptopygus antarcticus* (Isotomidae) from the maritime Antarctic (Block and Duman, 1989).

Apart from cold tolerance, other aspects of the thermal biology of Antarctic Collembola have received less attention. As may be expected, Antarctic Collembola are able to maintain activity at low temperatures, with reported chill coma temperatures ( $\approx$ critical thermal minimum) ranging

from near zero in *G. hodgsoni* (Janetschek, 1967) to  $-10^{\circ}\text{C}$  in *C. antarcticus* (Sømme and Block, 1982). Thermal preferences are often surprisingly high: in the region of  $10^{\circ}\text{C}$  for *G. hodgsoni* (Fitzsimons, 1971) and  $9\text{--}13^{\circ}\text{C}$  for warm-acclimated *C. antarcticus* (Hayward et al., 2003). Upper thermal limits have rarely been measured, although Fitzsimons (1971) reported an upper lethal temperature of  $+29.5^{\circ}\text{C}$  for *G. hodgsoni*. With the exceptions of an investigation of the potential of Hsp70 as an ecotoxicological biomarker (Staempfli et al., 2002) and the demonstration of the induction of Hsp70 in response to desiccation (Bayley et al., 2001), molecular responses to thermal or other environmental stresses (see Chown and Nicolson, 2004, for review) have not been investigated in Collembola.

Low precipitation, sub-zero temperatures, and high water vapour deficit mean that desiccation is a major stress for terrestrial Antarctic organisms (Kennedy, 1993). Cutaneous respiration, permeable cuticles and a large surface area:volume ratio conspire to make Collembola susceptible to desiccation (Hopkin, 1997), and significant interspecific variation in behaviour, cuticle structure and consequently rate of water loss mean that there is significant among-species variation in desiccation tolerance (Hopkin, 1997; Hertzberg and Leinaas, 1998). Hayward et al. (2004) suggested that the fine-scale distributions of *Friesea grisea* and *C. antarcticus* at Rothera on the Antarctic Peninsula may be a consequence of their different rates of or tolerances to water loss. Collembola are generally poor osmoregulators (Hopkin, 1997). Haemolymph osmolalities of most Collembola under normal conditions fall between 200 and  $400\text{ mOsm kg}^{-1}$  (Hopkin, 1997; Sinclair and Chown, 2002). Early spring haemolymph osmolalities of up to  $1755\text{ mOsm kg}^{-1}$ , decreasing to ca.  $500\text{ mOsm kg}^{-1}$  in summer, were reported in *G. hodgsoni* (Sinclair and Sjørnsen, 2001a). Block and Harrison (1995) report much lower osmolalities of ca.  $284\text{ mOsm kg}^{-1}$  for *C. antarcticus*.

An essential element of environmental physiology is determination of the conditions that organisms actually encounter (Bale, 1987; Kingsolver, 1989; Danks, 1991; Sinclair et al., 2003c). In terrestrial Antarctic habitats, microclimatic temperatures have long been collected as part of ecological and physiological studies (e.g. Pryor, 1962; Janetschek, 1963, 1967; Rudolph, 1963), and this practice has been significantly advanced by the development and availability of automatic dataloggers (e.g. Block, 1985; Sømme, 1986; Friedmann et al., 1987; Davey et al., 1992; Sinclair and Sjørnsen, 2001a). Although most records are limited to temperature data, these are of value in assessing the magnitude and frequency of thermal stresses in the field. Indeed, recent assessments of daily temperature variation prompted greater temporal resolution of cold hardiness studies, revealing diurnal changes in the SCPs of arthropods in response to this variation (Worland and Convey, 2001; Sinclair et al., 2003b). Integrating microclimate temperature recordings and physiological information is therefore essential for understanding the likelihood



that organisms experience physiologically stressful conditions in the field, the variability of these stresses at small and large spatial scales, and their importance in influencing population dynamics and consequently the abundance and distribution of the species concerned. The extent to which organisms are operating at the edge of their physiological tolerances is of great significance for understanding the changes that are likely to be induced by rapid climatic change (Holt et al., 1997; Kingsolver and Huey, 1998; Sinclair, 2001a).

Although apparently extensive, as the brief review above suggests, physiological and ecological investigations of Antarctic Collembola have been dominated by research on *C. antarcticus* in the Maritime Antarctic, and this work has contributed substantially to modern understanding of springtail biology in the Antarctic. However, comprehensive investigations of the biology of one species in a single region is inadequate for understanding the environmental physiology of a higher taxon, and its likely responses to climate change (Chown et al., 2002a). This is especially true for the wide range of species found across Antarctica: a continent whose regions' climates vary dramatically, both currently and in predicted responses to global climate change (Convey, 2001; Doran et al., 2002; Walther et al., 2002). Here, we therefore extend previous studies of the environmental physiology of the Antarctic springtail fauna by investigating the thermal biology and water relations of three species of Collembola at Cape Hallett, North Victoria Land; an area for which little information is currently available. Furthermore, we investigate protein expression in one of the species, and present high-resolution microclimate temperature data for a summer at Cape Hallett, placing the physiological measurements in an appropriate environmental context.

## 2. Methods

### 2.1. Study site and animals

This work was conducted at the 72 ha Cape Hallett ice-free area (72°19'S, 170°13'E; Antarctic Specially Protected Area No. 106, <http://www.cep.aq/apa/>) in North Victoria Land, Antarctica. The site consists of basalt scree, moraines and beach deposits (Harrington et al., 1967). Approximately half of the area is occupied by an Adelie Penguin (*Pygoscelis adeliae*) colony, and is consequently devoid of terrestrial arthropods (Sinclair, Scott, Klok, Terblanche, Marshall, Reyers and Chown, in preparation). The rest of the habitat may be divided into 'algal flats' (low-lying undulating moraine with abandoned penguin mounds, moss and algal communities and often abundant flowing or standing surface water, sometimes several centimetres deep during the height of snowmelt); 'scree slope', a steep, unstable area 20–250 m a.s.l. which is dominated by lichen communities, with scattered algae and mosses; and 'ridge' habitat, above snowfields at approximately 300 m a.s.l., an exposed habitat with a distinct

community of lichens. Data presented here are from animals sampled from the algal flats and lower scree slope habitats. The position of the scree slope, and the Hallett Peninsula which rises to 1770 m behind it, result in a diurnal sun–shade cycle during the summer with consequent large daily temperature variations (Sinclair et al., 2003b).

Three species of Collembola occur at Cape Hallett (Wise and Shoup, 1967). *Friesea grisea* (Schäffer) (Neanuridae) is sparsely distributed throughout the entire area, with foci at the edges of moss banks. *Cryptopygus cisantarcticus* Wise (Isotomidae) is found largely in the moist algal flats; and *Isotoma klovstadi* Carpenter (Isotomidae) is extremely abundant in vegetated areas of the scree slope (Pryor, 1962; Wise and Shoup, 1967; Sinclair et al., 2003b). All three species are also present in soil at the ridge site (Sinclair et al., in preparation).

Collembola were collected from beneath rocks using aspirators, and placed in dry plastic containers (for immediate use, e.g. SCP determinations) or onto moist Plaster-of-Paris, if they were to be stored for more than a few minutes. During daytime in mid-summer, *I. klovstadi* could be found in large aggregations grazing on the surface of moss. These 'foraging' individuals were collected from the moss surface with an aspirator for some SCP experiments. Field-fresh animals were always kept in the shade on snow at the camp (ca. 0 °C), and used within 1 h of collection. Springtails for acclimation (generally *I. klovstadi*) were placed in plastic vials with a piece of moist filter paper and a mesh-covered perforated lid, which was placed within a larger sealed container containing 25–40 ml distilled water, maintaining 100% relative humidity (RH). Acclimation to 5 °C in 24 h light was carried out as described by Sinclair et al. (2003b) in a Sable Systems PTC-1 Peltier-effect Temperature Cabinet (Sable Systems Inc., Henderson, Nevada, USA) with white light provided by a Black Diamond Ion LED lamp (Black Diamond Equipment, Salt Lake City, UT, USA).

With the exception of protein electrophoresis, all of the data were gathered on site from a tent-based field laboratory at Cape Hallett between 9 November 2002 and 27 January 2003. Generators typically operated for 14 h per day and could not be operated during blizzards (ca. 10/77 days), limiting the practicability of extended acclimation treatments.

### 2.2. Supercooling points

SCPs, (see Block and Young, 1979, for a general description) were measured in freshly caught Collembola within a few minutes of their return to the laboratory. Two or three springtails were affixed with silicone grease to the tip of a 40-gauge Type T thermocouple connected to an eight channel Grant Squirrel SQ800 data logger (Grant Instruments, Cambridge, UK). Temperature measurements were made continuously and averaged over 4 s intervals. The thermocouples and springtails were placed in 1.5 ml

microcentrifuge tubes in an aluminium block cooled by a Peltier module as described by Sinclair et al. (2003b). Springtails were cooled at  $0.5\text{ }^{\circ}\text{C min}^{-1}$  from 0 to  $-40\text{ }^{\circ}\text{C}$  (preliminary experiments indicated that SCP was not affected by varying the cooling rate between 0.8 and  $0.05\text{ }^{\circ}\text{C min}^{-1}$ ), and SCPs were derived from the latent heat of crystallisation (Block and Young, 1979), which was clearly discernible for each individual attached to the thermocouple. No pre-freeze mortality or survival of freezing was observed in preliminary observational experiments, where groups of springtails individually placed on thermocouples were cooled until half the individuals had frozen, and movement observed in unfrozen individuals upon rewarming (B.J. Sinclair, unpublished observations). Thus, it is assumed that, as for other Antarctic Collembola (Cannon and Block, 1988; Sinclair and Sjørnsen, 2001a), the SCP is a valid measure of the acute lower lethal temperature in the species at Cape Hallett.

SCPs (usually of 20–30 individuals) were measured at regular intervals throughout the season, from late-November to mid-January for a total of five measurements for each species. Because substantial diurnal variation in SCP was found in *I. klovstadi* and *C. cisantarcticus* (Sinclair et al., 2003b), SCPs of all three species were measured during the ‘day’ (when the study site was in the sun, between 11:00 and 16:30 h) and ‘night’ (when the study site was in the shade, between 23:30 and 04:30 h) on two consecutive nights and days (unless interrupted by poor weather), and the data from the 2 days or nights pooled for each sampling point. Although the December data reported here were used by Sinclair et al. (2003b) to characterise the diurnal changes in SCP, this is the first presentation of the full seasonal spectrum of the SCP data.

SCPs of *F. grisea* were unimodal, but strongly skewed (Sinclair et al., 2003b), so the absolute SCPs during night and day were compared between sampling periods using Generalized Linear Models (GLZ, Poisson distribution and identity link) in Statistica (v. 6.1, Statsoft, Tulsa, Oklahoma, USA). SCP distributions of *I. klovstadi* and *C. cisantarcticus* were approximately bimodal, and were split into high and low groups (HG and LG, respectively), with cut-offs of  $-16\text{ }^{\circ}\text{C}$  for *C. cisantarcticus* and  $-18\text{ }^{\circ}\text{C}$  for *I. klovstadi* (see Sinclair et al., 2003b, and references therein for rationale). The distributions of high and low groups of SCPs were approximately normal, and the means of the high and low groups were compared among and within sampling dates using ANOVA on Statistica. Counts of individuals in high and low groups were compared using GLZ (Poisson distribution and identity-link) in Statistica. Daytime SCP distributions of foraging and non-foraging individuals of *I. klovstadi* were combined for this comparison.

### 2.3. Upper and lower lethal temperatures

Upper and lower lethal temperatures (ULT and LLT, respectively) for each species were determined on field-fresh

animals in mid-December 2002 using a cumulative technique modified from that of Worland et al. (1992). Collembola were placed in 1.5 ml microcentrifuge tubes in groups of 10 individuals (with moist filter paper to prevent desiccation for ULT determinations), and placed in a plastic bag in a Grant LTD-20 water bath controlled by a PZ-1 programmer (Grant Instruments, Cambridge, UK) for equilibration for 15 min at  $5\text{ }^{\circ}\text{C}$ . For ULTs, the temperature of the bath was then increased at  $0.1\text{ }^{\circ}\text{C min}^{-1}$  to a set high temperature, where the temperature was held for 1 h, after which 3 tubes for each species were removed to tent temperature (ca.  $10\text{--}15\text{ }^{\circ}\text{C}$ ) and then  $5\text{ }^{\circ}\text{C}$  for recovery. The temperature was then increased at  $0.1\text{ }^{\circ}\text{C min}^{-1}$  with one hour hold periods and 3 tubes were removed for each species at  $1\text{ }^{\circ}\text{C}$  increments. After preliminary experiments to determine a suitable range of temperatures, tubes were removed at temperatures from 28 to  $35\text{ }^{\circ}\text{C}$  for *C. cisantarcticus* ( $n = 8$  temperatures), from 31 to  $37\text{ }^{\circ}\text{C}$  for *I. klovstadi* ( $n = 7$  temperatures), and from 31 to  $39\text{ }^{\circ}\text{C}$  for *F. grisea* ( $n = 9$  temperatures). A similar protocol was followed for LLTs, with cooling rates of  $0.1\text{ }^{\circ}\text{C min}^{-1}$ , and 3 tubes of each species were removed at  $-8$  and  $-10\text{ }^{\circ}\text{C}$ , and thereafter at 5 degree intervals to  $-30\text{ }^{\circ}\text{C}$  ( $n = 6$  temperatures). Tubes were removed to  $5\text{ }^{\circ}\text{C}$  for recovery. Logistic regression models of survival (see Quinn and Keogh, 2002) of the form:

$$p(\text{alive}) = 1 - \frac{e^{a+bx}}{1 + e^{a+bx}} \quad (1)$$

were fitted to the ULT and LLT data using the nonlinear estimation module of Statistica, and standard errors were calculated from error estimates on the slope and intercept of the fitted equation. ULT50 and LLT50 (the high and low temperatures, respectively, where 50% mortality is expected) were estimated from the fitted equation.

### 2.4. Critical thermal minimum and maximum

Critical thermal minima (CTmin) and critical thermal maxima (CTmax) were determined on four occasions across the summer. Field-fresh springtails were contained in 5 mm diameter circular plastic enclosures on a cold stage cooled by a Grant LTD20 water bath and PZ1 controller, and observed with a dissecting microscope. Each species was assessed separately, and all measurements were made by the same observer (CJK). Fifteen field-fresh animals were placed individually in the enclosures and allowed to equilibrate at  $0\text{ }^{\circ}\text{C}$  for 15 min (escapes were excluded from observation, reducing sample size in some instances). The stage was then cooled at  $0.25\text{ }^{\circ}\text{C min}^{-1}$  to  $1\text{ }^{\circ}\text{C}$  below the lowest CTmin<sub>onset</sub>, where they were held for 5 min before rewarming. CTmin<sub>onset</sub> was defined during cooling as the temperature at which animals could not maintain coordinated movement even when agitated by vigorously tapping the base of the cold stage. During rewarming the CTmin<sub>recovery</sub> was defined as the resumption of coordinated movement including righting and walking—mere movement of legs and antennae

was discounted. CT<sub>max</sub> was determined in a similar fashion for the same individuals with a warming rate of 0.25°C min<sup>-1</sup>, and was defined as the onset of muscular spasms following the vigorous activity of the animals at high temperatures (Lutterschmidt and Hutchison, 1997). Freezing was observed as a violent stiffening of the body accompanied by extension of the furca and legs in some individuals, and if more than 50% of the individuals froze during a CT<sub>min</sub> run, the frozen individuals were removed, and new individuals introduced to the chamber for CT<sub>max</sub> determination. Critical thermal limits were compared between species and across the season using ANOVA in Statistica, followed by Tukey's HSD post hoc comparison. The within-species relationship between CT<sub>min<sub>onset</sub></sub>, CT<sub>min<sub>recovery</sub></sub> and CT<sub>max</sub> was examined using Pearson's correlation coefficient on data for all individuals (regardless of sampling date) for which data from the three parameters was available ( $n = 34, 57$  and  $54$  for *I. klovstadi*, *F. grisea* and *C. cisantarcticus*, respectively).

### 2.5. Haemolymph osmolality

Haemolymph osmolality was determined optically using a calibrated Clifton Nanoliter Osmometer (Clifton Technical Physics, NJ, USA), using standard methods (Sjursen and Sømme, 2000). Haemolymph was obtained from the drop exuded after the amputation of a leg or antenna of an animal held under liquid paraffin to prevent evaporation. Individual samples of haemolymph from *I. klovstadi* were used, whereas samples pooled from the haemolymph of 3–4 individuals were used for *C. cisantarcticus*. Haemolymph from *F. grisea* rapidly coagulated and oxidation turned the fluid opaque, and an approximate determination of osmolality was made on only one occasion for this species. Haemolymph osmolality was determined on six occasions between late-November 2002 and mid-January 2003 for *I. klovstadi* and *C. cisantarcticus* ( $n = 10$  replicates per determination, apart from the initial sample, when  $n = 25$  for *I. klovstadi* and  $n = 15$  for *C. cisantarcticus*). Haemolymph was also examined for thermal hysteresis activity, indicated by a discrepancy between the melting and freezing points of a single ice crystal. Hysteresis in mOsm kg<sup>-1</sup> was converted into °C (1000 mOsm = 1.86 °C), on occasions where it was found. Haemolymph osmolality and thermal hysteresis activity were compared across the season using ANOVA.

### 2.6. Desiccation resistance

Desiccation resistance was compared between species using a modification of the method of Hertzberg and Leinaas (1998). All experiments were conducted from 29 November to 1 December 2002. Collembola were acclimated for 3 days at 5 °C in 100% RH with moist filter paper before being placed individually into 1.5 ml micro-centrifuge tubes ( $n = 20$  individuals per species) with a large crystal of dry silica gel attached to the interior of the

lid (treatment—approximately 5% RH) or a piece of moist filter paper (control—approximately 100% RH). The tubes were then placed in a sealed plastic bag in a Grant LTD20 water bath (Grant Instruments, Cambridge UK) at 20 °C. After 30 (*C. cisantarcticus*) or 60 min had elapsed, the tubes were removed every 30 (*I. klovstadi*) or 15 (*F. grisea* and *C. cisantarcticus*) minutes, and survival (coordinated movement) of each individual scored. Once a treatment individual had been classified as dead, it was removed and placed in 100% RH at 5 °C to look for recovery after 24 h. A logistic regression model of survival was fitted as for ULT and LLT data, and the time to 50% mortality (analogous to the LLT50 or ULT50) and standard errors estimated.

### 2.7. Osmoregulation in *Isotoma klovstadi*

We examined the recovery from desiccation of *I. klovstadi*. *Isotoma klovstadi* was acclimated in groups of 100 at 5 °C at 100% RH for 3 days, before being exposed *en masse* to silica gel at 5 °C for 3 h. Collembola (in containers) were then removed from silica gel, and either supplied with moist filter paper in 100% RH ('Free Water' treatment), or placed in 100% RH without direct access to water ('Water Vapour' treatment). Measurements of SCPs ( $n \approx 16$  individuals), haemolymph osmolality ( $n = 5$  individuals) and gravimetric water content were made before desiccation, at the conclusion of the 3 h desiccation, and at 1, 3, 6, 12 and 24 h after the conclusion of desiccation (Free Water), and at 4 and 26 h after conclusion of desiccation (Water Vapour). Gravimetric water content was measured for 3 groups of 25–30 springtails per treatment as described by Sinclair et al. (2003b). Comparisons were made between groups using ANOVA (osmolality), Kruskal–Wallis Rank ANOVA (SCP medians) and analysis of covariance (ANCOVA) with dry mass as the covariate (water content).

### 2.8. Total protein analysis

*I. klovstadi*, the largest and most abundant springtail at the site, was selected for qualitative protein analysis by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE, Laemmli, 1970) on field-fresh individuals on six occasions throughout the summer. *I. klovstadi* for electrophoresis were also acquired from experiments investigating response to and recovery from desiccation (see below), and rapid cold-hardening (based on the procedures of Nunamaker (1993), see Sinclair et al. (2003b) for full rationale and methodology). Briefly, groups of field-fresh *I. klovstadi* were exposed in groups of 20 individuals to 2 h treatments in 1.5 ml tubes as controls (5 °C, 100% RH), or at 28 (with moist filter paper), 0, -5 or -9 °C (all in dry tubes); as well as -5 °C followed by a 2 h recovery period at +5 °C ('-5gap'), three hours in a container with low relative humidity provided by silica gel ('desiccation') and a slow cooling treatment (+5 to -2 °C at 1 °C h<sup>-1</sup>).



Approximately 5 mg of field-fresh or acclimated and treated *I. klovstadi* were weighed to  $\pm 1 \mu\text{g}$  and immediately homogenised in 50  $\mu\text{l}$  SDS-PAGE sample-treatment buffer (2% SDS, 10% glycerol, 100 mM Dithiothreitol (DTT), 60 mM Tris [pH 6.8] and 0.001% bromophenol blue (Laemmli, 1970), and stored at sub-zero temperatures (below  $-5^\circ\text{C}$  in the field for up to 2 months, below  $-20^\circ\text{C}$  for transport from Antarctica to New Zealand (1 week), and thereafter below  $-50^\circ\text{C}$  until processing). In the laboratory in South Africa, samples were boiled for 5 min and diluted with SDS-PAGE loading buffer (100 mM Tris (pH 6.8), 5%  $\beta$ -mercaptoethanol, 4% SDS, 0.2% Bromophenol Blue, 20% Glycerol) to a final amount of 0.35 mg fresh mass/lane. Proteins were separated by SDS-PAGE using the BioRad Mini-PROTEAN II System (BioRad, Hercules, CA, USA) and a 10% polyacrylamide resolving gel. The size of protein bands was estimated with reference to standard molecular mass markers (Broad Range SDS-PAGE markers, BioRad). Western analysis was performed according to Towbin et al. (1979). The enhanced chemiluminescence system (ECL; Amersham) was used to detect heat shock proteins during Western analysis using mouse anti-Hsp70 and mouse anti-Hsp90 antibodies.

## 2.9. Microclimate recordings

### 2.9.1. Measurement of microclimate data

Microclimate temperatures were measured every 10 min throughout the summer beneath rocks representative of those turned to collect each species. *I. klovstadi* habitat was monitored at two different sites (ca. 120 m apart) at the same elevation. Temperature at the first site was monitored by two channels of a Hobo H-8 logger with TMCx-HA thermistor probes (Onset Computer Corporation, Pocasset, MA, USA) under two stones (184 and 107  $\text{cm}^2$ , 22 and 64 mm thick, respectively). Temperatures at site 1 were monitored from 16 November 2002–5 January 2003, before the receding snowbank made access to the logger across the unstable scree slope overly destructive to the habitat. The second *I. klovstadi* site consisted of three stones, of 26–151  $\text{cm}^2$  and between 12 and 74 mm thick. Temperatures were logged by calibrated iButton Thermochron data loggers (accurate to  $\pm 0.5^\circ\text{C}$ , Dallas Semiconductors, Dallas, TX, USA) or probes from a Hobo H-8 logger as above. At the two *I. klovstadi* sites, stone surface temperatures were also monitored with an unshielded TMx-HA thermistor probe at each site.

*C. cisantarcticus* stones were located in a moist area of the algal flat, near the southern edge of the penguin colony, whereas the *F. grisea* stones were in a drier area, close to *I. klovstadi* site 2 (see Sinclair et al., 2003b, for a description of these stones) Temperatures at *F. grisea* and *C. cisantarcticus* sites were monitored with calibrated iButtons as above. The two most distant sites (*I. klovstadi* site 1 and *C. cisantarcticus*) were approximately 400 m apart.

### 2.9.2. Analysis of microclimate data

Summary statistics for each data logger location were calculated using Microsoft Excel. Microclimate data were also analysed with VisualBasic macros in Microsoft Excel (Microsoft, Seattle, WA, USA), using the general approach described by Sinclair (2001a, b). A current version of the macros (v. 2.0.2) is available upon request from B.J. Sinclair ([celatoblatta@yahoo.co.uk](mailto:celatoblatta@yahoo.co.uk)). Three basic types of summaries were extracted from the data: cooling rates across a specific threshold (calculated in all cases for the 60 min preceding the threshold); the number of cycles that cross a threshold; the period of time (in minutes) spent above or below a threshold. All cooling rates calculated by the macro were checked manually, and only those calculated for a period of monotonically decreasing temperature were used in analyses.

General biological thresholds were analysed across the entire dataset for each logger, but comparisons between loggers and habitats were made only for the month of December 2002, for which a complete dataset was available for all 11 logger sites. Biologically relevant thresholds were derived from the physiological data described elsewhere in the paper. ULT5 and ULT50 (upper temperatures at which 5% and 50% of individuals are expected to die) were calculated using the logistic regression equations for the upper thermal limit data, while SCP5 and SCP50 (temperatures at which 5 and 50% of individuals are expected to freeze upon acute exposure) were derived from cumulative SCP distributions of the entire SCP data set for each species. To determine the likelihood of potentially lethal thermal events during the summer, the number of times in each complete dataset that the temperature dropped below  $0^\circ\text{C}$  (freeze–thaw cycles); the number of times it dropped below the SCP5 and SCP50 for each species of springtail and the number of times the temperature increased above the ULT5 and ULT50 for each species were counted. The cooling rate for the hour preceding the crossing of a threshold, with thresholds of  $+10$ ,  $+5$ ,  $0$ ,  $-5$  and  $-10^\circ\text{C}$ , was also calculated to make comparisons of cooling rates across the temperature spectrum. Cooling rates at  $0^\circ\text{C}$  were compared between habitats using a generalized linear model (Gamma distribution, Identity-link; Statistica). Counts of freeze–thaw cycles across  $0^\circ\text{C}$  and  $-3.2^\circ\text{C}$ , as well as the total time spent above  $0$  and  $+10^\circ\text{C}$  were compared between habitats using a Kruskal–Wallis rank ANOVA followed by non-parametric multiple comparisons in Statistica. A correlation matrix of area and volume of stones (area was calculated using the method of Sinclair et al., 2001) with the above thresholds was constructed, and the relationship between stone size and microclimate thermal characteristics tested using Spearman's rank correlation in Statistica.

## 2.10. Relationships between physiological and climatic variables

The effects of prevailing microclimate temperature conditions on physiological variables measured on field



fresh animals were examined using Pearson's correlation coefficients. Central measures of cold tolerance (mean day and night HG and LG SCP for *I. klovstadi* and *C. cisantarcticus*, and median SCP for *F. grisea*); maximum and minimum SCP, mean CT<sub>min<sub>onset</sub></sub>, CT<sub>min<sub>recovery</sub></sub> and CT<sub>max</sub>; and mean haemolymph osmolality were used. Microclimate temperature variables were calculated from data logger data from the representative sites for that species (see above). Means of microclimate variables were calculated as the mean of the means recorded from the three loggers at a site, whereas the maximum or minimum was the absolute maximum or minimum across all three loggers at the site. Mean, maximum, minimum and range (max–min) temperatures were calculated for 1, 3 and 5 days preceding the measurement of the physiological variable and used for the correlation analysis. Significance of all multiple comparisons was corrected using a table-wide step-up false discovery rate correction (García, 2004).

### 3. Results

#### 3.1. Supercooling points

SCPs were unimodal (but strongly skewed, skewness ± SEM = 1.56 ± 0.16) in *F. grisea*, and bimodal in *I. klovstadi* and *C. cisantarcticus* (Fig. 1). SCPs of *F. grisea* did not change significantly across the season, nor between 'day' and 'night' (Table 1; see also Sinclair et al., 2003b). High- and low-group SCPs of *C. cisantarcticus* also did not change between night and day (Table 1). In one period (26 Nov–2 Dec 2002, which also had the lowest mean temperature), *I. klovstadi* low group mean was significantly lower during the night than during the day (which accounts for the apparent third mode in the overall SCP distribution, Fig. 1), but in all other cases the means did not differ between day and night (Table 1). The HG:LG ratio of both *I. klovstadi* and *C. cisantarcticus* differed substantially between day and night (Day/night-High/Low interaction: *I. klovstadi*: Wald  $\chi^2 = 7.37$ ,  $p = 0.007$ ,  $df = 1$ ; *C. cisantarcticus*: Wald  $\chi^2 = 8.43$ ,  $p = 0.004$ ,  $df = 1$ ), with a strong night-time bias towards LG (see also Sinclair et al., 2003b). From November to January, the proportion of HG *C. cisantarcticus* during the day increased markedly (Table 1). A similar pattern was observed for *I. klovstadi* if foraging animals were pooled in the daytime sample, with a larger proportion of HG animals in December and January than in November (Table 1). SCP parameters of each species of springtail were not significantly correlated with any of the environmental temperature indices measured (Pearson's correlation coefficient,  $p > 0.05$  in all cases).

#### 3.2. Upper and lower lethal temperatures

The highest temperatures survived were 34, 34 and 38 °C for *I. klovstadi*, *C. cisantarcticus* and *F. grisea*, respectively. Upper lethal temperatures and fitted logistic regression

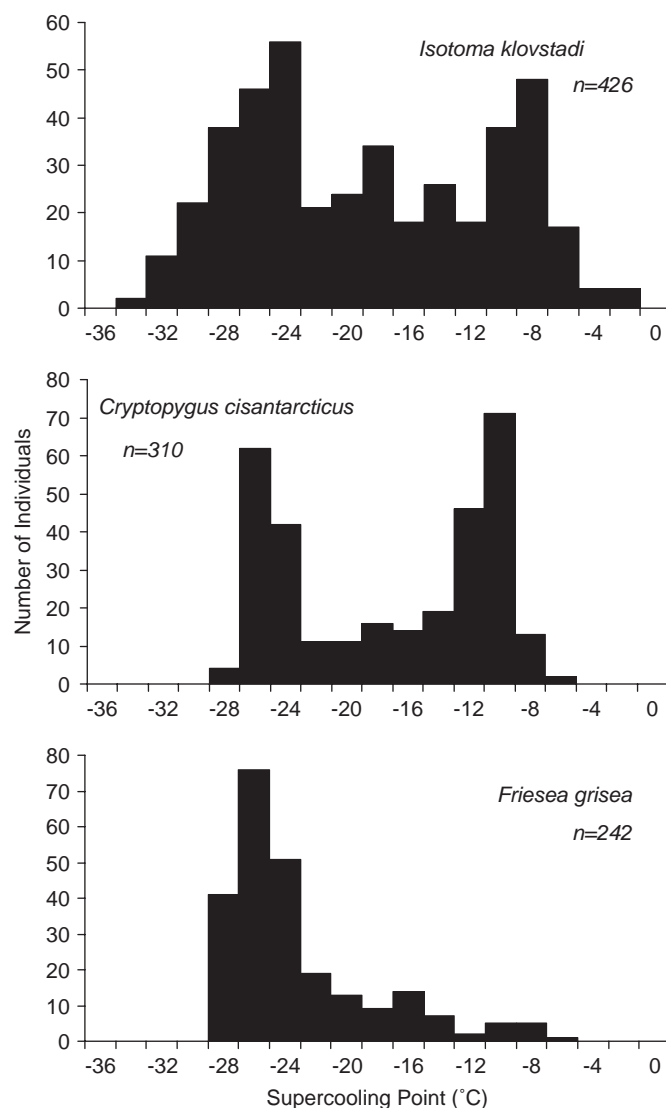


Fig. 1. Combined supercooling point distributions for three species of Collembola at Cape Hallett, Antarctica.

models for all three species are shown in Fig. 2a. The standard errors from the model suggest no significant differences between the ULT50s of the three species. However, *I. klovstadi* has a very rapid, steep response to high temperatures, whereas there is a greater intraspecific variability in the other species, resulting in shallower fitted curves (Fig. 2a). This means that although the maximum temperature survived for *I. klovstadi* and *C. cisantarcticus* is identical, high temperature mortality begins at 29 °C in *C. cisantarcticus*, but at 33 °C in *I. klovstadi*. The rank order of the ULT50 is *C. cisantarcticus* < *I. klovstadi* < *F. grisea*.

*C. cisantarcticus* was less cold tolerant than the other species (LLT50s were -13.6, -19.1 and -19.8 °C for *C. cisantarcticus*, *I. klovstadi* and *F. grisea* respectively, Fig. 2b). As may be expected from the SCPs, the LLT and LLT50 of *I. klovstadi* and *F. grisea* were similar, thus indicating that the rank order of the three species in terms

**Table 1**  
 Summary of supercooling point distributions and means for three species of Collembola at Cape Hallett, Antarctica, during the 2002/2003 summer. Low group included those individuals with SCP < -18 °C (*Isotoma klovstadi*) or < -16 °C (*Cryptopygus cisantarcticus*). *Friesea grisea* did not display a bimodal SCP distribution, so median and quartiles are shown. Within species, means with different letters differ significantly (ANOVA: Tukey's HSD  $p < 0.05$ ; *I. klovstadi*: Date × high/low interaction  $F_{4,407} = 10.05, p < 0.001$ , Date × Day/night interaction  $F_{4,407} = 3.93, p = 0.011$ ; *C. cisantarcticus*: Date × Day/Night interaction  $F_{4,291} = 3.05, p = 0.018$ ; GLZ: *F. grisea*: Between Dates: Wald  $\chi^2 = 3.985, p = 0.41, df = 4$ ; Between day and night: Wald  $\chi^2 = 8.040, p = 0.09, df = 4$ )

	Time of day (n)	HG:LG ratio	Min (°C)	Max (°C)	HG mean ± SEM (°C)	LG mean ± SEM (°C)		
<i>Isotoma klovstadi</i>								
13–17 November 2002	Night (9)	1 : 2	-34.4	-9.4	-11.8 ± 1.2 <sup>wx</sup>	-30.4 ± 2.0 <sup>a</sup>		
	Day (31)	1 : 2.1	-32.2	-14.2	-15.6 ± 0.4 <sup>w</sup>	-27.9 ± 0.7 <sup>a</sup>		
26 November–2 December 2002	Night (52)	1 : 7.7	-33.6	-14.2	-15.8 ± 0.5 <sup>wx</sup>	-27.3 ± 0.6 <sup>a</sup>		
	Day (65)	1 : 1.2	-31.8	-6.8	-13.5 ± 0.6 <sup>wx</sup>	-22.7 ± 0.6 <sup>c</sup>		
12–13 December 2002	Night (31)	1 : 2.1	-24.0	-7.8	-13.6 ± 0.7 <sup>w</sup>	-20.7 ± 0.5 <sup>c</sup>		
	Day (36)	17 : 1	-19.2	-3.6	-9.0 ± 0.4 <sup>w</sup>	-18.7 <sup>abc</sup>		
21–24 December 2002	Night (41)	1 : 12.6	-30.0	-8.0	-11.1 ± 1.6 <sup>wx</sup>	-26.5 ± 0.4 <sup>a</sup>		
	Day (43)	1 : 4.4	-30.2	-10.0	-12.4 ± 0.8 <sup>x</sup>	-26.1 ± 0.5 <sup>ab</sup>		
	Foraging (20)	1 : 0	-12.4	-6.4	-9.0 ± 0.4	—		
14–15 January 2003	Night (33)	1 : 4.5	-30.8	-8.6	-12.0 ± 0.8 <sup>wx</sup>	-24.1 ± 0.5 <sup>a</sup>		
	Day (38)	1 : 1.9	-31.4	-8.6	-11.1 ± 0.4 <sup>x</sup>	-25.7 ± 0.8 <sup>ab</sup>		
	Foraging (28)	1 : 0	-11.2	-3.2	-8.2 ± 0.4	—		
<i>Cryptopygus cisantarcticus</i>								
26–29 November 2002	Night (42)	1 : 1.6	-27.4	-6.8	-10.5 ± 0.6 <sup>mm</sup>	-24.5 ± 0.5 <sup>no</sup>		
	Day (40)	1 : 1.7	-27.2	-10.8	-12.8 ± 0.4 <sup>mm</sup>	-24.5 ± 0.7 <sup>no</sup>		
2–3 December 2002	Night (17)	1.1 : 1	-27.4	-9.2	-12.4 ± 0.8 <sup>mm</sup>	-22.6 ± 1.5 <sup>no</sup>		
	Day (13)	1 : 1.2	-27.0	-9.6	-12.4 ± 0.9 <sup>mm</sup>	-24.6 ± 1.0 <sup>no</sup>		
12–13 December 2002	Night (29)	1 : 2.2	-27.4	-10.0	-12.0 ± 0.5 <sup>mm</sup>	-23.0 ± 0.9 <sup>no</sup>		
	Day (33)	1.8 : 1	-27.8	-9.8	-11.1 ± 0.3 <sup>mm</sup>	-26.5 ± 0.3 <sup>o</sup>		
21–24 December 2002	Night (39)	1.1 : 1	-28.0	-9.6	-12.5 ± 0.4 <sup>mm</sup>	-24.3 ± 0.7 <sup>no</sup>		
	Day (37)	1.8 : 1	-28.6	-10.2	-11.5 ± 0.2 <sup>mm</sup>	-25.1 ± 0.9 <sup>no</sup>		
14–15 January 2003	Night (33)	1 : 1.8	-28.6	-9.6	-12.5 ± 0.7 <sup>mm</sup>	-24.4 ± 0.8 <sup>no</sup>		
	Day (28)	4.6 : 1	-23.4	-9.6	-11.7 ± 0.3 <sup>mm</sup>	-21.6 ± 0.9 <sup>n</sup>		
<i>Friesea grisea</i>					Median	Lower quartile	Upper quartile	
2–3 December 2002	Night (16)		-28.6	-14.2	-27.1	-28.1	-26.0	
	Day (24)		-29.2	-16.8	-25.6	-27.5	-23.6	
5–7 December 2002	Night (10)		-25.0	-16.4	-21.0	-24.0	-20.4	
	Day (14)		-29.4	-19.0	-28.3	-29.2	-25.6	
12–13 December 2002	Night (36)		-29.6	-9.4	-26.2	-27.8	-21.6	
	Day (31)		-29.8	-10.4	-26.0	-28.4	-20.0	
21–23 December 2002	Night (40)		-28.0	-9.4	-25.8	-26.6	-23.5	
	Day (30)		-28.2	-8.8	-25.6	-26.8	-20.4	
14 January 2003	Night (22)		-27.6	-7.6	-25.5	-26.6	-19.8	
	Day (20)		-29.0	-9.8	-25.1	-26.9	-22.3	

of lower lethal temperature is *C. cisantarcticus* > *I. klovstadi* = *F. grisea*. Lower lethal temperatures corresponded closely with concurrently measured SCPs for *I. klovstadi*

and *C. cisantarcticus*, but not for *F. grisea* (Median SCP = -14.6, -18.6, and -25.8 °C for *C. cisantarcticus*, *I. klovstadi* and *F. grisea*, respectively).

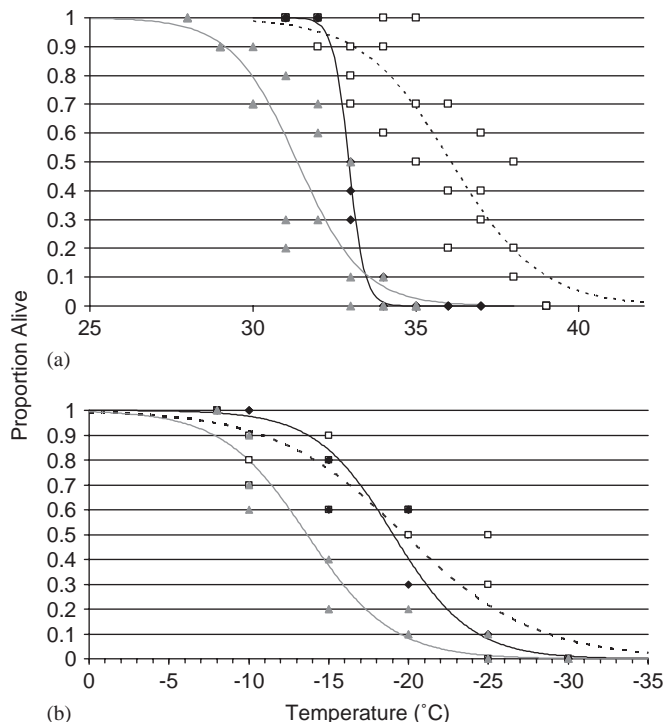


Fig. 2. (a) High temperature-related mortality (points) and fitted logistic regression models (lines) for field-fresh Collembola at Cape Hallett, Antarctica. Black diamonds, solid black line: *Isotoma klovstadi* (Goodness-of-fit test, 1 df:  $\chi^2 = 219.2$ ,  $p < 0.0001$ ;  $a = -136.3$ ,  $b = 4.137$ , ULT50 = 33, SEM could not be calculated); open squares, dashed line: *Friesea grisea* ( $\chi^2 = 129.8$ ,  $p < 0.0001$ ;  $a = -26.5$ ,  $b = 0.736$ , ULT50 = 36.1, 28.5/45.8 lower/upper SEM); grey triangles, solid grey line: *Cryptopygus cisantarcticus* ( $\chi^2 = 146.9$ ,  $p < 0.0001$ ;  $a = -31.1$ ,  $b = 0.993$ , ULT50 = 31.4, 24.6/40.0). (b) Low temperature-related mortality (points) and fitted logistic regression models (lines) for field-fresh Collembola at Cape Hallett, Antarctica. Black diamonds, solid black line: *Isotoma klovstadi* ( $\chi^2 = 150.5$ ,  $p < 0.0001$ ;  $a = -7.8$ ,  $b = -0.411$ , LLT50 = -19.1, -19.1/-19.0); open squares, dashed line: *Friesea grisea* ( $\chi^2 = 94.7$ ,  $p < 0.0001$ ;  $a = -4.8$ ,  $b = -0.245$ , LLT50 = -19.8, -19.8/-19.7); grey triangles, solid grey line: *Cryptopygus cisantarcticus* ( $\chi^2 = 122.9$ ,  $p < 0.0001$ ;  $a = -5.2$ ,  $b = -0.377$ , LLT50 = -13.6, -13.7/-13.6).  $a$  and  $b$  refer to coefficients in Eq. (1).

### 3.3. Critical thermal limits

Critical thermal minima and maxima of all three species are summarised in Table 2. All three species had sub-zero CTmin<sub>onset</sub>, and individuals of all three species were observed to freeze during CTmin runs (*I. klovstadi*: 20/60; *C. cisantarcticus*: 6/60; *F. grisea*: 3/60 individuals). In the case of *I. klovstadi*, 15 of the 20 animals that froze did so at a temperature at which they were still active. All *C. cisantarcticus* and *F. grisea* individuals that froze did so at a temperature lower than their CTmin<sub>onset</sub>. CTmin<sub>onset</sub> did not differ significantly between *C. cisantarcticus* and *F. grisea*, but mean CTmin<sub>onset</sub> was significantly lower in *I. klovstadi* ( $F_{2,146} = 286.35$ ,  $p < 0.001$ ). CTmin<sub>onset</sub> did not vary across the season in *F. grisea* or *C. cisantarcticus* (Table 2), but in *I. klovstadi*, CTmin<sub>onset</sub> was significantly higher in the later parts of the summer compared with the

sampling dates in November and early December (species  $\times$  date interaction:  $F_{6,146} = 8.83$ ,  $p < 0.001$ ).

CTmin<sub>recovery</sub> differed between all three species and showed the same rank-order pattern as CTmin<sub>onset</sub> ( $F_{2,134} = 314.72$ ,  $p < 0.001$ ; Table 2). By contrast with CTmin<sub>onset</sub>, CTmin<sub>recovery</sub> was significantly lower in the last two sampling times in *F. grisea* and *C. cisantarcticus* than in the first, although CTmin<sub>recovery</sub> did not differ across the season in *I. klovstadi* (Table 2; species  $\times$  date:  $F_{6,134} = 3.55$ ,  $p = 0.003$ ). For individuals for which all critical limit measures were available, CTmin<sub>recovery</sub> was significantly positively correlated with CTmin<sub>onset</sub> in all three species (Pearson's correlation coefficient,  $p < 0.001$  in all cases).

Prior exposure to a CTmin run did not affect the mean CTmax of *I. klovstadi* collected on 20 December (when the most individuals froze; mean of CTmin individuals =  $32.4 \pm 0.2^\circ\text{C}$ ,  $n = 4$ , mean of fresh replacements =  $32.4 \pm 0.2^\circ\text{C}$ ,  $n = 11$ ), so CTmax of both CTmin and replacement individuals were pooled. *Cryptopygus cisantarcticus* had a significantly lower CTmax than *I. klovstadi*, which in turn had a CTmax significantly lower than that of *F. grisea* (Table 2;  $F_{2,144} = 176.2$ ,  $p < 0.001$ ). CTmax did not change within the season in *F. grisea*, but there was a significant change in both *I. klovstadi* and *C. cisantarcticus* (Table 2; species  $\times$  date:  $F_{6,144} = 8.7$ ,  $p < 0.001$ ). In *I. klovstadi*, this change was not consistent across the season (Table 2), whereas in *C. cisantarcticus* the mid-season sampling dates had higher CTmax, which coincided with the warmest part of the summer (see below). CTmax was significantly negatively correlated with CTmin<sub>onset</sub> in *I. klovstadi* ( $r = -0.44$ ,  $p = 0.009$ ,  $n = 34$ ), although not in the other two species (Pearson's correlation coefficient,  $p > 0.1$ ), and CTmin<sub>recovery</sub> and CTmax were not correlated in any of the species (Pearson's correlation coefficient,  $p > 0.1$ ).

### 3.4. Haemolymph osmolality and thermal hysteresis activity

Haemolymph osmolality and thermal hysteresis activity of *I. klovstadi* and *C. cisantarcticus* were measured on six occasions throughout the summer season (Table 3). Haemolymph osmolality of *C. cisantarcticus* increased steadily from November to January, and contrasted with thermal hysteresis activity, which decreased as the season progressed (Table 3). Haemolymph thermal hysteresis activity in *C. cisantarcticus* was low (less than  $0.5^\circ\text{C}$  of hysteresis), but present throughout the season in both *I. klovstadi* and *C. cisantarcticus* (Table 3). In contrast to *C. cisantarcticus*, osmolality and thermal hysteresis in *I. klovstadi* did not show any systematic changes across the season, although values for individual sampling dates differed significantly (Table 3). Haemolymph osmolality of both *C. cisantarcticus* and *I. klovstadi* was not significantly correlated with environmental temperature conditions (Pearson's correlation coefficient,  $p > 0.05$  in both cases).

*F. grisea* had rapidly coagulating and readily oxidised haemolymph which resulted in fouling of the sample wells

Table 2

Critical thermal minima and maxima of three species of Collembola at Cape Hallett, Antarctica, over the 2002/2003 summer. Within species, mean values with the same superscript letters do not differ significantly, an absence of letters within a group indicates no significant difference (see text for statistics). Individuals that froze during the CTmin assessments were excluded from the analyses presented here

Date	CTmin <sub>onset</sub> (°C)				CTmin <sub>recovery</sub> (°C)				CTmax (°C)			
	n	Mean±SE	Min	Max	n	Mean±SE	Min	Max	n	Mean±SE	Min	Max
<i>Isotoma klovstadi</i>												
14 Nov 2002	13	-13.3 <sup>a</sup> ±0.2	-14.1	-11.0	13	-11.8±0.3	-13.2	-9.2	13	33.2 <sup>p</sup> ±0.3	31.5	34.8
5 Dec 2002	10	-13.2 <sup>a</sup> ±0.3	-14.4	-11.0	10	-11.3±0.4	-13.5	-9.6	9	34.5 <sup>q</sup> ±0.2	33.6	35.5
20 Dec 2002	8	-12.0 <sup>b</sup> ±0.3	-12.8	-10.7	4	-10.6±0.8	-11.9	-8.1	15	32.4 <sup>r</sup> ±0.1	31.7	33.3
9 Jan 2003	9	-11.9 <sup>b</sup> ±0.3	-12.7	-10.2	8	-10.9±0.3	-11.9	-9.5	8	33.4 <sup>p</sup> ±0.3	31.8	34.3
<i>Cryptopygus cisantarcticus</i>												
28 Nov 2002	15	-6.9±0.4	-8.4	-3.2	15	-5.0 <sup>ab</sup> ±0.3	-7.5	-1.5	15	28.7 <sup>p</sup> ±0.2	26.9	29.9
6 Dec 2002	15	-7.4±0.2	-9.3	-5.9	14	-4.6 <sup>a</sup> ±0.2	-6.7	-3.3	14	31.6 <sup>q</sup> ±0.3	29.3	33.6
21 Dec 2002	15	-7.9±0.3	-9.4	-5.6	13	-5.7 <sup>ab</sup> ±0.4	-7.8	-3.4	13	31.8 <sup>q</sup> ±0.4	28.8	33.4
12 Jan 2003	15	-7.4±0.2	-8.7	-5.8	12	-6.1 <sup>b</sup> ±0.3	-7.4	-4.4	12	29.8 <sup>p</sup> ±0.2	28.8	31.1
<i>Friesea grisea</i>												
1 Dec 2002	15	-8.1±0.3	-9.3	-6.3	15	-3.8 <sup>a</sup> ±0.2	-4.9	-2.8	15	34.9±0.2	33.8	36.4
7 Dec 2002	15	-7.7±0.3	-9.1	-5.1	15	-4.2 <sup>a</sup> ±0.2	-5.1	-2.9	15	35.1±0.4	32.3	37.8
20 Dec 2002	15	-8.2±0.3	-9.9	-5.9	13	-5.4 <sup>b</sup> ±0.4	-7.1	-2.0	13	34.7±0.5	31.0	36.8
15 Jan 2003	15	-8.7±0.2	-9.9	-7.4	14	-5.4 <sup>b</sup> ±0.4	-7.5	-3.1	14	34.2±0.5	28.9	36.0

Table 3

Mean haemolymph osmolality and thermal hysteresis activity of field-fresh *Isotoma klovstadi* and *Cryptopygus cisantarcticus* in the 2002/2003 summer at Cape Hallett, Antarctica. Within species, values with the same letter are not statistically significantly different (osmolality: *I. klovstadi*:  $F_{5,69} = 10.43$ ,  $p < 0.0001$ , *C. cisantarcticus*:  $F_{5,59} = 9.09$ ,  $p < 0.0001$ ; thermal hysteresis: *I. klovstadi*:  $F_{5,69} = 11.93$ ,  $p < 0.0001$ , *C. cisantarcticus*:  $F_{5,59} = 11.30$ ,  $p < 0.0001$ )

Date	N	Mean haemolymph osmolality (mOsm±SE)	Minimum (mOsm)	Maximum (mOsm)	Mean haemolymph thermal hysteresis (°C±SE)
<i>Isotoma klovstadi</i>					
19 Nov 2002	25	698 <sup>ac</sup> ±4	665	745	0.29 <sup>p</sup> ±0.01
2 Dec 2002	10	680 <sup>ab</sup> ±11	601	735	0.41 <sup>q</sup> ±0.03
13 Dec 2002	10	663 <sup>b</sup> ±9	627	701	0.45 <sup>q</sup> ±0.03
23 Dec 2002	10	665 <sup>b</sup> ±7	632	706	0.34 <sup>pr</sup> ±0.02
6 Jan 2003	10	717 <sup>cd</sup> ±13	669	811	0.40 <sup>qr</sup> ±0.02
18 Jan 2003	10	735 <sup>d</sup> ±7	601	771	0.32 <sup>pr</sup> ±0.02
<i>Cryptopygus cisantarcticus</i>					
25 Nov 2002	15	700 <sup>ab</sup> ±9	666	774	0.38 <sup>pq</sup> ±0.02
2 Dec 2002	10	672 <sup>a</sup> ±8	619	701	0.39 <sup>p</sup> ±0.01
13 Dec 2002	10	711 <sup>abc</sup> ±6	689	739	0.32 <sup>qr</sup> ±0.01
23 Dec 2002	10	726 <sup>bc</sup> ±11	673	793	0.27 <sup>r</sup> ±0.02
6 Jan 2003	10	751 <sup>c</sup> ±12	686	802	0.28 <sup>r</sup> ±0.01
17 Jan 2003	10	752 <sup>c</sup> ±13	709	861	0.29 <sup>r</sup> ±0.01

and the micropipette, so haemolymph osmolality was determined on only a single occasion. Mean (±SEM) osmolality ( $n = 5$ ) on 27 November was  $800 \pm 36$  mOsm. Some thermal hysteresis ( $0.44 \pm 0.02$  °C) was also observed in *F. grisea*, but oxidation of the haemolymph made the observation of individual crystals difficult.

### 3.5. Desiccation resistance, desiccation recovery and osmoregulation

Control animals of all three species (held above distilled water) showed less than 10% mortality over more than 5 h in the experimental set-up, confirming that observed

mortality in individuals held in the presence of silica gel was a result of desiccation stress rather than handling. The maximum time survived above silica gel was 90 min for *C. cisantarcticus*, 210 min for *I. klovstadi* and 225 min for *F. grisea* (Fig. 3). Time to 50% mortality was significantly greater in the latter species than in *C. cisantarcticus*, and did not differ significantly between *I. klovstadi* and *F. grisea* (based on overlapping 95% confidence intervals).

The 3 h desiccation treatment resulted in a significant loss of body water in *I. klovstadi*, which was slowly recovered in the presence of liquid water, but not in a saturated atmosphere (Fig. 4A), suggesting that *I. klovstadi* is unable to absorb atmospheric water vapour. Desiccation

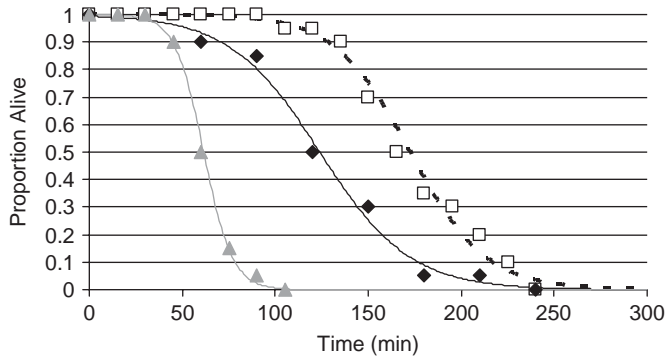


Fig. 3. Time to mortality of three Collembola species from Cape Hallett, Antarctica, when held at 20 °C above silica gel. Lines are fitted logistic regression curves. Black diamonds, solid black line: *Isotoma klovstadi* (Goodness of fit test, 1 df:  $\chi^2 = 120.3$ ,  $p < 0.0001$ ); open squares, dashed line: *Friesea grisea* ( $\chi^2 = 243.7$ ,  $p < 0.0001$ ); grey triangles, solid grey line: *Cryptopygus cisantarcticus* ( $\chi^2 = 151.1$ ,  $p < 0.0001$ ).

also led to increased haemolymph osmolality, which showed a similar pattern of recovery (free water) or lack of recovery (water vapour; Fig. 4B). Three hours after access to free water, water content of springtails had increased to pre-desiccation levels. Paradoxically, haemolymph osmolality was high during this period, although haemolymph osmolality was significantly negatively correlated with water content in general (Pearson's  $r = -0.77$ ,  $p = 0.026$ ,  $n = 8$ ).

After both acclimation and desiccation, there was a strong bias towards low group SCPs (Fig. 5). A switch to high group SCPs took place upon removal to recovery conditions, which was manifest both in animals with access to free water and those only with access to water vapour (Fig. 5).

With the exception of a 53–58 kDa protein species recorded during recovery (but not before or immediately after desiccation), no evidence was found of production of novel protein species by *I. klovstadi* during exposure to or recovery from desiccation (Table 4; see below).

### 3.6. Total protein analysis

The most common and qualitatively abundant protein species detected in total protein extracts of *I. klovstadi* was in the region of 79–84 kDa (Fig. 6, Table 5). High levels of a higher molecular mass species (152–162 kDa) were also present in many samples (Fig. 6, Table 5). Other protein species were present (Fig. 4), although not in high quantities, and there were no systematic changes in presence/absence across the season (Table 5) or in response to heat or desiccation stress (Table 6). In preliminary Western analyses, both the 79–84 kDa and 152–162 kDa species in some samples cross-reacted with a mouse anti-Hsp70 antibody. The 79–84 kDa species also cross-reacted with a mouse anti-Hsp90 antibody (results not shown).

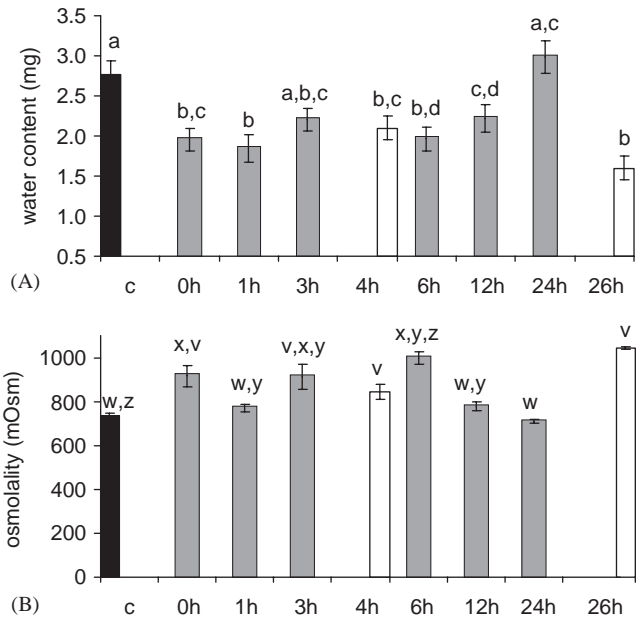


Fig. 4. Least-square mean water content (A, after ANCOVA with dry body mass as covariate;  $\pm$  standard error) and mean haemolymph osmolality (B,  $\pm$  standard error) of *Isotoma klovstadi* before (solid bars) and after desiccation (0h, hatched bars), and during recovery with liquid water available (hatched bars) and in 100% relative humidity (open bars). Similar letters indicate homogeneous groups (Tukey's HSD  $p < 0.05$ ; Water content:  $F_{8,17} = 9.282$ ,  $p < 0.0001$ ,  $n = 3$  for each treatment; Osmolality:  $F_{8,32} = 15.502$ ,  $p < 0.0001$ ,  $n = 5$  for all treatments except 3h and 26h, for which  $n = 3$ ).

### 3.7. Thermal microenvironments at Cape Hallett

Fig. 7 indicates the high level of daily variability in temperatures at Cape Hallett, and the extent to which snow cover buffers the environmental temperatures from the otherwise strong daily cycles. The maximum microclimate temperature recorded at Cape Hallett during the 2002/03 summer was 36.6 °C at *Isotoma* site 1 at 15h10 on 10 December 2002. The minimum temperature was  $-12.9$  °C at the same site on 2 December 2002. Summary temperature data for the three habitats at Cape Hallett for the summer of 2002/2003 are presented in Figs. 8 and 9 and in Table 7. The average thermal environment remained remarkably stable over the 10 week period of recording, with minimum temperatures at the end of the summer generally about 5 °C warmer than those measured at the beginning of the season (Fig. 8). Concomitant air temperatures varied from a mean of  $-6.6$  °C (max 0.6, min  $-13.5$ ) in the last 2 weeks of November 2002 to a mean of  $-1.1$  °C (max 6.1, min  $-9.6$ ) in the first 2 weeks of January 2003 (data from NIWA automatic weather station, Cape Hallett; Ian Hawes, pers. comm.).

The *I. klovstadi* habitats on the scree slope were the only data logger sites where microclimate temperatures approached potentially lethal levels during the summer, with temperatures crossing the SCP5 threshold of all three



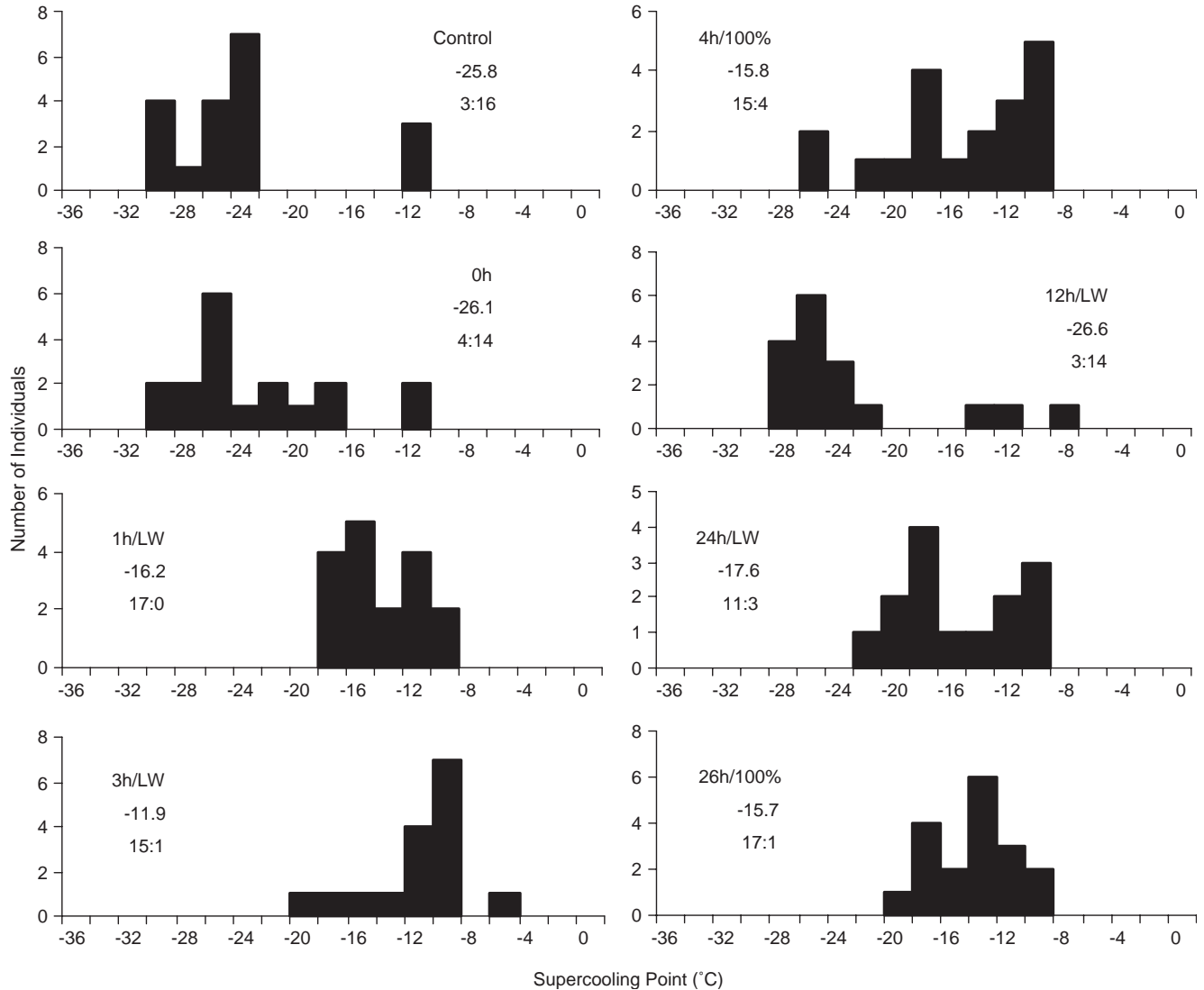


Fig. 5. Supercooling point distributions of *Isotoma klovstadi* in response to desiccation and recovery in the presence of liquid water (LW) and in 100% RH. Median and HG:LG ratio is given for each.

Table 4

Summary of presence/absence of protein species from SDS-PAGE of laboratory-acclimated *Isotoma klovstadi* collected at Cape Hallett, Antarctica, in response to desiccation and during recovery (hours) in the presence of liquid water or 100% RH

Protein band size (kDa)	Control (2)	0 h (2)	Recovery with liquid water available					Recovery with only water vapour available	
			1 h (2)	3 h (3)	6 h (2)	12 h (3)	24 h (3)	4 h (3)	26 h (3)
171–188	1	1	0	2	2	1	0	1	0
142–163	2	1	0	2	1	3	1	1	0
131–143	1	1	2	0	0	0	0	0	0
109–125	1	1	2	2	0	1	1	1	1
95–104	0	0	0	0	0	0	0	0	0
78–88	2	2	2	3	2	3	3	3	3
81	0	0	0	0	0	0	0	0	0
64–75	1	2	0	1	0	1	0	1	1
53–58	0	0	2	1	0	1	1	1	1
48–52	1	1	2	2	1	2	2	2	2
43–47	1	0	0	1	1	2	1	1	2

Numbers in parentheses indicate number of samples from each treatment.

species on a number of occasions during the summer (Table 8). Microclimate temperatures did not drop below the SCP50 or LLT of any species. Temperatures above

the ULT5 and ULT50 of all three species were also recorded in this habitat (Table 8). ULT thresholds for *I. klovstadi* and *F. grisea* (both of which occupy this habitat) were only occasionally crossed, but the thresholds for *C. cisantarcticus* were frequently experienced. The maximum cooling rates recorded in microclimates over the summer were at the *I. klovstadi* site 1, where a cooling rate of  $0.24\text{ }^{\circ}\text{C min}^{-1}$  was recorded for the hour preceding 16h15 on 10 December 2002 across  $10\text{ }^{\circ}\text{C}$  (part of a cycle in which the microclimate temperature decreased from  $25.2$  to  $-1.0\text{ }^{\circ}\text{C}$  in 12 h,  $0.04\text{ }^{\circ}\text{C min}^{-1}$ ). The maximum cooling rate over 1 hour at the  $0\text{ }^{\circ}\text{C}$  threshold was  $0.17\text{ }^{\circ}\text{C min}^{-1}$  at 20h15 on 16 November 2002, part of a cycle in which the temperature decreased from  $12.9$  to  $-7.9\text{ }^{\circ}\text{C}$  in 9 h ( $0.04\text{ }^{\circ}\text{C min}^{-1}$ ). Cooling rates in the two algal flats habitats were relatively stable across temperature thresholds, whereas in scree slope habitat, cooling rate was slower at lower temperatures (Fig. 9).

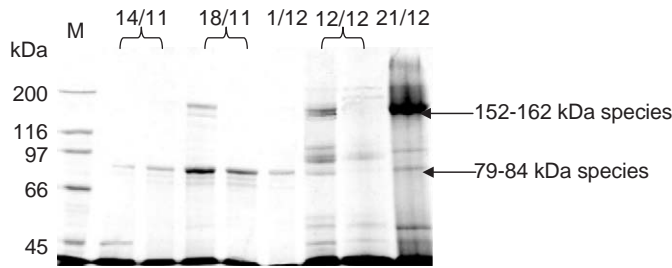


Fig. 6. Coomassie-stained SDS-PAGE gel (10% polyacrylamide) of total protein extracts from 0.035 mg fresh mass *Isotoma klovstadi* collected at Cape Hallett, Antarctica, across the 2002/2003 summer (the dates of sampling are indicated above each lane or lanes). M: molecular mass marker lane (the size of the markers are indicated on the left-hand side of the gel). The positions of the major species detected are indicated by arrows on the right-hand side of the gel.

Table 5

Summary of presence/absence of protein species from SDS-PAGE of field fresh *I. klovstadi* collected at Cape Hallett, Antarctica, across the 2002/2003 summer

Protein Band Size (kDa)	Date						Overall number of samples (17)
	14/11 (2)	18/11 (4)	1/12 (3)	12/12 (3)	21/12 (3)	6/1 (2)	
171–188	0	1	1	1	1	1	5
142–163	0	3	1	3	3	2	12
131–143	0	3	0	2	1	0	6
109–125	0	1	1	2	2	1	7
95–104	0	2	1	3	3	1	10
78–88	2	4	3	2	3	2	16
81	2	2	1	1	0	0	6
64–75	1	4	0	1	0	2	8
53–58	0	0	3	1	0	0	4
48–52	0	3	2	3	2	1	11
43–47	1	1	1	3	2	1	9

Numbers in parentheses indicate number of samples at each date.

Table 6

Summary of presence/absence of protein species from SDS-PAGE of lab-acclimated *Isotoma klovstadi* collected at Cape Hallett, Antarctica, in response to temperature and desiccation treatments

Protein band size (kDa)	Treatment								Overall number of samples (25)
	Control (3)	Des (4)	Slow (4)	28 °C (2)	0 °C (5)	-5 °C (2)	-5g (3)	-9 °C (2)	
171–188	0	1	2	1	1	1	1	0	7
142–163	0	0	0	0	0	0	0	0	0
131–143	0	0	0	0	0	0	0	0	0
109–125	3	2	1	1	2	2	2	2	15
95–104	2	3	2	1	2	2	3	2	17
78–88	2	4	4	2	5	2	3	2	24
81	0	0	0	0	0	0	0	0	0
64–75	1	0	0	0	0	0	0	0	1
53–58	2	3	1	2	2	2	3	3	18
48–52	1	1	0	0	0	1	1	1	5
43–47	1	1	0	1	1	1	1	1	7

Numbers in parentheses indicate number of samples from each treatment. Des = desiccation treatment; slow = slow cooling from  $+5$  to  $-2\text{ }^{\circ}\text{C}$  ( $1\text{ }^{\circ}\text{C h}^{-1}$ );  $-5\text{g}$  = 2 h treatment at  $-5\text{ }^{\circ}\text{C}$  followed by 2 h at  $+5\text{ }^{\circ}\text{C}$ ; other treatments indicate a 2 h exposure to a given temperature.



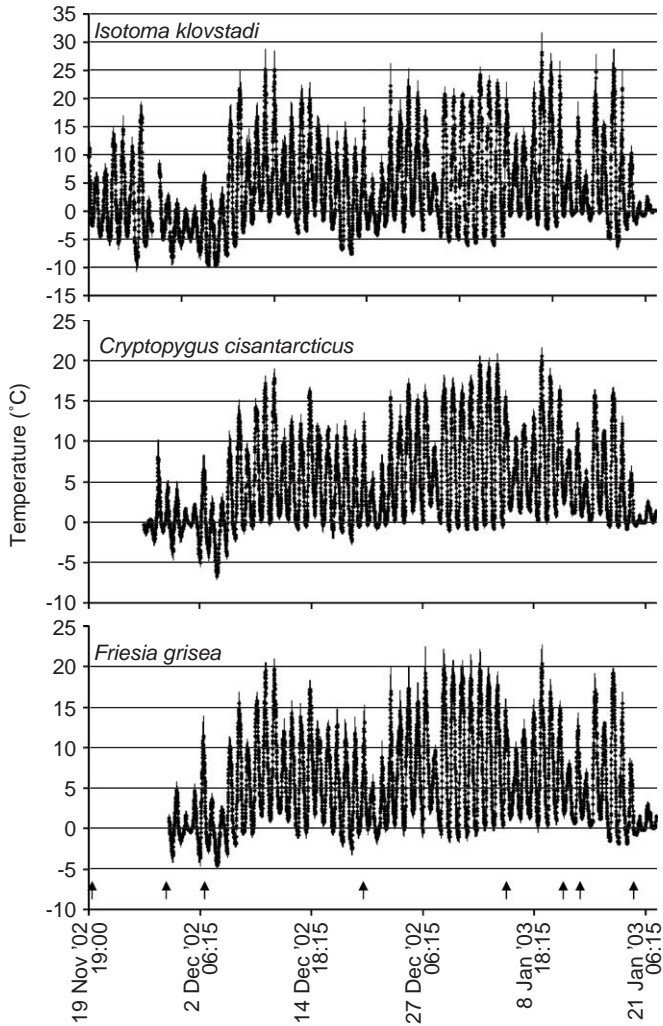


Fig. 7. Mean  $\pm$  standard error microclimate temperatures measured at 10 min intervals at representative sites for three species of Collembola at Cape Hallett, Antarctica, during the 2002/2003 summer. *Isotoma klovstadi*: 5 locations; *Friesia grisea* and *Cryptopygus cisantarcticus*: 3 locations. Arrows indicate major snowfall events.

Direct comparisons of the thermal environments offered by the three different habitats were made during the month of December, for which a full set of data were available for all three sites (Table 9). Cooling rates at 0 °C were fastest in the *Isotoma* habitat, and did not differ substantially between the other two habitats (Table 9). The greatest number of freeze-thaw cycles and events below the -3.2 °C threshold (the highest recorded SCP) were also experienced in the scree slope habitat. The amount of time spent above 0 °C was lowest for *Isotoma* habitat, but there was no difference between habitats in terms of time spent above 10 °C (Table 9). The only significant relationship between stone characteristics and microclimate temperature statistics was a negative relationship between stone area (but not volume) and total time spent above 10 °C (Spearman's  $r = -0.618$ ,  $p < 0.05$ ,  $n = 11$ ;  $p > 0.05$  in all other correlations).

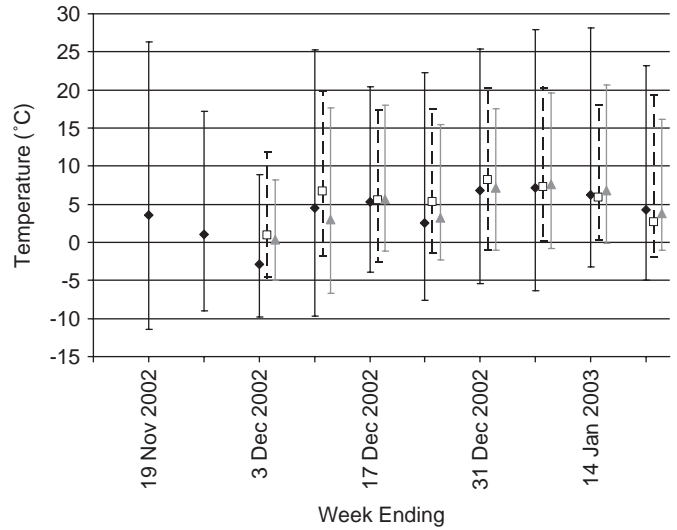


Fig. 8. Weekly mean, mean minimum and mean maximum microclimate temperatures over the 2002/2003 summer at Cape Hallett, Antarctica. Black diamonds: representative *Isotoma klovstadi* habitat ( $n = 5$  locations); open squares = *Friesia grisea* ( $n = 3$  locations); grey diamonds: *Cryptopygus cisantarcticus* ( $n = 3$  locations).

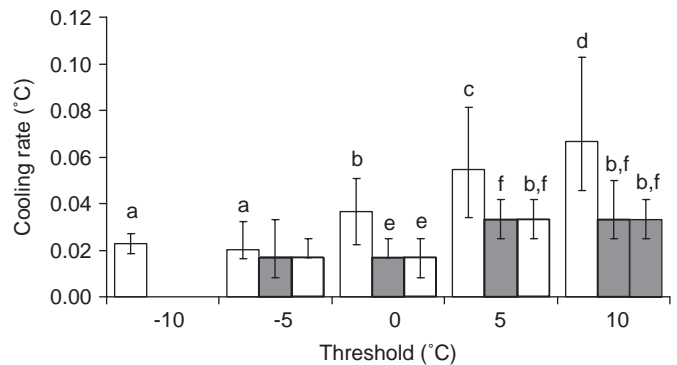


Fig. 9. Median microclimate cooling rates ( $\pm$  quartiles) across threshold temperatures for the 2002/2003 summer at Cape Hallett, Antarctica. Open bars: representative *Isotoma klovstadi* habitat (5 locations); hatched bars: *Friesia grisea*; stippled bars: *Cryptopygus cisantarcticus*. Similar letters indicate homogeneous groups (within *I. klovstadi* comparisons, GLZ, Wald  $\chi^2 = 310.6$  (4 df),  $p < 0.0001$ ; between species comparisons (0, 5 and 10 °C only) Wald  $\chi^2 = 436.1$  (2 df),  $p < 0.0001$ ; threshold  $\times$  species interaction (0, 5 and 10 °C only) Wald  $\chi^2 = 37.67$  (4 df),  $p < 0.0001$ ).

#### 4. Discussion

The extremely low winter temperatures experienced by Collembola during winter at Cape Hallett (see Pryor, 1962) are likely to be a primary stressor driving their physiological evolution. However, we show here that potentially lethal high and low temperatures do occur in the microhabitats during the summer. Thus, Collembola living at Cape Hallett must show resistance adaptations (*sensu* Precht, 1958) to survive in their environment, even during the summer. Although the period from November to January is one of large changes to the physical

Table 7

Summary of microclimatic temperatures (°C) collected over the 2002/2003 summer from locations representative of habitat for three species of Collembola at Cape Hallett, Antarctica. *Isotoma klovstadi* site 2 is approximately 100 m north of site 1, and has a similar aspect and elevation. Mean, mean minimum and mean maximum were only calculated for *I. klovstadi* site 2 because of sample size, and the two *I. klovstadi* sites were not combined because of the truncated logging period for site 1

	<i>Cryptopygus cisantarcticus</i>	<i>Friesea grisea</i>	<i>Isotoma klovstadi</i> Site 1	<i>I. klovstadi</i> Site 2
Number of loggers	3	3	2	3
Start date	25 Nov 2002	28 Nov 2002	16 Nov 2002	19 Nov 2002
End date	22 Jan 2003	22 Jan 2003	5 Jan 2003	22 Jan 2003
Mean $\pm$ SEM	4.6 $\pm$ 0.3	5.3 $\pm$ 0.2		4.5 $\pm$ 0.7
Median	3	4	2.0	1.6
Lower quartile	8.5	9.5	10.2	8.2
Upper quartile	0	0.5	-2.9	-1.5
Mean maximum ( $\pm$ SEM)	20.7 $\pm$ 1.0	21.5 $\pm$ 1.7		33.4 $\pm$ 3.9
Absolute maximum	22.5	24.5	36.6	35
Mean minimum ( $\pm$ SEM)	-6.7 $\pm$ 0.4	-4.7 $\pm$ 0.4		-10.7 $\pm$ 0.7
Absolute minimum	-7.5	-5.5	-12.9	-12

Table 8

Occurrence of biological temperature thresholds in microclimates representative of *Isotoma klovstadi* habitat (sites 1 and 2 combined) at Cape Hallett, Antarctica over the 2002/2003 summer. SCP5 is the temperature at which 5% of individuals would be expected to freeze (from cumulative SCP data); ULT5 and ULT50 are the temperatures at which 5% and 50% of individuals would be expected to die from acute heat stress (from ULT data). Number of loggers refers to the number of data loggers that recorded temperatures outside the threshold,  $N = 5$  data loggers.

		Species		
		<i>Isotoma klovstadi</i>	<i>Friesea grisea</i>	<i>Cryptopygus cisantarcticus</i>
SCP5	Threshold value	-7.6 °C	-11.2 °C	-9.7 °C
	Mean no. events	11.6	1.2	5
	Max, min no. events	23, 2	3, 0	10, 1
	No. loggers	5	3	5
ULT5	Threshold value	33 °C	32.1 °C	28.4 °C
	Mean no. events	3	3	9.2
	Max, min no. events	8, 0	8, 0	23, 0
	No. loggers	2	2	2
ULT50	Threshold value	32.2 °C	36.1 °C	31.4 °C
	Mean no. events	1.8	0.4	5.6
	Max, min no. events	5, 0	2, 0	15, 0
	No. loggers	2	1	2

Table 9

Comparison of microclimatic conditions for representative habitats of three species of Collembola at Cape Hallett, Antarctica, during December 2002. Mean  $\pm$  SE is presented, with the exception of surface values, for which only two locations were monitored.  $N = 5$  locations for *Isotoma klovstadi* site, and 3 for *Cryptopygus cisantarcticus* and *Friesea grisea* sites. Comparisons were only made between habitats (not including surface) using GLZ (cooling rates) or Kruskal–Wallis rank ANOVA (2 df,  $n = 11$ ), and means with similar superscript letters are not significantly different. Maximum cooling rate is at the 0 °C threshold

	Surface	Site			
		<i>Isotoma</i>	<i>Cryptopygus</i>	<i>Friesea</i>	
Maximum cooling rate (°C min <sup>-1</sup> )	0.104	0.118 <sup>a</sup> $\pm$ 0.017	0.05 <sup>b</sup> $\pm$ 0.008	0.042 <sup>b</sup> $\pm$ 0.008	Wald $\chi^2 = 118.7$ , $p < 0.001$
No. freeze-thaw cycles	51.5	35.8 <sup>a</sup> $\pm$ 0.7	21.3 <sup>b</sup> $\pm$ 6.2	22 <sup>b</sup> $\pm$ 6.0	$H = 7.549$ , $p = 0.023$
No. cycles below -3.2 °C	36.5	23.8 <sup>a</sup> $\pm$ 1.9	4.3 <sup>b</sup> $\pm$ 0.9	3.3 <sup>b</sup> $\pm$ 0.9	$H = 7.813$ , $p = 0.020$
Time above 0 °C (min)	25995	28326 <sup>a</sup> $\pm$ 778	33393 <sup>b</sup> $\pm$ 1222	35310 <sup>b</sup> $\pm$ 1390	$H = 7.879$ , $p = 0.020$
Time above 10 °C (min)	8023	10724 $\pm$ 1797	6663 $\pm$ 1031	9513 $\pm$ 1743	$H = 0.776$ , $p = 0.679$

environment, including the break-up of sea ice surrounding Cape Hallett and the beginning of snow melt, the microclimate temperature environment changes relatively

little (Figs. 7 and 8). Coupled with the availability of water from large snowbanks and regular precipitation events at Cape Hallett (Sinclair, Klok, Scott and Terblanche, pers.

obs.), this stability of microclimate conditions results in a long growing season by continental Antarctic standards (see Janetschek, 1967; Sømme, 1986; Marshall et al., 1995; Sinclair and Sjørnsen, 2001a, for examples from elsewhere on the continent).

#### 4.1. Thermal tolerances

In common with all Antarctic Collembola studied thus far (Cannon and Block, 1988), the correspondence of LLT50 and median SCP indicate that all three species of Collembola at Cape Hallett are freeze avoiding. *C. cisantarcticus* and *I. klovstadi* both demonstrate diurnal variation in SCP, while *F. grisea* does not (see also Sinclair et al., 2003b). The lowest SCPs recorded for each species (Table 1) are lower than those recorded during summer in species from the Antarctic peninsula (Sømme and Block, 1982; Cannon and Block, 1988) and not dissimilar to those recorded for *G. hodgsoni* during the summer at Cape Bird (5° south of Cape Hallett, Sinclair and Sjørnsen, 2001a). Although when collected under stones they had a strongly bimodal SCP distribution, individuals of *I. klovstadi* collected while grazing on the surface of moss had unimodally high SCPs, which lends weight to the hypothesis that high SCPs result from food in the gut (Cannon and Block, 1988; Sinclair et al., 2003b). *C. cisantarcticus* SCPs were higher than those described for acclimated individuals of the continental *Cryptopygus sverdrupi* (Sømme, 1986), but lower than those reported for field-fresh *C. antarcticus* from the Antarctic Peninsula (Worland and Convey, 2001). *F. grisea* showed a consistently unimodal pattern of 'low group' SCPs. This contrasts with the substantial (and nearly unimodal) high SCP group that Block (1982) reported for *F. grisea* from Adelaide Island in the maritime Antarctic. *F. grisea* is the only circum-Antarctic species of Collembola (Wise, 1967). Given the likely low or negligible rate of cross-continental dispersal, the presumed antiquity of the separations between species across the continent (Marshall and Pugh, 1996) and the differing environmental conditions between the continental and maritime Antarctic regions (Block, 1994), it is not surprising to find substantial differences between populations on opposite sides of the continent. As a predator, *F. grisea* may not ingest large quantities of ice nucleators (or may have proteolytic enzymes that destroy them), which may account for the unimodality of its SCP. *F. grisea* was the only species where LLT50 and median SCP differed, suggesting that although it may have a strong tolerance to acute low temperatures, it is susceptible to prolonged exposure. However, tolerance of prolonged periods at subzero temperatures is a necessity for surviving the Antarctic winter, so *F. grisea* presumably must undergo some level of seasonal acclimatisation that increases its chill tolerance (see Sømme, 1996, for further discussion of the effects of prolonged low temperature exposure). Such a separation of tolerances to acute and prolonged low temperature has been shown in temperate Collembola

and other insects (Nedved, 1998; Hanc and Nedved, 1999), and although noted for Antarctic species (see Pryor, 1962; Fitzsimons, 1971; Convey and Worland, 2000), has not yet been explored in any depth.

Low levels of thermal hysteresis activity were detected in all three species of Collembola at Cape Hallett. These low levels are certainly not enough to account for the high degree of supercooling, but are similar to those described for summer-acclimated individuals of several alpine Collembola (Zettel, 1984). Although thermal hysteresis activity declined in *C. cisantarcticus* during the season, it never entirely disappeared, in contrast to *G. hodgsoni*, where haemolymph thermal hysteresis activity disappears entirely during the summer (although whole-animal homogenates still inhibit recrystallisation, Sinclair and Sjørnsen, 2001a). It is likely that, during summer, thermal hysteresis proteins may still be advantageous for preventing gut nucleation or inoculative freezing in Antarctic Collembola, but in *G. hodgsoni* are intracellular or associated with tissues, rather than the haemolymph. If high haemolymph osmolality is directly related to cold tolerance, osmolality may have been expected to decline over the summer as it does in *G. hodgsoni* (Sinclair and Sjørnsen, 2001a). However, no consistent patterns were observed in *I. klovstadi*, and osmolality actually increased in *C. cisantarcticus*. Similarly, there was no strong seasonal pattern in cold tolerance for either species (see below).

SCPs within high and low groups did not change systematically across the summer in any of the species we examined. However, in *C. cisantarcticus* and *I. klovstadi*, there was a seasonal shift in the HG:LG ratio, such that there were more individuals in the high group towards the end of summer (Table 1). This suggests that, in summer, the relative levels of cold tolerance in the two modes are fixed, and the animals respond to ameliorating or deteriorating thermal conditions by switching between groups, as has been demonstrated on a diurnal scale (Sinclair et al., 2003b). Seasonally, this shift between the groups could be a result of an active physiological change, or simply a coincidental shift as the proportion of the population that is actively feeding or moulting at a given time changes across the season (Worland, 2005). The substantial shift towards the low group in *I. klovstadi* in late December coincided with a period of low temperatures and snow (see Sinclair et al., 2003b, for discussion). Nevertheless, the extent to which the shifts in SCP from HG to LG are mediated by temperature, feeding or moulting is unclear from our data and the mechanisms for the observed SCP shifts are currently unknown for any Antarctic collembolan (Cannon and Block, 1988; Worland and Convey, 2001; Worland, 2005).

Critical thermal minima of all three species were well below 0 °C, and the mean CT<sub>min-onset</sub> of -12 to -13 °C for *I. klovstadi* (Table 2) is among the lowest chill coma temperatures recorded in any arthropod (but see Fitzsimons, 1971; Kohshima, 1984). It is unique to find a freeze avoiding species which is still potentially active at the SCP,

which suggests that the low chill-coma temperature in *I. klovstadi* may be an adaptation used by cold-susceptible foraging individuals to avoid potentially lethal temperatures.  $CT_{min_{onset}}$  of *I. klovstadi* was significantly higher in the height of summer than earlier in the season (Table 2). This is consistent with observations of acclimatisation of  $CT_{min_{onset}}$  in response to season and altitude in other insect species (e.g. Klok and Chown, 2003). Critical thermal minima of *C. cisantarcticus* ( $-3$  to  $-9^{\circ}C$ ) were slightly higher than the  $-6$  to  $-10^{\circ}C$  reported for *C. antarcticus* on Signy island (Sømme and Block, 1982), although the latter authors used a much faster cooling rate ( $1^{\circ}C\ min^{-1}$ ) than was used here.

Within-individual  $CT_{min_{recovery}}$  was strongly correlated with  $CT_{min_{onset}}$ , and *I. klovstadi*, which had the lowest  $CT_{min_{onset}}$  also had the lowest  $CT_{min_{recovery}}$ . Although this has important ecological consequences, the correlation may be the result of a common mechanism (possibly  $Na^{+}/K^{+}$ -ATPase activity, see Hosler et al., 2000; Sinclair et al., 2004) determining both onset and recovery. However, significant (and systematic) seasonal changes in  $CT_{min_{recovery}}$  of *C. cisantarcticus* and *F. grisea* were not mirrored in  $CT_{min_{onset}}$  (Table 2), suggesting that acclimatisation of the two parameters may not be directly coupled (see Chown and Nicolson, 2004 for a review of measures of thermal tolerance and their variation). *C. cisantarcticus* and *F. grisea* showed a similar trend in  $CT_{min_{recovery}}$  over the summer season, with a decrease in  $CT_{min_{recovery}}$  in the second part of the season, in spite of a lack of change in  $CT_{min_{onset}}$ .

Because of the assumption that low temperatures are the most important environmental stress for terrestrial Antarctic arthropods, upper lethal tolerances have rarely been investigated (but see Janetschek (1967) and Fitzsimons (1971) for data on *G. hodgsoni* from South Victoria Land). However, our microclimate data from the scree slope sites where both *I. klovstadi* and *F. grisea* are present indicate that potentially lethal high temperatures can and do occur in the field at Cape Hallett (Table 8). Both acute ( $CT_{max}$ ) and prolonged (2h exposure in moist conditions) exposure to high temperatures indicate that all three species of Collembola can survive temperatures in excess of  $30^{\circ}C$ , and that the rank order of tolerance to high temperatures is *F. grisea* (most tolerant) > *I. klovstadi* > *C. cisantarcticus* (least tolerant). These upper temperature limits are in a similar range to those described for Arctic Collembola (Hodkinson et al., 1996). Overall, *F. grisea* is the species most tolerant to temperature extremes, followed by *I. klovstadi* and *C. cisantarcticus*. However, *I. klovstadi* has the widest thermal activity window (difference between mean  $CT_{min}$  and mean  $CT_{max} = 47.7^{\circ}C$  in December 2002), possibly allowing better behavioural exploitation of the thermal landscape than *F. grisea* ( $43^{\circ}C$  in December 2002) or *C. cisantarcticus* ( $39.7^{\circ}C$ ). Hayward et al. (2004) reported that *F. grisea* from Rothera on the Antarctic peninsula has greater environmental tolerances and shows less movement across the habitat than *C. antarcticus*, and suggest that the lower tolerances of *C. antarcticus* necessitate

movement to avoid potentially lethal conditions. At Cape Hallett, *F. grisea* has greater tolerances than *C. cisantarcticus*, which is consistent with Hayward et al.'s (2004) report. Assuming that other factors such as food availability and interspecific competition are equal, it seems likely that, during the summer, high temperatures may prevent *C. cisantarcticus* from occupying the scree slope habitat.

#### 4.1.1. Desiccation, recovery and osmoregulation

Haemolymph osmolality of all three species of Collembola in this study (ca.  $700\ mOsm\ kg^{-1}$ ) was higher than that measured in summer-acclimatised Collembola in Antarctica (Block and Harrison, 1995; Sinclair and Sjørnsen, 2001a) and elsewhere (Zettel, 1984; Sinclair and Chown, 2002), which tend to be below  $500\ mOsm\ kg^{-1}$ . Winter-adapted Collembola show osmolalities comparable to (or higher than) those we measured (Meier and Zettel, 1997; Sinclair and Sjørnsen, 2001a), suggesting that the high osmolality of the Cape Hallett species may be associated with their retention of cold hardiness during the summer. There was a significant increase in haemolymph osmolality of both *C. cisantarcticus* and *I. klovstadi* across the season (Table 3), which may correspond to changing water availability as the local sources of water (snowbanks) were depleted by January (not all habitats receive meltwater from the larger, permanent snowbanks, B.J. Sinclair, pers. obs.).

Collembola are highly susceptible to desiccation stress (Hopkin, 1997). Low water availability in their environment means that continental Antarctic Collembola might be expected to be more desiccation tolerant than their mesic counterparts. Nevertheless, water stress is thought to substantially influence distributions of and interactions between Antarctic arthropods at both small (Janetschek, 1967; Sinclair and Sjørnsen, 2001b; Hayward et al., 2004) and large (Kennedy, 1993) scales, as well as being potentially important in terms of species' responses to precipitation pattern changes as a result of climate change (Convey et al., 2003). Of the three species examined at Cape Hallett, *C. cisantarcticus* was the least desiccation tolerant, and mortality begins much earlier in *I. klovstadi* than *F. grisea*, although the maximum tolerances of the two are similar. This is consistent with both the small-scale distribution of the three species (*C. cisantarcticus* is not found in the much drier scree slope habitat), and (unlike the SCP data) is concordant with data from the Antarctic Peninsula, where Hayward et al. (2004), infer from distribution responses that *F. grisea* is more desiccation tolerant than *C. antarcticus*.

We were able to investigate osmoregulation and recovery from desiccation stress more thoroughly in *I. klovstadi* than in *C. cisantarcticus* or *F. grisea*. Like most Collembola (Hopkin, 1997), *I. klovstadi* is a poor osmoregulator, with haemolymph osmolality increasing considerably after desiccation. Recovery of body water was reasonably rapid when liquid water was available, but there was a lag in the reduction of haemolymph osmolality. In *Drosophila melanogaster*, the regulation of ions is an important aspect of



recovery from desiccation stress (Folk et al., 2001), so the observed lag in *I. klovstadi* could result from a mechanism preventing loss of ions during desiccation in the less sophisticated collembolan osmoregulatory system. Water content and osmolality did not change when the Collembola were only allowed access to a saturated atmosphere, suggesting that, unlike Collembola from mesic environments (Bayley and Holmstrup, 1999; Holmstrup et al., 2001), *I. klovstadi* is unable to absorb atmospheric water vapour. After acclimation and desiccation, SCPs were largely in the low group (as may be expected for starved animals, Sinclair et al., 2003b), but there was a shift to the high group upon removal from the desiccating conditions. This SCP shift occurred in animals with access to both free water and water vapour, suggesting that it is not simply an effect of drinking (as has been described for *C. antarcticus*; Cannon et al., 1985), but is part of the recovery process. The SCP of *I. klovstadi* kept in constant dark or constant light under standard thermal regimes did not differ between night and day (B.J. Sinclair, unpublished observations), ruling out the possibility of confounding effects from an endogenous circadian rhythm in SCP.

#### 4.1.2. Total protein analysis

With the exception of a ca. 53–58 kDa species apparently expressed during recovery from desiccation stress (Table 4), there were no consistent qualitative changes in *I. klovstadi* protein levels in response to temperature or desiccation treatments. The most consistently detected protein band was approximately 83 kDa in size, and in some preliminary Western analyses on certain samples, this protein species exhibited cross-reaction with a Mouse anti-Hsp90 antibody, as did the larger (ca. 140 kDa) species that was present in a large proportion of samples. Hsp90 is the most commonly expressed constitutive heat shock protein in animal cells (Csermely et al., 1998), and in *Drosophila melanogaster*, Hsp90 is expressed as an 83 kDa protein (Sollars et al., 2003), suggesting that the protein species observed here could well be a constitutively produced heat shock protein. However, Hagner-Holler et al. (2004) show that arthropod haemocyanins (also generally in the 70–90 kDa size range) are expected to be common in lower arthropods, so the bands in this size range in the *I. klovstadi* homogenates could represent several, different, constitutively produced proteins. The insect antifreeze proteins that have been described are in the region of 9–13 kDa (Duman, 2001) and are therefore smaller than would have been detected using our methods. Clearly a proteomics approach, including investigation of post-translational changes, could be particularly interesting in interpreting stress responses in this and other Antarctic Collembola.

#### 4.2. Physiological drivers of range limitation on small and large scales?

Antarctic terrestrial arthropods are often studied to elucidate their ‘heroic’ adaptations to extreme conditions

(Edwards, 1986; Sømme and Block, 1991; Sømme, 1995). Here we use microclimate data to show that environmental tolerances are indeed relevant in the summer, but with the caveat that the adaptations we describe are likely inadequate for survival of winter temperatures. An automatic weather station positioned on the algal flats recorded a minimum air temperature in the 2002 winter of  $-40^{\circ}\text{C}$ , and a minimum soil temperature (buffered by snow cover) of  $-34.6^{\circ}\text{C}$ . In 2003, the recorded winter minimum was  $-36^{\circ}\text{C}$  for air, with a soil minimum of  $-34.7^{\circ}\text{C}$  (Ian Hawes, pers. comm.). Pryor (1962) spent a winter investigating microclimates at a site within 20 m of the location of our *I. klovstadi* dataloggers, and showed that winter-acclimatised *I. klovstadi* are capable of surviving experimental temperatures below  $-50^{\circ}\text{C}$ , suggesting that there must be substantial seasonal changes to the physiology of this species. Although winter sampling is unlikely, Cape Hallett is not so inaccessible to preclude possible future early spring or late autumn investigations that might shed light on these phenomena.

Winter conditions at Cape Hallett do not appear to be significantly less severe than those at Cape Bird, some 600 km further south (Janetschek, 1967; Sinclair and Sjørnsen, 2001a; Gordon, 2003), and while the growing season is shorter further south, there is no overt climatic or physiological reason preventing North Victoria Land Collembola from surviving in more southerly habitats. Although interactions between water and temperature seem likely to restrict the distribution of *C. cisantarcticus* at Cape Hallett (cf. Hayward et al.’s (2004) observations of *C. antarcticus*), its presence at the ridge site requires further investigation. By contrast, *I. klovstadi* and *F. grisea* are able to tolerate relatively severe environmental stresses and are accordingly found further south in North Victoria Land (Frati et al., 1997). The North Victoria Land coastline is characterised by large glaciers sometimes extending several kilometres beyond the coast, and these (combined with the associated katabatic winds) are likely effective barriers for southward dispersal of Collembola. Assuming that the absence of *I. klovstadi* and *C. cisantarcticus* at more southerly locations is a consequence of lack of dispersal (and not of availability of food resources, competition with resident microarthropods or too short a growing season), human-mediated transfer of arthropod species between locations in Victoria Land during research and logistic activities may be the biggest current risk to terrestrial arthropod communities in the Ross Sea Region.

#### 4.3. What is (and is not) known about Antarctic collembolan ecophysiology?

Although there is a wealth of physiological information for arthropods in the primary literature, in reality, most species that are represented in the matrix are there by virtue of a single investigated parameter, and the geographic extent of these studies is restricted, particularly in the

Table 10  
Summary of available physiological information for Collembola from the Antarctic Continent. If more than one study presents similar data, data from the most recent study were included

Species	Location	Acclimation	SCP (°C)	Water content / desiccation	Sugars and Polyols	CTmin CTmax (°C)	ULT (°C)	Preferred temperature	Metabolic rate (W/g)	Haemolymph osmolality (mOsm kg <sup>-1</sup> )	Diurnal variation	Microclimate temperatures	References
<b>Hypogastruridae</b>													
<i>Gomphiocephalus hodgsoni</i>	Ross Island	FF	−35.4 (−38.0)	nd	G,T,U	nd	29.5	8–11.5	nd	500 1650	nd	s,w	(Fitzsimons 1971; Sinclair and Sjørnsen 2001)
<b>Isotomidae</b>													
<i>Cryptopygus antarcticus</i>	Antarctic Peninsula	FF	−26.0 (−30.0)	w,d <sup>a</sup>	G,Gu,M	−8.3 <sup>a</sup>	nd	1–13	2.05E-03 (5°C)	282 397 <sup>a</sup>	y	s	(Block 1979, 1982; Block and Harrison 1995; Block et al., 1990; Hayward et al. 2003; Sømme and Block 1982; Worland and Convey 2001)
<i>C. cisantarcticus</i>	Cape Hallett	FF	−26.5 (−28.6)	d	nd	−7.9 31.8	34	nd	nd	700 750	y	s,w	This study, (Pryor 1962; Sinclair et al. 2003)
<i>C. sverdrupi</i>	Dronning Maud Land	FF A <sup>b</sup>	−28.6 (−31.0) −34.6 (−38.0)	d	nd F,G,Gu,M,S,T	nd	nd	nd	nd	nd	nd	s	(Sømme 1986)
<i>Isotoma klovstadi</i>	Cape Hallett	FF	−30.4 (−34.4)	d	nd	−13.3 34.5	34.0	nd	2.00E-02 (18°C) 1.77E-03 (−4°C)	650 750	y	s,w	This study, (Pryor 1962; Sinclair et al. 2003; Strong et al. 1970)
<i>I. (Folsomotoma) otooculata</i>	Antarctic Peninsula	FF	−20.8 (−25)	nd	nd	−4.8 <sup>a</sup>	nd	nd	1.48E-03 (5°C)	nd	nd	nd	(Block 1982; Sømme and Block 1982)
<b>Neauridae</b>													
<i>Friesea grisea</i>	Cape Hallett, Antarctic Peninsula	FF FF	−28.3 (−29.8) −24.4 (−27)	d w	nd nd	−8.7 35.1 nd	38.0 nd	Nd nd	nd nd	800 nd	n nd	s,w nd	This study, (Pryor 1962; Sinclair et al. 2003) (Block 1982; Hayward et al. 2004)

Data from Signy Island were included only if there were no data from the Antarctic continent for a species. Field fresh data were used by preference. nd – no data; Acclimation: FF—field fresh; A—subject to acclimation. SCP—Lowest Low Group mean or median (lowest recorded); Water content/desiccation: w—water content reported, d—comparative or other study of desiccation rate; Sugars and Polyols that were identified as important by the authors: F—fructose; G—glycerol, Gu—glucose, M—mannitol, S—sorbitol T—trehalose, U—unidentified carbohydrate; Lowest mean onset of chill coma (CTmin), highest mean CTmax; ULT—highest temperature survived; Preferred temperature—total range of preferred temperatures recorded under all acclimation conditions; Metabolic rate—converted to W/g assuming an RQ of 0.72, temperature of measurement in parentheses; Haemolymph osmolality—lowest and highest reported mean; Diurnal variation: y—yes, n—no; Microclimate temperatures: s—summer measurements, w—winter measurements. nd—no data throughout.

<sup>a</sup>Measured at Signy Island.

<sup>b</sup>Acclimated at −8°C for 1–2 months.

Southern Hemisphere (Chown et al., 2002b; Sinclair et al., 2003a). Within the Southern Hemisphere, Antarctica would seem to be an exception—physiological studies of supercooling in Antarctic Collembola have reached textbook status (Hopkin, 1997; Wharton, 2002; Chown and Nicolson, 2004), and many other aspects of physiology have been reported, including metabolic rates (Strong et al., 1970; Block and Young, 1978) and water balance (Block et al., 1990; Block and Harrison, 1995; Convey et al., 2003). However, the majority of these studies have been on a single species, *C. antarcticus* (Isotomidae), and many of those were conducted on the comparatively warm and wet Signy Island (Cannon and Block, 1988). Even so, a new strategy of cold hardening—rapid SCPs change—was recently reported for this hitherto well-understood species (Worland and Convey, 2001). With the inclusion of the three species from Cape Hallett, physiological information is now available for seven species of Antarctic Collembola in three families (Table 10), out of approximately 15 described species on the continent. However, only SCPs are available for all seven species, and good winter data are lacking for all species except *C. antarcticus* on Signy Island (Cannon and Block, 1988). Some parameters (e.g. ULT) have been measured only rarely, and metabolic rate has not been measured in any Antarctic Collembola since the 1970s. In addition, we note that there is substantially less physiological information available for the (more diverse) terrestrial Antarctic mite fauna. Given differential rates of climate change across the continent, and the requirement for signatory nations of the Madrid Protocol for Environmental Protection to the Antarctic Treaty (see [www.ce-paq](http://www.ce-paq)) to monitor and conserve the Antarctic biota, we find the present situation extraordinary. We hope that the forthcoming International Polar year (2007) will present an opportunity to improve basic knowledge of and mechanisms underlying the distribution and abundance of springtails across Antarctica. This information will certainly be required before we might pretend to disentangle the effects of natural and anthropogenic change on this important component of the Antarctic biota.

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### References

- Bale, J.S., 1987. Insect cold hardiness: freezing and supercooling—an ecophysiological perspective. *Journal of Insect Physiology* 33, 899–908.
- Bayley, M., Holmstrup, M., 1999. Water vapor absorption in arthropods by accumulation of myoinositol and glucose. *Science* 285, 1909–1911.
- Bayley, M., Petersen, S.O., Knigge, T., Kohler, H.R., Holmstrup, M., 2001. Drought acclimation confers cold tolerance in the soil collembolan *Folsomia candida*. *Journal of Insect Physiology* 47, 1197–1204.
- Block, W., 1979. Oxygen consumption of the Antarctic springtail *Parisotoma otooculata* (Willem) (Isotomidae). *Revue d'Ecologie et de Biologie du Sol* 16, 227–233.
- Block, W., 1982. Supercooling points of insects and mites on the Antarctic Peninsula. *Ecological Entomology* 7, 1–8.
- Block, W., 1985. Cold Resistance of two continental Antarctic microarthropods. *Cryo-Letters* 6, 391.
- Block, W., 1994. Terrestrial ecosystems: Antarctica. *Polar Biology* 14, 293–300.
- Block, W., Duman, J.G., 1989. Presence of thermal hysteresis producing antifreeze proteins in the Antarctic mite, *Alaskozetes antarcticus*. *Journal of Experimental Zoology* 250, 229–231.
- Block, W., Harrison, P.M., 1995. Collembolan water relations and environmental change in the maritime Antarctic. *Global Change Biology* 1, 347–359.
- Block, W., Young, S.R., 1978. Metabolic adaptations of Antarctic terrestrial microarthropods. *Comparative Biochemistry and Physiology* 61A, 363–368.
- Block, W., Young, S.R., 1979. Measurement of supercooling in small arthropods and water droplets. *Cryo-Letters* 1, 85–91.
- Block, W., Harrison, P.M., Vannier, G., 1990. A comparative study of patterns of water loss from two antarctic springtails (Insecta, Collembola). *Journal of Insect Physiology* 36, 181–187.
- Cannon, R.J.C., Block, W., 1988. Cold tolerance of microarthropods. *Biological Reviews* 63, 23–77.
- Cannon, R.J.C., Block, W., Collett, G.D., 1985. Loss of supercooling ability in *Cryptopygus antarcticus* (Collembola: Isotomidae) associated with water uptake. *Cryo-Letters* 6, 73–80.
- Chown, S.L., Nicolson, S.W., 2004. *Insect Physiological Ecology. Mechanisms and Patterns*. Oxford University Press, Oxford.
- Chown, S.L., Addo-Bediako, A., Gaston, K.J., 2002a. Physiological variation in insects: large-scale patterns and their implications. *Comparative Biochemistry and Physiology B* 131B, 587–602.
- Chown, S.L., Addo-Bediako, A., Gaston, K.J., 2002b. Physiological variation in insects: large-scale patterns and their implications. *Comparative Biochemistry and Physiology B—Biochemistry & Molecular Biology* 131, 587–602.
- Convey, P., 2001. Terrestrial ecosystem responses to climate changes in the Antarctic. In: Walther, G.R., Burga, C.A., Edwards, P.J. (Eds.), *Fingerprints of Climate Change*. Plenum, New York.
- Convey, P., Worland, M.R., 2000. Refining the risk of freezing mortality for Antarctic terrestrial microarthropods. *CryoLetters* 21, 333–338.
- Convey, P., Pugh, P.J.A., Jackson, C., Murray, A.W., Ruhland, C.T., Xiong, F.S., Day, T.A., 2002. Response of antarctic terrestrial microarthropods to long-term climate manipulations. *Ecology* 83, 3130–3140.



- Convey, P., Block, W., Peat, H.J., 2003. Soil arthropods as indicators of water stress in Antarctic terrestrial habitats? *Global Change Biology* 9, 1718–1730.
- Csermely, P., Schneider, T., Söti, C., Prohászka, Z., Nardai, G., 1998. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. *Pharmacology & Therapeutics* 79, 129–168.
- Danks, H.V., 1991. Winter habitats and ecological adaptations for winter survival. In: Lee, Jr, R.E., Denlinger, D.L. (Eds.), *Insects at Low Temperature*. Chapman & Hall, New York, pp. 231–259.
- Davey, M.C., Pickup, J., Block, W., 1992. Temperature variation and its biological significance in fellfield habitats on a maritime Antarctic island. *Antarctic Science* 4, 383–388.
- Doran, P.T., Priscu, J.C., Lyons, W.B., Walsh, J.E., Fountain, A.G., McKnight, D.M., Moorhead, D.L., Virginia, R.A., Wall, D.H., Clow, G.D., Fritsen, C.H., McKay, C.P., Parsons, A.N., 2002. Antarctic climate cooling and terrestrial ecosystem response. *Nature* 415, 517–520.
- Duman, J.G., 2001. Antifreeze and ice nucleator proteins in terrestrial arthropods. *Annual Review of Physiology* 63, 327–357.
- Edwards, J.S., 1986. How small ectotherms thrive in the cold without really trying. *Cryo-Letters* 6, 388–390.
- Ewing, H.E., 1922. Notes on the occurrence and distribution of antarctic land arthropods (springtails and mites: Collembola and Acarina). *Entomological News* 33, 76–79.
- Fitzsimons, J.M., 1971. Temperature and three species of Antarctic arthropods. *Pacific Insects Monographs* 25, 127–135.
- Folk, D.G., Han, C., Bradley, T.J., 2001. Water acquisition and partitioning in *Drosophila melanogaster*: effects of selection for desiccation-resistance. *Journal of Experimental Biology* 204, 3323–3331.
- Frati, F., Fanciulli, P.P., Carapelli, A., Dallai, R., 1997. The Collembola of northern Victoria Land (Antarctica): distribution and ecological remarks. *Pedobiologia* 41, 50–55.
- Friedmann, E.I., McKay, C.P., Nienow, J.A., 1987. The Cryptoendolithic microbial environment in the Ross Desert of Antarctica: Satellite-Transmitted continuous nanoclimate data, 1984 to 1986. *Polar Biology* 7, 273–287.
- García, L.V., 2004. Escaping the Bonferroni iron claw in ecological studies. *Oikos* 105, 657–663.
- Gordon, S., 2003. Site Description & Literature Review of Cape Hallett & Surrounding Areas. Antarctica New Zealand, Christchurch.
- Gressitt, J.L., ed. 1967. *Entomology of Antarctica*, vol. 10. American Geophysical Union, Washington, DC.
- Hagner-Holler, S., Schoen, A., Erker, W., Marden, J.H., Rupprecht, R., Decker, H., Burmester, T., 2004. A respiratory hemocyanin from an insect. *Proceedings of the National Academy of Sciences of the United States of America* 101, 871–874.
- Hanc, Z., Nedved, O., 1999. Chill injury at alternating temperatures in *Orchesella cincta* (Collembola: Entomobryidae) and *Pyrrhochorista aptera* (Heteroptera: Pyrrhocoridae). *European Journal of Entomology* 96, 165–168.
- Harrington, H.J., Wood, B.L., McKellar, I.C., Lensen, G.J., 1967. Topography and geology of the Cape Hallett district. Victoria Land, Antarctica New Zealand Geological Survey Bulletin 80, Wellington.
- Hayward, S.A.L., Worland, M.R., Convey, P., Bale, J.S., 2003. Temperature preferences of the mite, *Alaskozetes antarcticus*, and the collembolan, *Cryptopygus antarcticus* from the maritime Antarctic. *Physiological Entomology* 28, 114–121.
- Hayward, S.A.L., Worland, M.R., Convey, P., Bale, J.S., 2004. Habitat moisture availability and the local distribution of the Antarctic Collembola *Cryptopygus antarcticus* and *Friesea grisea*. *Soil Biology & Biochemistry* 36, 927–934.
- Hertzberg, K., Leinaas, H.P., 1998. Drought stress as a mortality factor in two pairs of sympatric species of Collembola at Spitsbergen, Svalbard. *Polar Biology* 19, 302–306.
- Hodkinson, I.D., Coulson, S.J., Webb, N.R., Block, W., 1996. Can high Arctic soil microarthropods survive elevated summer temperatures? *Functional Ecology* 10, 314–321.
- Holmstrup, M., Sjørnsen, H., Ravn, H., Bayley, M., 2001. Dehydration tolerance and water vapour absorption in two species of soil-dwelling Collembola by accumulation of sugars and polyols. *Functional Ecology* 15, 647–653.
- Holt, R.D., Lawton, J.H., Gaston, K.J., Blackburn, T.M., 1997. On the relationship between range size and local abundance: back to basics. *Oikos* 78, 183–190.
- Hopkin, S.P., 1997. *Biology of the Springtails (Insecta: Collembola)*. Oxford University Press, Oxford.
- Hosler, J.S., Burns, J.E., Esch, H.E., 2000. Flight muscle resting potential and species-specific differences in chill-coma. *Journal of Insect Physiology* 46, 621–627.
- IPCC, 2001. *Climate Change 2001: The Scientific Basis*. Technical Summary IPCC, Geneva.
- Janetschek, H., 1963. On the terrestrial fauna of the Ross-Sea area, Antarctica. *Pacific Insects* 5, 305–311.
- Janetschek, H., 1967. Arthropod ecology of South Victoria Land. In: Gressitt, J.L. (Ed.), *Entomology of Antarctica*. American Geophysical Union, Washington, DC, pp. 205–293.
- Kennedy, A.D., 1993. Water as a limiting factor in the Antarctic terrestrial environment: a biogeographical synthesis. *Arctic and Alpine Research* 25, 308–315.
- Kingsolver, J.G., 1989. Weather and the population dynamics of insects—integrating physiological and population ecology. *Physiological Zoology* 62, 314–334.
- Kingsolver, J.G., Huey, R.B., 1998. Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist* 38, 545–560.
- Klok, C.J., Chown, S.L., 2003. Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* 78, 401–414.
- Kohshima, S., 1984. A novel cold-tolerant insect found in a Himalayan glacier. *Nature* 310, 225–227.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Canadian Journal of Zoology* 75, 1553–1560.
- Marshall, D.J., Newton, I.P., Crafford, J.E., 1995. Habitat temperature and potential locomotor activity of the continental Antarctic mite, *Maudheimia petronia* Wallwork (Acari: Oribatei). *Polar Biology* 15, 41–46.
- Marshall, D.J., Pugh, P.J.A., 1996. Origin of the inland Acari of continental Antarctica, with particular reference to Dronning Maud Land. *Zoological Journal of the Linnean Society* 118, 101–118.
- Meier, P., Zettel, J., 1997. Cold hardiness in *Entomobrya nivalis* (Collembola, Entomobryidae): annual cycle of polyols and antifreeze proteins, and antifreeze triggering by temperature and photoperiod. *Journal of Comparative Physiology B* 167, 297–304.
- Nedved, O., 1998. Modelling the relationship between cold injury and accumulated degree days in terrestrial arthropods. *Cryo-Letters* 19, 267–274.
- Nunamaker, R.A., 1993. Rapid Cold-Hardening in *Culicoides variipennis sonorensis* (Diptera, Ceratopogonidae). *Journal of Medical Entomology* 30, 913–917.
- Parnesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37–42.
- Peck, L.S., 2002. Ecophysiology of Antarctic marine ectotherms: limits to life. *Polar Biology* 25, 31–40.
- Peck, L.S., Webb, K.E., Bailey, D.M., 2004. Extreme sensitivity of biological function to temperature in Antarctic marine species. *Functional Ecology* 18, 625–630.
- Precht, H., 1958. Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In: Prosser, C.L. (Ed.), *Physiological Adaptation*. American Academy for the Advancement of Science, Washington.

- Pryor, M.E., 1962. Some environmental features of Hallett Station, Antarctica, with special reference to soil arthropods. *Pacific Insects* 4, 681–728.
- Quinn, G.P., Keogh, M.J., 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- Rudolph, E.D., 1963. Vegetation of Hallett Station, Victoria Land, Antarctica. *Ecology* 44, 585–586.
- Sinclair, B.J., 2001a. Biologically relevant environmental data: macros to make the most of microclimate recordings. *Cryo-Letters* 22, 125–134.
- Sinclair, B.J., 2001b. Field ecology of freeze tolerance: interannual variation in cooling rates, freeze-thaw and thermal stress in the microhabitat of the alpine cockroach *Celatoblatta quinque maculata*. *Oikos* 93, 286–293.
- Sinclair, B.J., Sjørnsen, H., 2001a. Cold tolerance of the Antarctic springtail *Gomphiocephalus hodgsoni* (Collembola, Hypogastruridae). *Antarctic Science* 13, 271–279.
- Sinclair, B.J., Sjørnsen, H., 2001b. Terrestrial invertebrate abundance across a habitat transect in Keble Valley, Ross Island, Antarctica. *Pedobiologia* 45, 134–145.
- Sinclair, B.J., Chown, S.L., 2002. Haemolymph osmolality and thermal hysteresis activity in 17 species of arthropods from sub-Antarctic Marion Island. *Polar Biology* 25, 928–933.
- Sinclair, B.J., Lord, J.M., Thompson, C.M., 2001. Microhabitat selection and seasonality of alpine invertebrates. *Pedobiologia* 45, 107–120.
- Sinclair, B.J., Addo-Bediako, A., Chown, S.L., 2003a. Climatic variability and the evolution of insect freeze tolerance. *Biological Reviews* 78, 181–195.
- Sinclair, B.J., Klok, C.J., Scott, M.B., Terblanche, J.S., Chown, S.L., 2003b. Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. *Journal of Insect Physiology* 49, 1049–1061.
- Sinclair, B.J., Vernon, P., Klok, C.J., Chown, S.L., 2003c. Insects at low temperatures: an ecological perspective. *Trends in Ecology & Evolution* 18, 257–262.
- Sinclair, B.J., Klok, C.J., Chown, S.L., 2004. Metabolism of the sub-Antarctic caterpillar *Pringleophaga marioni* during cooling, freezing and thawing. *Journal of Experimental Biology* 207, 1287–1294.
- Sjørnsen, H., Sømme, L., 2000. Seasonal changes in tolerance to cold and desiccation in *Phauloppia* sp. (Acari, Oribatida) from Finse, Norway. *Journal of Insect Physiology* 46, 1387–1396.
- Sollars, V., Lu, X.Y., Xiao, L., Wang, X.Y., Garfinkel, M.D., Ruden, D.M., 2003. Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution. *Nature Genetics* 33, 70–74.
- Sømme, L., 1986. Ecology of *Cryptopygus sverdrupi* (Insecta: Collembola) from Dronning Maud Land, Antarctica. *Polar Biology* 6, 179–184.
- Sømme, L., 1995. *Invertebrates in Hot and Cold Arid Environments*. Springer, Berlin.
- Sømme, L., 1996. The effect of prolonged exposures at low temperatures in insects. *Cryo-Letters* 17, 341–346.
- Sømme, L., Block, W., 1982. Cold hardiness of Collembola at Signy Island, maritime Antarctic. *Oikos* 38, 168–176.
- Sømme, L., Block, W., 1991. In: Lee, R.E., Denlinger, D.L. (Eds.), *Adaptations to alpine and polar environments in insects and other terrestrial arthropods*. Chapman & Hall, New York, pp. 318–359.
- Staempfli, C., Becker-van Slooten, B., Tarradellas, J., 2002. Hsp70 instability and induction by a pesticide in *Folsomia candida*. *Biomarkers* 7, 68–79.
- Strong, F.E., Dunkle, R.L., Dunn, R.L., 1970. Low-temperature physiology of Antarctic arthropods. *Antarctic Journal of the United States* 5, 123.
- Tillbrook, P.J., 1977. Arthropod ecology of South Victoria Land. In: Llano, G.R. (Ed.), *Antarctic Terrestrial Biology*. American Geophysical Union, Washington, DC, pp. 935–946.
- Towbin, H., Staehlin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America* 76, 4350–4354.
- Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. *Nature* 416, 389–395.
- Wharton, D.A., 2002. *Life at the Limits: Organisms in extreme environments*. Cambridge University Press, Cambridge.
- Wise, K.A.J., 1967. Collembola (Springtails). In: Gressitt, J.L. (Ed.), *Entomology of Antarctica*. American Geophysical Union, Washington, DC, pp. 123–148.
- Wise, K.A.J., Gressitt, J.L., 1965. Far southern animals and plants. *Nature*, London 207, 101–102.
- Wise, K.A.J., Shoup, J., 1967. Distribution of Collembola at Cape Hallett. In: Gressitt, J.L. (Ed.), *Entomology of Antarctica*. American Geophysical Union, Washington, DC, pp. 325–330.
- Worland, M.R., 2005. Factors that influence the supercooling point of the sub-Antarctic springtail *Tullbergia antarctica*. *Journal of Insect Physiology* 51, 881–894.
- Worland, M.R., Convey, P., 2001. Rapid cold hardening in Antarctic microarthropods. *Functional Ecology* 15, 515–524.
- Worland, M.R., Block, W., Rothery, P., 1992. Survival of sub-zero temperatures by two South Georgian beetles (Coleoptera, Perimylopidae). *Polar Biology* 11, 607–613.
- Zachariassen, K.E., Husby, J.A., 1982. Antifreeze effect of thermal hysteresis agents protects highly supercooled insects. *Nature* 298, 865–867.
- Zachariassen, K.E., Kristiansen, E., 2000. Ice nucleation and antinucleation in nature. *Cryobiology* 41, 257–279.
- Zettel, J., 1984. Cold hardiness strategies and thermal hysteresis in Collembola. *Revue d'Écologie et de Biologie du Sol* 21, 189–203.