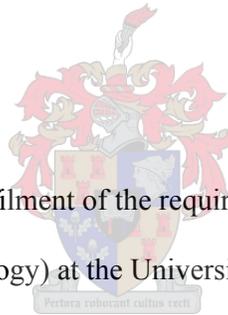


# **Physiological variation in insects: metabolic scaling and gas exchange patterns**

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Supervisor: Prof. S. L. Chown

April 2005

## **Declaration**

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

**Signature.....**

**Date .....**

## Abstract

Physiological diversity in insects was investigated in terms of gas exchange patterns and metabolic rate. Since the majority of hypotheses that propose to explain the occurrence of Discontinuous Gas Exchange Cycles (DGCs) are adaptive, the possibility of adaptation was explored in two ways. First, since repeatability is one of the three prerequisites for natural selection, and rarely tested in insects, the repeatability of gas exchange characteristics and metabolic rate were tested in a *Perisphaeria* sp. cockroach. Four major gas exchange patterns were recognized and these patterns were found to be extremely variable within this species. However, repeatability was still generally high for the majority of the gas exchange characteristics and metabolic rate. Moreover, between individual variability generally accounted for a large proportion of the variance, supporting the repeatability findings. Therefore, both metabolic rate and gas exchange patterns might indeed be adaptive traits in this *Perisphaeria* sp. cockroach. Second, the distribution of the three gas exchange patterns (DGCs, cyclic and continuous) across the insect phylogeny was investigated. Data were extracted from the literature and gas exchange patterns for eight insect orders, representing mainly the endopterygotes, were measured. Using standard respirometry techniques, data for a total of ten additional orders were added, which represented both apterygote orders, six exopterygote orders and two endopterygote orders that were previously uninvestigated. In addition, four of the five adaptive hypotheses were tested by investigating the occurrence of DGCs in winged or wingless insects, subterranean and non-subterranean insects, and insects from mesic and xeric environments. Results indicate that DGC has evolved at least five times in the insects and that the cyclic gas exchange pattern is likely to be basal. No support was found for the chthonic, hybrid and oxidative damage hypotheses, while DGCs were associated with xeric environments. This analysis provides some support for the hygric hypothesis. In addition,

the prediction of the fractal network model for metabolic scaling which states that both intra- and interspecific scaling coefficients should be identical and equal to 0.75, was tested. Three species of polymorphic worker ants were used to determine the intraspecific scaling coefficient. Data from the literature were added to provide metabolic rate information on 42 species of the Formicidae. The interspecific scaling coefficient was determined in several ways: the traditional method (Ordinary Least Squares regression models), with the two respirometry techniques analyzed separately or in combination (e.g. open and closed system), and using phylogenetic independent contrasts with the same sets of data. Intraspecific coefficients were significantly different from 0.75. The slope of the interspecific regression obtained using Ordinary Least Squares regression models was not significantly different from 0.75. However, when phylogenetic relatedness was accounted for the slope differed significantly from 0.75. Therefore, the fractal network model was not supported by these findings, since slope values are not equal and also not statistically equivalent to 0.75. Overall, variability of both metabolic rate and gas exchange patterns in insects is higher than generally suggested by the literature. These findings provide much scope for future work.

## Opsomming

Hier word die fisiologiese diversiteit van insekte ondersoek in terme van hul gaswisselings patrone asook hul metaboliese tempo. Die oorgrote meerderheid van die hipoteses wat voorgestel is om die ontstaan en bestaan van Diskontinue Gaswisseling in insekte te verklaar, is op 'n evolusionêre grondslag gebaseer. Vir hierdie rede word die moontlikheid van Diskontinue Gaswisseling as 'n aanpassing op twee maniere ondersoek. Herhaalbaarheid is een van die voorvereistes van aanpassing (in terme van evolusie) en dit is tot dusver slegs 'n paar keer in insekte getoets is. Daarom word die herhaalbaarheid van die gaswisselings komponente, asook die metaboliese tempo hier in 'n *Perisphaeria* sp. kokkerot ondersoek. Vier hoof gaswisselings patrone is geïdentifiseer en die bevinding was dat die patrone geweldig kan varieer binne hierdie spesie. Ten spyte hiervan was die herhaalbaarheid steeds hoog vir die oorgrote meerderheid van die gaswisselings komponente asook vir die metaboliese tempo. Verder het die variasie tussen individue tot 'n groot proporsie van die totale variasie bygedra, wat die betekenisvolheid van die hoë herhaalbaarheid beklemtoon. Dus, beide metaboliese tempo sowel as gaswisselings komponente kan moontlik as 'n aanpassing beskou word in hierdie kokkerot. Tweedens, die verspreiding van die drie gaswisselings patrone (Diskontinue Gaswisseling, siklies en kontinu) in die insekte is ondersoek. Gaswisselings patrone vir agt van die dertig insek ordes is vanuit die literatuur verkry, alhoewel dit meestal verteenwoordigend van die Endopterygota is. Data vir tien addisionele ordes wat nog nooit voorheen ondersoek is nie is bygevoeg, insluitende beide die ordes van Apterygota en ses ordes van die Exopterygota, asook twee ordes van die Endopterygota. Verder is vier van die vyf hipoteses met betrekking tot aanpassing ondersoek deur die teenwoordigheid van Diskontinue Gaswisseling in gevleuelde en ongevleuelde insekte, ondergrondse en bogrondse insekte, asook insekte van gematigde en droë omgewings te ondersoek. Resultate dui aan dat

Diskontinue Gaswisseling ten minste vyf keer onafhanklik in insekte ontstaan het en dat die sikliese patroon heel moontlik basaal is. Die resultate bied geen ondersteuning vir die hipoteses wat gegrond is op gaswisseling in ondergrondse omgewing of oksidasie beskerming nie. Daar is wel ondersteuning vir die hipotese aangaande die rol van water verlies in die ontstaan en onderhoud van Diskontinue Gaswisseling. Addisioneel is daar ondersoek ingestel na die verwagting wat deur die fraktale netwerk model vir metaboliese glyskale gestel word, wat lui dat beide die binne- sowel as tussen-spesies koëffisiënte identies aan mekaar en gelyk aan 0.75 sal wees. Drie polimorfiese mier spesies is gebruik om die binne-spesies koëffisiënt te bepaal. Data vanuit die literatuur is hier bygevoeg en sodoende was informasie vir 42 mier spesies van die Formicidae beskikbaar. Verskillende metodes is gebruik om die tussen-spesies koëffisiënt te bepaal: die tradisionele metode (sg. “OLS”-regressie), met die twee tegnieke om metaboliese tempo te bepaal (bv. oop en geslote sisteem metodes) apart asook saam, en deur gebruik te maak van filogeneties onafhanklike vergelykings vir dieselfde datastelle. Die binne-spesies koëffisiënte was almal betekenisvol verskillend van 0.75. Die tussen-spesies regressie waar gebruik gemaak is van ‘n “OLS”-regressie model was nie betekenisvol verskillend van 0.75 nie. Met filogenetiese verwantskappe egter in ag geneem, het die koëffisiënt wel betekenisvol verskil van 0.75. Dus word die fraktale netwerk model nie ondersteun deur hierdie studie nie, aangesien die koëffisiënte nie identies aan mekaar is nie, en ook statisties verskil van 0.75. In die algemeen is die variasie in metaboliese tempo sowel as gaswisselings patrone aansienlik hoër as wat afgelei kan word uit die literatuur. Hierdie bevindinge stel ‘n basis vir heelwat verdere werk in die toekoms.

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# **Chapter 1**

## **General Introduction**

*Physiological diversity and evolution of physiological traits*

Understanding physiological diversity is critical for developing a sound knowledge of the way organisms respond to their environments, and how this response influences the evolution of diversity (Feder, 1987; Spicer and Gaston, 1999). Perhaps one of the most profound recent illustrations of this is the exploration of the importance of the “ $3/4$  scaling law” for understanding the distribution of diversity across the planet. West et al. (1997) developed a fractal network model suggesting that the all physiological scaling coefficients should be 0.75 or a power thereof. Further empirical work by Gillooly et al. (2001) found that whole-animal metabolic rates vary with body size with a scaling coefficient of 0.75 so supporting the model (see also Savage et al., 2004). Moreover, West et al. (2002) showed that molecules, cells and whole animal metabolic rate scale with an exponent of 0.75. Gillooly et al. (2001) also included the effect of temperature into the model using the “Universal Temperature Dependence” (UTD), since biochemical reaction rates (and therefore whole organism metabolic rate) are temperature dependant. Based on the findings of Gillooly et al. (2001), Allen et al. (2002) claimed that the biochemical kinetics of metabolism can be used to predict abundance and species diversity across the planet. The main assumptions of their study were that metabolic rate increases with body size with an exponent of  $3/4$ , whilst population densities per unit area decrease with body size with an exponent of  $-3/4$ . This entire suite of reasoning has come to be known as the “metabolic theory of ecology” (Brown et al., 2004).

Clearly, much of this understanding has come from investigations of interspecific variation in physiological and ecological parameters that vary with body size. However, there is also considerable physiological variation at other levels that might not be a function of size. This variation includes that associated with ontogeny, intra-individual variation through time, and variation between similar-sized individuals of the same species

(Spicer and Gaston, 1999). Despite the obvious importance of understanding physiological diversity at several levels, much of the focus has been on between-species comparisons (e.g. Jensen and Holm-Jensen, 1980; Nielsen, 1986; Feder et al., 1987; Chown and Gaston, 1999; Davis et al., 2000; Klok et al., 2002; Pither, 2003) and this trend has only recently started to change with greater focus on both between-population and individual level variability (see Bennett, 1987; Garland and Adolph, 1994; Jenkins, 1997, Klok and Chown, 2003). Understanding individual and population level variability is important because it is only in this way that the relationship between physiological variation and fitness can be comprehended, as well as the possibility of adaptation can be understood (Feder, 1987).

For a trait to be considered an adaptation, natural selection must have been responsible for the origin of a trait and must be responsible for its current maintenance (Coddington, 1988). Three requirements are essential for natural selection to take place, and therefore for a character to be considered adaptive (Bech et al., 1999; see also Endler, 1986; Ridley, 1996). First, variation in the characteristic should be related to variation in fitness. Second, repeatability of the variation should be high and significant. That is, variation between individuals should be greater than the variation within an individual. Repeatability can be defined as “the proportion of the total variance in multiple measurements of a trait that is due to differences among individuals” (Dohm, 2002). Third, the trait must be heritable.

Given the importance of basal metabolic rate (BMR) as a trait influencing a wide variety of environmental responses (see above, Chown and Gaston, 1999; Brown et al., 2004), as well as its relative ease of measurement, there has been much recent work on individual-level variation in BMR, as well as in field metabolic rate (FMR) and resting metabolic rate (RMR), especially in vertebrates (e.g. Hayes et al., 1992; Speakman et al.,

1994; Hayes et al., 1998; Bech et al., 1999; Chappell and Rogowitz, 2000; Terblanche et al., 2004). Generally, inter-individual variation accounts for 30% – 89% of the total variation in metabolic rate, suggesting not only that the trait can be subject of selection, but also that at least some of its variation might be adaptive.

Variation in metabolic rate is also thought to be of considerable adaptive significance in invertebrates such as insects (Chown and Gaston, 1999). For example, it is thought that elevated metabolic rates are a response to cold (MCA – metabolic cold adaptation, see Clarke, 1991 for general discussion and Addo-Bediako et al., 2002 for an insect example) and lower metabolic rates are a response to dry conditions to save water (e.g. Kestler, 1985; Lighton, 1994, 1996). Moreover, in insects the pattern of gas exchange is also thought to be an important response to the environment. Some insects, and other tracheated arthropod taxa, are known to exchange gas in a discontinuous fashion (Levy and Schneidermann, 1966a, b; Lighton, 1994, 1996; Wasserthal, 1996; Klok et al., 2002). These cycles, also known as discontinuous gas exchange cycles (DGCs), refer to the cyclic release of carbon dioxide and uptake of oxygen. The classical discontinuous pattern comprises three periods, namely the Closed-period (C-period), Flutter-period (F-period) and the Open-period (O-period) (see below for more detail on the periods). The duration and rate of these periods vary substantially between species and this variation is thought to be adaptive (Davis et al., 1999; Duncan and Byrne, 2000). The primary hypotheses for the occurrence of DGC are adaptive (Lighton, 1998; Bradley, 2000; see Chown et al., submitted ms. for discussion of all the hypotheses). However, there have been few attempts to explore variation in metabolic rate and gas exchange patterns between individuals to ascertain the extent to which they might be considered adaptive. Nonetheless, considerable variation in metabolic rate and the components of DGC within as well as among individuals has been reported (see Lighton, 1998 and Chown, 2001 for discussion and

examples). Only a few studies have examined variability at the individual level in insects (Chappell and Rogowitz, 2000; Rourke, 2000; Nespolo et al., 2003). This is perhaps not entirely surprising, given that investigations of the levels of physiological variation in insects are rare (for exceptions and discussion see Addo-Bediako et al., 2001; Chown, 2001, 2002).

### *Scaling*

Scaling is the study of how anatomical and physiological parameters change with body size (see Schmidt-Nielsen, 1984, for an overview). The allometric scaling equation is as follows:

$$Y = aM^b \quad (\text{Eq. 1})$$

where  $Y$  represents the standard metabolic rate (or other physiological variable),  $M$  represents the body mass,  $a$  represents the intercept and  $b$  the slope (i.e. rate of change of metabolic rate with change in body mass). Thus, when  $b$  is not equal to 1,  $M$  and  $Y$  are allometrically related, which means that the two variables do not change at a similar rate. Logarithmically transforming the equation simplifies calculating the slope as well as the intercept (Von Bertalanffy, 1957).

$$\text{Log } Y = \text{Log } a + b \cdot \text{log } x \quad (\text{Eq. 2})$$

The whole-animal metabolic scaling exponent ( $b$ ) has variously been estimated as 1.0, 0.67, and 0.75 for mammals (Schmidt-Nielsen, 1984). Kleiber (1932) suggested that when the log of the metabolic rate is plotted against the log of the mass for animals of different species,  $b = 0.75$  (see Schmidt-Nielsen, 1984, pp 58-60). This law is better known as the “ $3/4$  power law” for metabolic rates (Schmidt-Nielsen, 1984; Dodds et al., 2001). A fractal network model was developed by West et al. (1997) which explains why scaling coefficients should be 0.75. This model has enjoyed much attention since it was published,

and has strongly influenced the current literature (e.g. Dodds et al., 2001; Dreyer and Puzio, 2001; Fujiwara, 2003; Hochachka et al., 2003; Sántillan, 2003). The fractal network model is based on three main assumptions: 1) the circulatory system of an organism extends throughout the organism; 2) the energy used to transport nutrients is minimized and 3) the smallest tubules or capillaries in all animals have the same size. West et al. (1997) also assumed that each time a tube splits into smaller tubes, the cross-sectional area of the larger tube is the sum of the smaller cross-sectional areas of the two daughter tubes. These assumptions led West et al. (1997) to suggest that the transport system must be a fractal-like structure with area preserving branching, and that this fractal-like nature is common for all organisms. They concluded that this is the underlying reason behind the  $\frac{3}{4}$ -power scaling law despite physical difference in structural design. Thus, because of the transport systems of organisms, scaling coefficients within- as well as between species should be identical (0.75 or multiples thereof – West et al., 1997, 2002). By contrast, Dodds et al. (2001) recently re-analysed metabolic rate data collected from several papers (e.g. Bennett and Harvey, 1987; Heusner, 1991) and found that for both mammals (weighing 10 kg or less) and all birds, the scaling coefficient was closer to 0.66 than to 0.75, thus obeying the  $\frac{2}{3}$  power law rather than the  $\frac{3}{4}$  power law. Furthermore, White and Seymour (2003), correcting for phylogenetic non-independence in mammals, also concluded that metabolic rates scale as 0.66 rather than 0.75 in this group of animals. Savage et al. (2004) responded that neither of the two studies supporting the 0.66 coefficient had corrected for problems associated with different numbers of species in each size class, and proceeded to do so using a binning method that revealed a slope of 0.75. Thus it is clear that the empirical value of the scaling relationship remains hotly contested, at least in endothermic vertebrates. It is also clear that the theoretical foundations of the

metabolic theory of ecology are also being questioned, with vigorous criticism coming from Dodds et al. (2001), Kozłowski and Konarzewski (2004), and Clarke (2004).

In insects, much attention has been paid to the main trends in gas exchange patterns and metabolic rate, such as the nature of the gas exchange cycle and variation of metabolic rate with body size (e.g. Lighton, 1991; Lighton and Fielden, 1995; Duncan and Byrne, 2000; Duncan and Dickman, 2001; Shelton and Appel, 2001). In many instances, however, close attention has not been paid to the standardisation of methods (e.g. open- or closed-system respirometry, feeding status of animal, phylogeny) to ensure that scaling exponents are as reliable a reflection of the true values as possible (for discussion see Lighton and Fielden, 1995; Addo-Bediako et al., 2002). This has largely been due either to different goals of the works in question, difficulty of standardization, or poor attention to protocols for establishing SMR because SMR is a difficult concept for insects in the first place (they lack a thermoneutral zone). One result of this lack of standardization is that no consensus has yet been reached on what the representative scaling exponent for insect metabolic rates should be. Indeed, variation in the scaling exponents obtained from insect studies is great and they range from 0.5 to 0.95 (e.g. Lighton, 1991; Lighton and Duncan, 1995; Lighton and Fielden, 1995; Davis et al., 1999; Lighton et al., 2001; Klok et al., 2002). Lighton and Fielden (1995) generated a consensus scaling relationship for arthropods,  $SMR = 906M^{0.825}$ . However, it has been reported that ticks and scorpions do not conform to the consensus scaling relationship (Lighton and Fielden, 1995; Lighton et al., 2001), since the intercept seems to be lower, although the exponent is still similar to that which Lighton and Fielden (1995) proposed. Moreover, Duncan and Dickman (2001) and Terblanche et al. (2004) have pointed out that the consensus scaling relationship for insects is based largely on beetles and ants, and that other groups, such as flies, are under represented. In consequence, the scaling relationship could reasonably be considered to be biased towards

non-flying, or typically less energetic insects.

### *Discontinuous Gas Exchange*

Three major gas exchange patterns can be recognized in insects, namely continuous, cyclic and discontinuous gas exchange cycles (DGCs). Discontinuous gas exchange cycles are portrayed in the literature to be a common gas exchange pattern among the insects (Lighton, 1994, 1996). However, DGCs have only been reported for five insect orders (Punt, 1950; Levy and Schneiderman, 1966b; Kestler, 1985; Lighton, 1990; Lighton and Berrigan, 1995; Harrison, 1997; Davis et al., 1999; Rourke, 2000; Vogt and Appel, 2000; Duncan et al., 2002), and the majority of orders remain unexplored from this perspective. Therefore the true extent of the occurrence of DGC in insects is still unknown. Besides DGC, cyclic patterns have also been observed in quiescent insects (Duncan and Crewe, 1993; Shelton and Appel, 2000; Chown, 2001; Duncan and Dickman, 2001) while the continuous pattern is usually observed during activity.

The classical discontinuous pattern comprises three periods, namely the Closed-period (C-period), Flutter-period (F-period) and the Open-period (O-period). When the spiracles are open (O-period), gas-exchange takes place freely. Oxygen moves into the endotracheal space and carbon dioxide moves out. After equilibration of atmospheric and endotracheal air the spiracles are closed (the C-period) and no gas exchange takes place between the endotracheal space and the atmosphere. This means that the insect has to make use of oxygen in the endotracheal stores. CO<sub>2</sub> is stored in, and buffered by, the haemolymph (Lighton, 1994, 1996). Gradually, the oxygen reserves in the endotracheal spaces are depleted and the spiracular muscles induce a partial opening of the spiracle. During this F-period, the spiracles open and close rapidly (or flutter) and the PO<sub>2</sub> in the endotracheal space is normalized (Wigglesworth, 1972; Kestler, 1985; Lighton, 1998).

Small amounts of CO<sub>2</sub> also escape during this period. When the CO<sub>2</sub> concentration becomes too high, the spiracular muscles are affected by means of peripherally mediated sensory feedback (Lighton and Fielden, 1996), which causes them to open completely. Oxygen moves along the pressure gradient (from the outside into the endotracheal space) restoring equilibrium, thus initiating a new cycle.

The main hypotheses proposed to account for both the origin and maintenance of discontinuous gas exchange cycles are adaptive (Kestler, 1985; Slàma, 1988; Lighton, 1994, 1996; Lighton and Berrigan, 1995). The most favoured of these is that DGCs are an adaptation to limit respiratory water loss (reviews in Kestler, 1985; Lighton, 1994; Chown, 2002; Chown et al., submitted ms.). In an environment where water availability is limited, the DGC might be an effective way of minimizing respiratory water loss. When the spiracles are constricted (during the C-period), water vapour is unable to escape and respiratory water loss in this period is effectively zero (Lighton et al., 1993) However, recently there has been evidence suggesting that restricting water loss might not be why DGCs evolved, or certainly not the only reason (see Chown et al., submitted ms. for discussion). Indeed, there are several alternative hypotheses for the occurrence of DGC. These include the chthonic genesis hypothesis, which suggests that DGCs have originated to increase the ability of an insect to exchange gas under hypoxic and/or hypercapnic (typically underground) conditions. The hypothesis that includes both the water savings idea as well as the gas exchange facilitation is termed the hybrid exchange hypothesis (Chown et al., submitted ms.). Bradley (2000) hypothesised that DGCs evolved to reduce the free radicals that are produced during constant oxygen supply, thus reducing tissue damage (oxidative damage hypothesis). The last adaptive hypothesis is the strolling arthropod hypothesis, which suggests that the spiracles close to keep parasites from entering the tracheal system. A single non adaptive hypothesis has been proposed by

Chown and Holter (2000). According to this hypothesis, DGCs are an emergent property of the two interactive feedback systems (one for CO<sub>2</sub> and the other for O<sub>2</sub>) that are responsible for regulating spiracular opening when they have no demands placed on them (i.e. when the insect is at rest).

Although there is support for several of the adaptive hypotheses (reviewed in Chown and Nicolson, 2004), some suspicion exists that DGCs are so variable that they might not be considered adaptive, although the broader literature has generally not been particularly clear on this point. Indeed, Lighton (1998) and Chown (2001) recently pointed out that variation in the DGC can be considerable. These reports are something of a rediscovery of earlier findings. For example, Miller (1973) reported highly variable nerve activation (nerves that are responsible for the opening and closing of the spiracles) in cockroaches. A cursory inspection of the summary statistics given for the components of the DGC in many studies also show that there is substantial variation in the DGCs and their component parts (e.g. Davis et al., 1999; Duncan and Byrne, 2000, Duncan and Dickman, 2001), and Chown (2001) suggested that variability in gas exchange patterns might be the norm rather than the exception. Thus, although mention is now being more regularly made of variability in discontinuous gas exchange cycles, and the implications of this variation for the adaptive hypotheses proposed to explain DGCs, to date few formal analyses of variability in the components of the DGC have been undertaken (see Buck and Keister, 1955; Chappell and Rogowitz, 2000).

### **Aims and structure**

From the preceding discussion it is clear that much remains to be discovered about variability in insect gas exchange patterns and in insect metabolic rates (see also Chown and Nicolson, 2004). Addressing this major deficiency is of considerable importance given the implications of this variation (see above). Therefore, the primary aim of this thesis is to provide new information that can contribute to resolving some of the questions regarding variability in insect gas exchange and metabolism. Specifically, the following questions will be addressed:

- First, to provide the first careful test of the inherent assumption made by the adaptive hypotheses for DGC, that between-individual variability in these patterns is higher than within-individual variability, repeatability of gas exchange characteristics and metabolic rate in a *Perisphaeria* sp. cockroach are examined.
- Second, to determine whether DGCs have evolved once among the insects, or independently, (which in the latter case would add substantial weight to arguments in favour of the adaptive scenario) gas exchange patterns are examined for exemplar insects across a range of orders that have previously not been investigated. A parsimony analysis is then applied to investigate the likely course of evolution of gas exchange patterns across the insect orders.
- Finally, to address the question of how different scaling is likely to be at the within- and between-species levels, inter- and intraspecific metabolic scaling exponents for several species of ants are determined. This approach not only informs the question of overall variability in insect metabolic rates, but also provides insight into the applicability of the metabolic theory of ecology to insects.

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## Chapter 2

### **Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp.**

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## Introduction

For a trait to be considered an adaptation, natural selection must have been responsible for its origin and must be responsible for its maintenance, or, if one is prepared to blur the distinction between exaptation and adaptation, must be responsible for its maintenance (Coddington, 1988; Baum and Larson, 1991; Ketterson and Nolan, 1999). In turn, for natural selection to take place there are three prerequisites (Endler, 1986; Bech et al., 1999; Dohm, 2002). First, the trait in question must show consistent variation among individuals. Second, there must be a consistent relationship between that variation and variation in fitness. Third, the trait must be heritable. Whilst these assumptions, and particularly that of consistent among-individual variation, are being increasingly well explored for physiological traits in vertebrates (e.g. Huey and Dunham, 1987; Hayes and Chappell, 1990; Hayes et al., 1992; Chappell et al., 1995; Berteaux et al., 1996; Chappell et al., 1996; Bech et al., 1999; Fournier and Thomas, 1999; Koteja et al., 2000; McCarthy, 2000), the same is not true of invertebrates.

In insects, the genetic variability and heritability of several physiological traits have been investigated, mostly in *Drosophila* (usually *melanogaster*) and often in the context of selection experiments (e.g. Parsons, 1980; Hoffmann and Parsons, 1989a; Graves et al., 1992; Gibbs et al., 1997; Gibert et al., 1998; Hoffmann et al., 2003). Likewise, variation in traits among populations and as a consequence of acclimation has also been well explored, especially for thermal tolerances and desiccation resistance (Hoffmann, 1990; Hoffmann et al., 2001; Klok and Chown, 2003). Whilst these studies provide evidence that adaptation has probably been responsible for variation in thermal tolerances and desiccation resistance (see also Chown et al., 2002), explicit exploration of the assumptions underlying the hypothesis of adaptation remains scarce for most traits. This is especially true of metabolic rate and gas exchange characteristics. The few explicit studies that have been undertaken

have generally demonstrated a metabolic response to laboratory selection for desiccation resistance in *Drosophila melanogaster*, which implies that the conditions for selection must have been met (Hoffmann and Parsons, 1989a, b; Hoffmann and Parsons, 1993; Gibbs et al., 1997; Djawdan et al., 1998; Williams et al., 1998). Nonetheless, it is widely assumed that among-species and among-population variation in whole-organism metabolic traits in most insect taxa is adaptive (for review and examples see Lighton, 1996; Chown and Gaston, 1999; Addo-Bediako et al., 2001, 2002; Gibbs et al., 2003). Metabolic rate is of particular significance in this regard. Not only is it thought to be closely linked to variation in life history characteristics and body size (Hoffmann and Parsons, 1991; Graves et al., 1992; Kozłowski and Gawelczyk, 2002), but variation therein apparently also has a profound influence on broad-scale variation in diversity (Allen et al., 2002).

Adaptive explanations for variation in metabolic rate and the patterns in exchange underlying oxygen delivery and CO<sub>2</sub> removal in insects generally take two major forms. First, variation in metabolic rate is thought to take place in response either to dry conditions, when it is reduced to conserve water, or in response to short seasons, when it is elevated to enable more rapid development (for reviews and discussion see Chown and Gaston, 1999; Addo-Bediako et al., 2002; Chown, 2002). Second, alterations in gas exchange patterns are thought to have taken place to effect a respiratory water savings under dry conditions. In particular, it has long been thought that discontinuous gas exchange, which is present in many insect species at rest (Lighton, 1996, 1998), evolved as a means to limit respiratory water loss and that it continues to serve this major function (Levy and Schneiderman, 1966; Kestler, 1985; Sláma and Coquillaud, 1992; Lighton et al., 1993a; Duncan et al., 2002a). Discontinuous gas exchange is typically cyclic with each cycle consisting of a Closed (C) period, during which the spiracles are tightly closed, a Flutter (F) period, during which the spiracles partly open and close in rapid succession, and

an Open (O) period, during which the spiracles are open (Lighton, 1996). The principal explanations for the contribution of discontinuous gas exchange cycles (DGCs) to water economy are that spiracles are kept closed for a portion (the C-period) of the DGC thus reducing respiratory water loss to zero, and that a largely convective F-period restricts outward movement of water (Kestler, 1985). Moreover, it has also been argued that there is adaptive variation in the durations of the C-, F-, and O-periods to further reduce water loss. That is, a reduced O-period, and prolonged C-, and F-periods are likely to further restrict respiratory water loss (Lighton, 1990; Lighton et al., 1993b; Davis et al., 1999; Bosch et al., 2000; Duncan et al., 2002a; Duncan, 2003). Whilst several other hypotheses for the evolution and maintenance of DGCs have been proposed (Lighton and Berrigan, 1995; Lighton, 1998; Bradley, 2000), these are also largely adaptive in nature (though see Chown and Holter, 2000 for an exception).

Nonetheless, with the exception of the laboratory selection experiments on *D. melanogaster*, there have been few explicit attempts to investigate the assumptions underlying these claims for adaptation, which have largely been made on the grounds of comparative studies, of which the majority have not been undertaken in an explicitly phylogenetic context (see Chown, 2002; Chown and Gaston, 1999 for discussion and Davis et al., 1999; Duncan and Byrne, 2000; Addo-Bediako et al., 2001, and Gibbs et al., 2003 for recent studies). Whilst such comparative studies are useful, they are not without their problems (e.g. Leroi et al., 1994), and it is widely accepted that comparative work should be supported by more explicit investigations of the extent to which selection is responsible for variation in physiological traits (e.g. Huey and Kingsolver, 1993; Kingsolver and Huey, 1998). In this study, we therefore report an investigation of the repeatability of metabolic rate and the characteristics of discontinuous gas exchange cycles in an insect species that not only exchanges gases intermittently, but also shows

considerable variation in its gas exchange pattern. We reasoned that if these traits show significant repeatability in this species, then it is likely that repeatability will be even more pronounced in most other insect species, which are generally not as variable (see Chown, 2001).

## **Materials and methods**

### *Experimental animals and design*

Repeatability of metabolic rate, and cyclic gas exchange and its components were examined in the cockroach, *Perisphaeria* sp. (Blattodea, Blaberidae). In short-term pilot studies this species showed not only a clear DGC typical of many other insect species, but also several other gas exchange patterns that were not associated with activity, which generally disrupts typical DGC patterns (Lighton, 1994). Seventeen females and three males (males of species within this genus are rarely found - Picker et al. (2002)) were collected at altitudes higher than 950 m above sea level on Stellenbosch Mountain (33°57'S 18°53'E) and returned to the laboratory in Stellenbosch. Here they were held for two months before experiments commenced to exclude possible variation associated with microhabitat differences (Huey and Berrigan, 1996). Throughout this period and over the course of the experiments, the cockroaches were kept individually in marked petri dishes in a humidified incubator at  $22 \pm 1.0^\circ\text{C}$  (12L: 12D). They were fed dry dog- and horse-feed pellets *ad libitum* and sliced apple was provided on a regular basis. Subsequent culturing has shown that this species is able to reproduce successfully under these conditions (E. Marais, unpublished data).

Prior to each gas exchange assessment, the individual in question was starved for 24 h to reduce variability associated with specific dynamic action (McEvoy, 1984; Lighton, 1989; Duncan et al., 2002b). Assessments were made during the day only, in a

well-lit room, because we were concerned only with discontinuous gas exchange and standard metabolic rate. The species is nocturnal, and at night activity and metabolic rate are high (Fig. 1a). Assessments were also made in dry air because under these conditions a discontinuous gas exchange cycle would seem most likely as a means to conserve water (Quinlan and Hadley, 1993; Duncan et al., 2002b). Each individual was weighed (resolution of 0.0001 g), using an analytical balance (Mettler Toledo AX504), and placed into a 5 ml cuvette kept at  $20 \pm 0.2^\circ\text{C}$  using a Grant LTD20 water bath. Air, scrubbed of water (using Drierite) and  $\text{CO}_2$  (using soda lime) was pushed through the cuvette at a flow rate of  $200 \text{ ml}\cdot\text{min}^{-1}$  (regulated using a Sidetrack Mass Flow Controller) and into a calibrated infrared gas analyzer (Li-Cor Li7000) set in differential mode to measure  $\text{CO}_2$  production. A Sable Systems AD-1 activity detector was used to detect any movement of the cockroach in the cuvette during the experiment, and the output of the detector was fed into the auxiliary channel of the Li7000. The AD-1 presents activity as a value between  $-5$  and  $5$  Volts, where  $0$  Volts is an accurate indication that the specimen is inactive (for more detail see [www.sablesys.com/ad1.html](http://www.sablesys.com/ad1.html)). Inspection of several individuals confirmed lack of activity detected by the AD-1. To avoid the potential influence of pheromones on the behaviour of individuals, the cuvette was cleaned thoroughly with ethanol after each experimental trial. Each experimental assessment also lasted for at least three hours (see Chown, 2001 for rationale). The data file generated by the Li7000 software was exported, via Microsoft Excel, to DATACAN V (Sable Systems), which was used for initial analysis of the respirometry data (corrected to standard temperature and pressure).

Each individual was assessed five times: twice between 07h00 and 11h00, once between 11h00 and 14h00 and twice between 14h00 and 18h00. This was done because circadian patterns in metabolic rate have been found in other insect species (Takahashi-Del-Bianco et al., 1992). Typically, at least five days elapsed between each assessment of

an individual, and the order of assessment with regard to time of day was randomised. The interval between repeated measures is important because the shorter it is the greater the likelihood that a high repeatability will be found (Chappell et al., 1996; Bech et al., 1999). Although we have no data on lifespan for this species, we have cultured adults for more than a year, and other blaberids are known to have an adult lifespan of several years (Scholtz and Holm, 1985). Therefore, an interval of at least five days is appropriate for this species, though perhaps biased somewhat in the direction of higher repeatability. The total time over which the study was undertaken was approximately five months.

### *Analyses*

Because of the small number of males available, we generally restricted our analyses of repeatability to females. Somewhat surprisingly, we found four major patterns of gas exchange, of which three were intermittent and cyclic (see Results), and the fourth was continuous. Data from the continuous pattern were excluded because metabolic rate was significantly higher (approximately twofold) during this pattern of gas exchange than during the others (Repeated measures ANOVA  $F_{(3, 41)} = 6.79$ ,  $p = 0.0008$ , Tukey's HSD for unequal sample sizes, Table 1), even though the individuals were inactive. In consequence, investigations of the repeatability of gas exchange components were undertaken for each of the three major cyclic patterns, and across the dataset as a whole. The variables investigated were duration (s), CO<sub>2</sub> volume (μl), and CO<sub>2</sub> emission rate (μl.h<sup>-1</sup>) for each period, and mean V̇CO<sub>2</sub> and mean frequency of the cycles. Where the analyses were done across the three cyclic patterns, the Flutter period typical of discontinuous gas exchange was compared with the "interburst" period associated with the other forms of cyclic gas exchange. In these cases we also included data for males. Repeatability (r) was calculated using the intraclass correlation approach (Berteaux et al., 1996; Falconer and Mackay,

1996), based on analyses of variance and the equations provided by Lessells and Boag (1987). Because variation in body mass affects variation in metabolic rate and DGC characteristics in arthropods (Peters, 1983; Lighton, 1991; Lighton and Fielden, 1995; Davis et al., 1999), and because there was a reasonable range in the body mass of the specimens we examined (females: mean  $\pm$  S.E. mass is  $0.3397 \pm 0.0184$  g, range 0.1795 – 0.4643 g, males:  $0.2357 \pm 0.0425$  g, range 0.1793 – 0.3189 g) the effects of body size were taken into account in a second round of repeatability analyses. Usually, to do this, the residuals from the regression of body mass and the characteristic of interest are used (Berteaux et al., 1996; Fournier and Thomas, 1999). Here, this was not done. Rather, in all cases, body mass was included as a covariate in the initial analyses of variance (see Freckleton, 2002 for rationale). Where mass did not explain a significant portion of the variance in the independent variable,  $r$  was not determined including mass as a covariate. Confidence intervals for  $r$  were calculated using the formulae provided by Krebs (1999). A significant repeatability value of 1 indicates that individuals are perfectly consistent in their performance over time, whereas a non-significant repeatability value, or one of 0, indicates no consistent variation among individuals. In all cases a sequential Bonferroni test ( $\alpha = 0.05$ ) was used to correct table-wide significance values for multiple tests (Rice, 1989).

To further investigate the likely sources of variation in these traits, nested (hierarchical) analyses of variance (Sokal and Rohlf, 1995) were used. This method allows ready identification of the level at which most variation can be explained, and has been used for this purpose in several other studies (Berteaux et al., 1996; Chown et al., 1999; Addo-Bediako et al., 2002). For each of the major gas exchange patterns, variance was partitioned between error nested within (<) trial < time of day < individual < gender. Gender was not used as a level of partitioning in the pulsation pattern because males never showed the pattern. A similar analysis was also undertaken across all three cyclic gas

exchange patterns. In the case of frequency and mean metabolic rate, the trial level was excluded because metabolic rate and frequency are calculated across all the cycles, rather than just for each individual cycle as can be done for the characteristics of each of the periods. A sequential Bonferroni correction ( $\alpha = 0.05$ ) was also applied here.

**Table 1** Mean ( $\pm$  S.E.M.) of CO<sub>2</sub> volumes ( $\mu$ l), period durations (min), emission rates ( $\mu$ l/hr), total metabolic rate ( $\mu$ l/hr) and mass (g) for each of the four gas exchange patterns identified for *Perisphaeria* sp. (Blattodea, Blaberidae). Means were calculated using the values from each trial. The metabolic rate of the continuous gas exchange pattern differed significantly from the cyclic patterns (Repeated measures  $F_{3,41} = 6.79$ ,  $p < 0.0008$ ; Tukey's HSD for unequal sample sizes). Means with the same symbols do not differ significantly. N is the number of individuals.

Pattern	C-period	F-period/ Interburst	O-period/ Burst	N	Metabolic rate	Mass
<b>Emission volumes</b>						
DGC	0.3989 $\pm$ 0.034	0.877 $\pm$ 0.078	6.607 $\pm$ 0.333	13		
Interburst Burst		1.516 $\pm$ 0.307	5.585 $\pm$ 0.153	13		
Pulsation		0.0312 $\pm$ 0.010	0.298 $\pm$ 0.0001	9		
Continuous				13		
<b>Period duration</b>						
DGC	11.667 $\pm$ 0.588	6.504 $\pm$ 0.400	7.919 $\pm$ 0.358	13		
Interburst Burst		13.039 $\pm$ 0.474	8.986 $\pm$ 0.793	13		
Pulsation		0.466 $\pm$ 0.007	0.367 $\pm$ 0.047	9		
Continuous				13		
<b>Emission rate</b>						
DGC	2.148 $\pm$ 0.120	8.400 $\pm$ 0.600	50.940 $\pm$ 1.500	13	20.717 $\pm$ 2.314 A	0.3148 $\pm$ 0.0179
Interburst Burst		9.060 $\pm$ 2.160	46.140 $\pm$ 1.080	13	19.811 $\pm$ 2.751 A	0.2846 $\pm$ 0.0185
Pulsation		10.680 $\pm$ 0.780	48.900 $\pm$ 0.600	9	20.721 $\pm$ 1.891 A	0.3083 $\pm$ 0.0112
Continuous				13	36.178 $\pm$ 3.425 B	0.3887 $\pm$ 0.0148

## Results

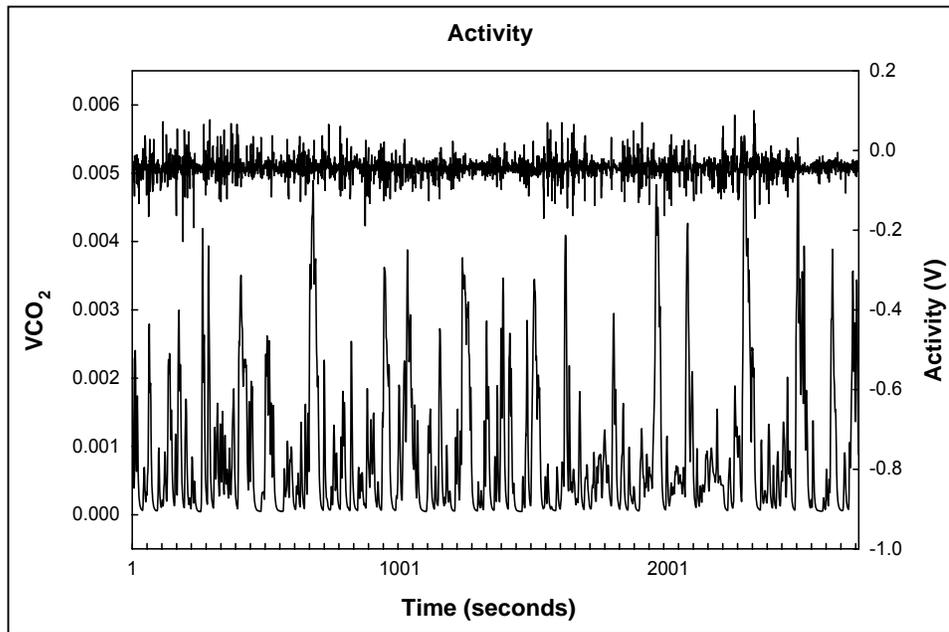
This *Perisphaeria* sp. showed four major gas exchange patterns at rest (confirmed by inspection and by the AD-1), here termed Continuous (Fig. 1b), DGC (Fig. 1c), Interburst-Burst (Fig. 1d), and Pulsation (Fig. 1e). Metabolic rate did not differ among the latter three patterns, although this was not the case with Continuous gas exchange (see Section 2b above). Whilst one individual showed all four patterns over the course of the study, most individuals showed two or three of them. However, neither females nor males preferred a particular pattern (females: log-linear analysis, ML  $\chi^2 = 65.3$ ,  $p = 0.05$ ,  $df = 48$ ; males: log-linear analysis, ML  $\chi^2 = 7.18$ ,  $p = 0.31$ ,  $df = 6$ ), although males never showed the Pulsation pattern. Time of day made no difference to the patterns that were shown (log-linear analysis, ML  $\chi^2 = 3.94$ ,  $p = 0.68$ ,  $df = 6$ ), and no individuals switched from one pattern to another over the course of a recording.

For most of the characteristics examined here repeatability was significant and large (Fig. 2, Bonferroni correction did not alter significance values appreciably). Within patterns, repeatability tended to be highest, as might be expected, with values for burst or O-period characteristics generally above 0.3 (with the exception of burst duration in the Pulsation pattern when mass was included as a covariate). By contrast, interburst or C-period characteristics tended to have lower repeatabilities (with the significant exception of emission rate, Fig. 2). Across patterns, repeatabilities were also high for the burst period and somewhat lower for the interburst period, with emission rate now having the lowest repeatability. This is not surprising because the three patterns differ in the extent to which individuals close their spiracles. In the DGC pattern the spiracles are held closed, whilst this is generally not the case in the other patterns (Fig. 1). There was consistent among-individual variation in metabolic rate (excluding mass:  $r = 0.51$  for males and females, 0.48 for females only; including mass  $r = 0.22$  for males and females, 0.29 for females only)

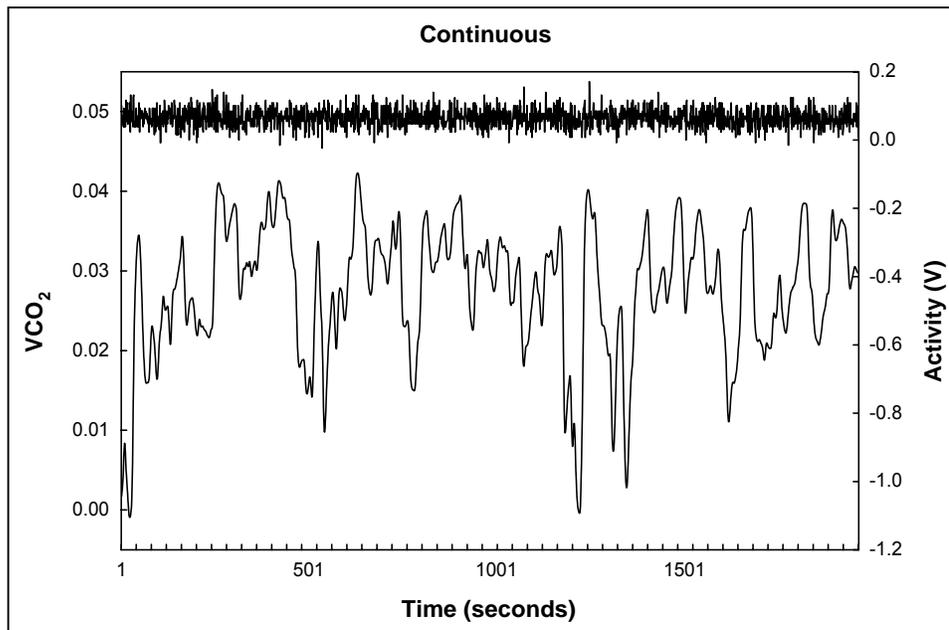
and frequency (excluding mass:  $r = 0.25$  for females only,  $0.31$  for males and females; including mass:  $r = 0.29$  for females only,  $0.35$  for males and females) (Appendix 1a). In general, repeatabilities tended to decline when mass was included as a covariate, but this was not always the case (Appendix 1b).

The nested analyses of variance generally bore out our repeatability results (Table 2). Moreover, they provided additional insight into the level at which variation that was not a function of individual identity was partitioned. Thus, it is clear that DGC patterns tended to vary much more between trials (the Trial term in Table 2) than within a given trial (the Error term in Table 2), whilst the converse was true of the Pulsation pattern and, to a lesser extent, of the Interburst-Burst pattern. In this context it is important to realize that the error term includes both error and variation between individual cycles in a particular trial. The nested ANOVAs also revealed that there is generally little variation amongst genders in most of the traits examined here. Although this does not appear to be the case when the analyses are undertaken across all three intermittent patterns, this is solely the consequence of the absence of a pulsation pattern in the males.

**A**

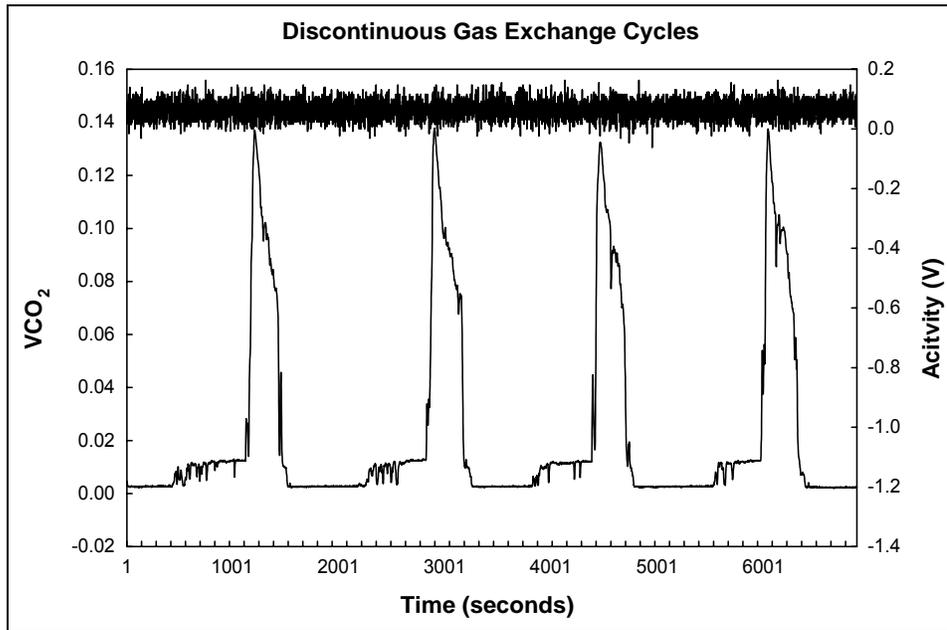


**B**

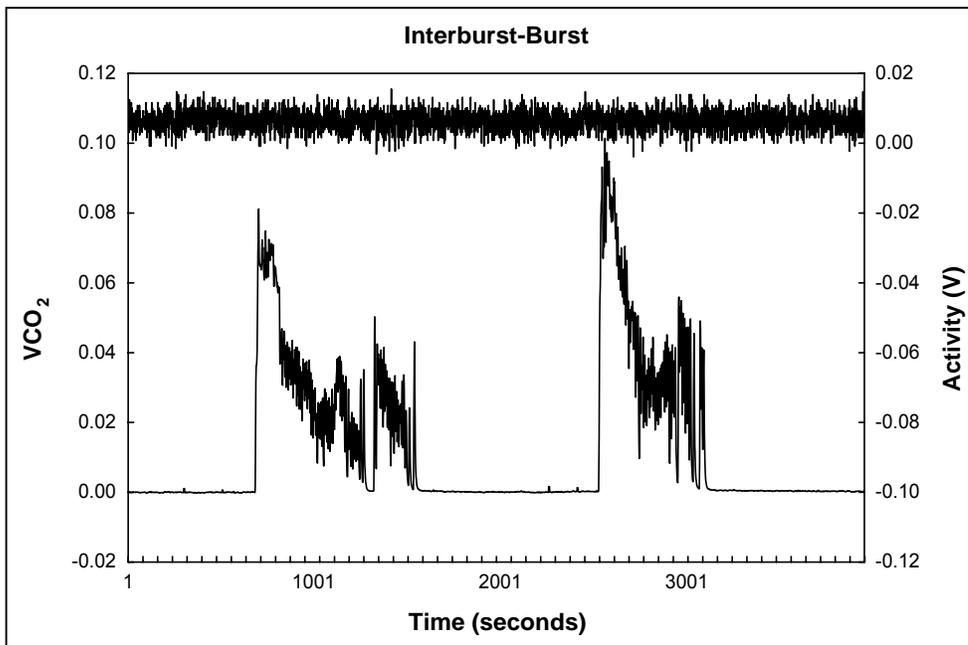


**Fig. 1 a and b**

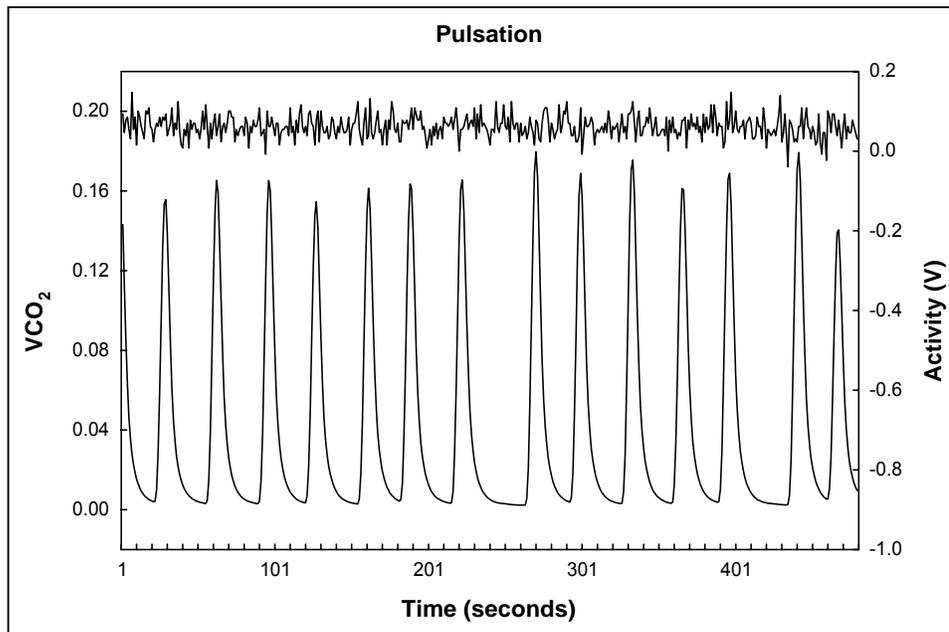
**C**



**D**

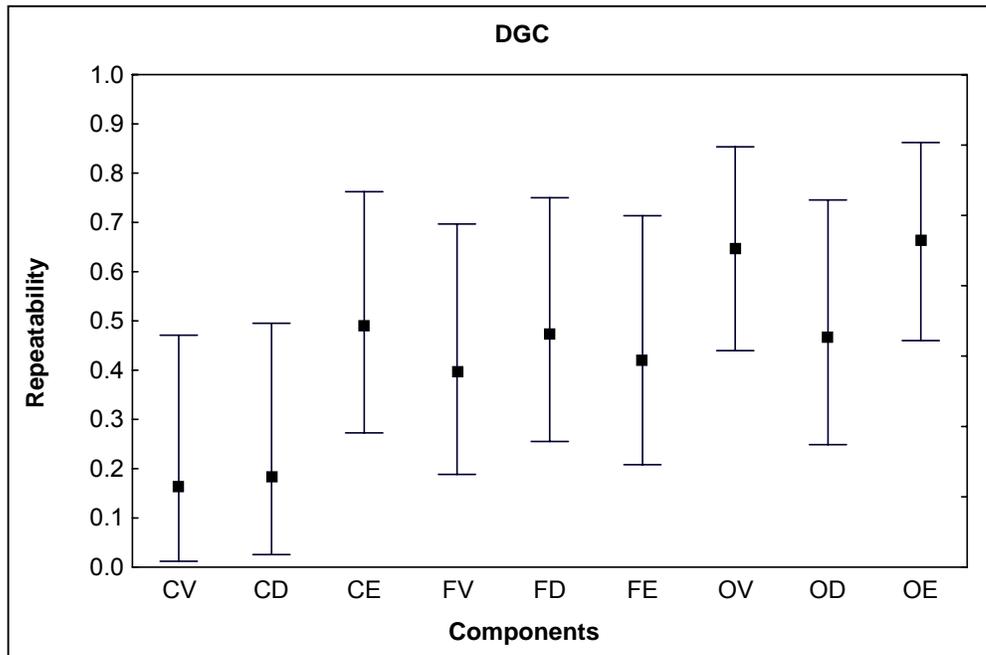


**Fig. 1 c and d**

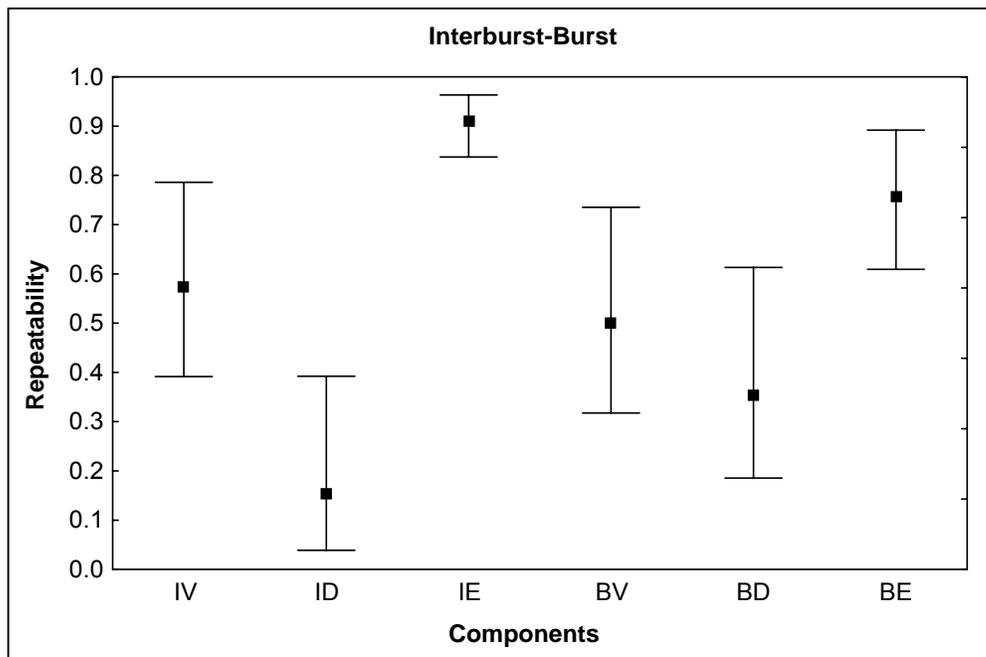
**E**

**Fig. 1.** Gas exchange patterns shown by *Perisphaeria* sp. (Blattodae, Blaberidae): a. Gas exchange during activity, b. Continuous gas exchange pattern, c. Discontinuous Gas Exchange Cycle pattern, d. Interburst-Burst pattern, e. Pulsation pattern. In each case,  $V\text{CO}_2$  (ml/h) is shown as the lower curve (left axis) and activity as the upper curve (right axis). Activity is interpreted as the variance of activity about the mean value, rather than the absolute value of this activity. It is negligible except in the case of 1a.

**A**

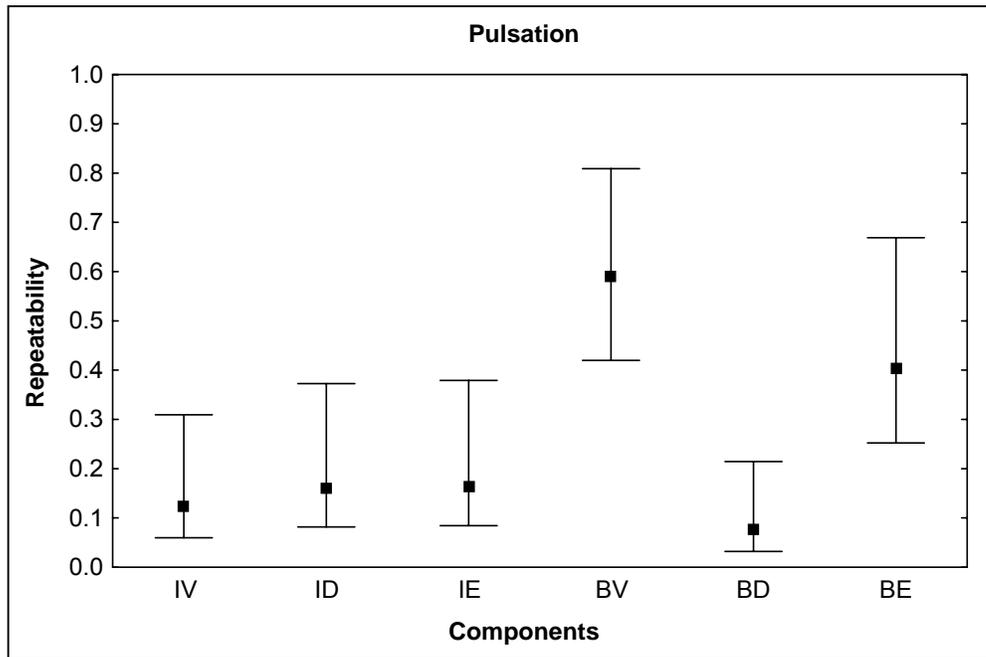


**B**

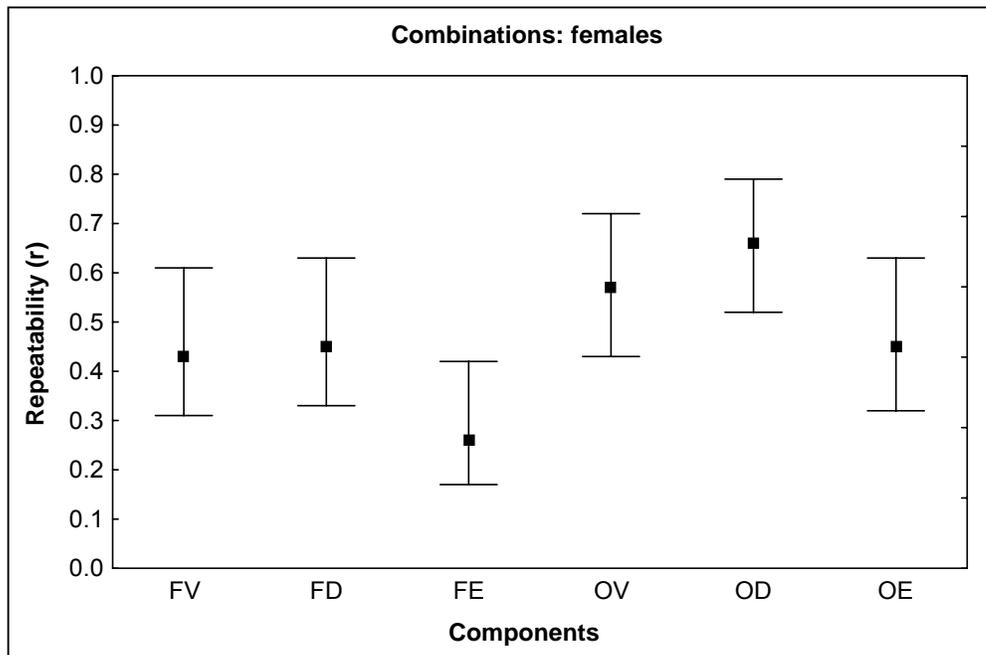


**Fig. 2 a and b**

**C**

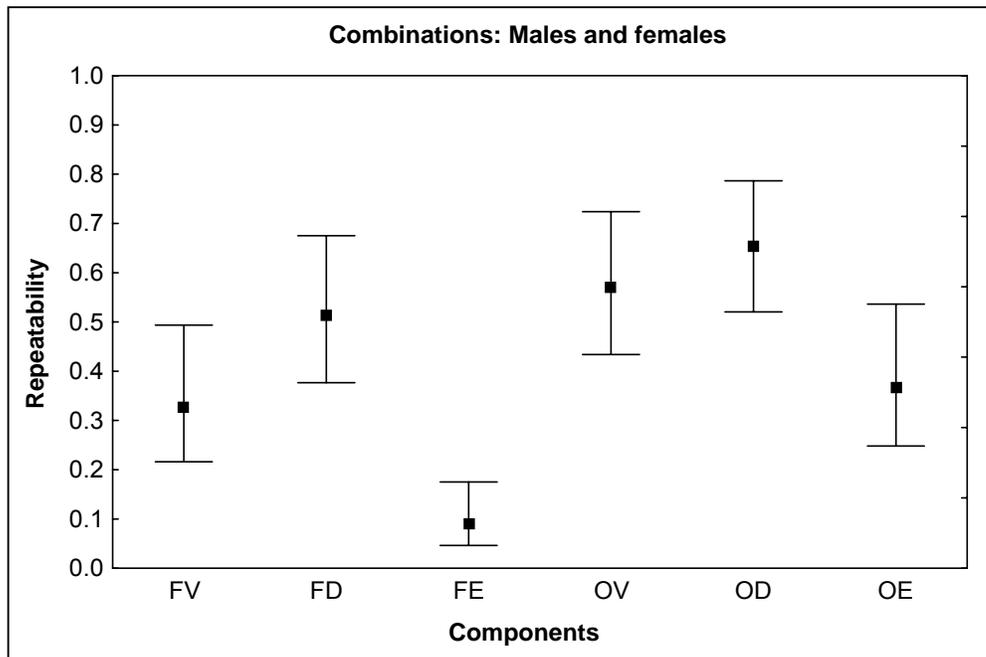


**D**

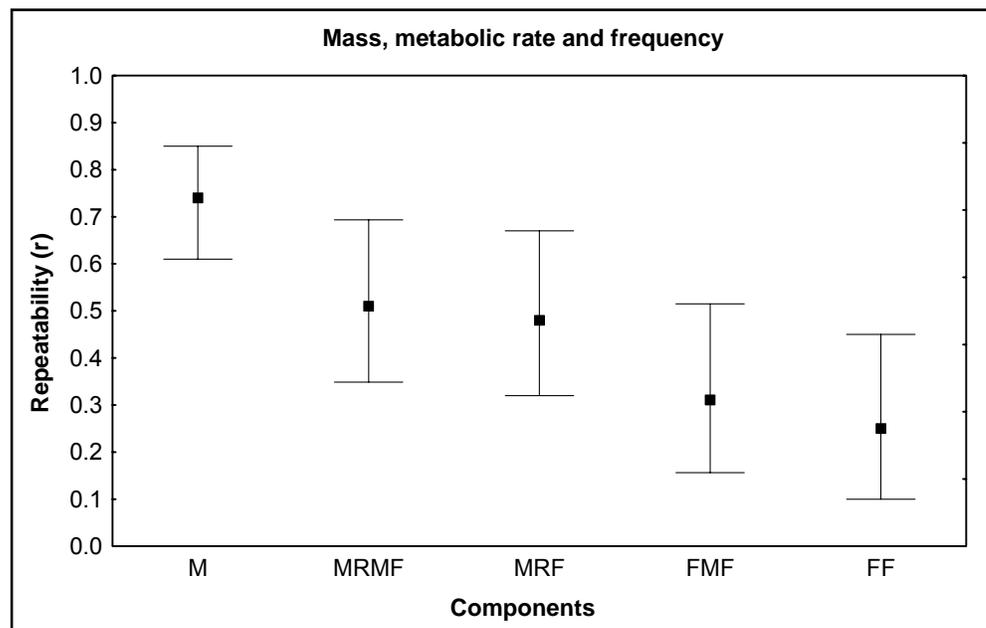


**Fig. 2 c and d**

**E**



**F**



**Fig. 2 e and f**

**Fig. 2.** Repeatability values ( $r$ ), and their upper and lower 95% confidence limits for all the components of the cyclic patterns, as well as for the components across patterns. a. Discontinuous Gas Exchange Cycle pattern, b. Interburst-Burst pattern, c. Pulsation pattern, d. the three gas exchange patterns combined for females only, e. the three gas exchange patterns combined for males and females, f. mass, metabolic rate and frequency. Mnemonics are as follows: Figs. a-e: O = O-period, C = C-period, F = F-period, I = Interburst period, B = Burst period, E = emission rate, V = volume, D = duration; Fig. f: M = mass, MRMF = metabolic rate for males and females combined, MRF = metabolic rate for females only, FMF = frequency for males and females combined, FF = frequency for females only.

## Discussion

The presence of four, or at the least three, very different patterns of gas exchange at rest is unusual for most insects, but perhaps not entirely so for cockroaches. Although most studies acknowledge that there is some variation in gas exchange characteristics (excluding that associated with body size and/or treatment temperature – see Lighton, 1991; Davis et al., 1999; Rourke, 2000), the majority have not found the range of variation within a single species documented here (see Lighton, 1998 and Chown, 2001 for discussion). The only investigations that have suggested that there might be pronounced variability within a species have been those on *Blaberus* cockroaches by Miller (1973, 1981), who investigated patterns in CNS firing associated with spiracle control, although he thought that some of the variability was probably associated with activity. Nonetheless, among different taxa a range of periodic gas exchange patterns has been found, varying from purely cyclic, with no spiracle closure, to discontinuous gas exchange cycles of the kind originally documented so carefully for lepidopteran pupae (Punt et al., 1957; Levy and Schneiderman, 1966; Lighton, 1988, 1991; Duncan and Byrne, 2000; Shelton and Appel, 2000, 2001). Our findings for *Perisphaeria* indicate either that the variety of gas exchange patterns documented in insects does not represent as much of a major difference between taxa as comparative studies might suggest (Lighton, 1998), or that basal taxa such as cockroaches and termites differ substantially from more derived groups.

Despite this variety in gas exchange patterns, repeatability values for metabolic rate, frequency, and the other gas exchange characteristics were generally high and always significant when examined across the three patterns that were typical of animals at rest with low metabolic rates. These high repeatabilities were not a consequence of pronounced differences between the genders, with the notable exception of the absence of a Pulsation pattern in males. However, the exclusion of body size variation did tend to result in lower

repeatabilities. Although most studies first remove the effects of size variation before examining repeatability, it might also be argued that this should not be done. This is most readily demonstrated in the context of metabolic rate variation. Several models have demonstrated the importance of metabolic rate for body size evolution (e.g. Kozłowski and Weiner, 1997), and Kozłowski and Gawelczyk (2002) have clearly shown that the major factors influencing optimal size are the size dependence of production rate (which is influenced by metabolic rate, see Sibly and Calow, 1986) and the size dependence of mortality rate (which could be influenced by metabolic rate, see Chown and Gaston, 1999). Thus, it seems much more likely that selection will act on the metabolic rate of an animal of a given size than on the residual variation of that trait once size has been taken into account. McNab (1999) arrived at a similar conclusion, pointing out "...that total units of metabolism are the ecologically and evolutionary relevant units." This argument can readily be applied to all of the other traits we examined, and indeed, in our view, to most other physiological and life history traits.

**Table 2** The distribution of variance of volume, duration and emission rates for periods (DGC: C-, F- and O-period; Interburst-Burst and Pulsation : Interburst- and Burst-period), frequency and metabolic rate. Tabulated values are percentages of the total variance accounted for at each successive level (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001). Significance values did not change appreciably following Bonferroni correction.

<b>Component</b>		<b>Gender</b>	<b>Individual</b>	<b>Time of Day</b>	<b>Trial</b>	<b>Error</b>
<b>DGC</b>						
Closed period	Log <sub>10</sub> (volume + 2)	10.0	0.1	0	69.8***	20.1
	Duration	0	19.9	0	52.2***	27.9
	Emission rate	41.8*	0	19.1	28.0***	11.1
Flutter period	Log <sub>10</sub> volume	4.2	31.0*	0	30.0***	34.8
	Duration	0	17.4	29.1	19.5**	34.0
	Emission rate	31.8*	24.8**	3.4*	0	40.0
Open period	Log <sub>10</sub> volume	5.1	46.8	0	34.9***	13.2
	Log <sub>10</sub> duration	0	36.4	0	30.6***	33.0
	Emission rate	0	62.2**	0	9.8*	28.0
<b>INTERBURST- BURST</b>						
Interburst	Log <sub>10</sub> volume	26.3	12.0	42.9*	3.0	15.8
	Duration	0	0	39.3	10.0	50.7
	Emission rate	0	89.5***	2.6	0	7.9
Burst	Log <sub>10</sub> volume	0	25.3	24.2	22.3**	28.2
	Duration	0	2.1	14.2	37.9**	45.8
	Log <sub>10</sub> emission rate	0	66.0**	10.6	9.2**	14.2
<b>PULSATION</b>						
Interburst	Log <sub>10</sub> volume	-	0.0	18.5	9.2***	72.3
	Log <sub>10</sub> duration	-	2.8	23.6*	0.1	73.5
	Log <sub>10</sub> emission rate	-	13.8*	0	7.9**	78.3
Burst	Log <sub>10</sub> volume	-	44.5*	13.0	17.3***	25.2
	Duration	-	4.5	5.1	0.4	90.0
	Emission rate	-	29.0*	0	30.7***	40.3
<b>Flutter period and interbursts for the three cyclic patterns</b>						
	Volume	0	18.7	26.5*	27.8***	27.0
	Duration	30.1*	28.1**	11.2	16.6***	13.9
	Emission rate	4.2	7.3*	8.7**	0	79.8
<b>Burst and open for the three cyclic patterns</b>						
	Log <sub>10</sub> volume	54.2**	14.4*	17.2**	12.5***	1.8
	Log <sub>10</sub> duration	54.8**	20.8**	13.3**	9.4***	1.9
	Log <sub>10</sub> emission rate	0	32.8**	4.3	28.4***	34.5
<b>Flutter period and interbursts for the three cyclic patterns Females</b>						
	Volume	-	21.7	30.4*	26.6***	21.3
	Duration	-	30.3**	21.1*	17.7***	30.9
	Emission rate	-	18.3**	16.9*	1.5	63.3
<b>Burst and open for the three cyclic patterns Females</b>						
	Log <sub>10</sub> volume	-	43.5**	30.2**	23.1***	3.3
	Log <sub>10</sub> duration	-	56.0***	23.2**	17.4***	3.5
	Log <sub>10</sub> emission rate	-	34.2***	6.4	24.6***	34.8
<b>Frequency</b>						
		23.7*	17.6*	7.2	-	51.5
<b>Metabolic rate (MR)</b>						
	Log <sub>10</sub> MR	17.1	40.2***	0.9	-	41.8

Therefore, we can conclude that for most of the characteristics we examined variation among individuals was typically significant, and often considerable. These results provide strong evidence that one of the conditions for considering natural selection an important process in the evolution both of gas exchange traits and standard metabolic rate has been met (Endler, 1986; Bech et al., 1999). They also provide a line of evidence, independent of that of comparative analyses, suggesting that variation in these traits among species and populations might well be adaptive. The only exceptions appeared to be the characteristics of the Closed period (in DGC) and Interburst period (in the other cyclic patterns), where repeatability was generally low. Thus, of the gas exchange characteristics examined, those associated with the Closed and Interburst periods are least likely to be the subject of selection. This finding is in keeping with evidence demonstrating that among species with discontinuous gas exchange cycles it is most often the F- and O-periods that vary in a way consistent with adaptive change (Lighton, 1988; Lighton et al., 1993a; Bosch et al., 2000; Duncan and Byrne, 2000; Chown and Davis, 2003; Duncan, 2003).

To date, no other studies have convincingly demonstrated consistent among-individual variation in standard metabolic rate and gas exchange characteristics in insects. Prior to this investigation, repeatability in one or more of these traits had only been examined on two occasions. Buck and Keister (1955) reported, but did not provide the statistics for, analyses of variance which apparently revealed that among-individual variation in O-period volume in diapausing moth pupae was larger than that within individuals, but that several other characteristics of the DGC showed “about as much variation between different cycles of a single pupa as between pupae”. Much later, Chappell and Rogowitz (2000) reported repeatability of standard metabolic rate and DGC characteristics for two species of longicorn beetles (see also Rogowitz and Chappell, 2000), but included both species in their analysis without distinguishing them, factored out body size before the

analyses, and considered their non-significant results a consequence of small sample size. Our work takes these initial, useful analyses a step further and demonstrates that, in general, both standard metabolic rate and gas exchange characteristics are significantly repeatable, so meeting one of the major requirements for selection.

Although repeatability estimates for physiological traits in insects and other arthropods are comparatively rare, our data are in keeping with the work that has been undertaken to date. For example, Chappell and Rogowitz (2000) found  $r$  values in the range of 0.26 to 0.57 for DGC characteristics in the longicorn beetles they examined. Our values for DGC characteristics not only span a broader range, but unlike theirs were also all significant. This difference is particularly important in the context of metabolic rate. Their analysis indicated a low and non-significant repeatability (0.38), whilst ours suggested that repeatability of metabolic rate was both higher (0.48 – 0.51) and significant. Nonetheless, it should be kept in mind that the inclusion of body mass as a covariate makes a considerable difference to the value of  $r$  in our analysis, but not to its significance (repeatability declined from 0.51 to 0.21 when the effects of mass were controlled for). Considering other physiological traits, in *Melanoplus* grasshoppers, repeatability of tethered flight duration varies between 0.6 and 0.7 (Kent and Rankin, 2001), whilst in *Rhizoglyphus* mites, repeatability of sperm competitive ability is much lower (0.22) (Radwan, 1998).

Our repeatability estimates for metabolic rate in *Perisphaeria* sp. were also well within the range of values typically found in vertebrates. For example, repeatability estimates ranged from 0.35 to 0.52 in breeding female kittiwakes measured over an interval of one year (Bech et al., 1999), and, in a variety of small mammals and birds, varied between 0.261 in meadow voles measured over an interval of 42 days (Berteaux et

al., 1996) and 0.64 in kittiwakes measured over the course of a single day (Fyhn et al., 2001).

In conclusion, we have provided evidence that at least one of the prerequisites for natural selection for metabolic rate and gas exchange characteristics in insects is satisfied, and therefore that variation in these traits might be considered adaptive. Whilst our work does not provide conclusive evidence for adaptation in these traits, when considered in conjunction with selection experiments (reviewed in Gibbs, 1999), and comparative analyses (reviewed in Chown and Gaston, 1999), it does make the argument for adaptive variation more compelling than it has been. In the past, investigations of variation in gas exchange characteristics in particular have suffered from an unduly Panglossian approach.

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## **Chapter 3**

### **Insect gas exchange patterns: A phylogenetic perspective**

## 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 in 1930, cf. Kestler, 1985) 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 (reviews in Kestler, 1985; Lighton, 1994; Chown, 2002).

Subsequent investigations cast doubt on this hypothesis, and there are now several competing explanations for the evolution of DGCs (reviewed in Chown et al., submitted ms.). These are the original, hygric (or water savings) hypothesis; the hybrid exchange hypothesis, which posits that the DGC evolved to restrict water loss and facilitate gas exchange under hypoxic and hypercapnic conditions (Lighton and Berrigan, 1995); the chthonic hypothesis, which suggests that DGCs evolved to facilitate gas exchange under hypoxic, hypercapnic, or both conditions; the oxidative damage hypothesis, which proposes that DGCs evolved to limit oxygen free radical damage to tissues (Bradley, 2000); the strolling arthropods hypothesis, which indicates that spiracular closure has evolved to limit tracheal parasitism (see Chown et al., submitted ms.); and the emergent property hypothesis, which is based on the idea that cyclic patterns of gas exchange can occur simply as a consequence of interacting feedback systems (Chown and Holter, 2000).

The hygric, chthonic and hybrid exchange hypotheses have been subject to several tests with mixed results (the distinction between the latter two ideas was often not drawn). For example, Lighton and Berrigan (1995) found strong support for the hybrid exchange hypothesis in their test comparing workers and alates of two *Messor* ant species, but Chown and Holter (2000) found that increasingly hypoxic conditions led the dung beetle *Aphodius fossor* to gradually abandon discontinuous gas exchange. Several other authors have questioned the chthonic hypothesis based largely on comparisons of gas exchange patterns of species that, given their preferred habitats, should show DGC, but apparently don't, or *vice versa* (Vogt and Appel, 1999; Duncan et al., 2002a). Likewise, based on the low proportional contributions of respiratory transpiration to total water loss, and the absence of discontinuous gas exchange under dry and warm conditions, where its benefits would apparently be most obvious, other studies have suggested that water saving is unlikely to be an important reason for the origin and/or maintenance of discontinuous gas exchange cycles (Hadley, 1994; Lighton, 1998; Chappell and Rogowitz, 2000). By contrast, a recent comparative analysis has shown that interspecific variation in components of the discontinuous gas exchange cycle might well represent a response to habitat water availability (Chown and Davis, 2003). To date, the oxidative damage, strolling arthropods and emergent property hypotheses remain either untested or the subject of few examinations.

A prominent feature of virtually all tests of the above 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. Vogt and Appel, 1999; Duncan et al., 2002a; 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 – see discussion in Coddington, 1988; Baum and Larson, 1991; Schluter, 2000), 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 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 98 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 likely 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 *c.* 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 underrepresented in the literature, with published information available for five cockroach species, six termite species, and five species of Orthoptera. No Apterygotes (*Zygentoma* and Archaeognatha) have been investigated.

In this chapter, I 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 the file-drawer issue cannot be resolved here, I comprehensively review patterns documented by the existing literature, provide information on several 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). Finally, using first order difference equations, the extent which periodic gas exchange patterns can be produced by two interacting feedback systems is examined. In undertaking this work I realise that a comprehensive comparative analysis of insect gas exchange patterns is still some way off. However, the aim here 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). 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 myself and Jaco Klok, 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, 2000a) and unpublished works (B. A. Klein et al., unpublished data) were obtained to verify these assessments.

### *Experimental investigations*

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 one 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  $22 \pm 1$  °C (12L : 12D photoperiod), with

access to water but not to food (with the exception of the hemipterans 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., 2002a). 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  $20 \pm 0.2^\circ\text{C}$ , using either a Grant LTD20 water bath or a Labcon (South Africa) temperature-controlled cabinet.

Air, scrubbed of  $\text{CO}_2$  (using soda lime) and water (using silica gel and then Drierite, OH, USA.) was passed through the cuvette (see Table 1 for flow rates; which were regulated using a Sidetrak Mass Flow Controller, Monterey, USA) and into a calibrated infrared gas analyzer (Li-Cor Li7000 or Li-Cor Li6262; Henderson, Nevada, USA) to measure  $\text{CO}_2$  production. A Sable Systems (Henderson, Nevada, 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  $+5$  Volts, where little deviation from the mean indicates that the animal is inactive, whereas 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 two hours. 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.  $\dot{V}\text{CO}_2$  traces 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. In these cases, I adopted the convention that the presence of regular bursts, distinguishable from the baseline  $\dot{V}\text{CO}_2$ , constitutes cyclic gas exchange, whilst all other patterns are continuous (compare Figs 1a and b). Summary statistics for the data were calculated following these assessments, based on the approach to cyclic and discontinuous gas exchange patterns adopted by Marais and Chown (2003).

### *Comparative Analyses*

Based on the data from the literature and the data generated in this study I 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 was 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.

**Table 1** Species examined for gas exchange patterns in this study. 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. Flow rates used for each of the species are also shown.

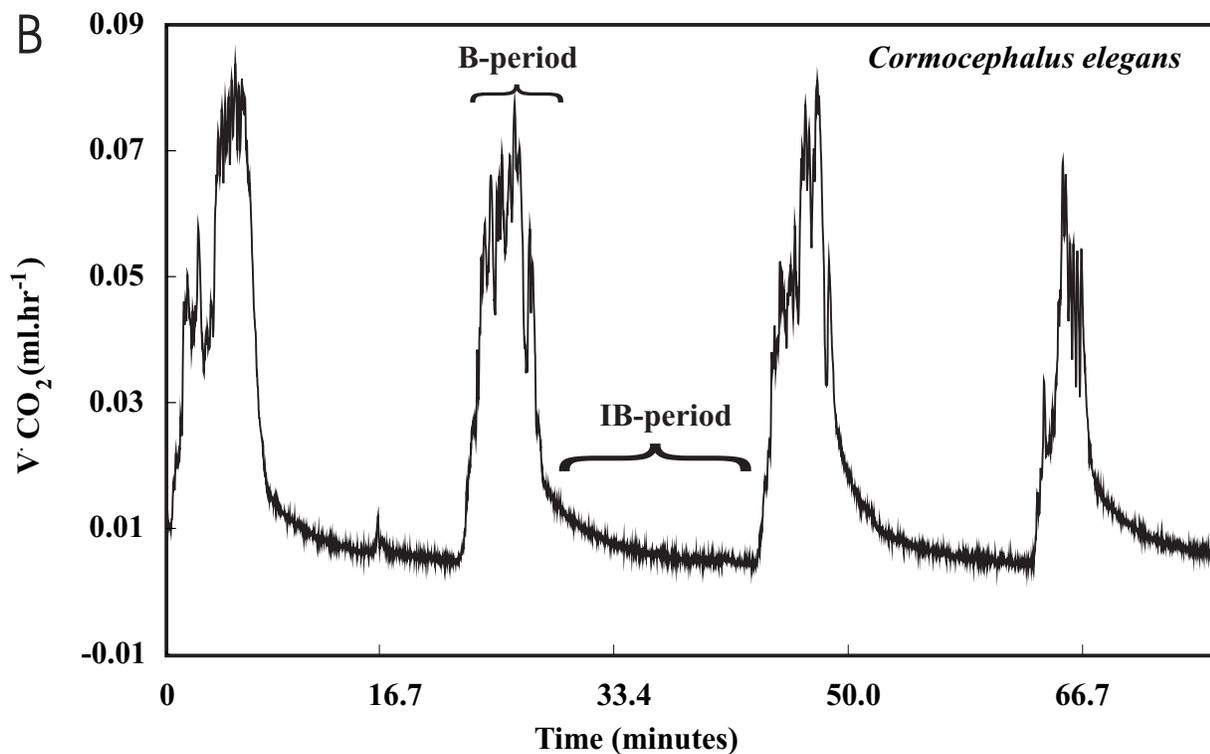
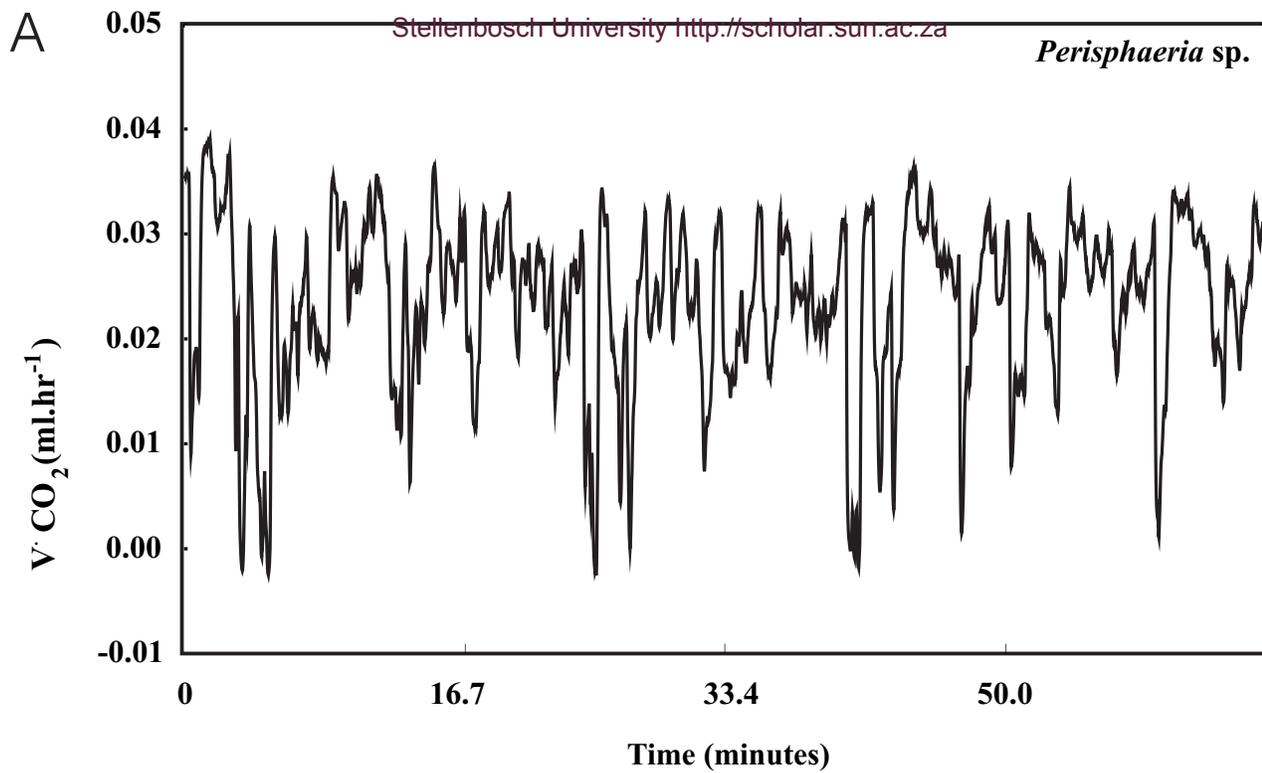
	<b>Locality</b>	<b>Flow Rate (ml/min)</b>
<b>Archaeognatha</b>		
Meinertellidae, sp.	Helderberg Nature Reserve, Somerset West, South Africa (34°02.579'S, 18°52.472'E)	75
<b>Zygentoma</b>		
Lepismatidae		
Lepismatidae sp. 1	Sutherland, South Africa (32°34.105'S, 20°57.747'E)	100
Lepismatidae sp. 2	Cederberg, South Africa (31°51.611'S, 18°55.122'E)	75-100
<i>Ctenolepisma longicaudata</i> (Echerich, 1905)	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	100
<b>Ephemeroptera</b>		
Heptrageniidae sp.	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	50
<b>Odonata</b>		
Coenagrionidae,		
<i>Ischnura senegalensis</i> (Rambur, 1842)	Jonkershoek, Stellenbosch, South Africa (33°57.814'S, 18°55.514'E)	75
Libellulidae		
<i>Trithemis arteriosa</i> (Burmeister, 1839)	Jonkershoek, Stellenbosch, South Africa (33°57.814'S, 18°55.514'E)	150
<b>Blattodea</b>		
Blaberidae, sp.	Cederberg, South Africa (31°51.611'S, 18°55.122'E)	200

Table 1 cont.

	Locality	Flow Rate (ml/min)
<b>Mantodea</b>		
Mantidae		
<i>Sphodromantis gastrica</i> Stål	Stellenbosch, South Africa (33°55.923'S, 18°51.812'E)	100
<b>Mantophasmatodea</b>		
Austrophasmatidae		
<i>Karoophasma biedouwensis</i> Klass et al., 2003	Cederberg, South Africa (32°05'S, 19°15'E)	50
<b>Phasmatodea</b>		
Phasmatidae		
<i>Extatosoma tiaratum</i> (Macleay, 1826)	Butterfly World, Klapmuts, South Africa, but originally from Australia	100
<b>Orthoptera</b>		
Pneumoridae		
<i>Bullacris intermedia</i> (Péringuey, 1916)	Zuurberg, South Africa (25°14'E, 33°48'S)	100
<b>Dermaptera</b>		
Labiduridae		
<i>Euborellia annulipes</i> (Lucas, 1847)	Stellenbosch, South Africa (33°55.923S, 18°51.812E)	75-100
<b>Hemiptera</b>		
Coreidae sp.		
	Nigel, South Africa (26°25.422'S, 28°28.349'E)	100
Lygaeidae sp.		
	Somerset West, South Africa (34°03.806'S, 18°49.473'E)	100
<b>Neuroptera</b>		
Chrysopidae		
<i>Chrysoperla</i> sp.	Somerset West, South Africa (34°03.806'S, 18°49.473'E)	50

Table 1 cont.

	<b>Locality</b>	<b>Flow Rate (ml/min)</b>
<b>Diptera</b>		
Glossinidae		
<i>Glossina morsitans</i> Westwood	FAO/IAEA, Vienna, Austria (Laboratory colony)	100
<b>Trichoptera</b>		
Leptoceridae		
<i>Leptocerina</i> sp.	Olifants River, Citrusdal (32°35'S, 18°40'E)	50
<b>Lepidoptera</b>		
Plutellidae		
<i>Plutella xylostella</i> (Linnaeus, 1758)	Somerset West, South Africa (34°03.806'S, 18°49.473'E)	50



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

*Modelling gas exchange patterns*

Chown and Holter (2000) suggested that discontinuous gas exchange cycles might have arisen as an emergent property of two interacting feedback systems regulating a single function (spiracle opening), rather than as an adaptation to a specific set of conditions (such as arid environments, or the threat of oxygen free radical damage). To assess this idea it was assumed that CO<sub>2</sub> and O<sub>2</sub> mediated control of spiracular opening (see Chown and Nicolson, 2004 for review) can be thought of as two feedback systems which regulate spiracle opening, but which also have effects on each other (see Levy and Schneiderman, 1966a; Burkett and Schneiderman, 1967, 1974; Ramirez and Pearson, 1989 for evidence that CO<sub>2</sub> has an effect on O<sub>2</sub> receptors and *vice versa*). Each feedback system was modelled as a first order difference equation (or logistic map) (see May and Oster, 1976; May, 1986), and then the two systems were coupled in a simple model that can be represented as follows:

$$\begin{array}{c}
 \begin{array}{c} \curvearrowright \\ \curvearrowleft \end{array} X_{t+1} = X_t \lambda_i (1 - X_t) \\
 X_{t+2} = X_{t+1} \lambda_j (1 - X_{t+1}) \begin{array}{c} \curvearrowright \\ \curvearrowleft \end{array}
 \end{array} \quad (1)$$

where  $X_t$  is the state of a receptor response  $X$  at time  $t$ ,  $\lambda(1-X_t)$  is a feedback system, the strength of which depends on  $\lambda$ , and  $\lambda_i$  and  $\lambda_j$  represent the two feedback systems. The strength of  $\lambda_i$  and  $\lambda_j$  was subsequently varied independently in a systematic fashion for each of the two systems, and a variety of behaviours of  $X$ , for  $t = 0$  to 200, was examined. The examinations were based on visual inspection of the resulting traces.

## Results

Reliable assessments of gas exchange patterns could be made for 98 species for which published data are available (and in one case we used unpublished information) (Table 2). Of these, 58 typically showed discontinuous gas exchange cycles, 35 showed cyclic gas exchange, and 31 continuous gas exchange. Three 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, my 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). My 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 (see McNab, 2003 for rationale). In virtually all of the species, there was substantial among-individual variation in gas exchange pattern at rest, such that several individuals showed continuous gas exchange while a few showed cyclic or discontinuous patterns, or *vice versa* (Table 3). In all cases, cyclic gas exchange patterns were clearly identifiable as such, as were discontinuous gas exchange cycles (Figs 2a to 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). It is clear that there are species within all of the orders examined that show continuous gas

exchange, many show cyclic gas exchange, and that 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 it is clear that there is substantial variation between species (Tables 2 and 3). 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.

If continuous and cyclic gas exchange are basal characteristics of the insects, it does seem likely that at least cyclic gas exchange might simply be an emergent property of any system that regulates spiracle cross-sectional area (or closing). Indeed, the model of interacting feedback systems that was developed here showed a remarkably wide range of behaviour that is typical of, though not necessarily identical to, that shown by insect gas exchange patterns. The behaviour of these systems ranged from highly variable, though cyclic (Fig. 4a), to strongly periodic and similar to cyclic gas exchange if not to some published DGC traces (Fig. 4b). Nonetheless, the model could not produce a gas exchange cycle with a typical F-period, and was therefore unable to simulate discontinuous gas exchange cycles.

**Table 2** Gas exchange patterns in insects, obtained from the literature (patterns from quiescent animals only are listed). In the case of the Lepidoptera, P represents Pupae, L represents Larvae. The classifications of subterranean or non-subterranean were made conservatively, such that in cases where the stage under consideration spends a reasonable part of its time below ground the group was considered subterranean. Namib Desert tenebrionids were not considered subterranean because it has been shown that their substrate is probably not a major barrier to gas exchange (Louw et al., 1986). Wing status is indicated by W (winged) and WL (Wingless), while general habitat is indicated by subterranean (S) –, non-subterranean (NS), xeric (X) and mesic (M) environment. Where species occurred more than once in the table, they were counted only once to obtain the total number of species investigated. Where the same species showed different patterns in different studies, or more than one pattern in the same study, the species was counted separately for each pattern (to obtain the value for the number of species that showed each pattern).

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Blattodea</b>					
<b>Family Blattidae</b>					
<i>Periplaneta americana</i>	DGC, Cyclic	Low	Wilkins, 1960	W	NS, M
<i>Periplaneta americana</i>	DGC	Medium	Kestler, 1991	W	NS, M
<i>Periplaneta americana</i>	DGC	Medium	Kestler, 1985	W	NS, M
<i>Periplaneta americana</i>	DGC	Medium	Machin et al., 1991	W	NS, M
<b>Family Blaberidae</b>					
<i>Blaberus craniifer</i>	Cyclic	Low	Ewards and Miller, 1986	W	NS, M

Table 2 cont.

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Blattodea (continued)</b>					
<i>Blaberus giganteus</i>	DGC, Cyclic	Low	Miller, 1973	W	NS, M
<i>Perisphaeria sp.</i>	DGC, Cyclic, Continuous	High	Marais and Chown, 2003	W and WL	NS, M
<i>Perisphaeria sp. 2</i>	DGC	High	Sinclair, unpublished data	WL	NS, M
<b>Isoptera</b>					
<b>Family Kalotermitidae</b>					
<i>Cryptotermes cavifrons</i>	Cyclic, Continuous	High	Shelton and Appel, 2001a	WL	NS, M
<i>Incisitermes minor</i>	Cyclic, Continuous	High	Shelton and Appel, 2001a	WL	NS, M
<i>Incisitermes minor</i>	Cyclic, Continuous	High	Shelton and Appel, 2000b	WL	NS, M
<i>Incisitermes tabogae</i>	Cyclic, Continuous	High	Shelton and Appel, 2001a	WL	NS, M
<b>Family Rhinotermitidae</b>					
<i>Coptotermes formosanus</i>	Continuous	High	Shelton and Appel, 2001b	WL	S, M
<i>Reticulitermes flavipes</i>	Continuous	High	Shelton and Appel, 2001b	WL	S, M
<b>Family Termopsidae</b>					
<i>Zootermopsis nevadensis</i>	Cyclic, Continuous	High	Shelton and Appel, 2000a	WL	NS, M

Table 2 cont.

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Orthoptera</b>					
<b>Family Acrididae</b>					
<i>Melanoplus sanguinipes</i>	DGC	High	Rourke, 2000	W	NS, M
<i>Romalea guttata</i>	Cyclic	High	Hadley and Quinlan, 1993	W	NS, M
<i>Romalea guttata</i>	DGC	Medium	Quinlan and Hadley, 1993	W	NS, M
<i>Schistocerca gregaria</i>	Cyclic, Continuous	Medium	Hamilton, 1964	W	NS, M
<b>Family Ramoleidae</b>					
<i>Taeniopoda eques</i>	DGC	High	Quinlan and Hadley, 1993	W	NS, X
<i>Taeniopoda eques</i>	DGC, Continuous	High	Harrison et al., 1995	W	NS, X
<b>Family Gryllidae</b>					
<i>Gryllus campestris</i>	Continuous	Medium	Punt, 1950	W	S, M
<b>Hemiptera</b>					
<b>Family Reduviidae</b>					
<i>Rhodnius prolixus</i>	Cyclic	High	Bradley et al., 2003	WL	NS, M
<i>Triatoma rubrofasciata</i>	Cyclic	Low	Punt, 1950	WL	NS, M
<b>Family Cicadidae</b>					
<i>Diceroprocta apache</i>	Continuous	High	Hadley et al., 1991	W	NS, X

Table 2 cont.

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Coleoptera</b>					
<b>Family Cerambycidae</b>					
<i>Phorocantha recurva</i>	DGC	High	Chappell and Rogowitz, 2000	W	NS, M
<i>Phorocantha semipunctata</i>	DGC	High	Chappell and Rogowitz, 2000	W	NS, M
<b>Family Tenebrionidae</b>					
<i>Akis goryi</i>	Continuous	High	Duncan et al., 2002a	WL	NS, M
<i>Blaps sulcata</i>	Continuous	High	Duncan et al., 2002a	WL	NS, M
<i>Cardiosis faimarei</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Epiphysa arenicola</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Heleus waitei</i>	Continuous	High	Duncan and Dickman, 2001	WL	NS, X
<i>Onymacris laeviceps</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Onymacris multistriata</i>	DGC	High	Duncan, 2003	WL	NS, X
<i>Onymacris plana</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Onymacris rugatipennis albatesselata</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Onymacris rugatipennis rugatipennis</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Onymacris unguicularis</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Onymacris unguicularis</i>	DGC	High	Louw et al., 1986	WL	NS, X
<i>Physadesmia globosa</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Pimelia canescens</i>	DGC	High	Duncan et al., 2002a	WL	NS, M/X
<i>Pimelia grandis</i>	Continuous	High	Duncan et al., 2002a	WL	NS, M
<i>Pimelia grandis</i>	DGC	High	Duncan et al., 2002b	WL	NS, X
<i>Psammodes striatus</i>	Continuous DGC	High	Lighton, 1988a	WL	NS, X

Table 2 cont.

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Coleoptera (continued)</b>					
<i>Pterohelaeus sp.</i>	Cyclic	High	Duncan and Dickman, 2001	WL	NS, X
<i>Scaurus puncticollis</i>	Continuous	High	Duncan et al., 2002a	WL	NS, M/X
<i>Stenocara gracilipes</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Trachyderma hispida</i>	Continuous	High	Duncan et al., 2002b	WL	NS, M
<i>Trachyderma philistina</i>	Continuous	High	Duncan et al., 2002a	WL	NS, X
<i>Zophosis complanata</i>	DGC	High	Duncan et al., 2002a	WL	NS, M/X
<i>Zophosis orbicularis</i>	DGC	High	Lighton, 1991	WL	NS, X
<i>Zophosis punctata</i>	DGC	High	Duncan et al., 2002a	WL	NS, M
<b>Family Scarabaeidae</b>					
<i>Anachalcos convexus</i>	DGC	High	Duncan and Byrne, 2000	W	S, M
<i>Aphodius fossor</i>	DGC	High	Chown and Holter, 2000	W	S, M
<i>Circellium bacchus</i>	Cyclic, DGC	High	Duncan and Byrne, 2000	WL	S, X
<i>Circellium bacchus</i>	DGC	High	Duncan and Byrne, 2002	WL	S, M
<i>Scarabaeus flavicornis</i>	DGC	High	Duncan and Byrne, 2000	W	S, X
<i>Scarabaeus galeus</i>	DGC	Medium	Davis et al., 1999	W	S, M
<i>Scarabaeus garipepinus</i>	DGC	High	Davis et al., 1999	WL	S, X
<i>Scarabaeus rusticus</i>	DGC	High	Davis et al., 1999	W	S, M
<i>Scarabaeus rusticus</i>	DGC, Cyclic	High	Duncan and Byrne, 2000	W	S, M
<i>Scarabaeus striatum</i>	DGC	High	Davis et al., 1999	WL	S, X
<i>Scarabaeus westwoodi</i>	DGC	Medium	Davis et al., 1999	W	S, M
<i>Sisyphus fasciculatus</i>	Cyclic	High	Duncan and Byrne, 2000	W	S, M

Table 2 cont.

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Coleoptera (continued)</b>					
<b>Family Carabidae</b>					
<i>Carabus nemoralis</i>	DGC	Low	Punt, 1950; Punt et al., 1957; Punt, 1956	WL	NS, M
<i>Carenum sp.</i>	DGC	High	Duncan and Dickman, 2001	WL	NS, X
<i>Cerotalis sp.</i>	DGC	High	Duncan and Dickman, 2001	WL	NS, X
<i>Hadrocarabus problematicus</i>	DGC	Low	Punt et al., 1957	WL	NS, ?
<b>Family Staphylinidae</b>					
<i>Staphylinus olens</i>	Continuous	Medium	Punt, 1950	WL	NS, M
<b>Family Trogidae</b>					
<i>Omorgus radula</i>	DGC	High	Bosch et al., 2000	WL	NS, M
<b>Family Curculionidae</b>					
<i>Bothrometopus randi</i>	Cyclic	High	Chown, 2001	WL	NS, M
<i>Ectemnorhinus similis</i>	Cyclic	High	Chown, 2001	WL	NS, M

Table 2 cont.

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Diptera</b>					
<b>Family Muscidae</b>					
<i>Glossina morsitans</i>	Continuous	High	Taylor, 1977	W	NS, M
<i>Glossina pallidipes</i>	Continuous	High	Terblanche et al., unpublished data	W	NS, M
<b>Family Drosophilidae</b>					
<i>Drosophila melanogaster</i>	Continuous (May show cyclic when active)	High	Williams et al., 1997	W	NS, M/X
<b>Family Culicidae</b>					
<i>Culex tarsalis</i>	Cyclic	High	Gray and Bradley, 2003	W	NS, X

Table 2 cont.

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Lepidoptera</b>					
<b>Family Arctiidae</b>					
<i>Arctia</i> sp. (hibernating L)	Cyclic	Medium	Punt, 1950		NS, ?
<b>Family Lasiocampidae</b>					
<i>Gonometa postica</i> (P)	DGC	High	Terblanche et al., unpublished data		NS, X
<b>Family Noctuidae</b>					
<i>Admetovis oxymoris</i> (P)	Continuous	Low	Buck and Keister, 1955		NS
<i>Agrotis</i> sp. (P)	DGC	Medium	Punt, 1950		NS, ?
<i>Agrotis</i> sp. (L)	Continuous	Medium	Punt, 1950		NS, ?
<b>Family Nymphalidae</b>					
<i>Vanessa urticae</i> (hibernating adult)	Cyclic	Medium	Punt, 1950		NS, M
<b>Family Papilionidae</b>					
<i>Ornithoptera priamus</i> (P)	DGC	High	Hetz et al., 1999		NS, M
<i>Papilio machaon</i> (L)	Continuous	High	Punt, 1950		NS, M
<i>Papilio machaon</i> (P)	DGC, Cyclic	Medium	Punt, 1950		NS, M
<i>Troides rhadamantus</i> (P)	DGC	High	Hetz et al., 1999		NS, M
<b>Family Pieridae</b>					
<i>Pieris rapae</i> (P and L)	Continuous	Medium	Punt, 1950		NS, M

Table 2 cont.

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Lepidoptera (continued)</b>					
<b>Family Pyralidae</b>					
<i>Galleria mellonella</i> (P)	Cyclic	Low	Kuusik et al., 1996		NS, M
<b>Family Saturniidae</b>					
<i>Agapema galbina</i> (P)	Cyclic, Continuous	Medium	Buck and Keister, 1955		S, X
<i>Hyalophora cecropia</i> (P)	DGC	Medium	Levy and Schneiderman, 1966a,b		S, M
<i>Hyalophora cecropia</i> (P)	DGC	Medium	Punt et al., 1957		S, M
<i>Hyalophora cecropia</i> (P)	DGC	Low	Brockway and Schneiderman, 1967		S, M
<i>Platysamia cecropia</i> (P)	DGC	Low	Schneiderman and Williams, 1955		S, M
<i>Platysamia cecropia</i> (L)	DGC	Medium	Schneiderman and Williams, 1955		NS, M
<i>Rothschildia orizaba</i> (P)	Cyclic, Continuous	Low	Buck and Keister, 1955		S, M
<b>Family Sphingidae</b>					
<i>Deilephila elpenor</i>	Continuous	High	Punt, 1950		NS, M
<i>Sphinx ligustri</i> (P)	DGC	Medium	Punt, 1950		NS, M

Table 2 cont.

<b>Hymenoptera</b>					
<b>Family Apidae</b>					
<i>Apis mellifera ligustica</i>	Cyclic	Medium	Lighton and Lovegrove, 1990	W	NS, M
<i>Bombus terrestris</i>	DGC	High	Beekman and van Stratum, 1999	W	NS, M
<b>Family Formicidae</b>					
<i>Camponotus detritus</i>	DGC	High	Lighton, 1990	WL	S, X
<i>Camponotus maculatus</i>	DGC	Medium	Duncan and Newton, 2000	WL	S, M
<i>Camponotus vicinus</i>	DGC	Medium	Lighton, 1992	WL	S, M/X
<i>Camponotus vicinus</i>	Cyclic	High	Lighton, 1988b	WL	S, M
<i>Camponotus vicinus</i>	DGC	High	Lighton and Garrigan, 1995	WL	S, M
<i>Cataglyphis bicolor</i>	DGC	High	Lighton, 1992	WL	S, X
<i>Cataglyphis bicolor</i>	DGC	High	Lighton and Wehner, 1993	WL	S, X
<i>Cataglyphis bicolor</i>	DGC	High	Lighton et al., 1993a	WL	S, X
<i>Leptogenys attenuata</i>	Cyclic	Medium	Duncan and Crewe, 1993	WL	S, M
<i>Leptogenys nitida</i>	Cyclic	Medium	Duncan and Crewe, 1993	WL	S, M
<i>Leptogenys schwabi</i>	Cyclic	Medium	Duncan and Crewe, 1993	WL	S, M
<i>Messor julianus</i> (alates/workers)	DGC, Cyclic	High	Lighton and Berrigan, 1995	W and WL	S, X
<i>Messor pergandei</i> (alates/workers)	DGC, Continuous	High	Lighton and Berrigan, 1995	W and WL	S, X
<i>Pogonomyrmex barbatus</i>	DGC, Cyclic, Continuous	High	Gibbs and Johnson, 2004	WL	S, X
<i>Pogonomyrmex rugosus</i>	DGC	High	Quinlan and Lighton, 1999	WL	S, X
<i>Pogonomyrmex rugosus</i>	DGC	High	Lighton et al., 1993b	WL	S, X
<i>Solenopsis invicta</i>	Cyclic	High	Vogt and Appel, 2000	WL	S, M

Table 2 cont.

Species	Gas exchange pattern	Confidence	Citation	Wing status	General habitat
<b>Hymenoptera (continued)</b>					
<b>Family Mutillidae</b>					
<i>Dasymutilla gloriosa</i>	Cyclic	High	Duncan and Lighton, 1997	WL	NS, X
<b>Family Vespidae</b>					
<i>Polistes arizonensis</i>	DGC	High	B.A. Klein, K.M.F. Larsen and A.G. Gibbs, unpublished data	W	NS, M

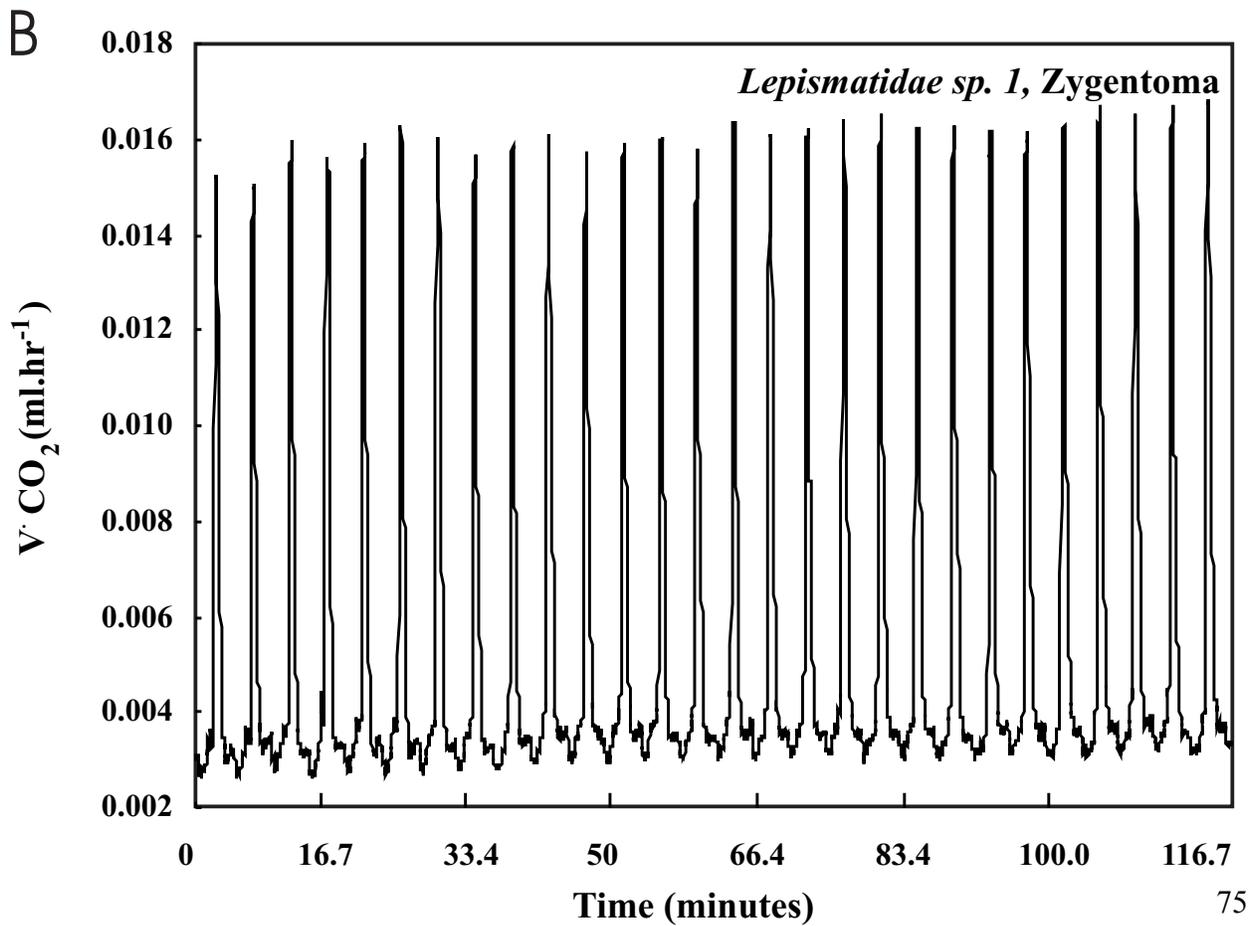
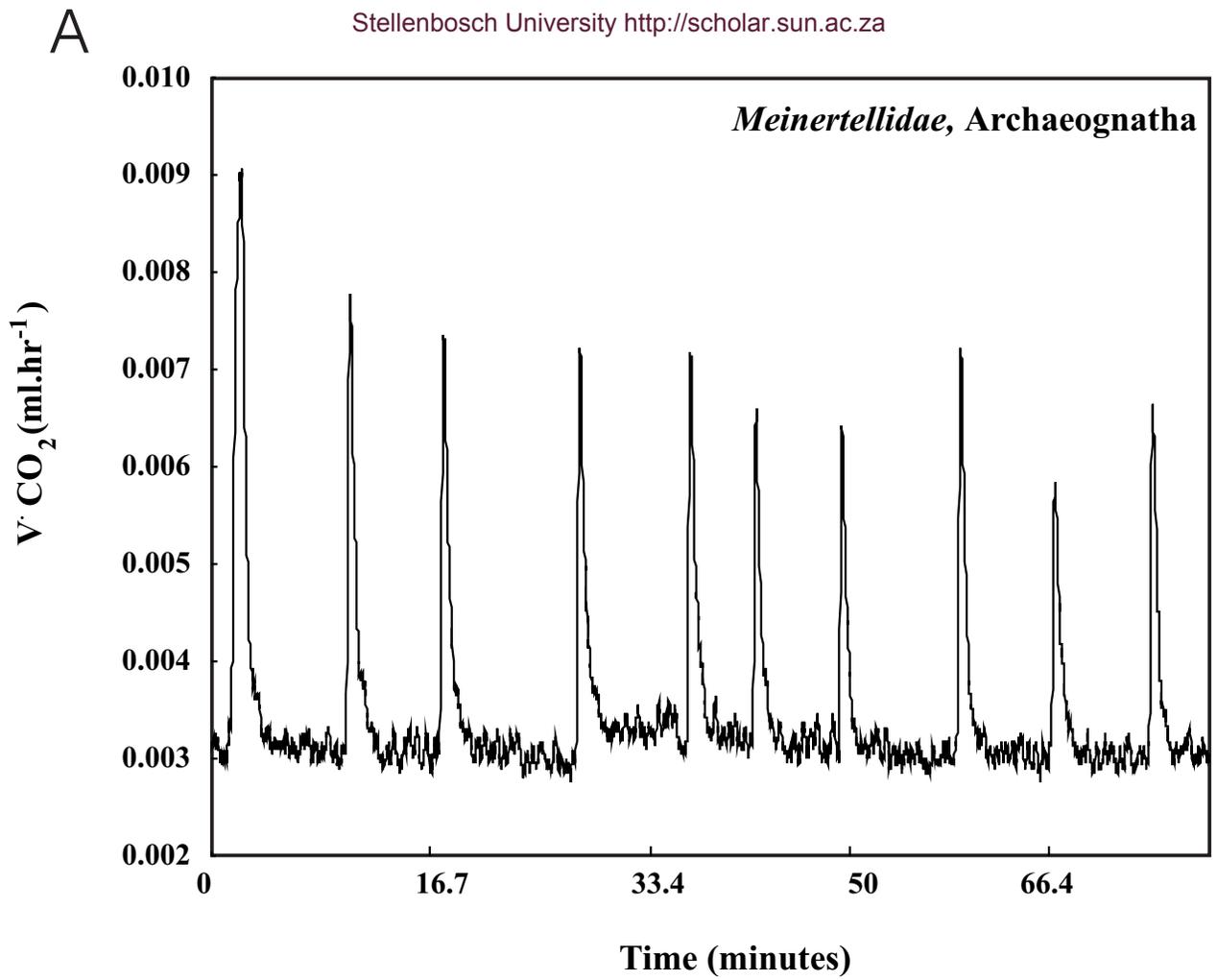


Fig. 2 a - b

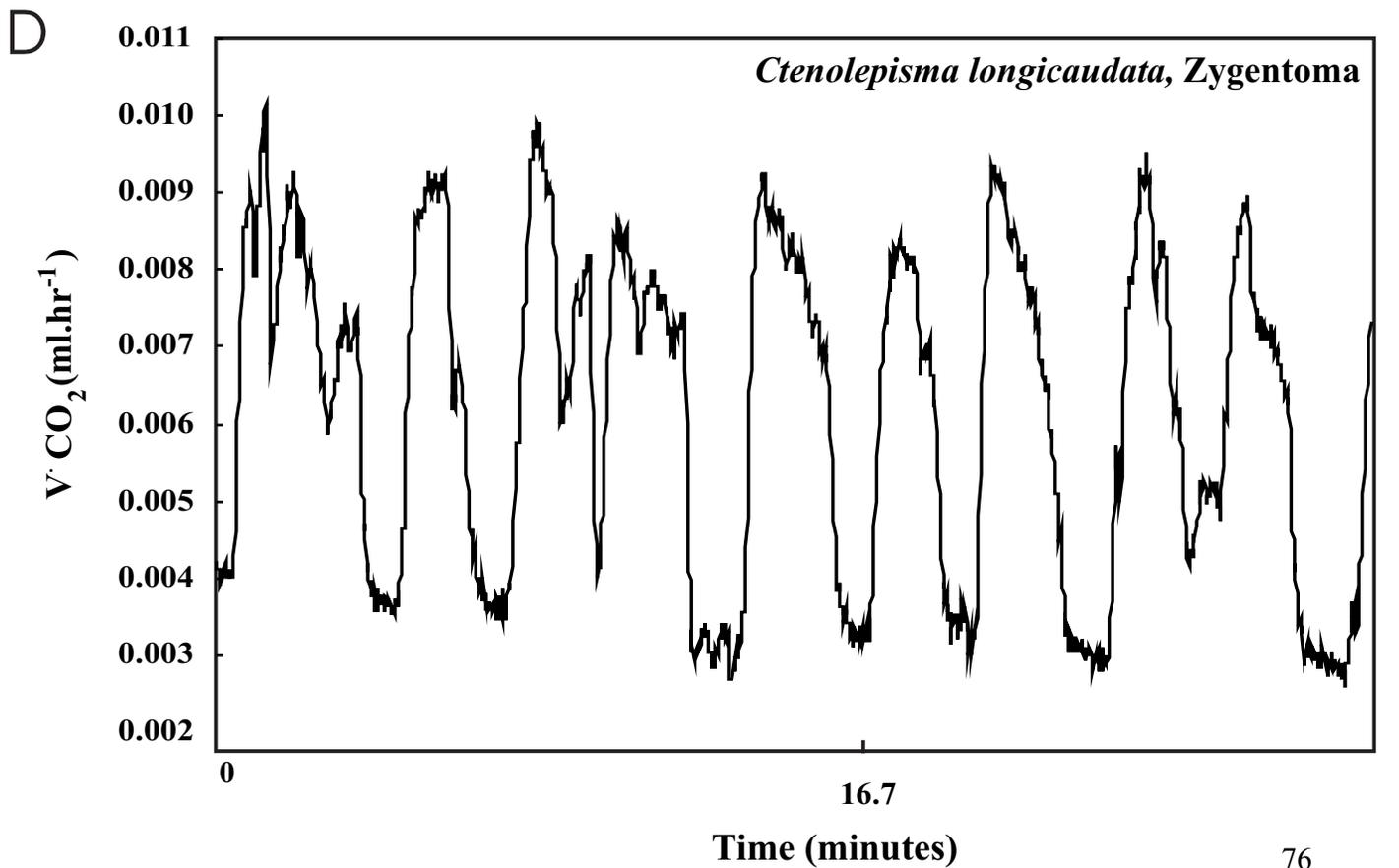
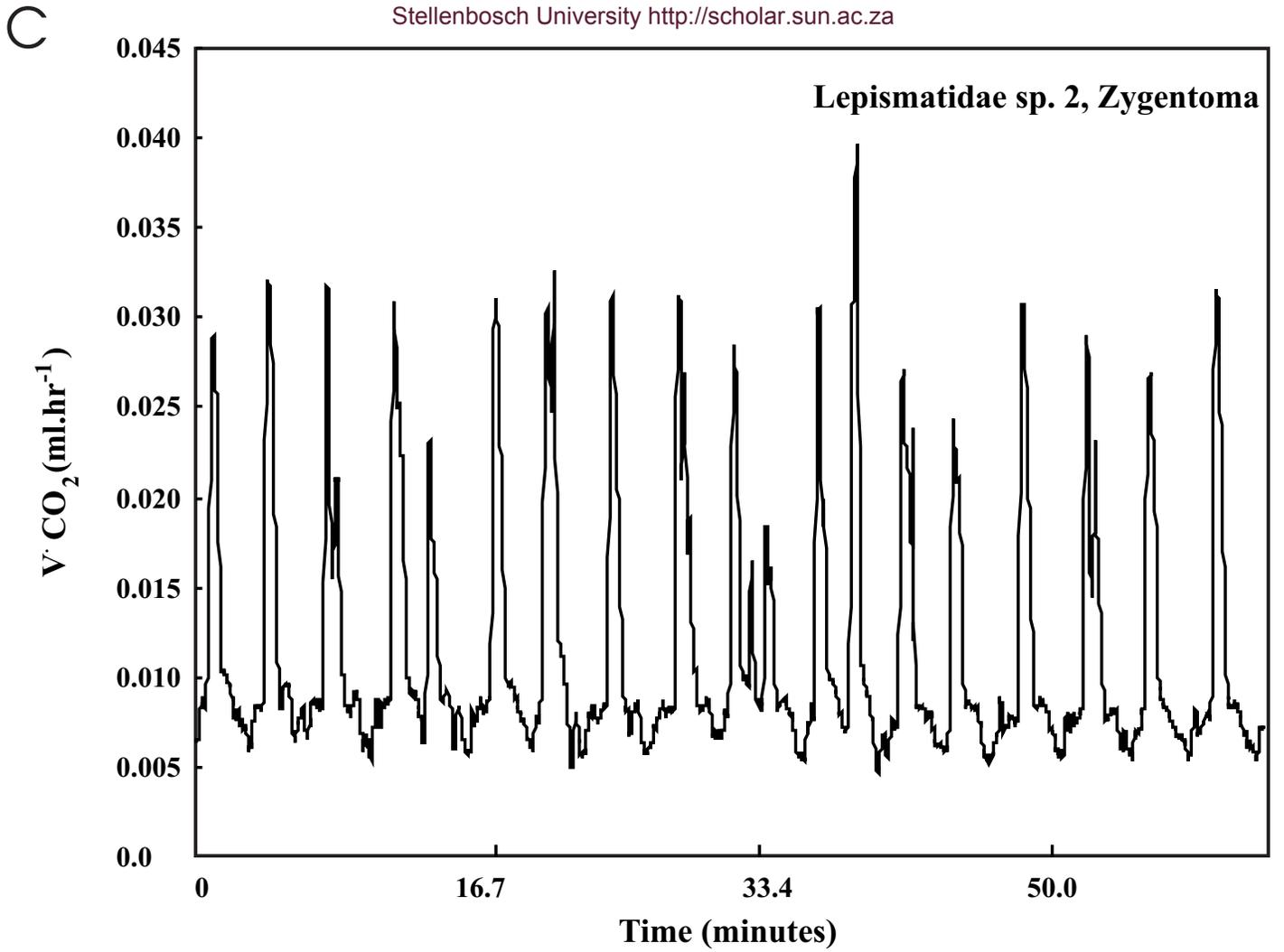


Fig. 2 c - d

E

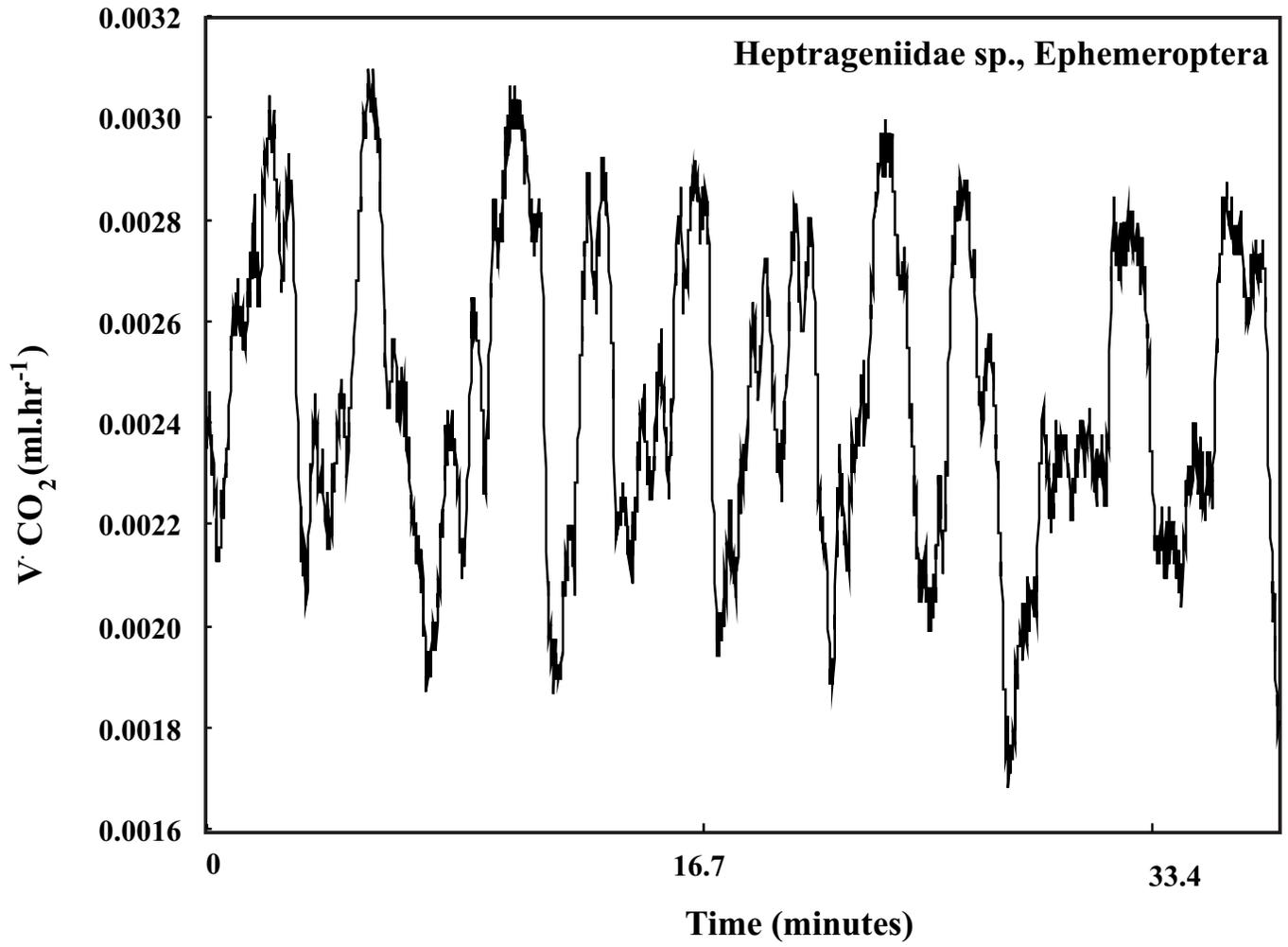


Fig. 2 e

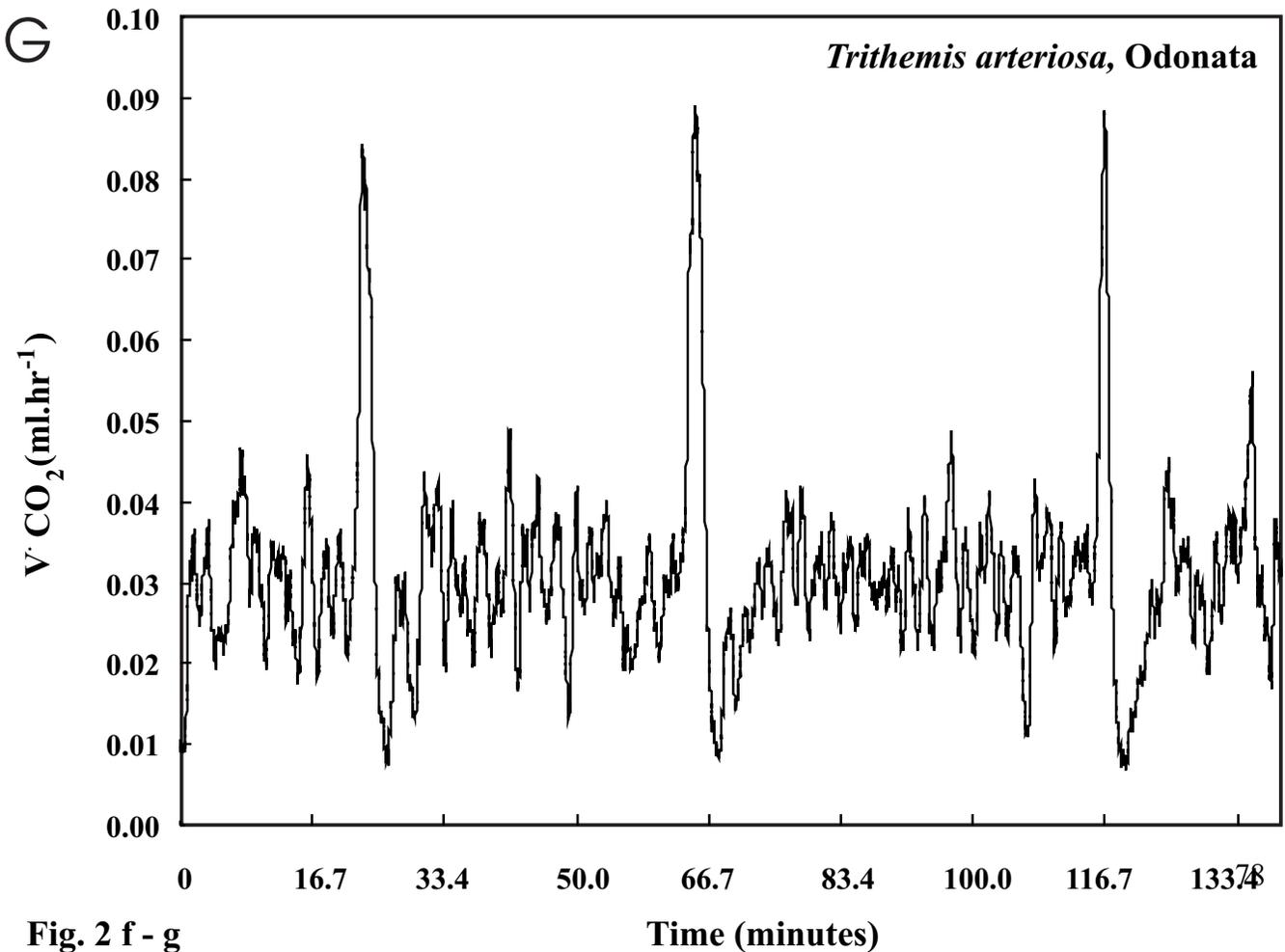
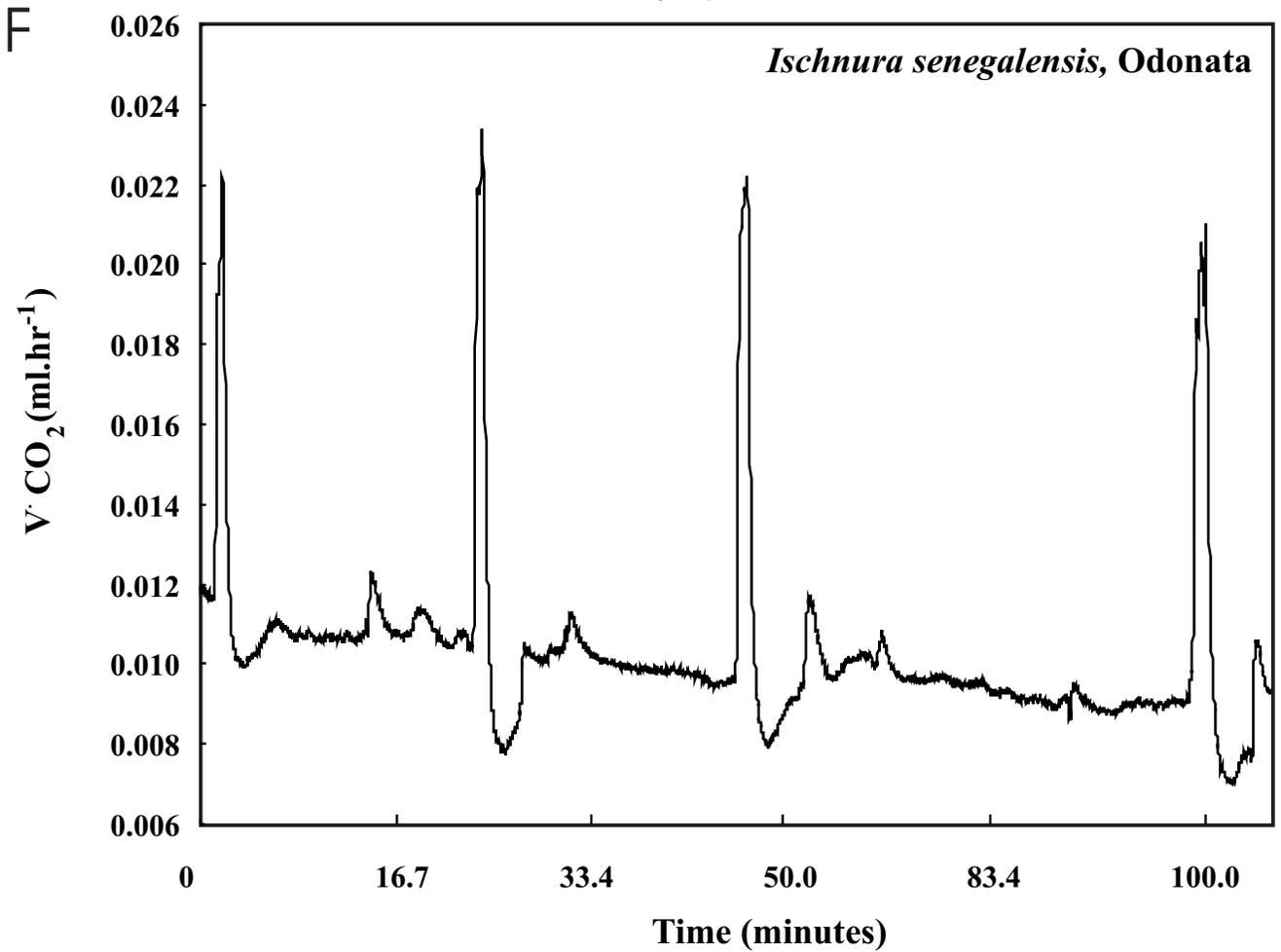


Fig. 2 f - g

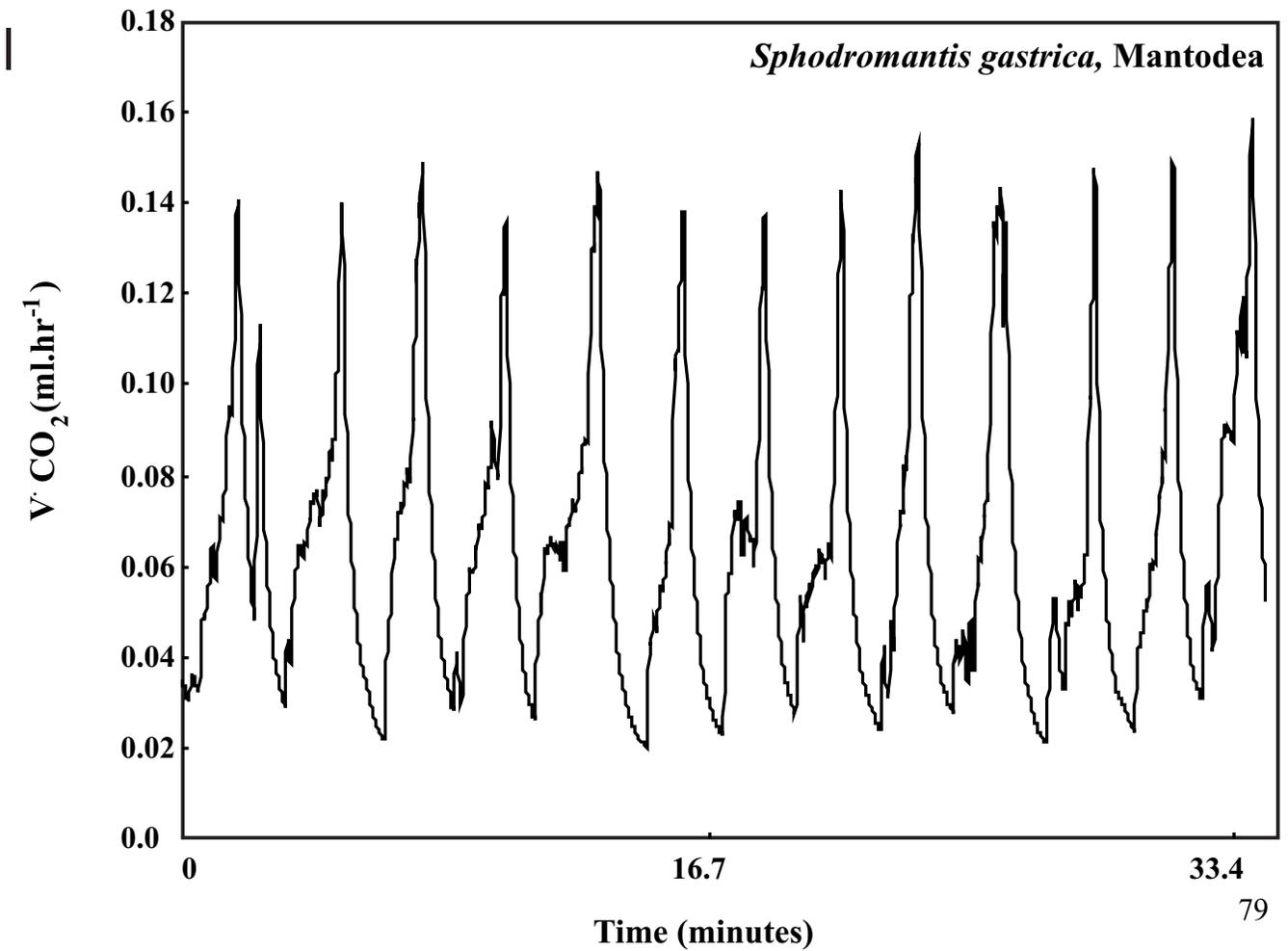
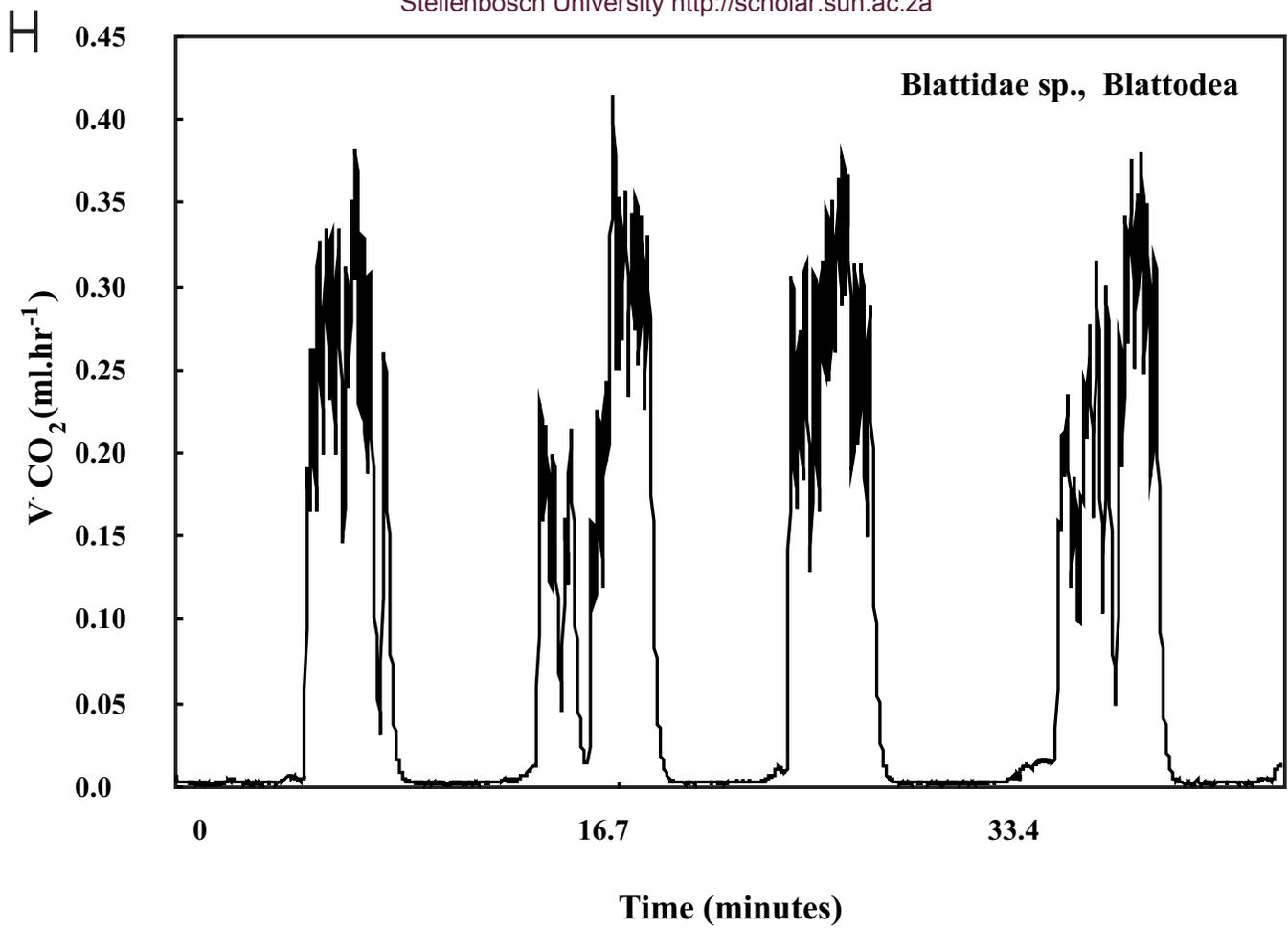


Fig. 2 h - i

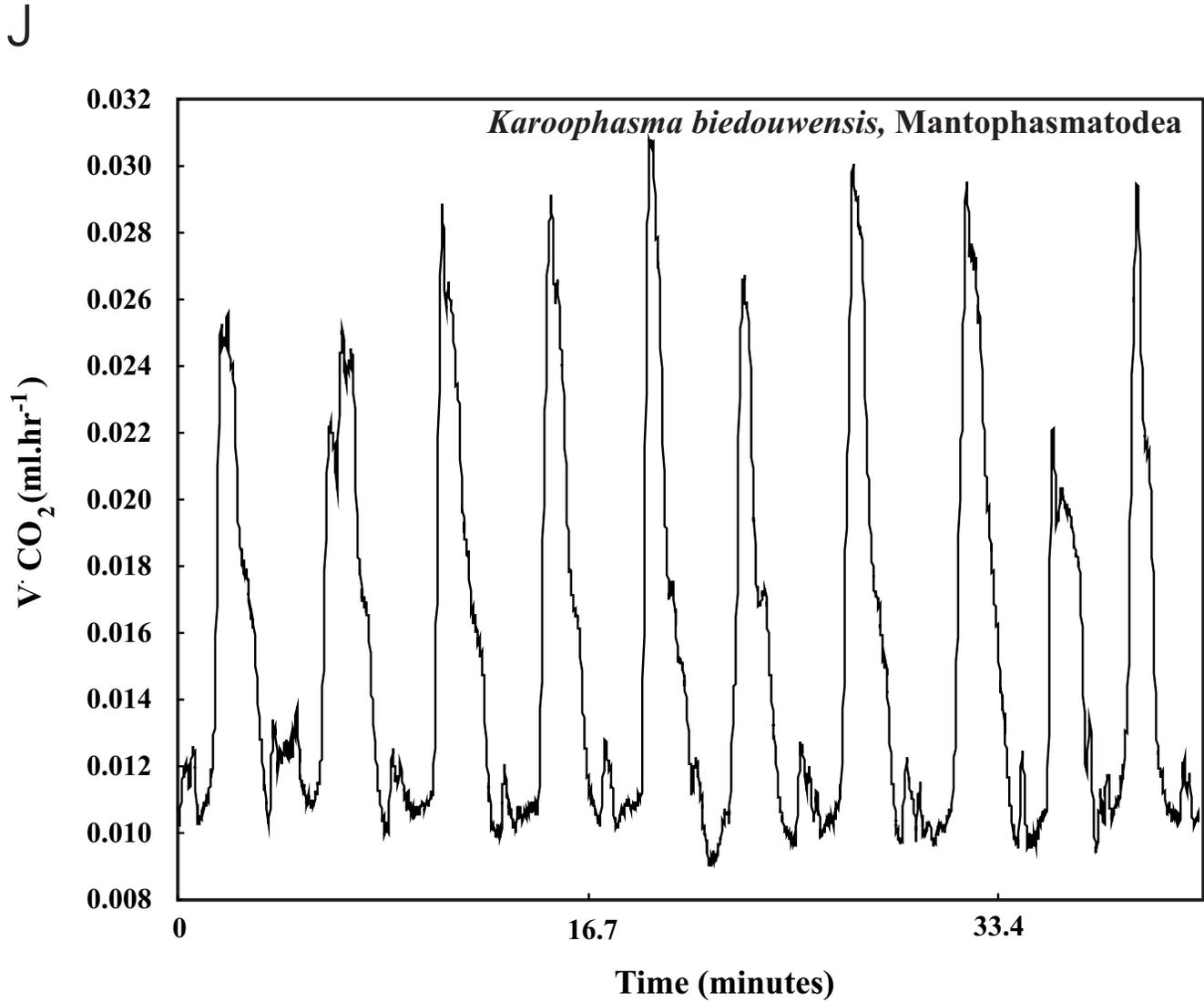
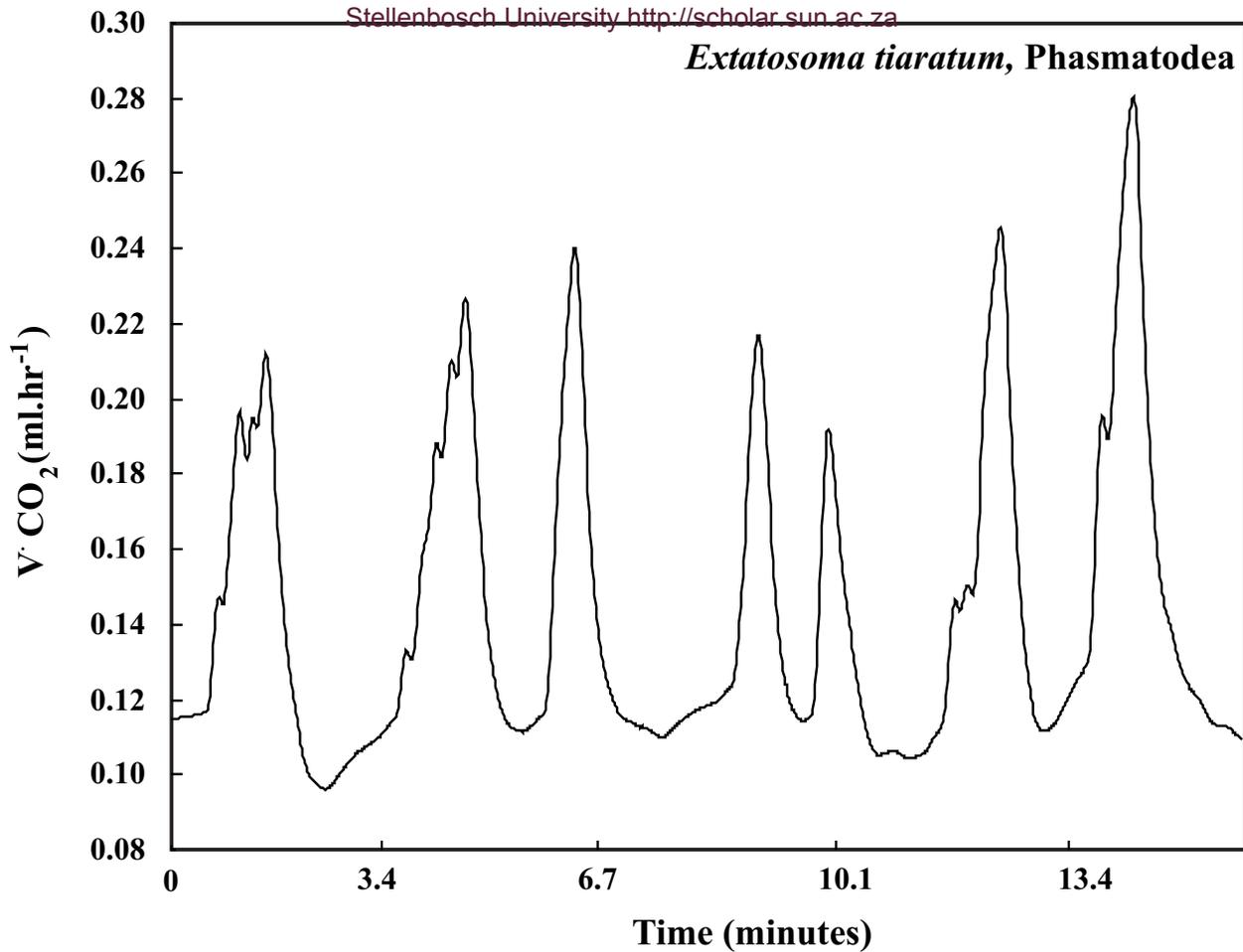


Fig. 2 j

K



L

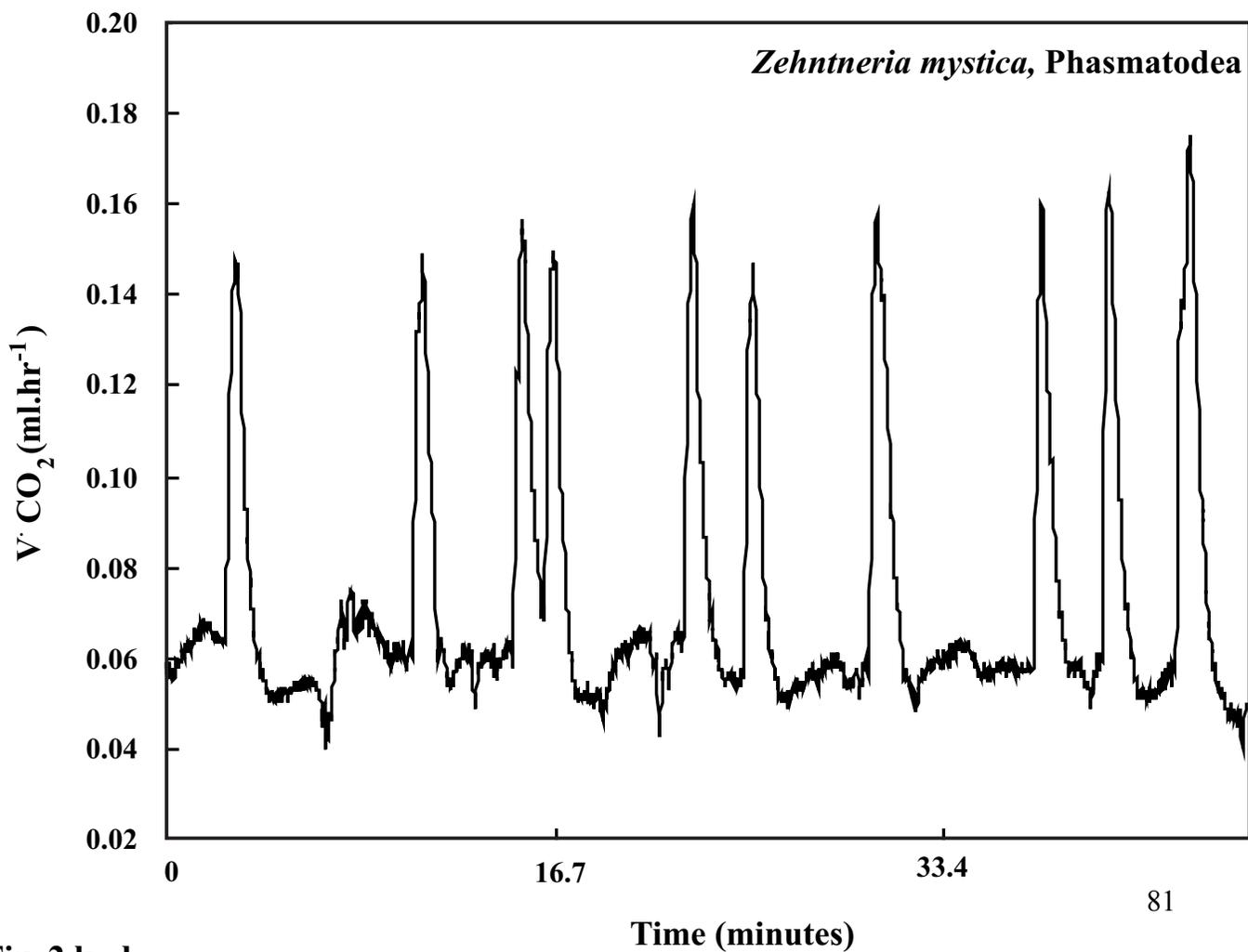


Fig. 2 k - l

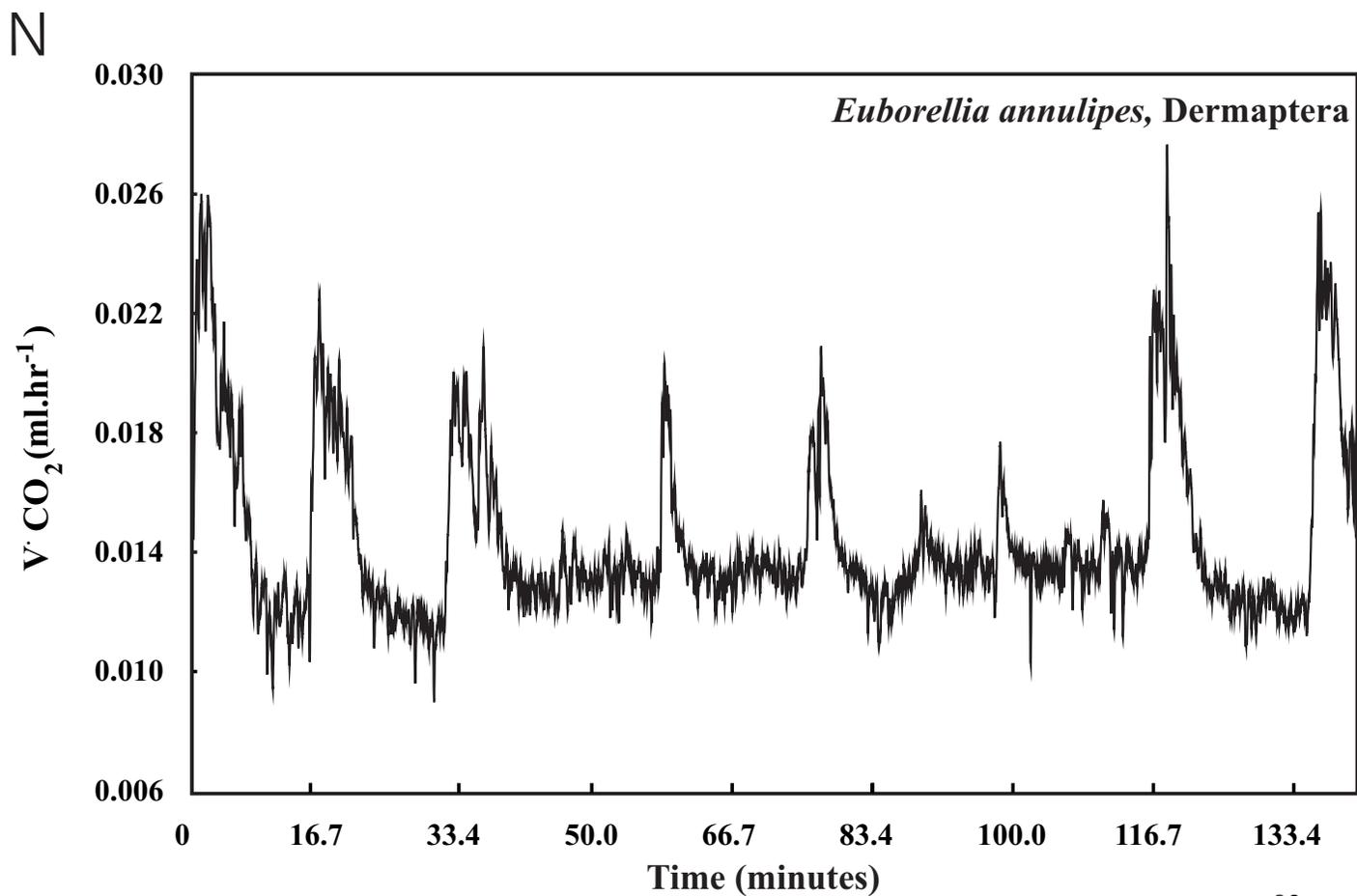
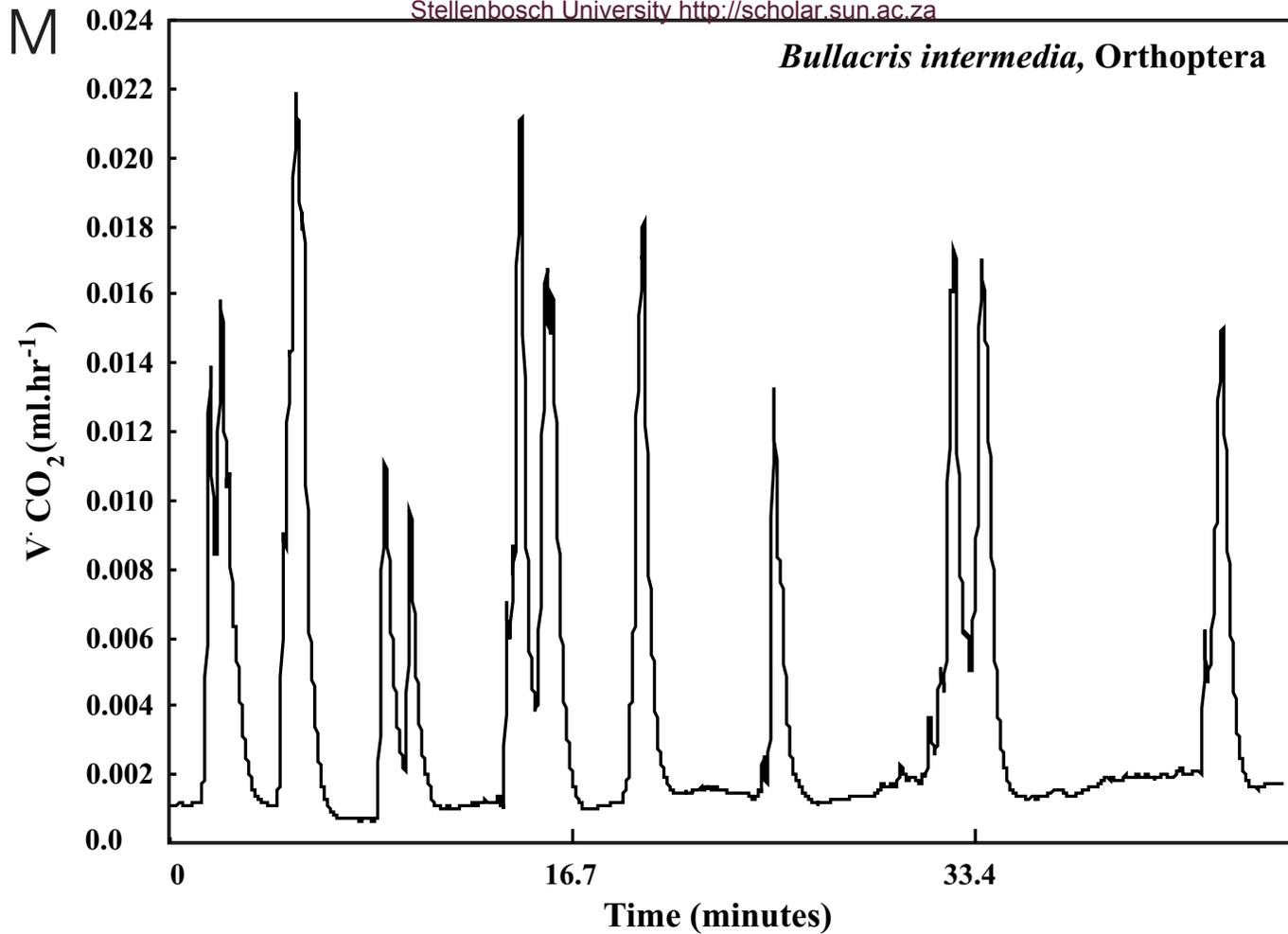


Fig. 2 m - n

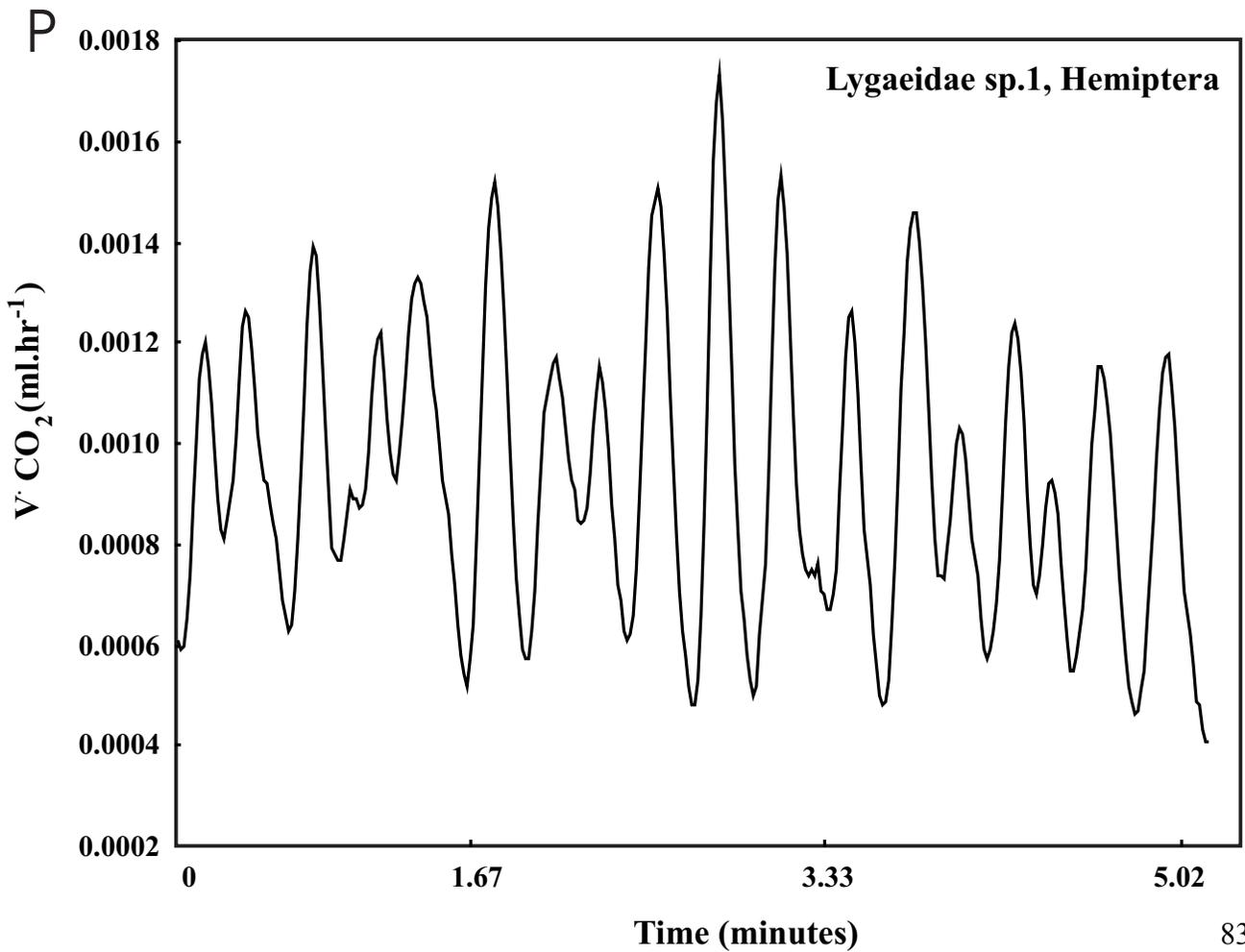
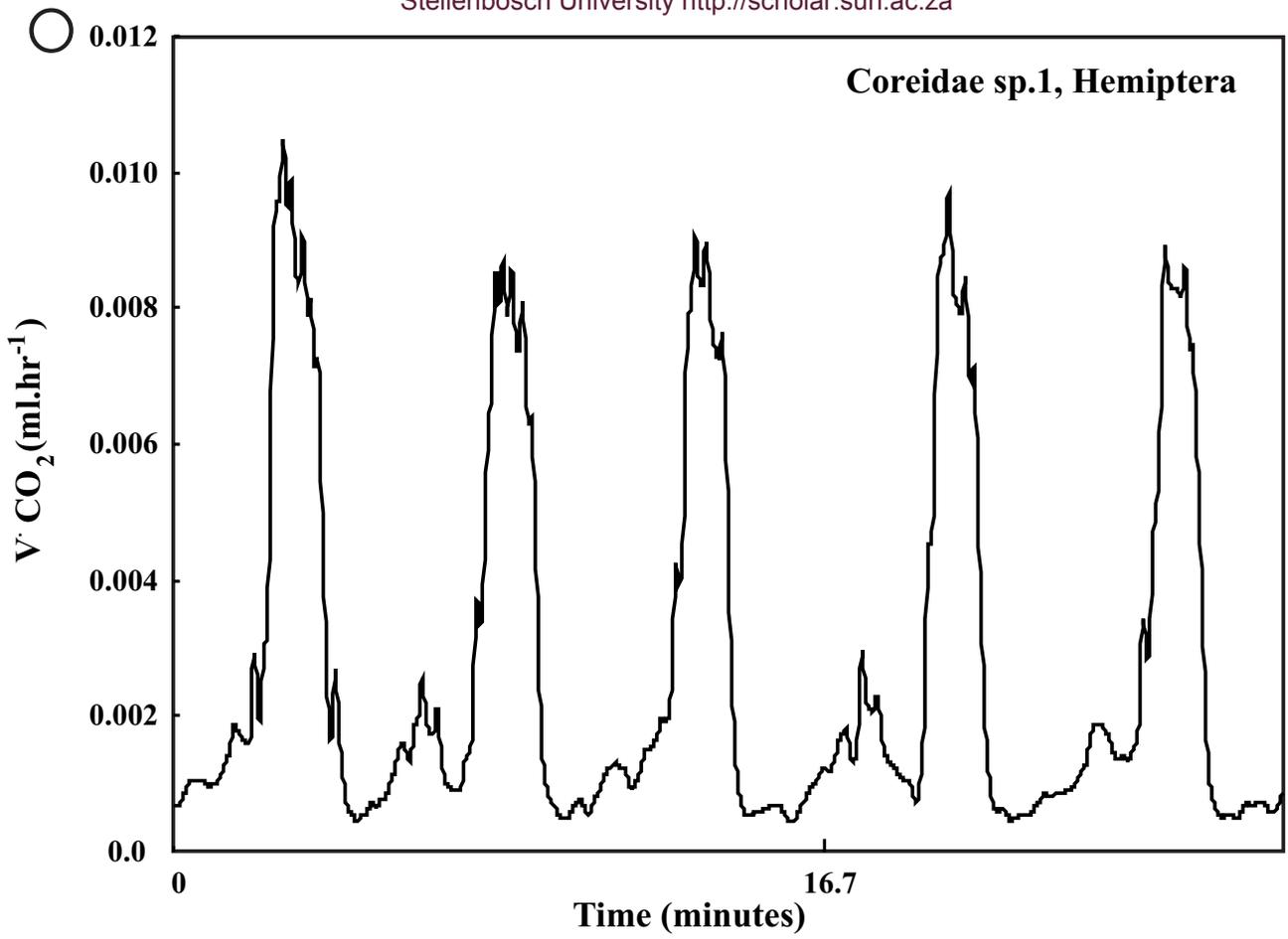


Fig. 2 o - p

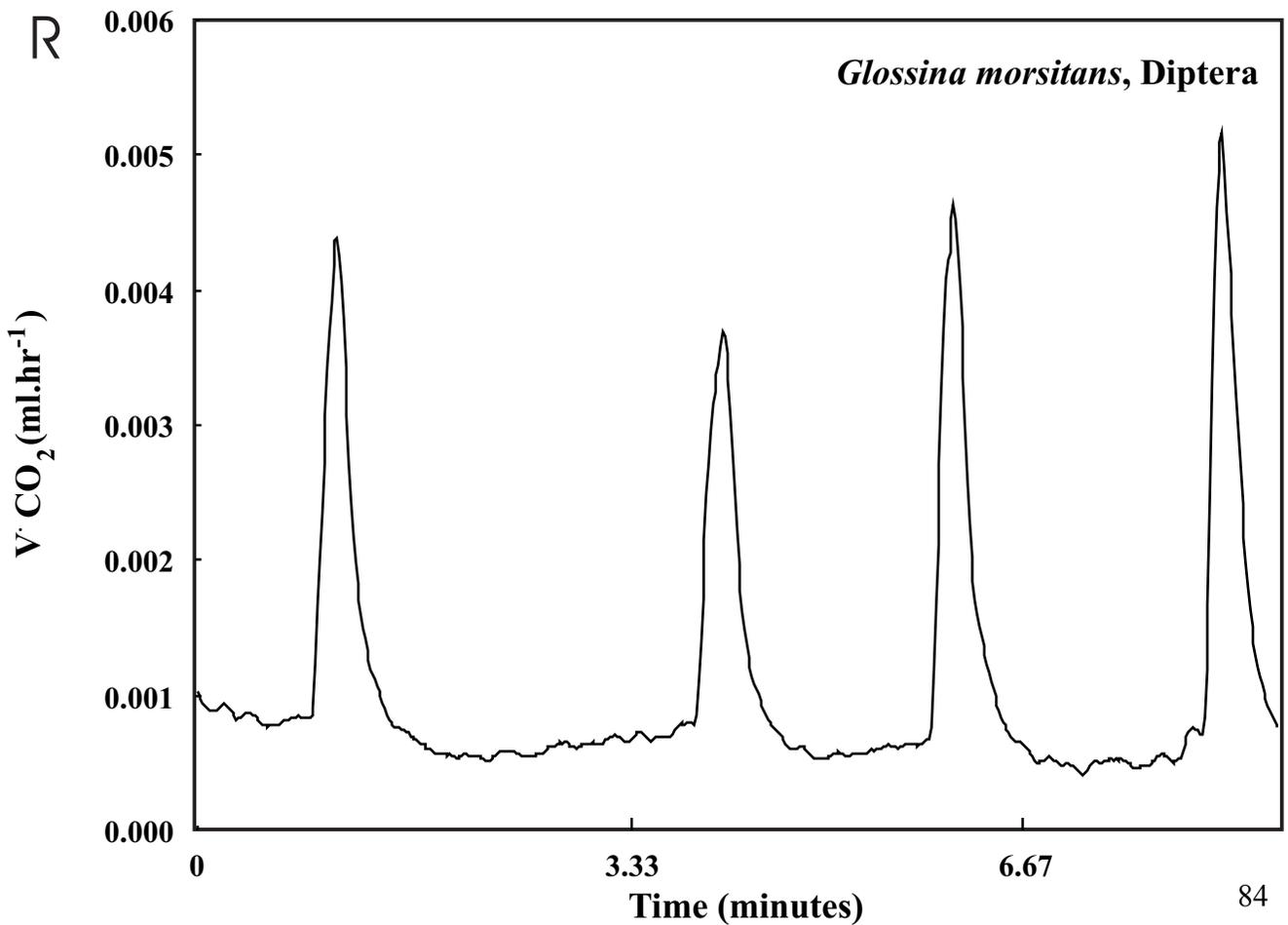
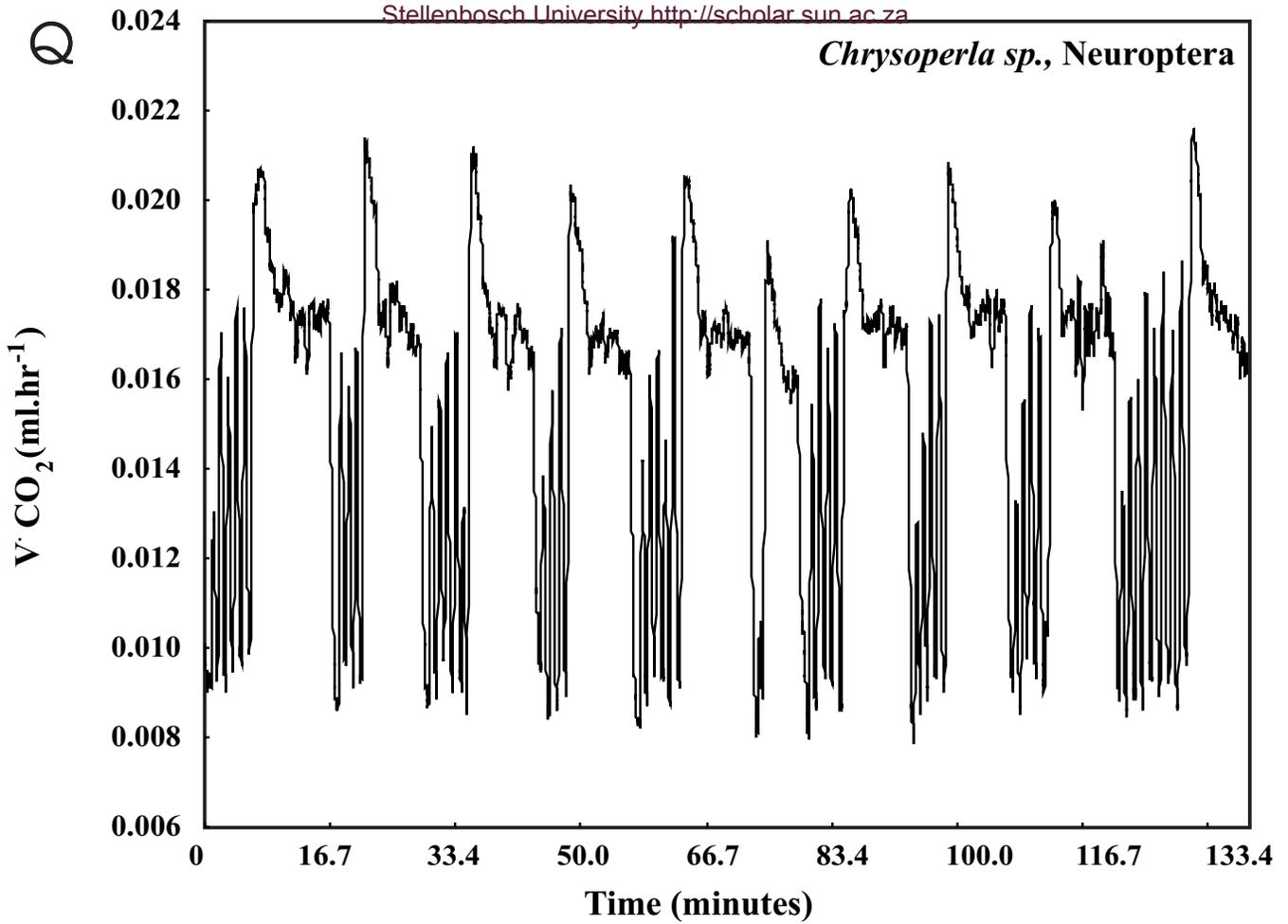


Fig. 2 q - r

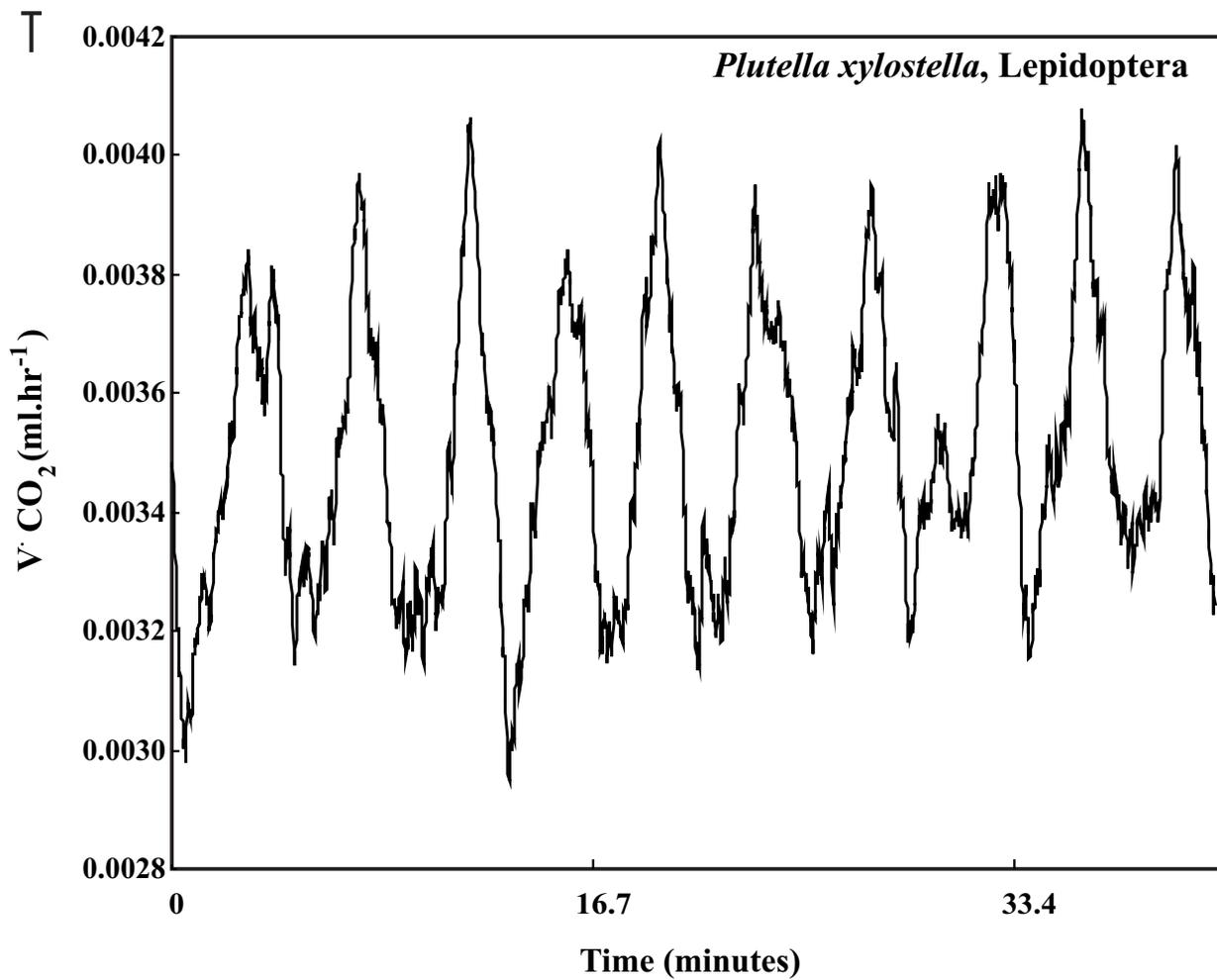
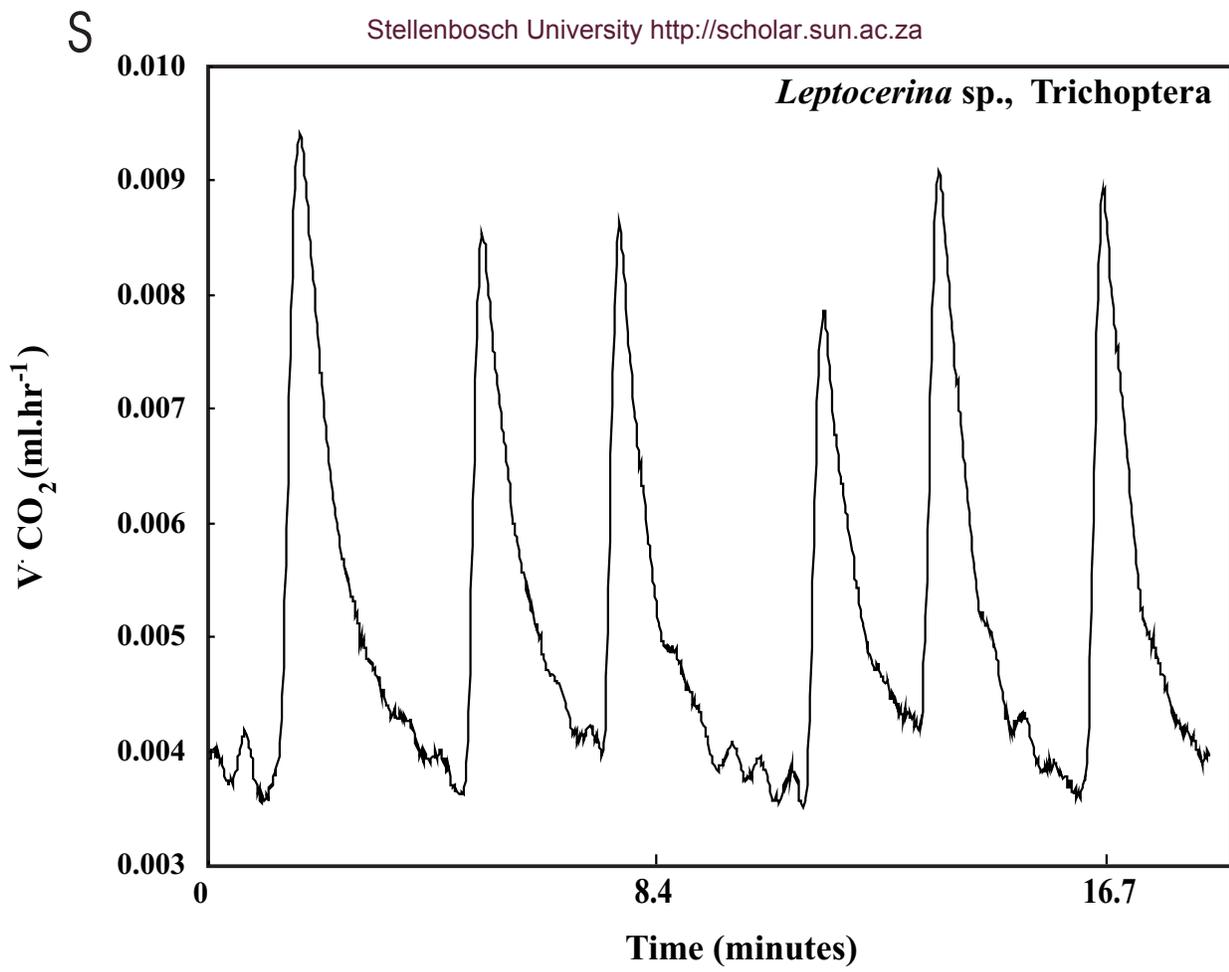


Fig. 2 s - t

**Fig. 2 a – t** Representative cyclic or discontinuous gas exchange patterns for all of the species and orders investigated in this study. *Zehntneria mystica* (Phasmatodea, Phasmatidae) (Fig. 2l) 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* (Fig. 2k), is a leaf-mimicking species which shows a swaying response to the slightest air flow, which explains the limited evidence for cyclic gas exchange in this species.

**Table 3** CO<sub>2</sub> emission volumes, period durations, emission rates, total metabolic rates and masses for each of the species investigated in this study.

	<b>Mean</b>	<b>Standard Deviation</b>	<b>N</b>
<b>Archaeognatha</b>			
<b>Meinertellidae sp.</b>			
Mass (mg)	12.75	5.18	4
Metabolic rate ( $\mu\text{l.h}^{-1}$ )	4.036	0.713	4
<b>Zygentoma</b>			
<b>Lepismatidae sp. 1</b>			
Mass (mg)	23.04	1.99	5
Metabolic rate ( $\mu\text{l.h}^{-1}$ )	3.89	0.884	5
Frequency (Hz)	3.70	0.521	5
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	0.179	0.078	5
Duration (sec)	218.43	45.87	5
Emission rate ( $\mu\text{l.min}^{-1}$ )	0.046	0.012	5
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	0.222	0.073	5
Duration (sec)	106.60	9.050	5
Emission rate ( $\mu\text{l.min}^{-1}$ )	0.120	0.032	5
<b>Lepismatidae sp. 2</b>			
Mass (mg)	17.18	0.72	6
Metabolic rate ( $\mu\text{l.h}^{-1}$ )	4.45 0	1.130	6
Frequency (Hz)	5.140	1.240	6

Table 3 cont.

	Mean	Standard Deviation	N
<b>Lepismatidae sp. 2 (continued)</b>			
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	0.143	0.070	6
Duration (sec)	172.0	35.84	6
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.052	0.017	6
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	0.166	0.041	6
Duration (sec)	108.74	13.34	6
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.102	0.026	6
<b>Lepismatidae</b>			
<i>Ctenolepisma longicaudata</i>			
Mass (mg)	26.64	5.46	9
Metabolic rate ( $\mu\text{l}\cdot\text{h}^{-1}$ )	2.430	0.492	9
Frequency (mHz)	5.627	3.501	7
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	0.047	0.017	8
Duration (sec)	90.61	21.31	8
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.029	0.005	8
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	0.124	0.029	8
Duration (sec)	134.86	12.41	8
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.054	0.010	8

Table 3 cont.

	Mean	Standard Deviation	N
<b>Ephemeroptera</b>			
<b>Heptageniidae sp.</b>			
Mass (mg)	5.61	5.98	12
Metabolic rate ( $\mu\text{l.h}^{-1}$ )	1.425	1.143	12
Frequency (mHz)	10.34	5.777	3
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	0.014	0.008	3
Duration (sec)	21.67	15.11	3
Emission rate ( $\mu\text{l.min}^{-1}$ )	0.041	0.006	3
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	0.067	0.045	3
Duration (sec)	89.0	64.67	3
Emission rate ( $\mu\text{l.min}^{-1}$ )	0.048	0.011	3
<b>Odonata,</b>			
<b>Coenagrionidae</b>			
<b><i>Ischnura senegalensis</i></b>			
Mass (mg)	21.16	4.58	10
Metabolic rate ( $\mu\text{l.h}^{-1}$ )	12.653	6.581	10
Frequency (mHz)	0.629	0.331	5
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	3.309	2.881	5
Duration (sec)	1160.84	908.49	5
Emission rate ( $\mu\text{l.min}^{-1}$ )	0.302	0.422	5

Table 3 cont.

	Mean	Standard Deviation	N
<i>Ischnura senegalensis</i>			
(continued)			
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	1.215	1.106	5
Duration (sec)	218.83	104.455	5
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.291	0.157	5
<b>Libellulidae</b>			
<i>Trithemis arteriosa</i>			
Mass (mg)	103.06	37.88	8
Metabolic rate ( $\mu\text{l}\cdot\text{h}^{-1}$ )	34.002	8.276	8
Frequency (mHz)	4.969		2
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	0.702		2
Duration (sec)	80.0		2
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.495		2
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	2.344		2
Duration (sec)	205.0		2
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.690		2

Table 3 cont.

	Mean	Standard Deviation	N
<b>Blattodea</b>			
<b>Blaberidae sp.</b>			
Mass (g)	2.54	0.94	6
Metabolic rate (ml.h <sup>-1</sup> )	0.091	0.044	6
<i>DGC</i>			
Frequency (mHz)	1.579		2
<i>C-period</i>			
Emission volume (μl)	0.306		2
Duration (sec)	144.5		2
Emission rate (μl.min <sup>-1</sup> )	0.130		2
<i>F-period</i>			
Emission volume (μl)	0.800		2
Duration (sec)	152.40		2
Emission rate (μl.min <sup>-1</sup> )	0.336		2
<i>O-period</i>			
Emission volume (μl)	11.71		2
Duration (sec)	261.5		2
Emission rate (μl.min <sup>-1</sup> )	2.686		2

Table 3 cont.

	Mean	Standard Deviation	N
<b>Blaberidae sp. (continued)</b>			
<b><i>Interburst-burst pattern</i></b>			
Frequency (mHz)	5.319	6.081	3
<b><i>Interburst</i></b>			
Emission volume ( $\mu\text{l}$ )	1.237	1.579	3
Duration (sec)	113.86	71.01	3
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.530	0.578	3
<b><i>Burst</i></b>			
Emission volume ( $\mu\text{l}$ )	14.55	12.82	3
Duration (sec)	340.86	358.38	3
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	2.733	1.705	3
<b>Mantodea</b>			
<b>Mantidae</b>			
<b><i>Sphodromantis gastrica</i></b>			
Mass (mg)	335.7	276.7	7
Metabolic rate ( $\text{ml}\cdot\text{h}^{-1}$ )	0.0644	0.0458	7
Frequency (mHz)	5.648	2.519	4
<b><i>Interburst</i></b>			
Emission volume ( $\mu\text{l}$ )	0.451	0.810	4
Duration (sec)	66.65	82.31	4
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.531	0.886	4

Table 3 cont.

	Mean	Standard Deviation	N
<i>Sphodromantis gastrica</i>			
(continued)			
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	3.683	4.027	4
Duration (sec)	214.35	119.81	4
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.806	0.515	4
<b>Mantophasmatodea</b>			
<b>Austrophasmatidae</b>			
<i>Karoophasma biedouwensis</i>			
Mass (mg)	98.62	83.50	14
Metabolic rate ( $\mu\text{l}\cdot\text{h}^{-1}$ )	23.96	14.87	14
Frequency (mHz)	3.45	1.01	14
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	0.416	0.239	11
Duration (sec)	120	69	11
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.216	0.010	11
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	1.254	0.566	11
Duration (sec)	192	63	11
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.394	0.144	11

Table 3 cont.

	Mean	Standard Deviation	N
<b>Phasmatodea</b>			
<b>Phamatidae</b>			
<i>Extatosoma tiaratum</i>			
Mass (g)	0.59	0.42	10
Metabolic rate ( $\mu\text{l}\cdot\text{h}^{-1}$ )	0.218	0.066	10
Frequency (mHz)	7.65	0.022	10
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	9.59		1
Duration (sec)	39.75		1
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	1.41		1
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	13.95		1
Duration (sec)	80.75		1
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	1.14		1
<b>Orthoptera</b>			
<b>Pneumoridae</b>			
<i>Bullacris intermedia</i>			
Mass (g)	1.69	1.19	5
Metabolic rate ( $\text{ml}\cdot\text{h}^{-1}$ )	0.297	0.135	5
Frequency (Hz)			
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	17.71		1
Duration (sec)	160.5		1
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	6.643		1

Table 3 cont.

	Mean	Standard Deviation	N
<i>Bullacris intermedia</i> (Continued)			
<i>Burst</i>			
Emission volume (ul)	1.40		1
Duration (sec)	90.75		1
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.926		1
<b>Dermaptera</b>			
<b>Labiduridae</b>			
<i>Euborellia annulipes</i>			
Mass (mg)	31.76	26.50	12
Metabolic rate ( $\mu\text{l}\cdot\text{h}^{-1}$ )	8.727	8.004	12
Frequency (Hz)	1.044		2
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	1.398	0.974	3
Duration (sec)	533.88	341.90	3
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.172	0.131	3
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	0.621	0.571	3
Duration (sec)	306.50	136.13	3
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.107	0.065	3

Table 3 cont.

	Mean	Standard Deviation	N
<b>Hemiptera</b>			
<b>Coreidae sp.</b>			
Mass (mg)	556.1	63.64	4
Metabolic rate (ml.h <sup>-1</sup> )	0.164	0.073	4
Frequency (mHz)	4.208	2.048	4
<i>Interburst</i>			
Emission volume (µl)	2.88	3.137	3
Duration (sec)	134.15	120.44	3
Emission rate (µl.min <sup>-1</sup> )	1.486	0.691	3
<i>Burst</i>			
Emission volume (µl)	12.03	2.164	3
Duration (sec)	172.92	39.28	3
Emission rate (µl.min <sup>-1</sup> )	4.233	0.522	3
<b>Lygaeidae sp.</b>			
Mass (mg)	19.66	3.94	9
Metabolic rate (ul.h <sup>-1</sup> )	11.033	18.726	9
Frequency (Hz)	48.786	3.418	4
<i>Interburst</i>			
Emission volume (µl)	0.003	0.002	4
Duration (sec)	4.50	1.461	4
Emission rate (µl.min <sup>-1</sup> )	0.036	0.014	4

Table 3 cont.

	Mean	Standard Deviation	N
<b>Lygaeidae sp. (continued)</b>			
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	0.026	0.009	4
Duration (sec)	21.50	6.207	4
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.075	0.032	4
<b>Neuroptera</b>			
<b>Chrysopidae</b>			
<i>Chrysoperla sp.</i>			
Mass (mg)	6.2	1.2	3
Metabolic rate ( $\mu\text{l}\cdot\text{h}^{-1}$ )	7.579	0.584	3
<b>Diptera</b>			
<b>Glossinidae</b>			
<i>Glossina morsitans</i>			
Mass (mg)	24.48	8.35	10
Metabolic rate ( $\text{ml}\cdot\text{h}^{-1}$ )	0.020	0.010	10
Frequency (Hz)	0.070	0.012	10
<i>Interburst</i>			
Emission volume ( $\mu\text{l}$ )	0.032	0.050	10
Duration (sec)	5.448	2.527	10
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.443	0.856	10
<i>Burst</i>			
Emission volume ( $\mu\text{l}$ )	0.087	0.046	10
Duration (sec)	15.994	1.710	10
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.324	0.167	10

Table 3 cont.

	Mean	Standard Deviation	N
<b>Trichoptera</b>			
<b>Leptoceridae</b>			
<i>Leptocerina sp.</i>			
Mass (mg)	5.20		2
Metabolic rate (ul.h <sup>-1</sup> )	3.532		2
Frequency (mHz)	10.21		2
<i>Interburst</i>			
Emission volume (ul)	0.054		2
Duration (sec)	51.5		2
Emission rate (µl.min <sup>-1</sup> )	0.045		2
<i>Burst</i>			
Emission volume (ul)	0.141		2
Duration (sec)	83.5		2
Emission rate (µl.min <sup>-1</sup> )	0.085		2
<b>Lepidoptera</b>			
<i>Plutella xylostella</i>			
Mass (mg)	3.52	0.93	13
Metabolic rate (ul.h <sup>-1</sup> )	3.751	0.578	13
Frequency (mHz)	0.005		1
<i>Interburst</i>			
Emission volume (ul)	0.062		1
Duration (sec)	68.75		1
Emission rate (µl.min <sup>-1</sup> )	0.053		1

Table 3 cont.

	<b>Mean</b>	<b>Standard Deviation</b>	<b>N</b>
<i>Plutella xylostella</i> (continued)			
<i>Burst</i>			
Emission volume (ul)	0.175		1
Duration (sec)	175.25		1
Emission rate ( $\mu\text{l}\cdot\text{min}^{-1}$ )	0.061		1

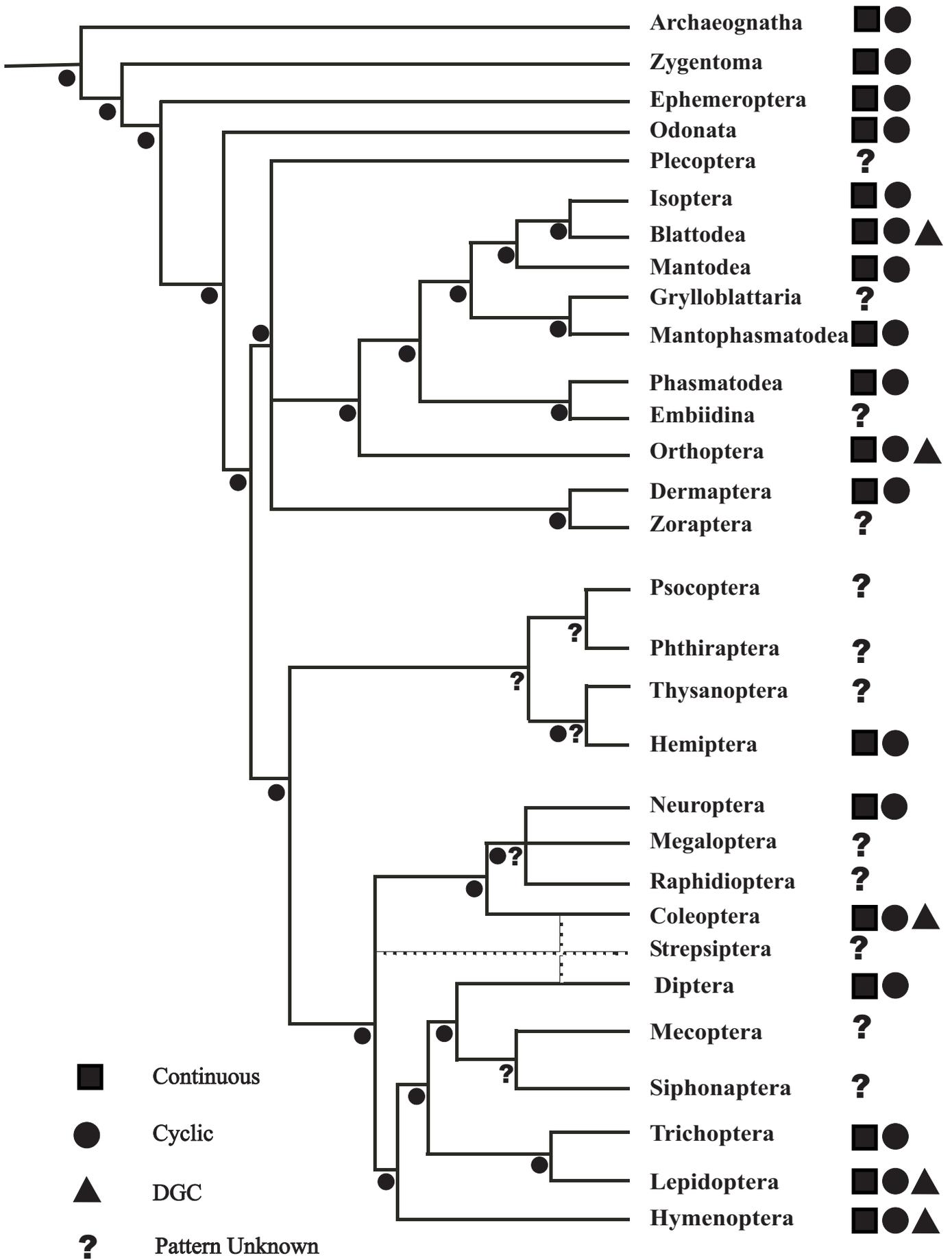
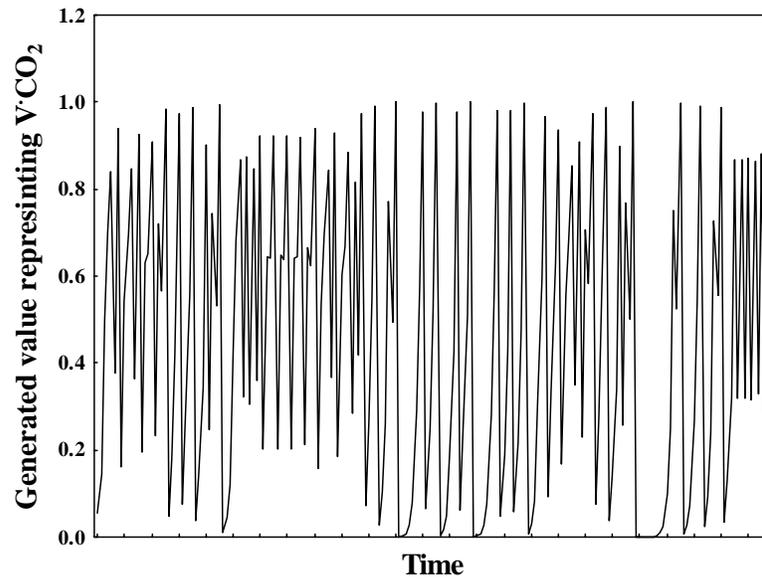
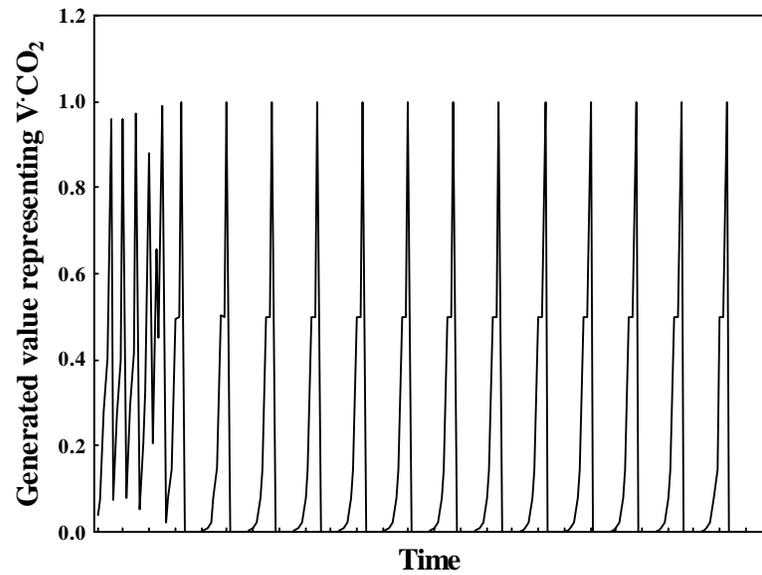


Fig. 3

**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: ■ = continuous gas exchange, ● = cyclic gas exchange, ▲ = 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 five of the 30 orders of insects, while continuous and cyclic patterns have been observed in all of the orders examined.

**A****B**

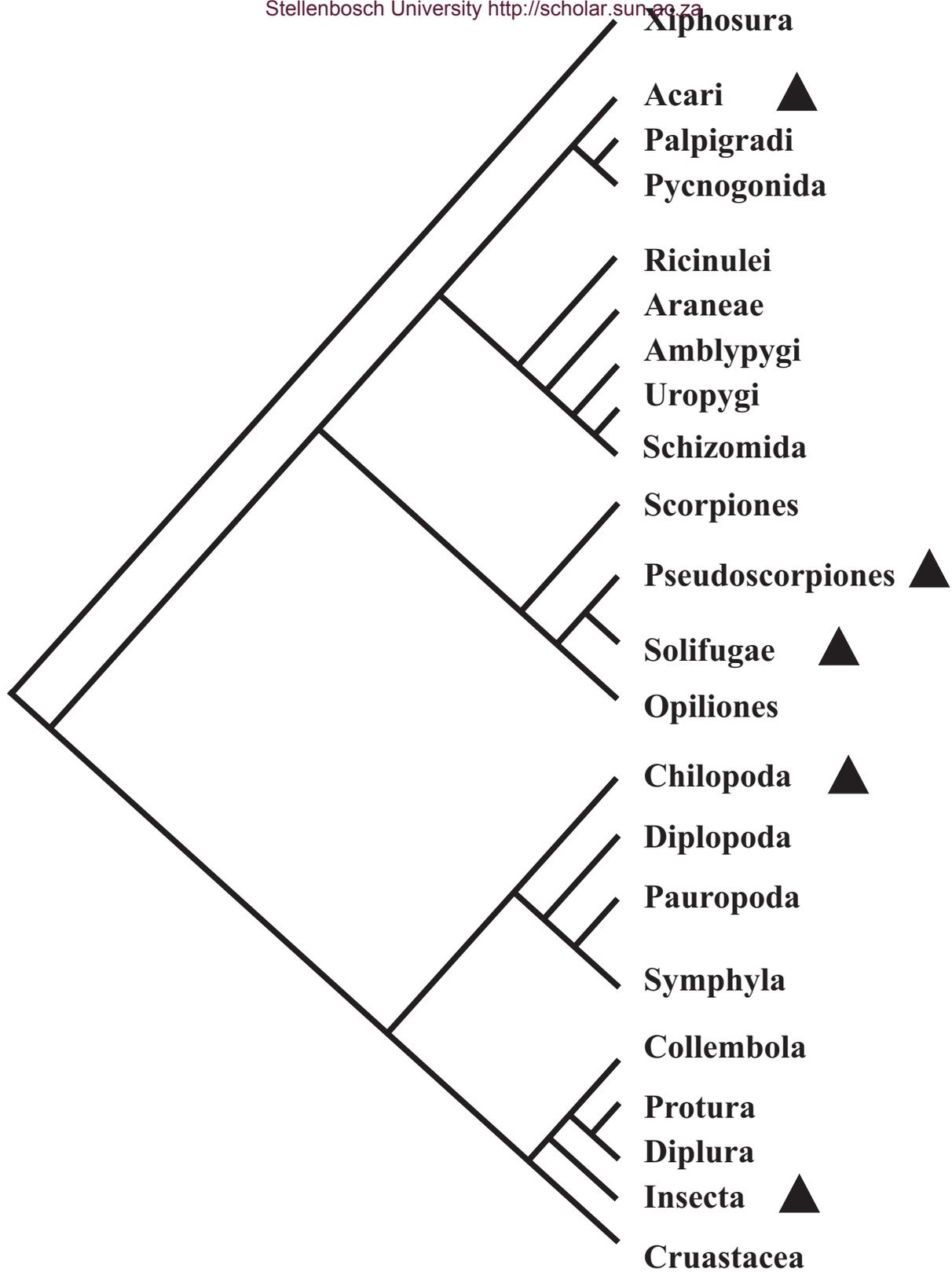
**Fig. 4** Outcome of the simulations using first order difference equations. Over a wide range of conditions the models produce patterns that are typical of cyclic gas exchange in insects, from (a) reasonably variable, though cyclic to (b) strongly cyclic and similar to some discontinuous gas exchange patterns (but with no F-period).

## Discussion

Gas exchange patterns have now been reported for 117 species from 18 insect orders. Although this represents an increase of nearly 125% in the number of orders for which data are available, it is clear that much remains to be learnt. 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., submitted ms.). 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 the DGC has evolved independently five times in the insects at the order level, this situation may change as additional species are investigated in each of the orders and as species from previously unstudied orders are examined. Even so, the independent evolution of the DGC 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 (Fig. 5), 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 (Tables 2 and 3) provides additional evidence for this idea, as does the fact that the coupled first order difference equations could not simulate this gas exchange pattern. 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. Levy and Schneiderman, 1966a, b; Kestler, 1985; Harrison, 1997) 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. Examination of Tables 2 and 3 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 ( $\chi^2 = 0.17$ ,  $p > 0.05$ ), by contrast with the predictions of the chthonic and hybrid exchange hypotheses (Lighton and Berrigan, 1995; Lighton, 1998). However, there are patterns of association between DGCs and xeric environments ( $\chi^2 = 5.67$ ,  $p < 0.05$ ), as might be predicted from the hygric hypothesis (Lighton, 1998; Chown and Nicolson, 2004). In essence, DGCs are equally frequent in xeric and mesic habitats (31 *vs.* 30 species), but non-DGC patterns are far more common in mesic than in xeric habitats (39 *vs.* 16 species). In other words, cyclic and continuous gas exchange at rest are much more likely in mesic than in xeric environments, than are DGCs, but DGCs can evolve in both kinds of environments. This suggests that xeric environments may indeed provide strong selection for DGC evolution (given that it seems to have evolved independently at least five times in the insects and four times in the arthropods), but that this is not the only reason for the evolution of DGCs.



**Fig. 5** The distribution of discontinuous gas exchange cycles (DGCs) across the Arthropod phylogeny (redrawn from Chown and Nicolson, 2004).

Unfortunately, there have been few tests of Bradley's (2000) oxidative damage hypothesis, though a forthcoming analysis of gas exchange in *Rhodnius prolixus* does provide evidence in favour thereof (T.J. Bradley and S.K. Hetz, personal communication). Nonetheless, there seems to be little pattern to DGCs from this perspective. Both winged and wingless species show DGCs ( $\chi^2 = 0.01$ ,  $p > 0.05$ ), despite their different oxygen demands (Reinhold, 1999; Addo-Bediako et al., 2002), and this gas exchange pattern is also present in both small and large species, which might be expected to differ in gas exchange modality (diffusion vs. convection) and relative tracheal cross-sectional area and length (Kestler, 1985). Likewise, the emergent property hypothesis does not seem to be supported for discontinuous gas exchange cycles. The coupled first order difference equations could not simulate the kinds of  $V \cdot CO_2$  traces that are characteristic of insects showing DGCs. Thus, the present data on the whole present some evidence for the hygric hypothesis, but little support for the others. At finer taxonomic resolutions such associations have also been identified (e.g. Duncan and Byrne, 2000; Chown and Davis, 2003), but these interspecific analyses have generally not been able to comply with the rigorous requirements for demonstrating adaptation (for discussion see Coddington, 1988; Brooks and McLennan, 1991; Reeve and Sherman, 1993; Davis et al., 2000; Schluter, 2000). Arguably, the reasons for the evolution of DGCs may also have to do with energy savings generally, and an analysis of the relationship between DGCs and the likelihood of diapause or quiescence would be particularly valuable in this regard (see Chown, 2002). Perhaps this might even be considered a sixth adaptive hypothesis (the energy conservation hypothesis).

By contrast with the case for DGC-like traces, the coupled equations reproduced patterns similar to cyclic gas exchange. This suggests that whenever there is a gas exchange

system where entry to it is modulated, such as by the regulation of spiracle cross-sectional area by both CO<sub>2</sub> and O<sub>2</sub> sensory systems (see Levy and Schneiderman, 1966a; Burkett and Schneiderman, 1967, 1974; Ramirez and Pearson, 1989 for examples and Miller, 1974; Chown and Nicolson, 2004 for review), cyclic gas exchange might result. The coupled equations also suggest that the actual pattern of gas exchange may well be highly dependent on the initial conditions of the system and the strength of the feedback, and could easily be altered by periods of enhanced demand on the system interrupting an otherwise quiescent state. If this were the case, gas exchange patterns should be highly variable within a species, should show considerable variation within individuals between repeated recordings, and should vary within an individual between periods of activity. Table 3 certainly suggests that the first prediction is supported, and Marais and Chown's (2003) investigation of a *Perisphaeria* sp. cockroach supports the second, with most individuals routinely showing two to three of the four gas exchange patterns found at rest. Moreover, additional investigations of this species (Fig. 6), and some of the cyclic gas exchange patterns presented for single individuals by Chown (2001), support the third prediction. In other words, between periods of activity the gas exchange pattern can change considerably. Thus, these predictions of the emergent property hypothesis all enjoy support. Partly basing this support on data from Marais and Chown (2003), who concluded that there is considerable repeatability in gas exchange patterns, and therefore greater among- than within-individual variation in the components of the DGC, does not constitute a contradiction of their work. They concluded that high repeatability means that gas exchange patterns and the components thereof satisfy one of the conditions for selection (see Endler, 1986; Bech et al., 1999), not that selection has already taken place.

Perhaps most significantly, 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), the first prediction of the emergent property hypothesis also seems to be supported because there is considerable variation among individuals in gas exchange pattern. This finding suggests that DGCs might likewise be an emergent property of the interacting CO<sub>2</sub> and O<sub>2</sub> setpoints, although they could not be modelled here. Therefore, there is good reason to undertake more sophisticated modelling work of interactions between the CO<sub>2</sub> and O<sub>2</sub> setpoints, as well as to examine, and, just as importantly, to report variation in gas exchange patterns within and between individuals (see Lighton, 1998; Chown, 2001 for additional discussion).

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., 1993c; 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 (reviews 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 (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 level of mechanistic investigations (Scheidermann and Williams, 1955; Lighton, 1988a; Lighton and Feener, 1989; Kestler, 1991; Lighton, 1992; Lighton et al., 1993c, d, Hetz et al., 1999; Chown and Holter, 2000; Lighton and Joos, 2002; Gibbs and Johnson, 2004). However, it has so far performed relatively poorly when it comes to laboratory selection and comparative approaches. It is therefore not surprising then that there has been poor resolution of the significance of the five competing hypotheses for the origin and maintenance of discontinuous gas exchange cycles, and that the pendulum of opinion continues to shift from one hypothesis to another depending on the study undertaken. Indeed, from the perspective of the hygric hypothesis it is becoming increasingly clear that interspecific analyses tend to provide support for it (Chown, 2002; Chown and Davis, 2003), whilst intraspecific analyses suggest the opposite (e.g. Hadley and Quinlan, 1993; Lighton and Berrigan, 1995; Chappell and Rogowitz, 2000; Rourke, 2000). Of course, these varying outcomes might be a consequence of the fact that DGCs have been pressed into service for more than one purpose, or have been modified several times from their original purpose to undertake some other task (Chown,

2002; Chown and Nicolson, 2004). However, it is difficult with the current information to come to a firm conclusion regarding either this idea or any of the alternatives. Remedying the situation does not mean undertaking 2-5 million studies to assess gas exchange patterns in all of the insects (Gaston, 1991). 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, 2001; Chown et al., 2003). This work could be combined with smaller scale comparative investigations, laboratory selection experiments (see Gibbs et al., 1997 for an excellent example of this combined approach), 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).

In conclusion, I have shown that broad scale analyses are providing some limited support for the hygric hypothesis, but that, despite much careful work, there is still a great deal to be done before a comprehensive understanding of the evolution of gas exchange patterns in insects will be realized. Fortunately, the work is proceeding apace, and the next few years are likely to see the kind of coherence that has long been hoped for (Lighton, 1996) emerging in this field.

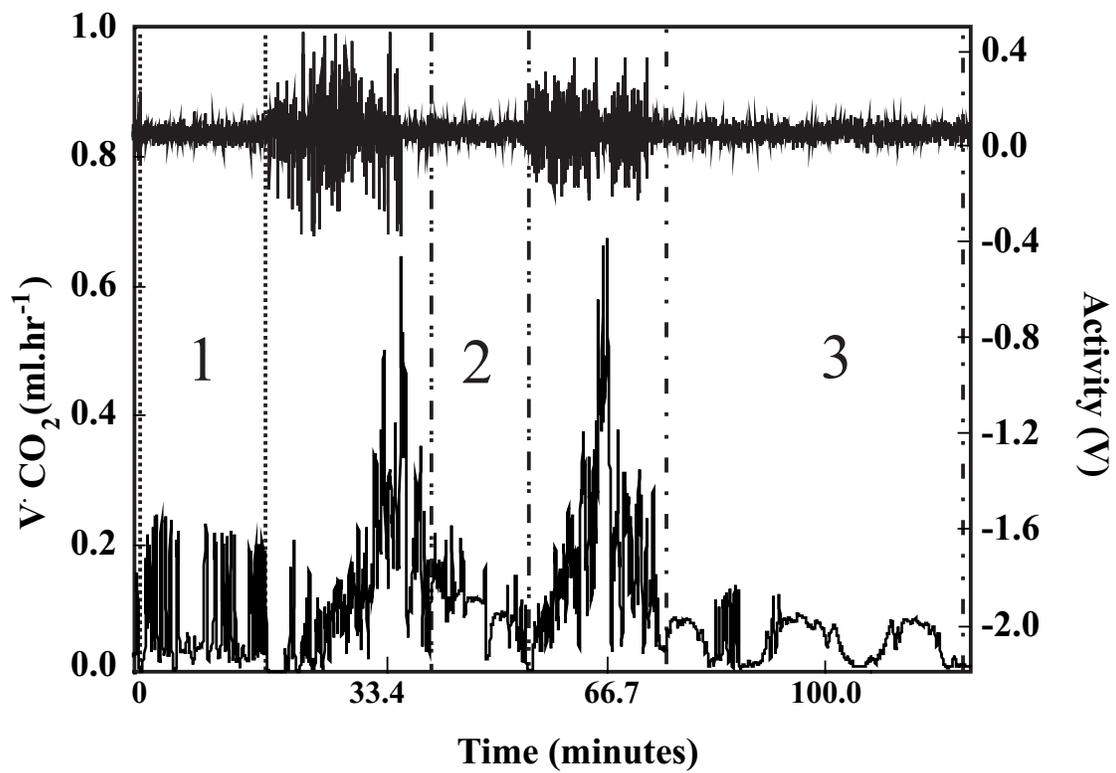
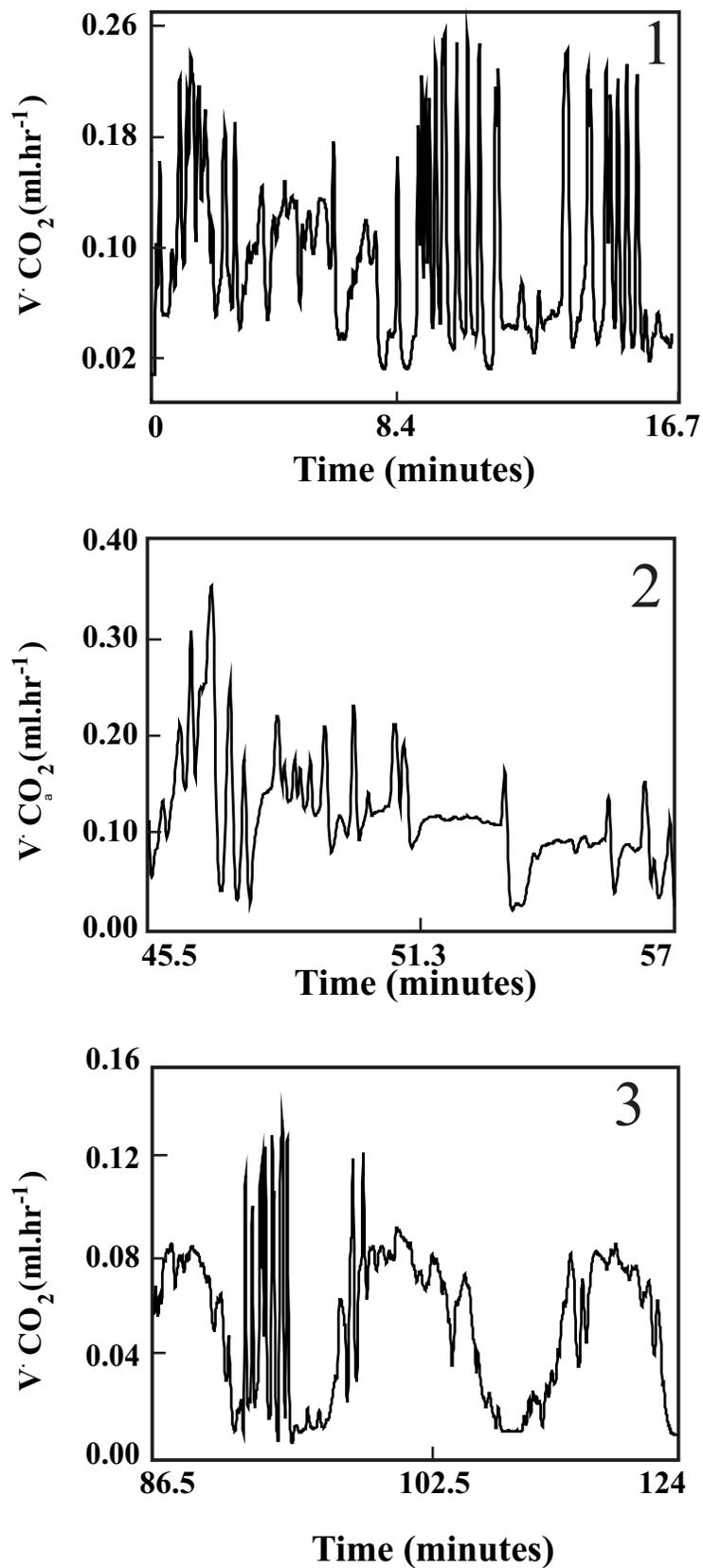


Fig. 6a



**Fig. 6** A gas exchange trace from a *Perisphaeria* sp. cockroach indicating substantial within-trace variation between the active periods. In (a) the entire trace is shown including periods of activity and inactivity (1, 2, and 3), while in (b) details of the periods of inactivity are shown.

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## **Chapter 4**

### **Investigation of the fractal network model: intra- and interspecific metabolic scaling in ants**

## Introduction

The relationship between physiological characteristics and body size can generally be described with the equation  $Y = aM^b$ , where  $Y$  is the physiological characteristic,  $a$  is the normalisation constant (the intercept of the log-log plot) and  $b$  is the scaling coefficient. The quarter power allometric scaling coefficient ( $b = 0.75$ ) was first proposed by Kleiber (1932) whose study was based on values from a few domesticated mammals and birds. Previously, the Euclidian model based on the body surface area to volume ratio was believed to represent the relationship between metabolic rate and body size best. Since Kleiber's work, many investigations have been made of what values the scaling coefficients should take and why this should be so (Stahl, 1967; Calder, 1981; Bartels, 1982; Heusner, 1982; Feldman and McMahon, 1983; Prothero, 1984; Schmidt-Nielsen, 1984; Hayssen and Lacy, 1985; Heusner, 1985). Recently, there has been a resurgence of interest in metabolic scaling (e.g. Cates and Gittleman, 1997; Lehmann et al., 2000; Dodds et al., 2001; Dreyer and Puzio, 2001; Frappell et al., 2001; Fujiwara, 2003; Hochachka et al., 2003; Santillán, 2003; Bokma, 2004; Brown et al., 2004; Horn, 2004; Savage et al., 2004; Suarez et al., 2004; Weibel et al., 2004), with both *Functional Ecology* and *Ecology* featuring special forums on the topic. This interest has been triggered by the recent work of West et al. (mainly 1997, but see also 1998, 1999, 2002), who have suggested that their fractal network model explains why physiological and even ecological characters (Gillooly et al., 2001; Allen et al., 2002; Brown et al., 2004) should scale with a coefficient of 0.75 or a multiple thereof. They state that this coefficient is a universal law.

The fractal network model is based on three key assumptions: 1) the branching of the circulatory or transport system is space-filling and fractal-like, 2) the smallest tubules or

capillaries in all organisms are of similar size, and 3) the energy used for nutrient transportation is always minimized. They concluded that the fractal-like structure of the system is common for all organisms and that this is the underlying reason behind the  $\frac{3}{4}$ -power scaling law.

However, there is considerable disagreement regarding the value of the metabolic scaling coefficient. The question keeps on arising: should the coefficient be 0.75, 0.67 or any specific value at all? Despite the fact that databases are larger and perhaps more complete, equipment more accurate, and statistical techniques perhaps more sophisticated, the value of the coefficient still remains unresolved. To date the empirical assumptions of the model have mainly been tested for birds (Stahl, 1967; Zar, 1969; Lasiewski and Calder, 1971; Prinzinger and Hänsler, 1980; Prinzinger et al., 1981; Bennett and Harvey, 1987) and mammals (Stahl, 1967; Bartels, 1982; Hayssen and Lacy, 1985; Frappell and Baudinette, 1995; Porter, 2001; Symonds and Elgar, 2002), with a handful of studies for reptiles (Andrews and Pough, 1985; Chappell and Ellis, 1987; Thompson and Withers, 1997; Maxwell et al., 2003).

Support for both 0.67 and 0.75 seems strong, although similar datasets often provide different results. For example, White and Seymour (2003) and Dodds et al. (2001) found that the coefficient is closer to  $\frac{2}{3}$  than to  $\frac{3}{4}$  for a large number of birds and mammals they investigated. However, Savage et al. (2004) reinvestigated the scaling coefficient for mammals (with similar data used as for the former two studies), and found support for the  $\frac{3}{4}$  coefficient. Furthermore, they criticized Dodds et al. (2001) for failing to take phylogenetic non-independence into account, and argued that White and Seymour's (2003) methods of doing so (averaging for mammal Orders) were biased for several reasons, but especially because of the reduction in mass range and sample size. Therefore, Savage et al. (2004) used a binning

technique claiming that “...closely related species are almost always similar in size...” and that “...it also prevents phylogenetic relatedness from having an undue influence” (p. 263). Each bin is 0.1 of the logarithmic mass (g) and the values within the bin are mean values for the species within the bin, thus each bin has a single data point. This technique supposedly corrects the data for the bias towards work on small mammals (< 1 kg). This is most certainly not the ideal approach, since the binning technique might be eliminating a great deal of the variance, which in turn will influence the slope. Moreover, binning similar-sized species is likely to lump a wide variety of not particularly closely related groups together, especially given the wide divergence between several orders that contain similar-sized species (see Murphy et al., 2001). Indeed, it is clear that the previous classification of the mammals (Novacek, 1992) might lead one to make such an assumption, whilst the more modern one (Murphy et al., 2001) would certainly not.

Although Savage et al. (2004) have shown that Maximal Metabolic Rate (MMR) should also scale with 0.75, Darveau et al. (2002) found that this is not the case. Darveau et al. (2002) reasoned that MMR has a higher coefficient than SMR/BMR because delivery rate of O<sub>2</sub> plays a major role at maximal MRs while a almost negligible role at “idling”. In addition, Weibel et al. (2004) also found that MMR scales with a coefficient of 0.872, which is statistically different from 0.75. Therefore, no consensus scaling coefficient has been determined for MMR.

Both Dodds et al. (2001) and Kozłowski and Konarzewski (2004) claimed that the assumptions of the fractal network model are inconsistent. Kozłowski and Konarzewski (2004) showed that the mathematics of the fractal network model contradicts one of its main assumptions. If the particular assumption (that all the terminal capillaries/ tubules are of

similar size) is not contradicted, then body size should be directly proportional to metabolic rate for the model to work mathematically, which completely contradicts the whole  $3/4$  scaling law. In addition, Kozłowski and Konarzewski (2004) disagree with one of the three major assumptions of the model, i.e. that all vessels/tubes should be self-similar space-filling fractals. Kozłowski and Konarzewski (2004) provide evidence that most plants, insects, reptiles and amphibians do not always conform to the assumption, and supply systems are not always self-similar or necessarily even form a branching network.

Thus, both the theoretical and the empirical assumptions of the fractal network model are subject to some doubt, and are certainly much more controversial than parts of the literature would suggest. In this regard, one of the most significant, testable empirical predictions of the fractal network model is that both the inter- and intraspecific scaling coefficients should be identical (0.75). By contrast, Kozłowski et al. (2003a) predict that a dataset with a phylogenetically wider range of species would be more likely to result in a scaling coefficient in the region of 0.75, whereas a single species could have a slope very different from 0.75. These two opposite predictions should be readily testable. However, as has been pointed out by several authors (e.g. Spicer and Gaston, 1999), intraspecific body size variation in most species (including mammals) is limited, which means that it is difficult to distinguish allometric variation from other forms of variation within a species (see Currie, 1993; Bosch et al., 2000). For some insects, such as bees, worker ants, and dung beetles (Emlen and Nijhout, 2000; Peat et al., in press) this is not the case, and therefore these species could be ideal for testing the above predictions. Indeed, if the fractal network model is to be considered universal, the model should also apply to insects because they constitute as much as 70% of the species richness on the planet.

A consensus scaling coefficient for metabolic rate has been calculated for insects by Lighton and Fielden (1995), and modified by Lighton et al. (2001). The latter also concluded that arthropods (excluding ticks and scorpions) should have a metabolic scaling coefficient of 0.856 at 25°C. The coefficient differs from both 0.75 and 0.67. These and other data were also used by Addo-Bediako et al. (2002) to investigate metabolic cold adaptation, concluding incidentally that the scaling coefficient is 0.75. However, it has been argued that the data used to derive these conclusions are not necessarily representative of the insects and/or arthropods, because they are dominated by species of beetles and ants, many of which are flightless and might therefore have lower metabolic rates than winged species (Reinhold, 1999). Duncan et al. (2002) raised this point explicitly and noted that additional data are required for insects, and Terblanche et al. (2004) provided support for these assertions by demonstrating exceptionally high resting metabolic rates for the fly *Glossina pallidipes*. In addition, studies reporting metabolic rate for insects and other arthropods have generally not been done with the aim of determining a scaling coefficient, and this means that standardization problems might arise in broader scale comparisons (see Addo-Bediako et al., 2002 for discussion), so biasing results further. In a recent review of these problems, Chown and Nicolson (2004) recommended that careful studies investigating scaling coefficients using standardized methods, at both the intra- and interspecific levels, should be undertaken.

Here this is partially done by investigating intra- and interspecific variation in standard metabolic rate in a variety of ant species using new data as well as data extracted from the literature. Because many ants are highly polymorphic, they are ideal for testing these hypotheses. Furthermore, all worker ants are non-reproductive female adults, thus restricting the variation that might arise for other reasons (see above), and if they are drawn from the

same colony are closely related too. Thus, here I use three species of ants to determine intraspecific scaling coefficients while adding data from the literature to determine an interspecific scaling coefficient, thus testing the prediction that both inter- and intraspecific scaling relationships should have a slope of 0.75.

## **Materials and Methods**

### *Intraspecific scaling: collection and laboratory experiments*

Many species of ants are highly polymorphic and adult ants are easy to distinguish from juveniles. Because of the great size variation in some ant species, polymorphic ants are suitable for examining intraspecific scaling coefficients. Harvester ants, *Messor capensis* (Hymenoptera, Formicidae, Myrmecinae) specimens were collected from Tierberg (Western Cape - 33°09.930'S 22°16.077'E), *Anoplolepis steinergroeveri* (Hymenoptera, Formicidae, Formicinae) specimens from the Cederberg Wilderness Area (Western Cape) (32°24.471'S 19°05.079'E) and a *Camponotus maculatus* (Hymenoptera, Formicidae, Formicinae) colony from the Stellenbosch mountain (33°58.161'S 18°53.870'E). All ants were kept in an insulated container during transport to prevent heat shock. Separate *M. capensis* sub-colonies were held at 22°C for three days before experiments started. Sub-colonies were held in containers filled with soil from their colony and were fed oats *ad libitum*. Pilot studies showed that starving an ant leads to a decline in metabolic rate and death within a relatively short time (see also Lighton, 1989). Therefore, ants were starved for approximately one hour before each trial to minimize the effect of specific dynamic action without causing extensive reduction in metabolic rate (see Chown and Nicolson, 2004 for rationale). Whole colonies of *A.*

*steinergroeveri* and *C. maculatus* were also kept for three days at c. 22- 25°C before experiments commenced.

Specimens were carefully handled using either light forceps or plastic containers for transfer. Each specimen was weighed at the start and at the end of each metabolic rate measurement (Mettler Toledo AX504 and a Mettler UMX2, Columbus, OH, USA, with resolutions of 0.1 mg and 0.1µg, respectively). When measuring gas exchange the incubator was kept dark to reduce activity. To keep the temperature of the cuvette and the air flowing through the cuvette constant, the tubing and the cuvette were placed in an incubator (Labcon, South Africa) set at  $20 \pm 0.2$  °C (mean  $\pm$  SD). Air, scrubbed of CO<sub>2</sub> (using soda lime) and water (using Drierite (Ohio, USA)) flowed through the cuvette at a flow rate of 100ml/min (regulated with a mass flow controller (Sidetrak Mass Flow Control Valve, Monterey, USA)). The cuvette was connected to a flow-through respirometry system (Sable Systems Inc., Henderson, Nevada, USA). The CO<sub>2</sub> production of the ants was recorded at one-second intervals using a calibrated infrared gas analyzer (Li7000 Li-Cor, Lincoln, Nebraska, USA). A Sable Systems AD-1 activity detector was used to detect any movement of the ant in the cuvette during the experiment. The AD-1 registers activity as a value between -5 and +5 Volts, where little deviation from the mean indicates that the animal is inactive, whereas a large deviation indicates high levels of activity (for detail see [www.sablesys.com/ad1.html](http://www.sablesys.com/ad1.html)). Data were eliminated from the analyses in cases where the ants showed activity. To avoid the potential influence of pheromones on the behaviour of individuals, the cuvettes were cleaned thoroughly with ethanol between all experimental trials. The recorded metabolic rate data were then exported from the Li7000 software via Microsoft Excel format to DATACAN V (Sable Systems). DATACAN V was used to calculate the mean metabolic rate (mlCO<sub>2</sub>.h<sup>-1</sup>)

(standard temperature and pressure). The mean metabolic rates were calculated from the 5 – 10 minutes where the metabolic rate was lowest at rest.

An Ordinary Least Squares (OLS) linear regression model was used to examine the relationship between  $\log_{10}$  metabolic rate (ml/hr) and  $\log_{10}$  mass (g) for each of the three ant species. Reduced Major-Axis (RMA) regression was not used because the measurement error in the body size is most likely less than 1/3 of the measurement error of the metabolic rate (McArdle, 1988).

Statistical comparisons of slopes against the parametric values of 0.75 and 0.67 were made using a Student's t-test (Zar, 1999). T-tests were used to compare each of the intraspecific slopes with the phylogenetically corrected interspecific slope, using the comparison of slopes from multiple regressions method (Zar, 1999)

#### *Interspecific scaling*

For the determination of an interspecific scaling coefficient for the Formicidae, a dataset from the Anglophone literature was compiled. Table 1 provides the list of the data found in the literature that was suitable for these analyses. Suitability was assessed as data on worker ants, with methods that were referable to standard techniques, and where ants were not active. The data were plotted and all outliers identified and excluded. These tended to be data that could not be accurately read off graphs where tabulated data in the original work were not available. Data from unknown genera were also eliminated. The data were converted from the original units given for MR to  $\mu\text{W}$  using the RQ given by the author or a RQ of 0.84 (Addo-Bediako et al., 2002). All masses were converted to grams. Data for closed and open system methods were analysed separately as well as together. The  $\log_{10}$  of the mean  $V \dot{CO}_2$  ( $\mu\text{W}$ ) released was

used as the dependent variable and body weight (g) as the independent variable. Analyses were based on raw data as well as on data corrected for phylogenetic non-independence (Felsenstein, 1985; Garland et al., 1992, 1999). Phylogenetic independent contrasts were used rather than the binning technique recommended by Savage et al. (2004) because of the effect binning has on the variation and because species that are distantly related might be grouped together. The phylogeny on which to base the phylogenetically independent analyses was obtained from Bolton (1994) and Grimaldi and Agosti (2000) (see also Parr et al., 2003). The  $\log_{10}$  data were converted to phylogenetically independent contrasts using the PDTREE module from the Phenotypic Diversity Analysis Program (PDAP - version 5.0 Garland et al., 1992, 1999). Polytomies were allocated a branch length of zero and branch lengths of one were assigned to all the other branches (e.g. branch length for genus level, subfamily level – see Fig. 1a-c) because branch lengths were not available from the source literature. The extent to which equal branch lengths corrected for phylogenetic non-independence was assessed by examining the relationships between the absolute values of the standardized contrasts and the square roots of their branch lengths (Garland et al., 1992; Díaz-Uriarte and Garland, 1998). These were found to be non-significant, thus confirming the fact that the contrasts had eliminated phylogenetic signal.

**Table 1** Species names with references and the method used to obtain metabolic rate data are indicated here. Information on whether the ants were collected in the field or whether laboratory colonies were used is also indicated. The additional information refers to methods that are not standard, such as using groups, or where data were read from a graph. These data were used to determine an interspecific slope of metabolic rate vs. body mass for Formicidae.

Species	Author(s)	Method	Lab/ Field	Temp.	Additional Information	Mass (g)	MR ( $\mu$ W)
<i>Anoplolepis steinergroeveri</i>	From this study	Open, Sable	Field	20		0.00495	9.952
<i>Aphaenogaster cockerelli</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.00472	82.540
<i>Atta laevigata</i>	Beraldo and Mendes, 1982	Closed, Warburg	Field	25		0.015	35.200
<i>Atta sexdens rubropilosa</i>	Hebling et al., 1992	Closed, Warburg	Lab	25		0.015	43.069
<i>Camponotus detritus</i>	Lighton, 1990	Open, Sable	Field	26		0.0429	83.548
<i>Camponotus fulvopilosus</i>	Lighton, 1989	Open, Applied Electro chemistry S-3A	Field	25	MR from equation	0.043	63.437
<i>Camponotus herculeanus</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.0269	169.824
<i>Camponotus laevigatus</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.0192	117.7483
<i>Camponotus maculatus</i>	From this study	Open, Sable	Field	20		0.0425	36.156
<i>Camponotus sericeiventris</i>	Lighton and Gillespie, 1989	Closed, constant volume	Field	28	Temp from 25 to 30	0.0402	90.133

Species	Author(s)	Method	Lab/ Field	Temp.	Additional Information	Mass(g)	uW
<i>Camponotus vafer</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.00451	58.769
<i>Camponotus vicinus</i>	Lighton and Garrigan, 1995	Open, Sable	Field	25	Decapitated	0.107	143.5
<i>Cataglyphis bicolor</i>	Lighton and Wehner, 1993	Open, IRGA TR-2	Lab	25	MR from equation	0.034	37.808
<i>Chelaner rothsteini</i>	Davison, 1987		Field	25	Dry Mass, 50 - 100 specimens measured at once	0.00025	1.092
<i>Eciton hamatum</i>	Bartholomew et al., 1988	Open, Ametek SA- 3/II	Field	25		0.006	25.323
<i>Forelius foetidus</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.0001	1.346
<i>Formica exsecta</i>	Jensen and Nielsen, 1975	Closed, Warburg	Field	25		0.00405	21.944
<i>Formica fusca</i>	Jensen and Nielsen, 1975	Closed, Warburg	Field	25		0.00441	9.646
<i>Formica occulta</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.0013	19.507
<i>Formica pratensis</i>	Jensen and Nielsen, 1975	Closed, Warburg	Field	25		0.00717	31.421
<i>Lasius alienus</i>	Jensen and Nielsen, 1975	Closed, Warburg	Field	25		0.00138	6.036
<i>Lasius flavus</i>	Jensen and Nielsen, 1975	Closed, Warburg	Field	25		0.00258	11.508
<i>Lasius niger</i>	Jensen and Nielsen, 1975	Closed, Warburg	Field	25		0.00174	6.092
<i>Lasius sitiens</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.00029	3.239

Species	Author(s)	Method	Lab/ Field	Temp.	Additional Information	Mass(g)	uW
<i>Leptogenys attenuata</i>	Duncan and Crewe, 1993	Open, Li-Cor	Field	25		0.004	12.32
<i>Leptogenys nitida</i>	Duncan and Crewe, 1993	Open, Li-Cor	Field	25		0.00172	5.229
<i>Leptogenys schwabi</i>	Duncan and Crewe, 1993	Open, Li-Cor	Field	25		0.00896	20.966
<i>Leptothorax acerovororum</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.00037	5.114
<i>Leptothorax unifasciatus</i>	Martin, 1991	Closed, Gilson Differential	Lab	20	40 ind., 1 year in lab only	0.000491	1.465
<i>Messor capensis</i>	From this study	Open, Sable	Field	20		0.0137	27.077
<i>Messor capitatus</i>	Nielsen and Baroni- Urbani, 1990	Closed, Micro- Warburg	Field	25	Conversion to fresh mass: x 0.363 by authors	0.00314	16.180
<i>Messor julianus</i>	Lighton and Berrigan, 1995	Open, Sable TR-3	Field	24		0.00509	6.648
<i>Messor pergandei</i>	Lighton and Berrigan, 1995	Open, Sable TR-3	Field	24		0.00719	13.095
<i>Myrmica alaskensis</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.00091	10.652
<i>Myrmica rubra</i>	Jensen and Nielsen, 1975	Closed, Warburg	Field	25		0.00276	6.826
<i>Paraponera clavata</i>	Fewel et al., 1996	Open, "Pre-Sable Systems"	Field	27		0.19	494.840
<i>Pogonomyrmex californicus</i>	Quinlan and Lighton, 1999	Open, Sable	Field	25		0.00592	9.308
<i>Pogonomyrmex maricopa</i>	Nielsen, 1986	Closed, Warburg	Field	25		0.00378	35.823

<b>Species</b>	<b>Author(s)</b>	<b>Method</b>	<b>Lab/ Field</b>	<b>Temp.</b>	<b>Additional Information</b>	<b>Mass(g)</b>	<b>uW</b>
<i>Pogonomyrmex occidentalis</i>	Quinlan and Lighton, 1999	Open, Sable	Field	25		0.00796	12.805
<i>Pogonomyrmex rugosus</i>	Quinlan and Lighton, 1999	Open, Sable	Field	25		0.0143	27.641
<i>Solenopsis invicta</i>	Vogt and Appel, 1999	Open, Sable TR-3	Field	25		0.00296	5.586
<i>Tetramorium caespitum</i>	Jensen and Nielsen, 1975	Closed, Warburg	Field	25		0.00069	2.426

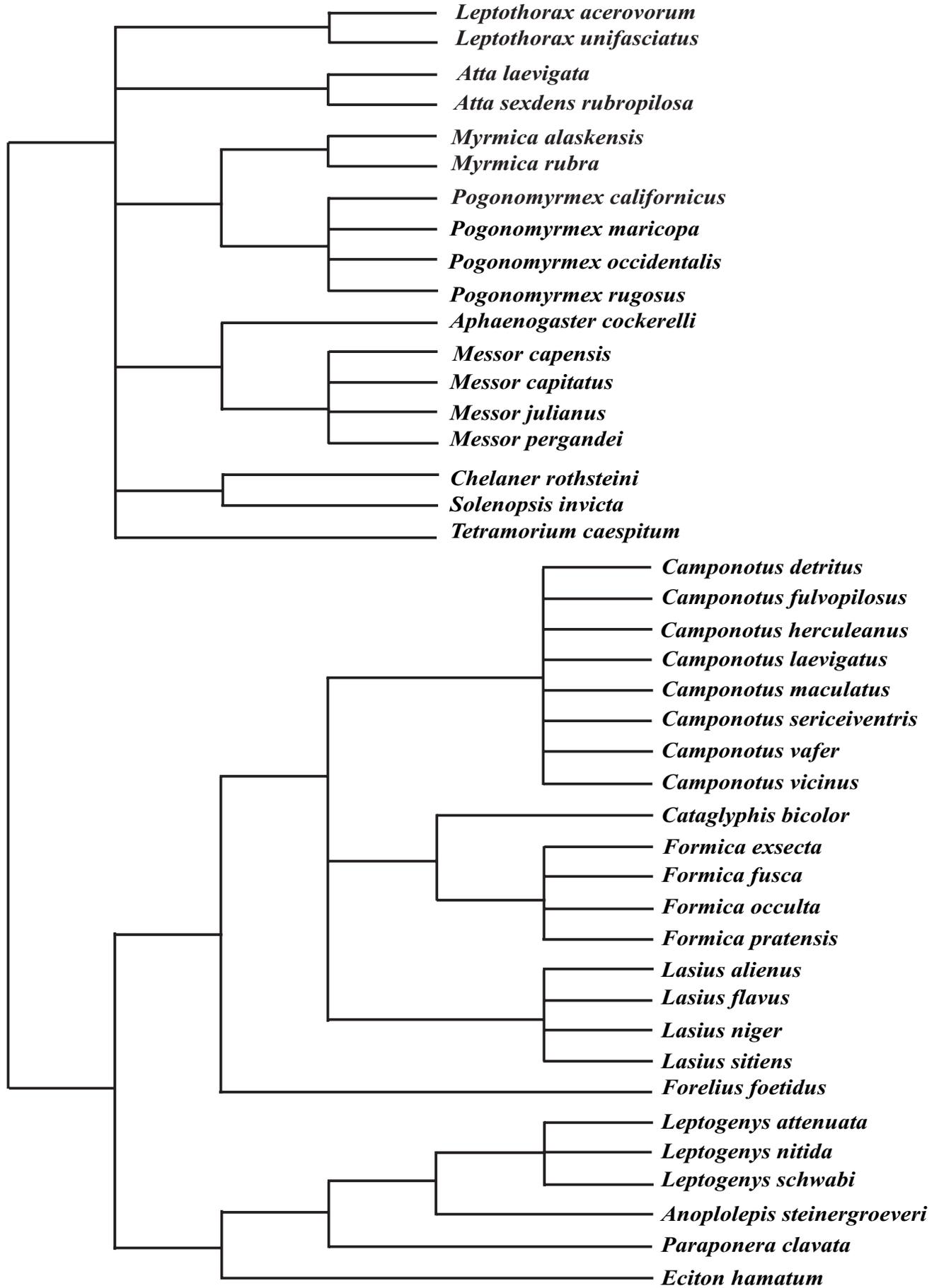


Fig. 1a

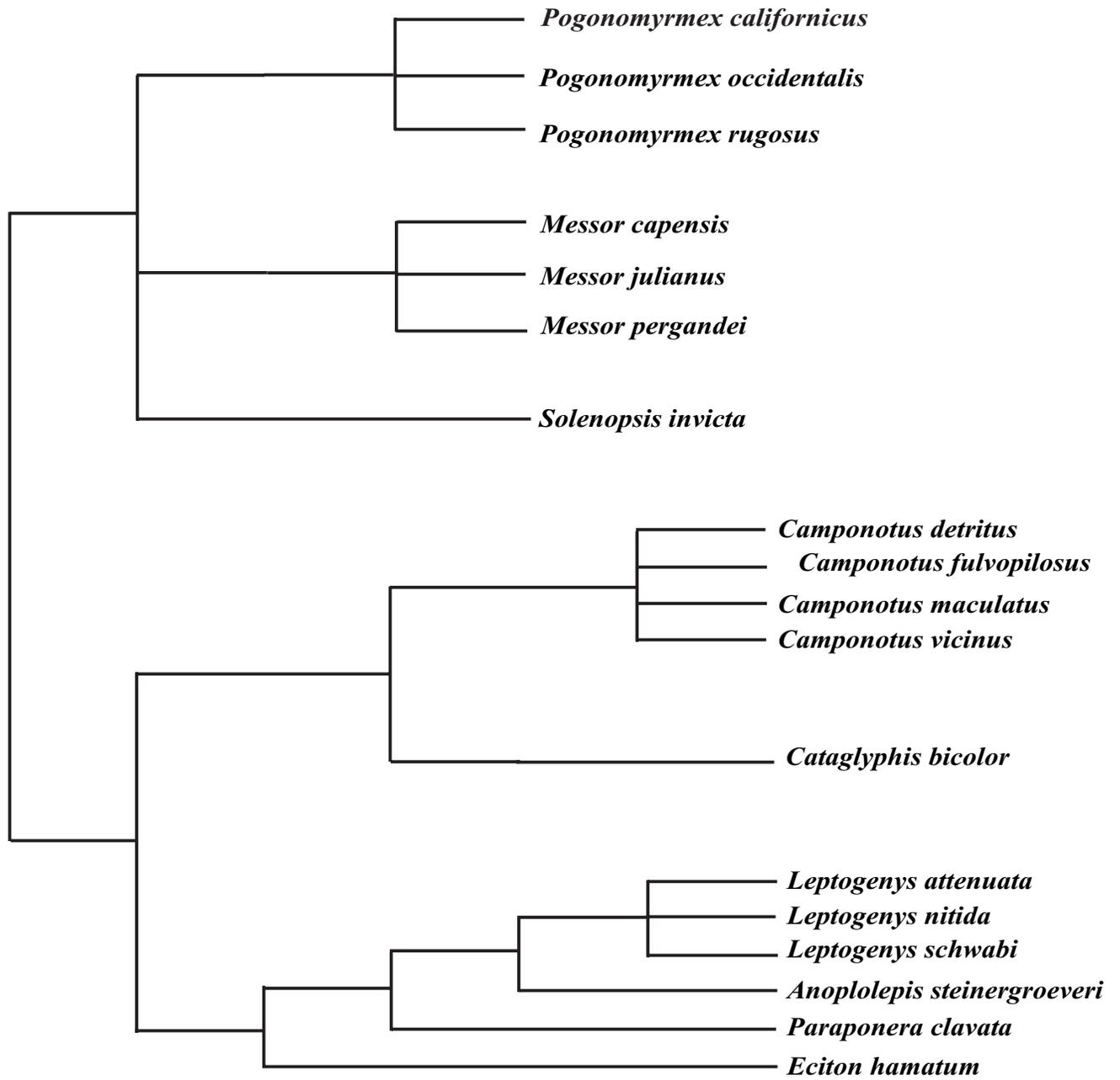
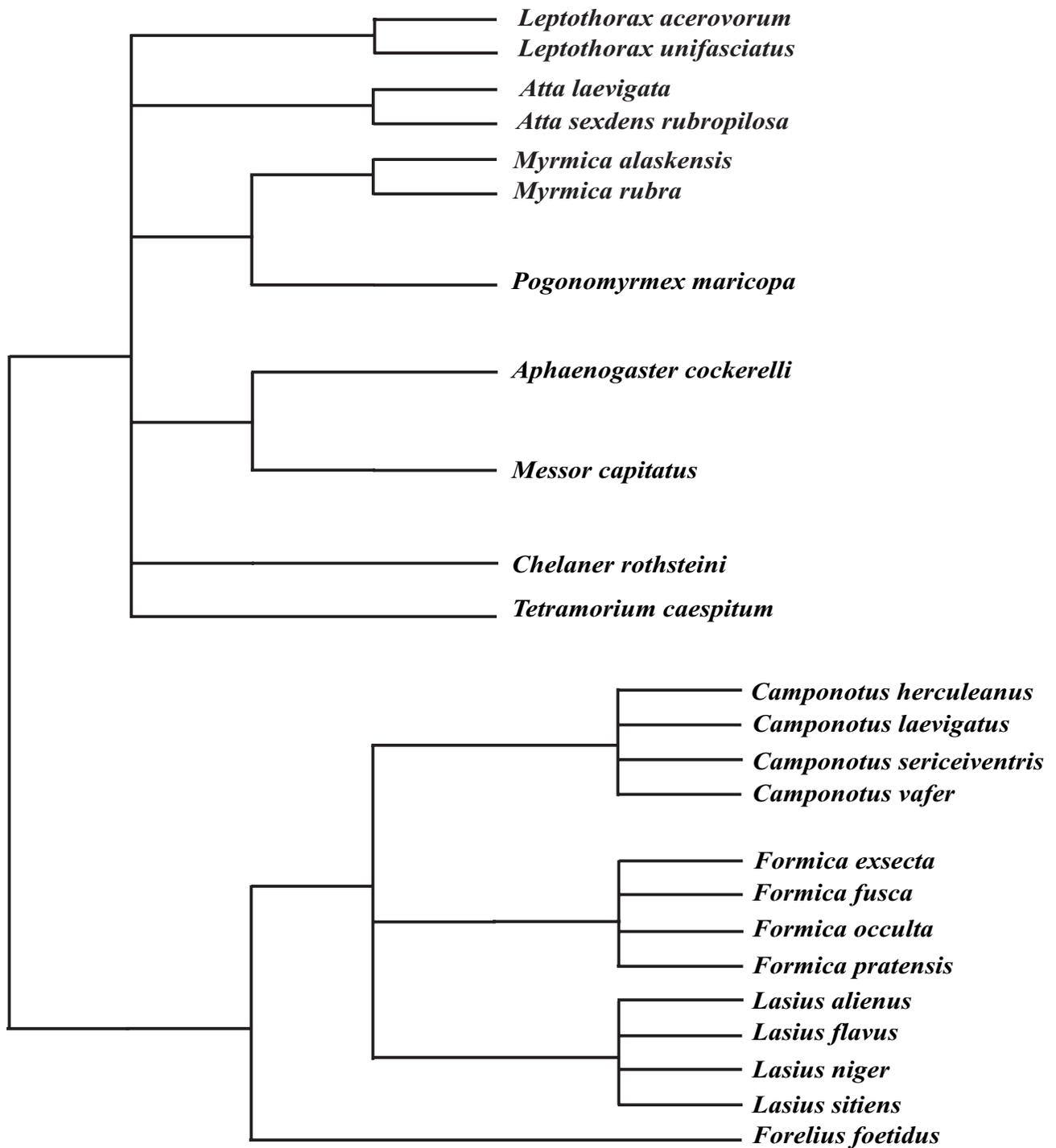


Fig. 1b



**Fig. 1c**

**Fig. 1** Phylogeny relationships of the Formicidae were obtained using Bolton (1994) and Grimaldi and Agosti (2000). Phylogenetic comparisons were made with combined methods (a), the open system (b) and the closed systems (c) separately.

## Results

### *Intraspecific scaling*

The intraspecific scaling coefficients derived from the OLS linear regression differed substantially for the three species investigated (see Table 2 and Fig. 2 a-c). The slopes for all three of the ant species differed significantly from a slope of 0.75 (*Anoplolepis steinergroeveri*:  $t = 4.556$ ,  $n = 38$ ,  $p < 0.001$ ; *Camponotus maculatus*:  $t = 3.177$ ,  $n = 51$ ,  $p < 0.005$ ; and *Messor capensis*:  $t = 2.463$ ,  $n = 49$ ,  $p < 0.02$ ) as well as from a slope of 0.67, with the exception of *Camponotus maculatus* that did not differ significantly from 0.67 (*Anoplolepis steinergroeveri*:  $t = 3.635$ ,  $n = 38$ ,  $p < 0.001$ ; *Camponotus maculatus*:  $t = 1.761$ ,  $n = 51$ ,  $p > 0.05$ ; and *Messor capensis*:  $t = 2.853$ ,  $n = 49$ ,  $p < 0.01$ ). The mean of the three intraspecific slopes was 0.728 (SE = 0.283) and was not different from 0.75 nor from 0.67 ( $t = 0.106$  and  $t = 0.189$  respectively,  $n = 3$ ,  $p > 0.05$ ).

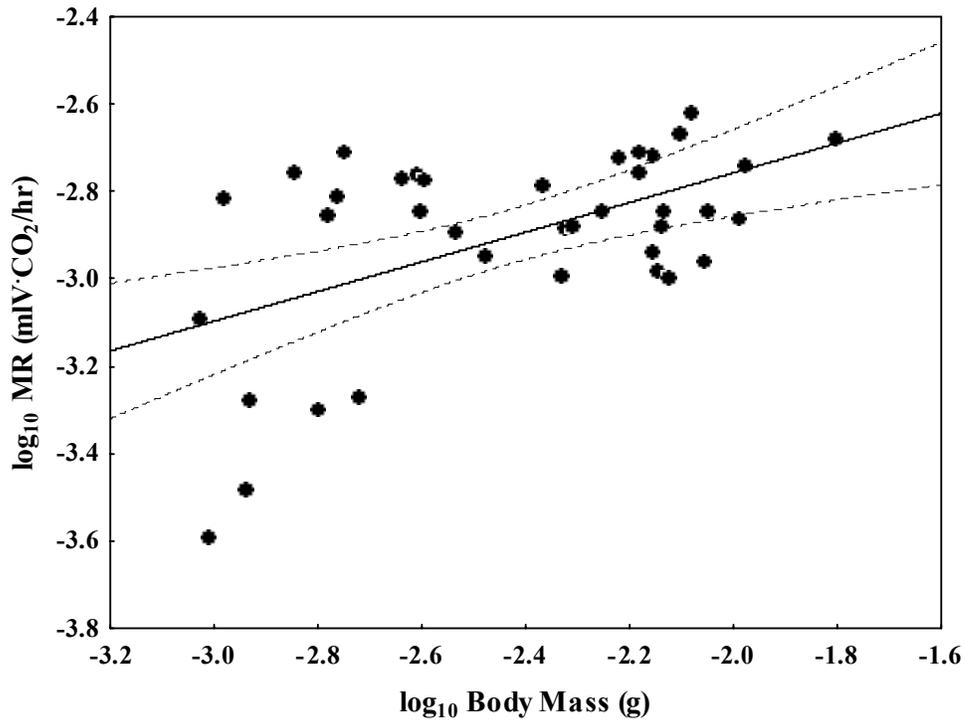
### *Interspecific scaling*

When the data for the open and closed methods were combined, the slope of 0.72 ( $R^2 = 0.77$ ; see Table 2 and Fig. 3 consensus slope) was not significantly different from 0.75 ( $t = 0.419$ ,  $n = 42$ ,  $p > 0.5$ ) nor from 0.67 ( $t = 0.937$ ,  $n = 42$ ,  $p > 0.2$ ). When analysing the open and closed system methods separately, the slope values were substantially different from the previous value and in each case the coefficient of determination also increased (open:  $b = 0.88$ ,  $R^2 = 0.91$  and closed:  $b = 0.84$ ,  $R^2 = 0.81$ , Fig. 3 and Table 2). Moreover, while both slopes were not significantly different from 0.75 (open system:  $t = 1.930$ ,  $n = 18$ ,  $p > 0.05$ , and closed system:  $t = 1.033$ ,  $n = 24$ ,  $p > 0.05$ ), the open system method differed significantly from 0.67 while the closed system did not (open system:  $t = 3.12591$ ,  $n = 18$ ,  $p < 0.01$ , and closed system:  $t = 2.00677$ ,  $n = 24$ ,  $p > 0.05$ ).

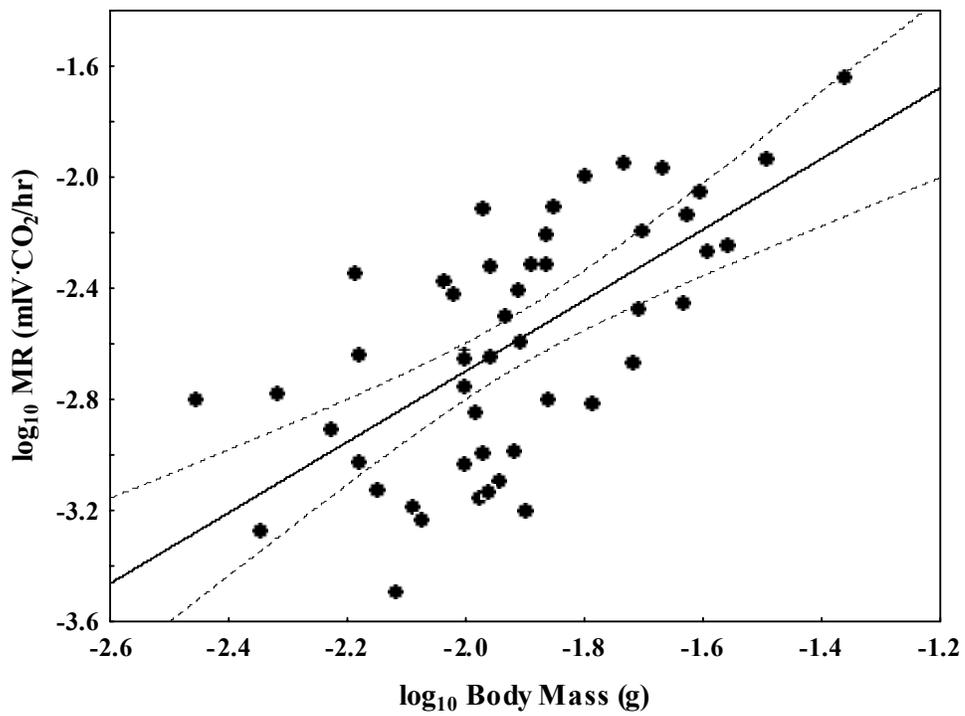
**Table 2** Summary statistics for the intra- and interspecific regressions of ant metabolic rate and body size. Standard errors of the estimates were not provided for the regressions from the phylogenetically corrected data.

	<b>F</b>	<b>df</b>	<b>p</b>	<b>R<sup>2</sup></b>	<b>slope</b>	<b>slope<sub>SE</sub></b>	<b>Intercept</b>	<b>Intercept<sub>SE</sub></b>	<b>Std. Error of estimate</b>
<b>Intraspecific</b>									
<i>Anoplolepis steinergroeveri</i>	14.23	1, 36	0.0005	0.28	0.34	0.09	-2.08	0.22	0.189
<i>Camponotus maculatus</i>	92.43	1, 49	<0.0001	0.65	0.56	0.06	-1.49	0.09	0.150
<i>Messor capensis</i>	35.80	1, 47	<0.0001	0.43	1.27	0.21	-0.15	0.41	0.331
<b>Interspecific</b>									
Combined data	139.9	1, 40	<0.0001	0.78	0.72	0.06	2.91	0.15	0.283
Open system method	162.3	1, 16	<0.0001	0.91	0.88	0.07	3.05	0.14	0.160
Closed system method	96.8	1, 22	<0.0001	0.82	0.84	0.09	3.33	0.23	0.279
<b>Phylogenetically corrected</b>									
Combined data	38.46	1, 20	<0.0001	0.49	0.55	0.09	0	0	
Open system method	175.39	1, 10	<0.0001	0.92	0.94	0.07	0	0	
Closed system method	26.22	1, 11	<0.001	0.54	0.63	0.12	0	0	

**A**

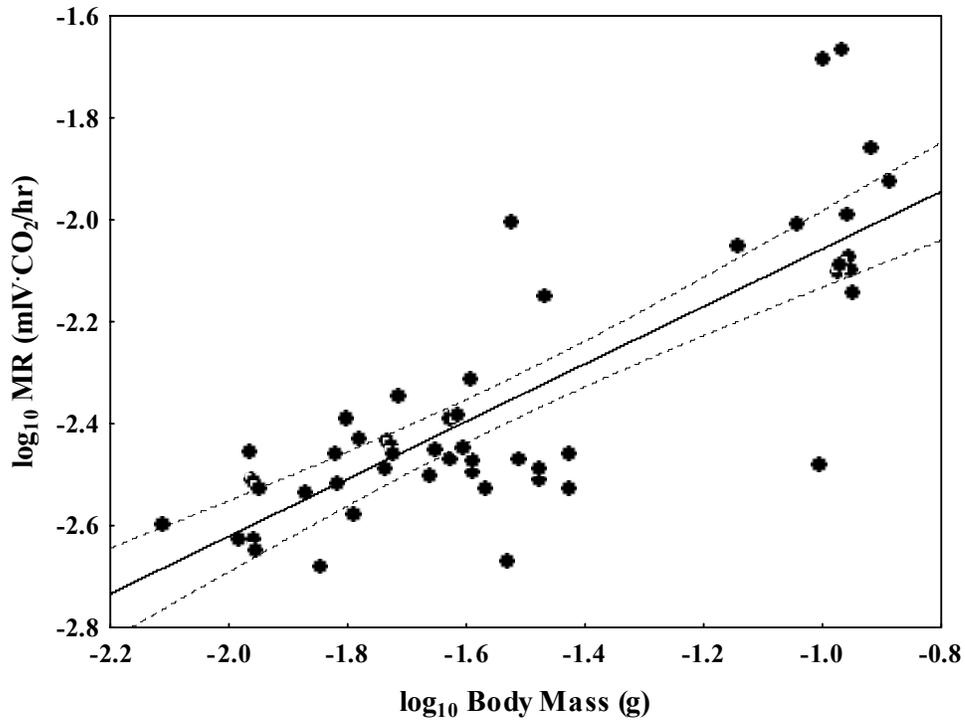


**B**



**Fig. 2 a and b**

C

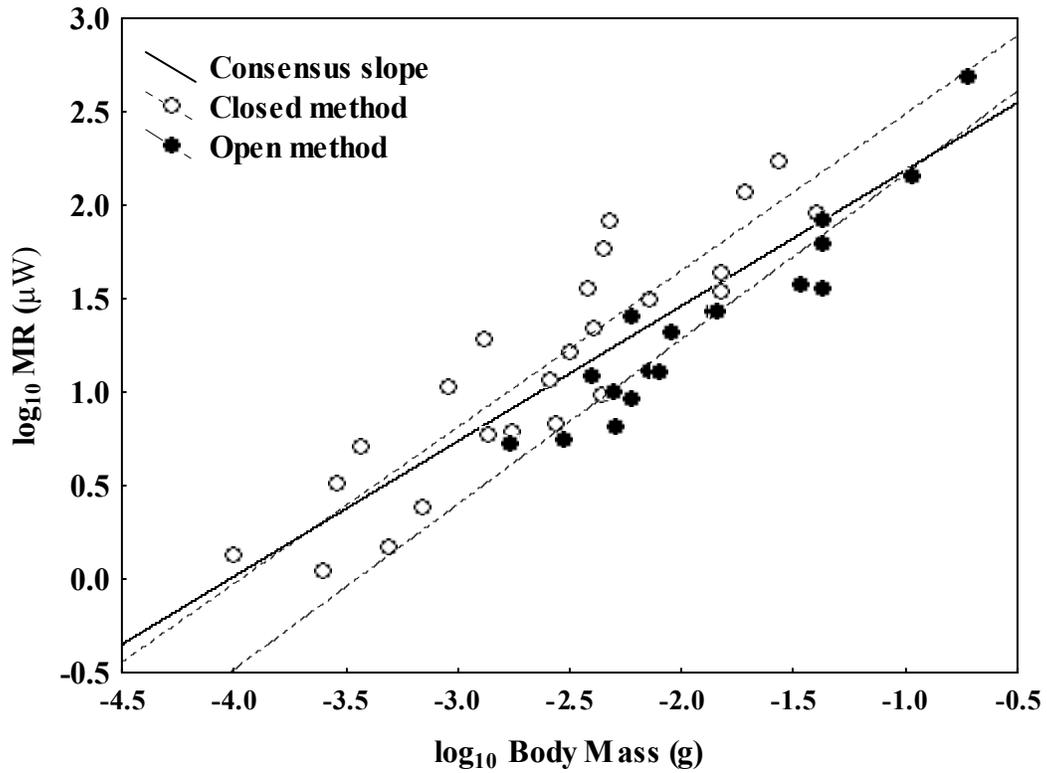


**Fig. 2** Intraspecific regressions of  $\log_{10}$  standard metabolic rate ( $\text{mIVCO}_2/\text{hr}$ ) on  $\log_{10}$  body mass (g) at 20°C: a) *Anoplolepis steinergroeveri* (n = 38), b) *Messor capensis* (n = 49) and c) *Camponotus maculatus* (n = 51). The 95% confidence limits are indicated by dotted lines.

Analyses using phylogenetic independent contrasts for both of the respirometry methods together resulted in a slope of 0.55 ( $F_{1, 20} = 38.46$ ,  $p < 0.0001$ ) that included 0.67 (note that  $n$  was reduced by 20 degrees of freedom because of polytomies;  $t = 1.274$ ,  $df = 20$ ,  $p > 0.02$ ) but excluded 0.75 ( $t = 2.200$ ,  $df = 20$ ,  $p < 0.05$ ). The open system data reanalysed with phylogenetic independent contrasts showed an increase in slope ( $b = 0.937$ ), and both theoretical slopes were excluded (0.67:  $t = 3.814$ ,  $df = 10$ ,  $p < 0.01$ ; 0.75:  $t = 2.640$ ,  $df = 10$ ,  $p < 0.05$ ). The closed system however, showed a decline in slope ( $b = 0.630$ ) and did not differ significantly from either 0.67 or 0.75 ( $t = 0.302$  and  $t = 0.977$  respectively,  $df = 11$ ,  $p > 0.2$ ).

When the slope from the phylogenetically independent analysis of the combined data ( $b = 0.55$ ) was set as a “parametric model”, and compared to the intraspecific slopes, there were no significant differences for any of the species (*A. steinergroeveri* :  $t_{40, 36} = 0.620$ ,  $p > 0.05$ ; *M. capensis* :  $t_{40, 47} = 0.053$ ,  $p > 0.05$  ; *C. maculatus* :  $t_{40, 49} = 0.2.432$ ,  $p > 0.05$ ).

A



**Fig. 3** The scaling relationship between  $\log_{10}$  SMR ( $\mu$ W) and  $\log_{10}$  Body Mass (g) investigated for Formicidae with the closed system method and the open system method separately and the consensus slope for these.

## Discussion

The present analysis does not support the prediction from the fractal network model (West et al., 1997) that both the intra- and interspecific slopes of the relationship between mass and metabolic rate should be identical and equivalent to 0.75. Rather, the fact that the intraspecific scaling relationships differ significantly from 0.75 suggests that at the intraspecific level, the slope of the relationship can assume a variety of values. This was predicted by the model of Kozłowski et al. (2003a, b), rather than that of West et al. (1997). West et al. (2002) claimed that a slope of 0.75 should be obtained irrespective of the hierarchical level at which the analysis is done. Therefore, this study found support rather for Kozłowski et al.'s (2003 a, b) model than for the fractal network model (West et al., 1997).

However, the fact that the intraspecific slopes for *Camponotus maculatus* and *Anoplolepis steinergroeveri* were less than the interspecific value contradicts the predictions made by Kozłowski et al. (2003b). This may be due to the fact that their model is for the optimization of different individuals within a species, whereas for two of the three species used in this study, single colonies were investigated where individuals are much more closely related than would be expected for other species (see Trivers and Hare, 1976; Gullan and Cranston, 2005) for a concise explanation of relatedness and the haplodiploid system of the social Hymenoptera). In the single case where ants from different colonies were used (*Messor capensis*), the intraspecific slope was greater than the interspecific one, and no different ( $t = 1.29$ ,  $p > 0.05$ ) from 1. The use of ants from a single colony holds no problems for the fractal network model though, because this model suggests that there should be identical scaling relationships from “molecules to monsters” (Brown et al., 2004).

The variances of the intraspecific relationships were relatively high, especially for *Anoplolepis steinergroeveri* ( $R^2 = 0.28$ ). This is not an uncommon finding for intraspecific

studies, and has been documented for both invertebrates and vertebrates. For example, Bosch et al. (2000) found little significance for intraspecific scaling relationships in the trogid beetle species *Omorgus radula*, and Lehmann et al. (2000) noted that metabolic rate was highly variable within the several *Drosophila* species they examined. Likewise, Thompson and Withers (1997) noted that the slopes of the metabolic-rate temperature relationships in the Goannas they studied had coefficients of determination as low as 0.02. Currie (1993) showed that the observed coefficient of determination in a log-log regression can be explained by the size range of the x-axis variable. An independent variable that spans a large size range will result in a high  $R^2$ , and *vice versa*. All three species of ants investigated here have body masses that span just over one order of magnitude, possibly explaining the low coefficients of determination observed. Nonetheless, all three the relationships were significant and clearly different from 0.75 and in two cases different from 0.67.

By contrast, the interspecific body mass range spans just over three orders of magnitude and, as predicted from Currie (1993), the coefficients of determination were relatively high ( $R^2 = 0.78$ ). However, whereas the intraspecific slopes differed from the proposed slopes (0.67 or 0.75), the interspecific slope did not differ from either of the two. Alternatively, when the effects of phylogeny were accounted, the interspecific slope also differed from 0.75, but not from 0.67. The importance of correcting for phylogenetic non-independence has been raised by many authors (reviews in Harvey and Pagel, 1991; Freckleton et al., 2002), and the need to do so is certainly apparent here. This provides support for the assertions by White and Seymour (2003) and by Savage et al. (2004) that phylogenetic non-independence must be accounted for during investigations of scaling (see McNab, 2003 for an alternative view). However, doing so by including species of the same size in particular bins and then calculating an average size and metabolic rate, as was done by Savage et al.

(2004), confounds body size and phylogeny (thinking about capybaras and dik-diks illustrates this problem). Rather, standard techniques, which take phylogenetic non-independence into account, and that have been explored thoroughly by various authors (e.g. Felsenstein, 1985; Harvey and Pagel, 1991; Garland et al., 1993, 1999; Garland and Ives, 2000; Freckleton et al., 2002) should be used. Nonetheless, it should be noted that both Bennett and Harvey (1987) and Symonds and Elgar (2002) found that controlling for phylogeny does not change the interspecific slope of metabolic rate on mass a great deal, and the value stays undistinguishable from 0.75 when investigating birds and mammals. This might be a consequence of the extent of the phylogenetic diversity included in the database. If this is the case then the predictions made by Kozłowski et al. (2003a), that a greater diversity should result in a convergence of the slope value on 0.75 would be confirmed. Effectively this means that a consensus scaling equation for ants is still some way off and this echoes statements made by Chown and Nicolson (2004, p. 86). Thus, careful investigations of both intra- and interspecific slopes are required.

The fact that the coefficients of the interspecific slopes also depend on the method of data collection certainly also supports the above assertion. Separating the methods leads to higher slopes than to those obtained using the combined data. At the interspecific level it is thus clear that the method of data collection has a considerable effect on the scaling coefficient. This effect is not wholly unexpected. Lighton and Fielden (1995) showed that closed and open system respirometry give rise to rather different values for metabolic rate, with the former tending to produce higher estimates owing to the fact that activity cannot be resolved using these systems (although they are intrinsically accurate). Addo-Bediako et al. (2002) also found that differentiating between open and closed systems altered the coefficient of the slope, although they did not pursue this issue in any detail. In a related vein, Duncan

and Dickman (2001) noted that many studies had concerned flightless beetles and ants which would also alter the value of the slope, although they did not draw attention to differences associated with the methods of data collection (see also Terblanche et al., 2004 for related comments). These findings add further weight to the idea that there is some way to go before a consensus scaling relationship for ants, and indeed for all insects, can be achieved.

In conclusion, this work has shown that a fundamental prediction of the fractal network model for metabolic scaling is not upheld for ants. This suggests that the model may not be as universal as its proponents have suggested (e.g. West et al., 1997; Brown et al., 2004). Work on other polymorphic species such as bumblebees and dung beetles is required to assess the extent to which this conclusion is general.

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## **Chapter 5**

### **Conclusions**

This study confirms that insects are much more variable in terms of gas exchange than the previous literature suggests, although Lighton (1998) and Chown (2001) had already raised this issue. This variability has been nicely illustrated in the *Perisphaeria* sp. where it was found that a single individual can show a repertoire of gas exchange patterns, varying from continuous respiration to classical DGCs, all at rest. Moreover, these patterns can be shown sequentially by an individual. Despite this high variability, gas exchange characteristics generally have high repeatabilities, and therefore fulfill one of the prerequisites for natural selection.

Bearing this great intra-individual variability in gas exchange patterns in mind, a range of species was examined to determine the phylogenetic distribution of gas exchange patterns. The previous literature suggested that DGCs are fairly common in insects (Chapman, 1998; Nation, 2002). However, here it was found that not only are DGCs absent in the Apterygota, but the pattern occurs in only two of the orders of the Expterygota that were investigated. Indeed, this study showed that it is mostly beetles, but also ants and Lepidopteran pupae, that have been investigated. By undertaking investigations of a range of exemplar taxa it was also demonstrated that DGCs probably evolved independently at least five times, therefore supporting the idea that DGCs might be adaptive (reviewed in Chown and Nicolson, 2004). In addition, the hygric hypothesis seemed to be supported by this study, while the oxidative damage hypothesis, the hybrid and the chthonic hypotheses were not. Of course, a phylogenetically independent investigation from the  $\chi^2$  test undertaken to examine these ideas would have been more appropriate, but this could not be undertaken owing to the absence of a species level phylogeny for the insects.

This study also sought to investigate variation in metabolic characteristics associated with body size. Apart from inherent variation, body size-related variation is one

of the most characteristic forms of physiological variability (Peters, 1983; Schmidt-Nielsen, 1983). It has also come to the fore owing to the metabolic theory of ecology (Brown et al., 2004). The fractal network model on which much of this metabolic theory is based (West et al, 1997) makes the prediction that the intra- and interspecific scaling coefficients should be the same. Here, it was shown that this prediction does not hold for ants, so substantially questioning the metabolic theory.

These outcomes raise several issues that should be addressed in the future:

- The repeatability of the gas exchange characteristics of more species should be assessed, since three species are insufficient to assess fully this prerequisite for natural selection. In addition, studies investigating the heritability of DGC and the relationship between its variation and fitness also need to be undertaken to address the extent to which the other prerequisites for natural selection, and thus adaptation, are met. Insight into the adaptive value of DGC can further be obtained by using the same species to investigate both the repeatability as well as the heritability of DGC in combination with selection experiments.
- Reflecting on the phylogenetic investigation, 12 of the 30 insect orders still remain uninvestigated, and on average less than five families per order have been investigated thus far. Far more studies are required with appropriate representation for each order to draw reliable conclusions on the distribution of DGC across the insects. Therefore, a proper broad scale comparative analysis of gas exchange patterns across the insect phylogeny would involve investigations of those orders that have not previously been investigated. Investigations within all the orders, with the possible exception of Coleoptera and Hymenoptera, need to be expanded because so

few taxa have been investigated in the other orders. Investigation of adult Lepidoptera would also prove useful for the same reasons.

- Mechanistic studies of species within the five orders that show DGC should be undertaken, and comparisons between species in families that do and do not show DGC should also be done. Because there is evidence for the origin of DGC five times independently in insects and four times in Arthropods, such mechanistic studies would reveal the extent to which the mechanisms underlying the DGC, such as the partial pressures of CO<sub>2</sub> and O<sub>2</sub> that trigger changes in spiracular behaviour, differ. It has already been demonstrated in this regard that the pseudoscorpions differ from other groups (Lighton and Joos, 2002).
- Finally, additional investigation of the fractal network model (West et al., 1997) is necessary and more insect species, with a large intraspecific body mass variation should be investigated. This would provide insight into the generality of the result that insects do not support the metabolic theory of ecology. Obvious candidates for such investigations are the bees (Peat et al., in press) and dung beetles (Emlen and Nijhout, 2000)

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## **Appendix 1**

### **Repeatability tables**

**Appendix 1a** ANOVA table used to calculate the repeatability values and their upper and lower 95% confidence limits for each of the components of the cyclic patterns, as well as comparable components across all three of the cyclic patterns, metabolic rate, frequency and body mass. Data were  $\log_{10}$  transformed in some cases to normalize the distributions. Repeatabilities were calculated for females unless indicated otherwise. Sample sizes for individuals used for each pattern are given in Table 1.

<b>Components</b>	<b>Source of variation</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F-ratio</b>	<b>p</b>	<b>r</b>	<b>Lower confidence limit</b>	<b>Upper confidence limit</b>
<b>DGC volume</b> C-period ( $\log_{10}(+2)$ )	Among groups	8	$1.01 \times 10^{-7}$	$1.26 \times 10^{-8}$	2.29	0.03	0.16	0.01	0.47
	Within groups	53	$2.92 \times 10^{-7}$	$5.51 \times 10^{-9}$					
	Total	61	$3.93 \times 10^{-7}$						
F-period ( $\log_{10}$ )	Among groups	8	4.32	0.54	5.37	$5.78 \times 10^{-5}$	0.40	0.19	0.70
	Within groups	53	5.33	0.10					
	Total	61	9.65						
O-period ( $\log_{10}$ )	Among groups	8	1.76	0.22	13.13	$3.2 \times 10^{-10}$	0.65	0.44	0.85
	Within groups	53	0.89	0.02					
	Total	61	2.65						

Appendix 1a cont.

<b>DGC duration</b>	Among groups	8	$1.58 \times 10^6$	$1.97 \times 10^5$	2.49	0.02	0.18	0.03	0.50
	Within groups	53	$4.21 \times 10^6$	$7.94 \times 10^4$					
	Total	61	$5.79 \times 10^6$						
C-period	Among groups	8	$2.19 \times 10^6$	$2.74 \times 10^5$	6.92	$3.34 \times 10^{-6}$	0.47	0.26	0.75
	Within groups	53	$2.09 \times 10^6$	$3.95 \times 10^4$					
	Total	61	$4.28 \times 10^6$						
F-period	Among groups	8	0.94	0.12	6.77	$4.41 \times 10^{-6}$	0.47	0.25	0.75
	Within groups	53	0.92	0.02					
	Total	61	1.86						
O-period ( $\log_{10}$ )	Among groups	8	$1.42 \times 10^{-8}$	$1.78 \times 10^{-9}$	7.38	$1.52 \times 10^{-6}$	0.49	0.27	0.76
	Within groups	53	$1.28 \times 10^{-8}$	$2.41 \times 10^{-10}$					
	Total	61	$2.70 \times 10^{-8}$						
<b>DGC emission rate</b>	Among groups	8	1.13	0.14	5.81	$2.55 \times 10^{-5}$	0.42	0.21	0.71
	Within groups	53	1.29	0.02					
	Total	61	2.42						
C-period	Among groups	8	$3.38 \times 10^{-6}$	$4.32 \times 10^{-7}$	14.07	$9.93 \times 10^{-11}$	0.66	0.46	0.86
	Within groups	53	$1.59 \times 10^{-6}$	$3.01 \times 10^{-8}$					
	Total	61	$4.97 \times 10^{-6}$						
F-period ( $\log_{10}$ )	Among groups	8							
	Within groups	53							
	Total	61							
O-period	Among groups	8							
	Within groups	53							
	Total	61							

Appendix 1a cont.

Components	Source of variation	df	SS	MS	F-ratio	p	r	Lower confidence limit	Upper confidence limit
<b>Interburst –Burst</b> volume	Among groups	10	5.23	0.52	10.09	$2.76 \times 10^{-11}$	0.50	0.32	0.74
	Within groups	93	4.82	0.05					
	Total	103	10.05						
Burst ( $\log_{10}$ )	Among groups	10	21.99	2.20	13.22	$4.14 \times 10^{-14}$	0.57	0.39	0.79
	Within groups	93	15.46	0.17					
	Total	103	37.45						
<b>Interburst –Burst</b> duration	Among groups	10	$3.39 \times 10^6$	$3.39 \times 10^5$	5.93	$6.92 \times 10^{-7}$	0.35	0.19	0.61
	Within groups	93	$5.31 \times 10^6$	$5.71 \times 10^4$					
	Total	103	$8.70 \times 10^6$						
Burst	Among groups	10	$6.74 \times 10^6$	$6.74 \times 10^5$	2.64	$7.00 \times 10^{-3}$	0.15	0.04	0.39
	Within groups	93	$2.37 \times 10^7$	$2.55 \times 10^5$					
	Total	103	$3.05 \times 10^7$						
<b>Interburst –Burst</b> emission rate	Among groups	10	5.30	0.53	29.28	$1.51 \times 10^{-24}$	0.76	0.61	0.89
	Within groups	93	1.68	0.02					
	Total	103	6.98						
Burst ( $\log_{10}$ )	Among groups	10	$4.26 \times 10^{-6}$	$4.26 \times 10^{-7}$	92.14	0	0.91	0.84	0.96
	Within groups	93	$4.30 \times 10^{-7}$	$4.62 \times 10^{-9}$					
	Total	103							

Appendix 1a cont.

Components	Source of variation	df	SS	MS	F-ratio	p	r	Lower confidence limit	Upper confidence limit
<b>Pulsation volume</b> Burst(log <sub>10</sub> )	Among groups	8	13.75	1.72	83.15	0	0.59	0.42	0.81
	Within groups	541	11.19	0.02					
	Total	549	24.94						
Interburst (log <sub>10</sub> )	Among groups	8	18.80	2.35	9.09	9.0 x 10 <sup>-12</sup>	0.12	0.06	0.31
	Within groups	541	1.39 x 10 <sup>2</sup>	0.26					
	Total	549	1.58 x 10 <sup>2</sup>						
<b>Pulsation duration</b> Burst	Among groups	8	4.33 x 10 <sup>3</sup>	5.41x 10 <sup>2</sup>	5.68	6.0 x 10 <sup>-7</sup>	0.08	0.03	0.21
	Within groups	541	5.16 x 10 <sup>4</sup>	95.38					
	Total	549	5.59 x 10 <sup>4</sup>						
Interburst (log <sub>10</sub> )	Among groups	8	42.19	5.27	11.95	9.0 x 10 <sup>-16</sup>	0.16	0.08	0.37
	Within groups	541	2.39 x 10 <sup>2</sup>	0.44					
	Total	549	2.81 x 10 <sup>2</sup>						
<b>Pulsation emission rate</b> Burst (log <sub>10</sub> )	Among groups	8	5.51	0.69	39.79	0	0.40	0.25	0.67
	Within groups	541	9.37	0.02					
	Total	549	14.88						
Interburst (log <sub>10</sub> )	Among groups	8	13.42	1.68	12.27	3.0 x 10 <sup>-16</sup>	0.16	0.08	0.38
	Within groups	541	73.66	0.14					
	Total	549	87.08						

Appendix 1a cont.

Components	Source of variation	df	SS	MS	F-ratio	p	r	Lower confidence limit	Upper confidence limit
<b>Metabolic rate</b> (Males and females) (log <sub>10</sub> )	Among groups	19	3.47	0.18	6.25	2.01 x 10 <sup>-9</sup>	0.51	0.35	0.69
	Within groups	80	2.33	0.03					
	Total	99	5.80						
<b>Metabolic rate</b> (Females) (log <sub>10</sub> )	Among groups	16	2.57	0.16	5.67	1.57 x 10 <sup>-7</sup>	0.48	0.32	0.67
	Within groups	68	1.93	0.03					
	Total	84	4.50						
<b>Frequency</b> (Males and females)	Among groups	19	1.98 x 10 <sup>4</sup>	1.04 x 10 <sup>3</sup>	3.37	5.9 x 10 <sup>-5</sup>	0.31	0.15	0.52
	Within groups	87	2.69 x 10 <sup>4</sup>	3.10 x 10 <sup>2</sup>					
	Total	106	4.68 x 10 <sup>4</sup>						
<b>Frequency</b> (Females)	Among groups	16	1.58 x 10 <sup>4</sup>	9.87 x 10 <sup>2</sup>	2.78	0.001	0.25	0.10	0.45
	Within groups	76	2.69 x 10 <sup>4</sup>	3.55 x 10 <sup>2</sup>					
	Total	92	4.27 x 10 <sup>4</sup>						
<b>Mass</b> (Males and females)	Among groups	19	0.64	0.03	15.12	3.93 x 10 <sup>-19</sup>	0.74	0.61	0.85
	Within groups	80	0.18	2.0 x 10 <sup>-3</sup>					
	Total	99	0.82						

Appendix 1a cont.

Components	Source of variation	df	SS	MS	F-ratio	p	r	Lower confidence limit	Upper confidence limit
<b>Flutter period and interbursts for the three cyclic patterns</b> (Males and females) Volume	Among groups	19	$2.54 \times 10^{-4}$	$1.34 \times 10^{-5}$	24.13	0	0.33	0.22	0.49
	Within groups	754	$4.17 \times 10^{-4}$	$5.53 \times 10^{-7}$					
	Total	773	$6.71 \times 10^{-4}$						
<b>Flutter period and interbursts for the three cyclic patterns</b> (Males and females) Duration	Among groups	19	$5.87 \times 10^7$	$3.09 \times 10^6$	50.82	0	0.51	0.38	0.68
	Within groups	754	$4.59 \times 10^7$	$6.08 \times 10^4$					
	Total	773	$10.46 \times 10^7$						
<b>Flutter period and interbursts for the three cyclic patterns</b> (Males and females) Emission rate	Among groups	19	$6.64 \times 10^{-6}$	$3.49 \times 10^{-7}$	5.65	$2.88 \times 10^{-13}$	0.09	0.05	0.18
	Within groups	754	$4.66 \times 10^{-5}$	$6.18 \times 10^{-8}$					
	Total	773	$5.32 \times 10^{-5}$						

Appendix 1a cont.

Components	Source of variation	df	SS	MS	F-ratio	p	r	Lower confidence limit	Upper confidence limit
<b>Open period and bursts for the three cyclic patterns</b> (Males and females) Volume ( $\log_{10}$ )	Among groups	19	$1.87 \times 10^2$	9.86	63.99	0	0.57	0.43	0.72
	Within groups	754	$1.16 \times 10^2$	0.15					
	Total	773	$3.04 \times 10^2$						
<b>Open period and bursts for the three cyclic patterns</b> (Males and females) Duration ( $\log_{10}$ )	Among groups	19	$2.10 \times 10^2$	11.0	89.83	0	0.65	0.52	0.79
	Within groups	754	92.7	0.12					
	Total	773	$3.03 \times 10^2$						
<b>Open period and bursts for the three cyclic patterns</b> (Males and females) Emission rate ( $\log_{10}$ )	Among groups	19	10.34	0.55	28.52	0	0.37	0.25	0.54
	Within groups	754	14.47	0.02					
	Total	773	24.81						

Appendix 1a cont.

Components	Source of variation	df	SS	MS	F-ratio	p	r	Lower confidence limit	Upper confidence limit
<b>Flutter period and interbursts for the three cyclic patterns</b> (Females) Volume	Among groups	16	2.56 x 10 <sup>2</sup>	16.01	31.52	0	0.43	0.31	0.61
	Within groups	700	3.55 x 10 <sup>2</sup>	0.51					
	Total	716	6.11 x 10 <sup>2</sup>						
<b>Flutter period and interbursts for the three cyclic patterns</b> (Females) Duration	Among groups	16	3.09 x 10 <sup>2</sup>	19.32	33.99	0	0.45	0.33	0.63
	Within groups	700	3.98 x 10 <sup>2</sup>	0.57					
	Total	716	7.07 x 10 <sup>2</sup>						
<b>Flutter period and interbursts for the three cyclic patterns</b> (Females) Emission rate	Among groups	16	31.43	1.96	15.12	0	0.26	0.17	0.42
	Within groups	700	90.88	0.13					
	Total	716	1.22 x 10 <sup>2</sup>						
<b>Open period and bursts for the three cyclic patterns</b> (Females) Volume (log <sub>10</sub> )	Among groups	16	1.39 x 10 <sup>2</sup>	8.71	52.87	0	0.57	0.43	0.72
	Within groups	700	1.15 x 10 <sup>2</sup>	0.17					
	Total	716	2.54 x 10 <sup>2</sup>						
<b>Open period and bursts for the three cyclic patterns</b> (Females) Duration (log <sub>10</sub> )	Among groups	16	1.60 x 10 <sup>2</sup>	10.01	77.30	0	0.66	0.53	0.79
	Within groups	700	90.64	0.13					
	Total	716	2.50 x 10 <sup>2</sup>						
<b>Open period and bursts for the three cyclic patterns</b> (Females) Emission rate (log <sub>10</sub> )	Among groups	16	10.46	0.65	33.45	0	0.45	0.32	0.63
	Within groups	700	13.68	0.02					
	Total	716	24.14						

**Appendix 1b** ANOVA table used to calculate the repeatability values and their upper and lower 95% confidence limits for each of the components of the cyclic patterns, as well as comparable components across all three of the cyclic patterns, metabolic rate, frequency and body mass, with body mass included as a covariate. Data were  $\log_{10}$  transformed in some cases to normalize the distributions. Repeatabilities were calculated for females unless indicated otherwise. Sample sizes for individuals used for each pattern are given in Table 1.

<b>Components</b>	<b>Source of variation</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F-ratio</b>	<b>p</b>	<b>r</b>	<b>Lower confidence limit</b>	<b>Upper confidence limit</b>
<b>DGC volume</b> F-period	Among groups	8	2.05	0.26	3.03	0.007	0.23	0.06	0.55
	Within groups	52	4.40	0.08					
	Total	60	6.45						
<b>DGC duration</b> C-period	Among groups	8	$2.02 \times 10^6$	$2.53 \times 10^5$	3.59	0.002	0.28	0.10	0.60
	Within groups	52	$3.66 \times 10^6$	$7.03 \times 10^4$					
	Total	60	$5.68 \times 10^6$						
F-period	Among groups	8	$1.50 \times 10^6$	$1.87 \times 10^5$	5.05	$1.14 \times 10^{-4}$	0.38	0.17	0.68
	Within groups	52	$1.93 \times 10^6$	$3.71 \times 10^4$					
	Total	60	$3.43 \times 10^6$						

Appendix 1b cont.

<b>Components</b>	<b>Source of variation</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F-ratio</b>	<b>p</b>	<b>r</b>	<b>Lower confidence limit</b>	<b>Upper confidence limit</b>
<b>Interburst –Burst volume</b> Burst ( $\log_{10}$ )	Among groups	10	1.50	0.15	3.63	$4.18 \times 10^{-4}$	0.22	0.09	0.48
	Within groups	92	3.80	0.04					
	Total	102	5.30						
<b>Interburst –Burst duration</b> Burst	Among groups	10	$3.86 \times 10^6$	$3.86 \times 10^5$	7.34	$1.9 \times 10^{-8}$	0.41	0.24	0.67
	Within groups	92	$4.84 \times 10^6$	$5.26 \times 10^4$					
	Total	102	$8.70 \times 10^6$						
<b>Interburst –Burst emission rate</b> Interburst	Among groups	10	$1.85 \times 10^{-6}$	$1.85 \times 10^{-7}$	42.73	$2.85 \times 10^{-30}$	0.82	0.70	0.92
	Within groups	92	$3.99 \times 10^{-7}$	$4.34 \times 10^{-9}$					
	Total	102	$1.27 \times 10^2$						

Appendix 1b cont.

Components	Source of variation	df	SS	MS	F-ratio	p	r	Lower confidence limit	Upper confidence limit
<b>Pulsation volume</b> Burst ( $\log_{10}$ )	Among groups	8	14.45	1.8	93.68	0	0.62	0.42	0.81
	Within groups	540	10.41	0.02					
	Total	548	24.86						
Interburst ( $\log_{10}$ )	Among groups	8	20.86	2.61	10.27	$2.08 \times 10^{-13}$	0.14	0.07	0.34
	Within groups	540	$1.37 \times 10^2$	0.25					
	Total	548	$1.58 \times 10^2$						
<b>Pulsation duration</b> Burst	Among groups	8	$4.14 \times 10^3$	$5.18 \times 10^2$	5.49	$1.12 \times 10^{-6}$	0.07	0.03	0.21
	Within groups	541	$5.10 \times 10^4$	94.38					
	Total	549	$5.52 \times 10^4$						
Interburst ( $\log_{10}$ )	Among groups	8	47.40	5.93	13.99	$1.48 \times 10^{-18}$	0.19	0.08	0.37
	Within groups	540	$2.30 \times 10^2$	0.42					
	Total	548	$2.77 \times 10^2$						
Emission rate Interburst ( $\log_{10}$ )	Among groups	8	13.37	1.67	12.59	$1.23 \times 10^{-16}$	0.17	0.10	0.41
	Within groups	540	71.41	0.13					
	Total	548	84.78						

Appendix 1b cont

<b>Components</b>	<b>Source of variation</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F-ratio</b>	<b>p</b>	<b>r</b>	<b>Lower confidence limit</b>	<b>Upper confidence limit</b>
<b>Metabolic rate</b> (Males and females) (log <sub>10</sub> )	Among groups	19	1.97	0.05	2.39	0.004	0.22	0.07	0.42
	Within groups	79	1.69	0.02					
	Total	98	2.66						
<b>Metabolic rate</b> (Females) (log <sub>10</sub> )	Among groups	16	0.92	0.06	3.05	7.14 x 10 <sup>-4</sup>	0.29	0.12	0.52
	Within groups	67	1.27	0.02					
	Total	83	2.19						
<b>Frequency</b> (Males and females)	Among groups	19	2.11 x 10 <sup>4</sup>	1.11 x 10 <sup>3</sup>	3.76	1.25 x 10 <sup>-5</sup>	0.35	0.19	0.55
	Within groups	86	2.55 x 10 <sup>4</sup>	2.96 x 10 <sup>2</sup>					
	Total	105	4.66 x 10 <sup>4</sup>						
<b>Frequency</b> (Females)	Among groups	16	1.71 x 10 <sup>4</sup>	1.07 x 10 <sup>3</sup>	3.16	3.96 x 10 <sup>-4</sup>	0.29	0.13	0.51
	Within groups	75	2.54 x 10 <sup>4</sup>	3.39 x 10 <sup>2</sup>					
	Total	91	4.25 x 10 <sup>4</sup>						

Appendix 1b cont

Components	Source of variation	df	SS	MS	F-ratio	p	r	Lower confidence limit	Upper confidence limit
<b>Flutter period and interbursts for the three cyclic patterns</b> (Males and females) Volume	Among groups	19	$2.54 \times 10^{-3}$	$1.34 \times 10^{-4}$	42.45	0	0.47	0.35	0.63
	Within groups	753	$2.4 \times 10^{-3}$	$3.19 \times 10^{-6}$					
	Total	772	$4.94 \times 10^{-3}$						
<b>Flutter period and interbursts for the three cyclic patterns</b> (Males and females) Duration	Among groups	19	$6.12 \times 10^7$	$3.22 \times 10^6$	54.35	0	0.53	0.41	0.68
	Within groups	753	$4.47 \times 10^7$	$5.90 \times 10^4$					
	Total	772	$10.59 \times 10^7$						
<b>Flutter period and interbursts for the three cyclic patterns</b> (Males and females) Emission rate	Among groups	19	$2.63 \times 10^{-5}$	$1.38 \times 10^{-6}$	15.60	0	0.24	0.16	0.38
	Within groups	753	$6.67 \times 10^{-5}$	$8.86 \times 10^{-8}$					
	Total	772	$9.30 \times 10^{-5}$						
<b>Open period and bursts for the three cyclic patterns</b> (Males and females) Volume ( $\log_{10}$ )	Among groups	19	$1.29 \times 10^2$	6.81	69.04	0	0.59	0.47	0.73
	Within groups	753	74.24	0.10					
	Total	772	$2.03 \times 10^2$						
<b>Open period and bursts for the three cyclic patterns</b> (Males and females) Duration ( $\log_{10}$ )	Among groups	19	$2.20 \times 10^2$	11.6	138.12	0	0.74	0.64	0.85
	Within groups	753	63.22	0.08					
	Total	772	$2.83 \times 10^2$						

Appendix 1b cont

Components	Source of variation	df	SS	MS	F-ratio	p	r	Lower confidence limit	Upper confidence limit
<b>Flutter period and interbursts for the three cyclic patterns (Females)</b> Volume	Among groups	16	$3.95 \times 10^2$	24.68	43.14	0	0.51	0.39	0.68
	Within groups	700	$3.99 \times 10^2$	0.57					
	Total	716	$6.11 \times 10^2$						
<b>Flutter period and interbursts for the three cyclic patterns (Females)</b> Duration	Among groups	16	$3.45 \times 10^2$	21.54	38.29	0	0.48	0.36	0.66
	Within groups	700	$3.93 \times 10^2$	0.56					
	Total	716	$7.07 \times 10^2$						
<b>Flutter period and interbursts for the three cyclic patterns (Females)</b> Emission rate	Among groups	16	34.94	2.18	14.23	0	0.25	0.16	0.41
	Within groups	700	90.88	0.15					
	Total	716	$1.07 \times 10^2$						
<b>Open period and bursts for the three cyclic patterns (Females)</b> Volume ( $\log_{10}$ )	Among groups	16	$1.10 \times 10^2$	6.94	74.25	0	0.65	0.52	0.79
	Within groups	700	65.39	0.09					
	Total	716	$1.75 \times 10^2$						
<b>Open period and bursts for the three cyclic patterns (Females)</b> Duration ( $\log_{10}$ )	Among groups	16	$1.67 \times 10^2$	10.47	121.93	0	0.75	0.65	0.86
	Within groups	700	60.13	0.09					
	Total	716	$2.27 \times 10^2$						

## **Appendix 2**

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

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

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### Abstract

Metabolic rate estimates as well as a measure of their repeatability and response to laboratory acclimation are provided for the amblypygid *Damon annulatipes* (Wood). This species (mean  $\pm$  S.E. mass :  $640 \pm 66$  mg) shows continuous gas exchange, as might be expected from its possession of book lungs, and at  $21^\circ\text{C}$  has a metabolic rate of  $30.22 \pm 2.87 \mu\text{l CO}_2 \text{ h}^{-1}$  ( $\approx 229.6 \pm 21.8 \mu\text{W}$ , R.Q. = 0.72). The intraclass correlation coefficient ( $r = 0.74\text{--}0.89$ ) indicated substantial repeatability in metabolic rate which did not change with laboratory acclimation over a period of 2 weeks. By contrast, absolute metabolic rate declined by *c.* 16–33%, although this was not a consequence of changes in mass (which were non-significant over the same period). Rather, it appears that a reduction in overall stress or activity in the laboratory might have been responsible for the decline in mass-independent metabolic rate. At the intraspecific level, metabolic rate scaled as  $\mu\text{W} = 342M^{0.857}$ , where mass is in grams. Metabolic rates of this species are in keeping with its sedentary behaviour such that for a given body size they are lower than those of most arthropods (spiders and insects), higher than the very sedentary ticks, and equivalent to scorpions. These findings have implications for the understanding of the evolution of metabolic rates in arthropods.

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**Keywords:** Acclimation; Gas exchange; Repeatability; Scaling

### 1. 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 and Gaston, 1999; Hochachka and Somero, 2002; Kozłowski and 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 and Chappell, 2000), time of day (Crozier, 1979; Takahashi-Del-Bianco et al., 1992), and fluctuations in temperature (Keister and Buck, 1964; Bartholomew et al., 1981; Cossins and Bowler, 1987). It also varies over longer time scales with age (Hack, 1997) and with season (acclimatization) (Forlow and 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 and Parsons, 1991; Berrigan and Partridge, 1997; Gibbs, 2002), but also their phylogenetic membership (Harvey and 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

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(Schmidt-Nielsen, 1984; Lighton and Fielden, 1995; Kozłowski and Weiner, 1997; Kozłowski and Gawelczyk, 2002). Nonetheless, many aspects of metabolic rate variation in arthropods remain poorly understood (Chown and 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 and Boag, 1987; Falconer and 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 and Keister, 1955), two boring beetles (Chappell and Rogowitz, 2000), an orthopteran (Nespolo et al., 2003), a highly variable cockroach (Marais and 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 and Hoffmann, 2000; Sgrò and 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 and 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 and Gaston, 1999). Typically, investigations of phenotypic flexibility (Huey and Berrigan, 1996; Huey et al., 1999; Piersma and Drent, 2003) of metabolic rates in arthropods compare responses to several different

environments after a fixed period (Stamou et al., 1995; Berrigan, 1997; Berrigan and Partridge, 1997; Djawdan et al., 1997; Fields et al., 1998; Bennett et al., 1999; Sokolova and 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 and Hoffmann, 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 and 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 and Duncan, 1995; Lighton and Fielden, 1995, 1996; Chown and 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 and 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, we repeatedly measure the standard metabolic rate of individuals of an amblypygid species, *Damon annulatipes* (Wood) (Arachnida: Amblypygi), to address these issues. Specifically, we 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. 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 we 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 and Chapman, 2000), and we therefore compare our 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.

## 2. Methods and materials

We collected *D. 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 3 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 2 weeks in the climate chamber with no experimental interference (except feeding), and then re-used once per day for another 5 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 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, Henderson, Nevada) at  $100 \text{ ml min}^{-1}$ . 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  $21.0 \pm 1.5$  °C. The entire system was stored within an air-conditioned laboratory that held temperature at  $24.0 \pm 3.0$  °C. We 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 min 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 to 120 min periods of experimental runs. In preliminary experiments (not used in the analyses) performed with a light on, we observed that after a restless period of approximately 10 min, individuals settle down and remain immobile for most of the remainder of the experiment (see also 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 mean  $\pm$  S.E. unless otherwise stated, and significance was set at  $P < 0.05$ . Repeatability and its confidence limits were calculated using the intraclass correlation coefficient approach (Lessells and 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. 4 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.

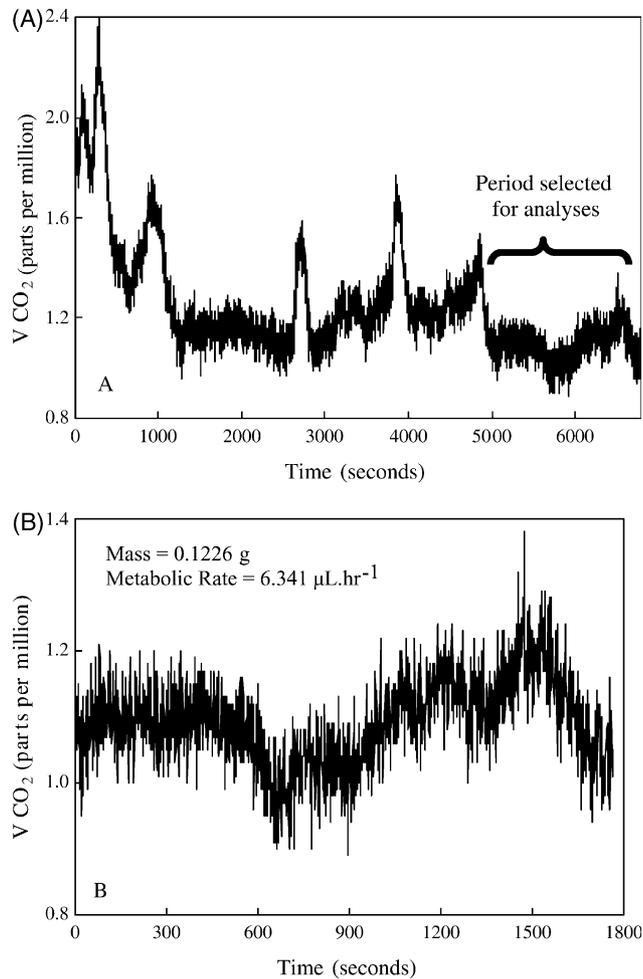


Fig. 1. (A) A typical metabolic rate recording for a *Damon annulatus* whip-spider (mass (g) = 0.1226), clearly showing an initial activity period during which the animal adopts a comfortable position in the respirometry cuvette. The obvious respiratory 'spike' (centre and left) represents an activity burst. The period after this peak would be selected for raw data transformation (from CO<sub>2</sub> parts per million to  $\mu\text{L CO}_2 \text{ h}^{-1}$ ) 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.

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

### 3. Results

*Damon annulatus* 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 ( $\mu\text{L CO}_2 \text{ h}^{-1}$ ) and re-calculated as microwatts using an R.Q. = 0.72, and rate of water loss ( $\text{mg H}_2\text{O h}^{-1}$ ) for individuals of *D. annulatus*, 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. annulatus* 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. annulatus*, mass-independent rates of water loss did not change after 2 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)  $\text{mg H}_2\text{O h}^{-1}$ ;  $F_{1,12} = 1.95$ ,  $P > 0.19$ ]).

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).

The intraspecific scaling relationship of body mass and metabolic rate was investigated using least squares linear regression. In *D. annulatus*, the scaling relationships of log metabolic rate on log body mass, in the form of  $\mu\text{W} = aM^b$ , was highly significant for the FF group ( $\mu\text{W}_{\text{FF}} = 342M^{0.857}$ ;  $F_{1,7} = 55.91$ ;  $r^2 = 0.889$ ;  $P < 0.001$ ) and the ACC group ( $\mu\text{W}_{\text{ACC}} = 258M^{0.897}$ ;  $F_{1,5} = 137.72$ ;  $r^2 = 0.965$ ;  $P < 0.001$ ; Fig. 3(A)). The slopes of the relationships of metabolic rate on mass

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

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

<sup>a</sup> FF<sub>All</sub> vs. ACC—no difference (repeated measures ANOVA:  $F_{1,67} = 0.000$ ;  $P > 0.99$ ).

<sup>b</sup> FF<sub>All</sub> vs. ACC—GLM, (covariate: body mass);  $F_{1,13} = 4.753$ ;  $P < 0.05$ .

<sup>c</sup> FF vs. ACC—GLM, (covariate: body mass);  $F_{1,13} = 4.78$ ;  $P = 0.0513$ .

<sup>d</sup> FF<sub>All</sub> vs. ACC—no difference (GLM, covariate body mass:  $F_{1,13} = 0.29$ ;  $P > 0.60$ ).

<sup>e</sup> FF vs. ACC—no difference (GLM, covariate body mass:  $F_{1,13} = 0.37$ ;  $P > 0.56$ ).

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:  $t_{1,5} = 1.351$ ;  $P > 0.2$ ).

#### 4. 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 and 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, we found that the repeatability of standard metabolic rate in *D. annulatipes* whip-spiders is both significant and high compared with that reported for insects ( $r$ : 0.4–0.6; Chappell and Rogowitz, 2000; Nespolo et al., 2003; Marais and 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 and 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 and Gaston, 1999; Chown and Nicolson, 2004).

The finding that metabolic rate repeatability remained unchanged with 2 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 and Keister, 1955; Chappell and Rogowitz, 2000; Nespolo et al., 2003; Marais and Chown, 2003), and our 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 and Hoffmann, 2000; Sgrò and 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

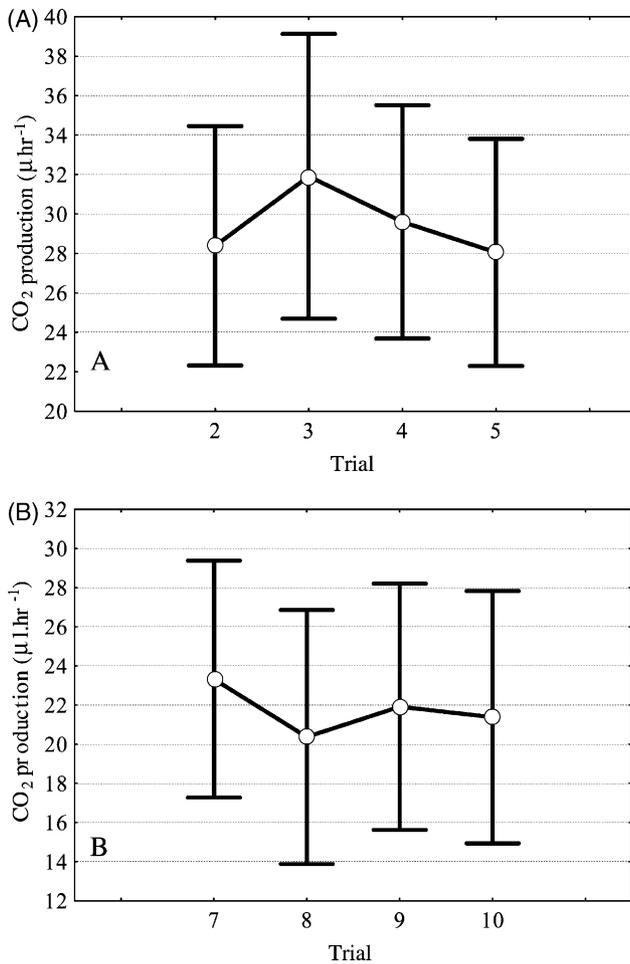


Fig. 2. Mean metabolic rate ( $\pm$ S.E.) in *Damon annulatipes* showed no systematic changes with experimental observation (test) in either the field-fresh (A) or acclimated (B) group. Metabolic rate was recorded at 21.0 °C, 0% R.H. and total darkness.

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 and Gaston, 1999). Finally, the similar intraspecific mass-scaling of metabolic rate in the FF and ACC animals (Fig. 3(A)) 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, we have refrained from additional interpretation thereof. However, we 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 we 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 and 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 for *T. marginemaculata* (Fig. 3(B)). 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.

Table 2

Results of analyses of variance to calculate repeatability ( $r$ ) of metabolic rate in *Damon annulatipes*. Overlap in the 95% confidence limits (LCL<sub>0.95</sub> and UCL<sub>0.95</sub>) 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 <sub>0.95</sub>	UCL <sub>0.95</sub>
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>a</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>a</sup> Repeatability calculated from test 3 and 8 (2 weeks apart) only in whip-spiders that completed all experiments.

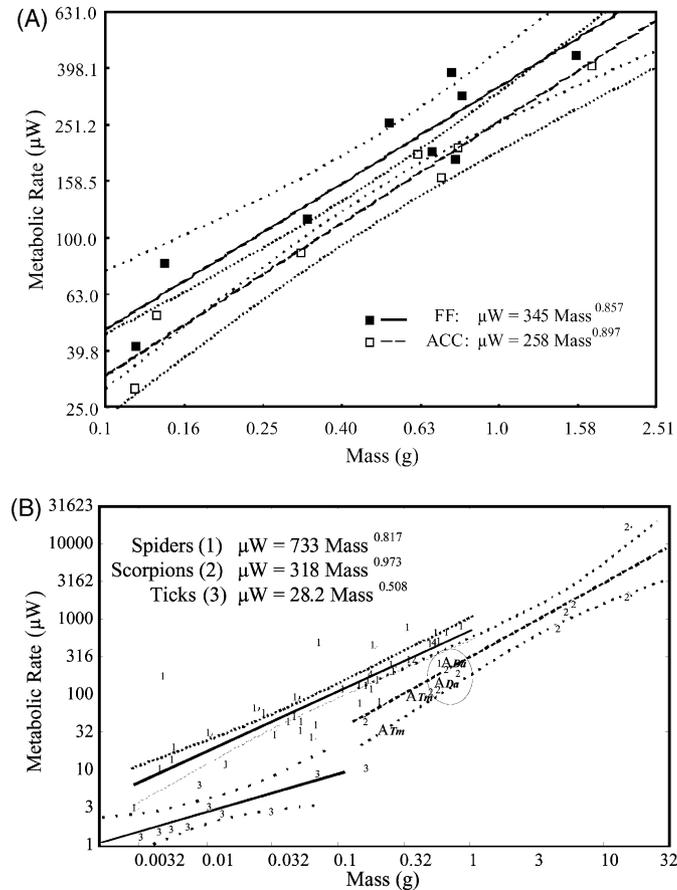


Fig. 3. (A) Intraspecific mass scaling relationship for metabolic rate in field fresh (FF: filled squares) and acclimated (ACC: open squares) *Damon annulatipes* whip-spiders. (B) Interspecific scaling relationship of metabolic rate for (1) spiders (Araneae), (2) scorpions (Scorpiones) and (3) ticks (Acari) taken from the literature (Anderson, 1970; Greenstone and Bennett, 1980; Anderson and Prestwich, 1982; Punzo, 1991; Kalarani et al., 1992; Lighton and Fielden, 1995; Anderson, 1996; Magano, 2000; Lighton et al., 2001). Solifugids (Solifugae) (4) are indicated (from Lighton and Fielden, 1996). *Damon annulatipes* (Amblypygi) (encircled, A<sub>Da</sub>) include field-fresh (upper symbol) and acclimated (lower symbol) respirometry recordings from this study, while A<sub>Tm</sub> 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  $\mu W = 513M^{0.875}$ , mass ( $M$ ) in g).

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 and 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 and Fielden, 1995; Lighton et al., 2001).

In conclusion, we 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.

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