Effects of thermal history on temperature-dependent flight performance in insects: *Ceratitis capitata* (Diptera: Tephritidae) as a model organism

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Declaration

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Abstract

Understanding the impact of environmental factors on locomotor performance and flight energetics is of fundamental importance to understanding evolution and ecology. Increased performance that leads to increased dispersal ability can result in increased migration distance to reach optimal habitats, increased gene flow between populations and an overall contribution to the survival of individuals as well as the structuring of species' geographic range sizes. The temperature-dependent nature of insects, in conjunction with predicted climate change and shifting optimal climatic ranges, could have important ecological and economic consequences such as increased invasion by alien and pest species. In this study, the influence of thermal history on temperature-dependent flight performance was investigated in a notorious invasive agricultural pest, *Ceratitis capitata* (Diptera: Tephritidae). Flies were exposed to one of four developmental acclimation temperatures (T_{acc}: 15, 20, 25, 30°C) during their pupal stage and tested at random at either of those temperatures (T_{test}) as adults in a full-factorial experimental design. Major factors influencing flight performance included sex, body mass, T_{test} and the interaction between T_{test} and T_{acc}. Performance increased with increasing T_{test} across all acclimation groups, e.g. at 15°C only 10% of all flies had successful flight, whereas at 30°C the success rate was 76.5%. Even though T_{acc} alone did not affect flight performance, it did have an effect in combination with T_{test}. The negative interaction term T_{test} x T_{acc}, in combination with a multiple comparison between T_{acc} groups at each T_{test}, indicated that flies acclimated to 15°C and 20°C performed better than those acclimated to 25°C and 30°C when tested at cold temperatures. This provides partial support for the 'colder is better' hypothesis. To explain these results, several key, flight-related traits were examined to determine if T_{acc} influenced flight performance as a consequence of changes in body or wing morphology, whole-animal metabolic rate or cytochrome c oxidase (CCO) activity. Although significant effects of T_{acc} could be detected in several of the traits examined, with emphasis on sex-related differences, increased flight performance could not be explained solely on the basis of changes in any one of these traits. To illustrate the potential applied value of this study, the main flight performance outcomes were also coupled with a degree-day (thermal development) model to determine if knowledge of flight ability could improve predicted population dynamics. The results and insights obtained from this study are broadly applicable to a variety of insect species and demonstrate that, by recognising the impact of environmental factors on locomotor performance and flight energetics, an increased understanding of the functioning, biology and evolution of flight-capable arthropods can be obtained.

Opsomming

Groter begrip van die impak van omgewingsfaktore op bewegingsprestasie en vlug-energetika is van fundamentele belang vir die verstaan van evolusie en ekologie. Verhoogde bewegingsprestasie wat lei tot verbeterde verspreidingsvermoë kan 'n toename in migrasieafstand om optimale habitat te bereik, verhoogde genevloei tussen populasies en 'n algehele bydrae tot die oorlewing van individue sowel as die strukturering van spesies se geografiese gebiedsgroottes tot gevolg hê. Die temperatuurafhanklike aard van insekte, tesame met voorspelde klimaatsverandering en die verskuiwing van optimale klimaatsones, kan belangrike ekologiese en ekonomiese gevolge, soos verhoogde indringing deur uitheemse en pes-spesies, inhou. Die invloed van termiese geskiedenis op die temperatuurafhanklike vlugprestasie van 'n berugte indringer-landboupes, *Ceratitis capitata* (Diptera: Tephritidae), word in hierdie studie ondersoek. Vlieë is blootgestel aan een van vier akklimasie-temperature (T_{acc}: 15, 20, 25, 30°C) tydens hul papiestadium en is op ewekansige wyse in 'n vol-faktoriale eksperimentele ontwerp by een van dieselfde toetstemperature (T_{test}) as volwassenes getoets. Belangrike faktore wat vlugprestasie beïnvloed het, sluit geslag, liggaamsmassa, T_{test} en die interaksie tussen T_{test} en T_{acc} in. Prestasie het verbeter met toenemende T_{test} oor al die akklimasiegroepe, bv. by 15°C het net 10% van alle vlieë suksesvol gevlieg, terwyl die sukseskoers by 30°C, 76,5% was. Selfs al het T_{acc} alleen nie die vlugprestasie beïnvloed nie, het dit 'n effek in kombinasie met T_{test} gehad. Die negatiewe interaksie term T_{test} x T_{acc}, in samewerking met 'n meervoudige vergelyking tussen T_{acc} groepe by elke T_{test}, het aangedui dat vlieë wat by 15°C en 20°C geakkimeer was, beter presteer het as dié wat by 25°C en 30°C geakklimeer was wanneer hulle by koue temperature getoets was. Dit bied gedeeltelike ondersteuning vir die "kouer is beter" hipotese. Om hierdie resultate te verklaar, is 'n paar sleutel vlugverwante eienskappe ondersoek om te bepaal of T_{acc} vlugprestasie a.g.v. veranderinge in die liggaam- of vlerkmorfologie, die hele-dier metaboliesetempo of sitochroom-c oksidase (CCO) aktiwiteit beïnvloed. Alhoewel beduidende effekte van T_{acc} op verskeie van hierdie eienskappe, veral wat geslagsverwante verskille betref, opgemerk is, kan verhoogde vlugprestasie nie uitsluitlik op grond van veranderinge in enige een van die eienskappe verduidelik word nie. Om die potensiële toepassingswaarde van hierdie studie te illustreer, is die hoof vlugprestasie uitkomste gekoppel aan 'n "degree-day" (termiese ontwikkeling)-model om te bepaal of kennis van vlugvermoë voorspellings van bevolkingsdinamika kan verbeter. Hierdie studie se resultate en insigte is oor die algemeen van toepassing op 'n verskeidenheid insek-spesies en toon dat erkenning van die invloed van omgewingsfaktore op bewegingsprestasie en vlug-energetika kan lei tot groter begrip van die funksionering, biologie en evolusie van geleedpotiges.

Acknowledgements

Dedication

Aan my ouers, Jasper en Dehlia Esterhuizen.

Sonder julle oneindige bron van liefde, tyd en ondersteuning sou
niks in my lewe moontlik wees nie.

Ewig dankbaar.

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Chapter 1 :

General Introduction

In order to better understand the evolutionary and ecological factors determining the geographic distribution of species, it is essential to consider dispersal ability and associated costs (reviewed in Bonte et al., 2012). Since dispersal ability can affect home range size, and hence, a species' geographic distribution, it also influences multiple levels of biological organisation (Gaston, 2009), e.g. gene-flow among populations (Ugelvig et al., 2012) as well as the fitness of individuals. Fitness, in turn, may be limited by biotic and abiotic opportunities in a species' home-range (McLoughlin et al., 2007) and the ability to respond to environmental pressures through migration or jump dispersal across disconnected patches of suitable habitat (Stevens et al., 2012). The act of dispersal may, however, be a trade-off between benefits, such as avoiding competition and finding food sources or optimal thermal environments (Huey and Slatkin, 1976; Richter and Cumming, 2006; Cote and Clobert, 2010) and possible costs, such as predation risk (Yoder et al., 2004). Bonte et al. (2012) summarises the four main types of costs that could be involved in dispersal trade-offs as:

1. energetic costs in the form of consuming metabolic energy;

2. the cost of time lost during locomotion that could have been spent on other activities;

3. the opportunity costs involved when leaving a familiar site and finally,

4. the risk that dispersal could result in injury or mortality.

In actively dispersing arthropods, the costs of dispersal can also result in indirect or future effects e.g. lower reproductive success due to energy allocation trade-offs via the finite or limited nature of energy resources (*cf.* Zera and Denno, 1997; Zera et al., 1999; Roff et al., 2003; discussed in Bonte et al., 2012). The dispersal and colonisation ability of species, especially insects, may also be limited by density-dependent factors in the expanding population front i.e. Allee effects, which refer to a decline in population growth rate due to e.g. the inability to locate mates, because of a decline in population abundance (Liebhold and Tobin, 2008).

1.1   Dispersal of invasive and pest species

The spread of invasive alien species (IAS) have recently become well discussed in the field of conservation ecology, evolutionary biology, population biology and genetics, with biological invasions being considered among the greatest threats to native biodiversity worldwide. In the United States, United Kingdom, Australia, South Africa, India and Brazil, the number of IAS range

from about 2000 to 50 000 (Pimentel et al., 2001). These species typically are introduced into new areas either accidentally, through the increase in global economic trade and human travel, or deliberately, for agricultural or ornamental purposes (Van Wilgen et al., 2001; Wilson et al., 2009; Chown et al., 2012). IAS can have detrimental effects on biota across the globe: they may directly cause extinctions by affecting the survival rate of native fauna and flora through competition and predation and can change the overall functioning and structure of ecosystems. They may also have indirect effects by altering nutrient cycling, hydrology, habitat structure or predator-prey interactions (Mack et al., 2000; Richardson and Van Wilgen, 2004; Simberloff et al., 2013). Besides the potentially severe negative ecological impact of IAS, there are also substantial economic costs associated with these biological invasions.

In South Africa, billions of South African Rands (ZAR) are spent annually on control, management and intervention strategies for IAS (Van Wilgen et al., 2012) and similarly large amounts are potentially lost due to decreases in ecosystem services instigated by these invasions (e.g. decrease in water availability in areas invaded by alien trees or a reduction in pollination based ecosystem services (Moran et al., 2000; Van Wilgen et al., 2001; Allsopp et al., 2008)). IAS are sometimes also agricultural pests and may be spread by increased global trade and travel (Kriticos et al., 2013), with potential impacts on crops, pastures and livestock health (Pimentel et al., 2000, 2001). This not only results in economic losses due to reduced productivity, but there are also costs involved in controlling these pests, totalling into billions of dollars in the United States annually (Mack et al., 2000; Pimentel, 2005). Additionally, if agricultural pests with invasive potential are detected in a country's fresh produce consignments, economic losses can occur due to fruit being banned from international export markets (Barnes, 2000).

Blackburn et al. (2011) proposed a unified framework for biological invasions, incorporating previous models to describe the different barriers that an alien species must overcome in order to become a successful IAS. From Fig. 1.1, it is evident that individuals from an alien species must 1) be moved beyond their native range (Step A, usually mitigated by humans, but not always) and 2) be able to disperse further than its location of introduction (Steps D-E, after steps B-C (survival and reproduction in a self-sustaining population) were successfully completed). From step D1 to step D2, a *"self-sustaining population in the wild, with individuals surviving a significant distance from the original point of introduction"* obtains the ability to successfully reproduce. Only once this point in the process is reached, the alien species can become recognised as an IAS with individuals *"dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence"* (Step E). In this framework, the ability to overcome the first and

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final obstacles standing in the way of successful invasion, i.e. transport and spread, is clearly linked to the dispersal ability of the organism in question. Bearing in mind the ecological and economic costs associated with invasions, it is therefore clear that it is important to understand the movement and dispersal patterns of IAS, as it may directly influence invasion potential.
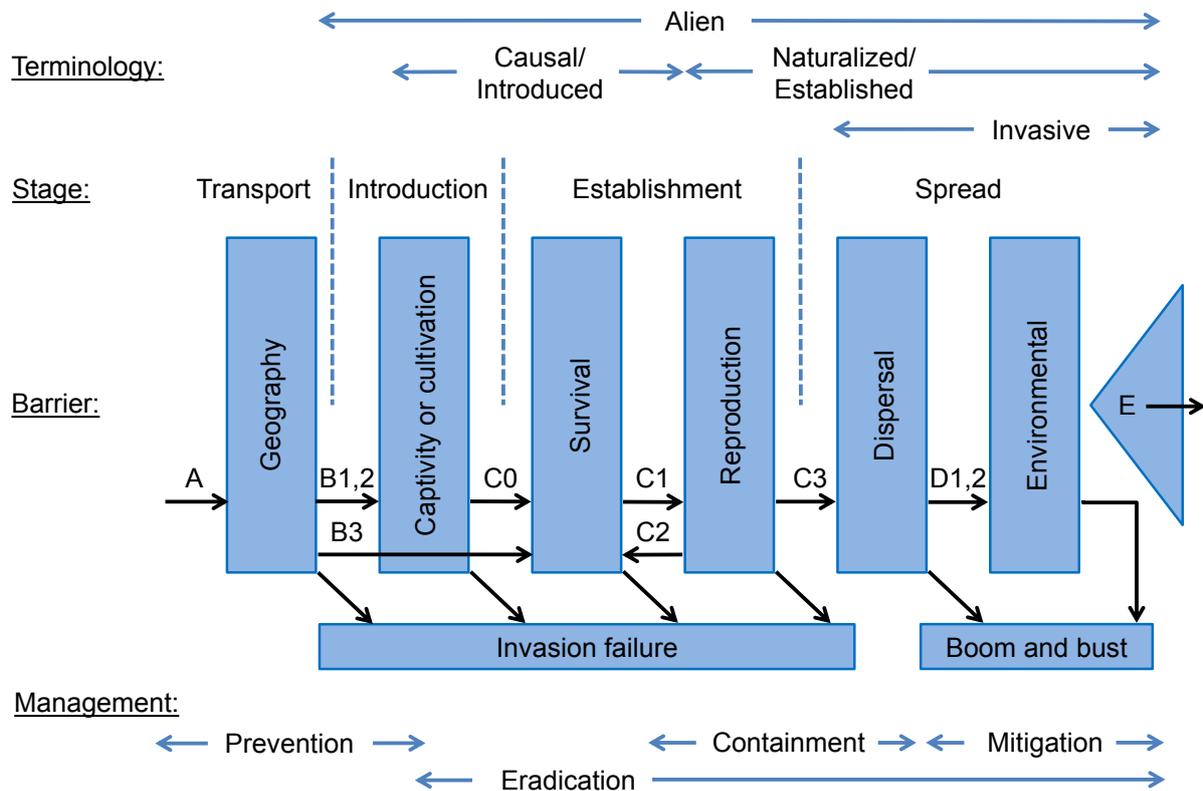
Figure 1.1. The proposed unified framework for biological invasions where step A (transport) and steps D-E (spread) are linked to dispersal ability and locomotor performance (redrawn from Blackburn et al., 2011).

1.2   Insect flight performance and its temperature-dependence

All locomotion is achieved by means of forces exerted by an organism on its immediate environment. Swimming and flying animals interact with their fluidic surroundings whereas a running animal pushes against a solid surface (Dickinson et al., 2000; Drucker and Lauder, 2001). The mechanics of insect flight is an intricate field of study (based heavily on engineering and physics) and the aerodynamic characteristics of insect flight have been studied in detail (e.g. Ellington, 1984a-f; Wakeling and Ellington, 1997a-c; Dickinson et al., 1999). When considering flight as a means of locomotion, although the underlying mechanisms are complex and differ

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greatly between organisms from different taxa (Norberg, 1976; Dickinson et al., 2000; Sane, 2003; Usherwood et al., 2011), the elementary principles of aerodynamic forces apply to all airborne animals. Simply put, if an insect cannot generate sufficient lift force, it will not be able to fly (Dudley, 2000). From a physiological point of view, the dispersal ability of an insect is thus directly related to the ability to create sufficient forces in its environment (reviewed in Weber, 2009). The factors influencing lift force production, and ultimately flight performance, may be intrinsic (physiological or morphological factors) or extrinsic (environmental factors), and will be discussed in subsequent sections.

1.2.1 Intrinsic factors influencing insect flight performance

Winged insects (pterygotes) can either have two pairs of functioning wings (a pair of fore wings and a pair of hind wings) or, depending on the insect order and sex, one of these pairs (either the fore- or hind wings) could have evolved into modified structures. For example, the front wings of beetles (Coleoptera) are modified to form elytra (hardened cases that protect the insect body) and the hind wings of flies (Diptera) are modified to form halteres (structures that function as stabilisers during flight) (Gullan and Cranston, 2010). The number of wings an insect has can influence flight performance with regards to speed, manoeuvrability and energy expenditure (Dudley, 2000). Many insect species are also totally wingless (apterygotes), but in other species, morphs with different wing lengths as well as wingless morphs occur. Insects from these species sometimes face a trade-off between increasing flight performance and another fitness-related trait. In sand cricket (*Gryllus firmus*) males, there is a trade-off between reproductive ability (determined as testes size) and flight capability (Saglam et al., 2008). It is also well established that longer flights typically result in reduced reproductive output (Baguette and Schtickzelle, 2006), which is generally regarded as a resource allocation trade-off (e.g. Zera and Mole, 1994; Zera and Denno, 1997; Karlsson and Johansson, 2008).

Wing morphology is another important element of insect flight. In principle, when all else is kept constant, an insect of any given weight can either have big wings that flap slowly or small wings that beat fast (Bartholomew and Heinrich, 1973). This difference can have dramatic consequences for energy expenditure during flight. Wing morphology, such as wing length, width, and area, also influence aspects of flight such as wing loading (the ratio of body weight to wing area) and aspect ratio (the ratio of wing length to wing width), which can affect aerodynamic ability and, ultimately, overall flight efficiency (Dudley, 2000; Norberg, 2002). In Fig. 1.2, the different combinations of, or trade-offs between, aspect ratio and wing loading is illustrated for birds, but the

same trends likely hold true for insects. Examples of organisms with inexpensive flight are shown in the top left corner, and in the bottom right corner the opposite is illustrated, i.e. organisms with very expensive flight or the inability to fly.
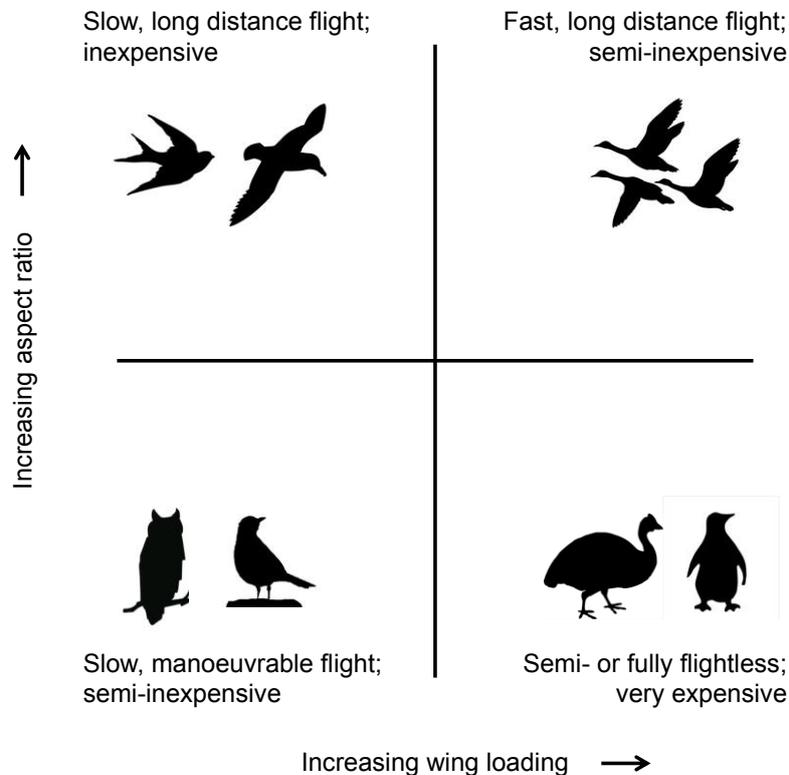
Figure 1.2. A schematic of potential trade-offs between wing loading, aspect ratio and aerodynamic performance (drawn with information adapted from Norberg (2002) and Stimpson (2012)).

Flight performance and endurance increases with increasing muscle percentage and, to achieve flight, insects must have flight muscles that contribute approximately 15% of their total mass (Marden, 2000). In insects, two types of flight muscles exist with fundamentally different characteristics: synchronous flight muscles which contract directly through nervous impulses and asynchronous flight muscles that oscillate at frequencies higher than the actual motoneuron activity (Harrison and Roberts, 2000). Insects that have asynchronous flight muscles (e.g. species from the orders Diptera and Hymenoptera) are thus capable of more energy efficient flight than insects with synchronous flight muscles (e.g. Lepidoptera and Orthoptera) (Dudley, 2000).

Apart from morphological features such as wing size, shape and number of wings, the overall size of an insect is also a crucial determinant of flight ability. Body size impacts not only wing loading via its effect on the mass component of the equation, but also influences metabolic rate

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through mass-scaling effects (Chown and Nicolson, 2004). Although larger insects have absolutely greater metabolic rate than smaller insects, their relative energy consumption (on a mass-specific basis) is in fact smaller (Niven and Scharlemann, 2005). This is true for resting as well as active or flying metabolic rates, i.e. smaller insects have relatively greater costs (Fig. 1.3). Flight efficiency (power output divided by power input) increases with body size, but as flying insects become larger their power requirements also increase at a greater rate than their metabolic energy production (Harrison and Roberts, 2000), thereby influencing flight performance.
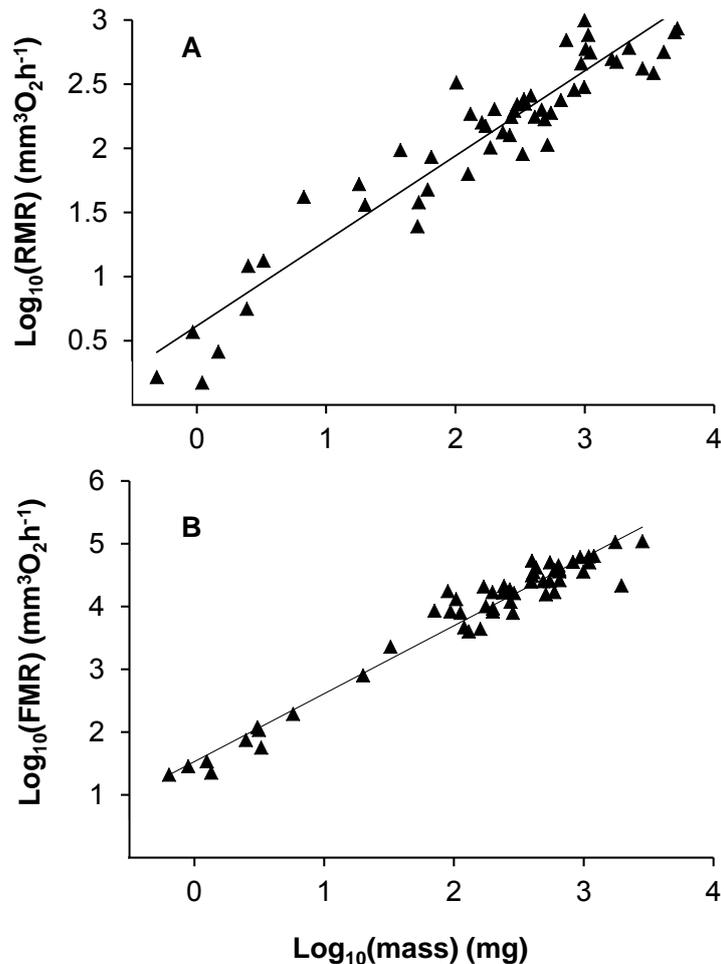
Figure 1.3. The linear relationships between A) resting metabolic rate (RMR) and body mass and B) flying metabolic rate (FMR) and body mass in insects. RMR scaled with body mass to the power of 0.66 ($M^{0.66}$) and FMR scaled with $M^{1.10}$ (redrawn from Niven and Scharlemann, 2005).

Different modes of transportation come at different costs, with flight in insects being about 30 times more energetically demanding than running (Harrison and Roberts, 2000). Short duration flights are also much more energetically costly than the same distance of pedestrian transport, but

the opposite is also true, with long distances being more efficiently covered using flight than by crawling or walking (Velasco and Millan, 1998). Whichever means of transport is chosen, no organism can move without sufficient energy resources at its disposal. Within flight muscles of insects, energy or ATP production takes place at a very high tempo in the mitochondrial Krebs cycle and electron transport chain (Harrison and Roberts, 2000). Fuel to sustain metabolic processes can be stored as lipids in the body. Although lipids are not the only energy source used by insects during flight (e.g. proline in Glossinidae (Bursell, 1963)), it can provide the energy needed for various biological activities such as reproduction and locomotion (Lease and Wolf, 2011). Storing energy as lipid reserves can come with both costs and benefits however, with increasing gut and lipid reserves leading to higher long-term mating success, but simultaneously causing a decrease in space available for flight muscles (Marden, 1989).

1.2.2   Extrinsic factors influencing insect flight performance

Given the diversity of habitats occupied by insect species worldwide, it is likely that species have physiologically adapted to survive and perform best in their specific habitat or environmental niche (Chown and Nicolson, 2004). Local and regional abiotic factors, such as temperature, moisture and solar radiation, can have a dramatic influence on insect species geographic distribution through limitations in energy availability (Turner et al., 1987; Hawkins et al., 2003). Since the early work of Krogh (1916) and Wigglesworth (1935) it has been repeatedly demonstrated that temperature also has pervasive effects on physiological performance and fitness in ectotherms, influencing e.g. wing beat frequency, running speed, metabolic and developmental rates. Ambient temperature can also affect muscle physiology, mechanical power output of muscles and changes in enzyme activity in flight muscles (Lehmann, 1999; Lachenicht et al., 2010). For example, cytochrome c oxidase (CCO), the final enzyme in the mitochondrial electron transport chain, may, through temperature changes, be a rate-limiting step in the process of energy production for flight (Capaldi, 1990; McMullen and Storey, 2008).

The diversity, fitness and survival of insect species are currently under threat by various factors such as IAS, habitat loss, degradation and fragmentation through urbanisation and agricultural activities, as well as anthropogenic climate change (Pryke and Samways, 2008; Gullan and Cranston, 2010). Fitness comprises many physiological attributes which are sensitive to temperature (Angilletta, 2009). For example, Berwaerts and Van Dyck (2004) showed that flight acceleration for both male and female *Pararge aegeria* butterflies depended on temperature, with faster flight acceleration at optimum temperatures (T_{opt}) compared to the suboptimal temperatures.

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Field observations also show that abiotic conditions can directly influence aspects of locomotion and dispersal. In codling moth (*Cydia pomonella*), for example, temperature has a positive linear relationship with moth capture rates across a thermally benign window (16-30°C) (Chidawanyika and Terblanche, 2011).

To better understand the potential environmental constraints on ectotherm performance, the theoretical concept of the thermal performance curve (TPC, Fig. 1.4) can be used (Angilletta, 2009). Two characteristics of the performance curve should be noted: first, this is an asymmetric curve, meaning that e.g. for a 3°C increase in temperature above T_{opt} there will be a greater decline in performance, and consequently higher risk of overheating, compared with a 3°C decrease below T_{opt} (Martin and Huey, 2008). Second, above and below T_{opt}, the organism's performance declines to zero at critical temperatures. These end-points are sometimes described as the maximum and minimum critical thermal limits (CT_{max} and CT_{min}), and the difference between these limits is the thermal range across which the insect can operate (Huey et al., 1989; Angilletta, 2009).
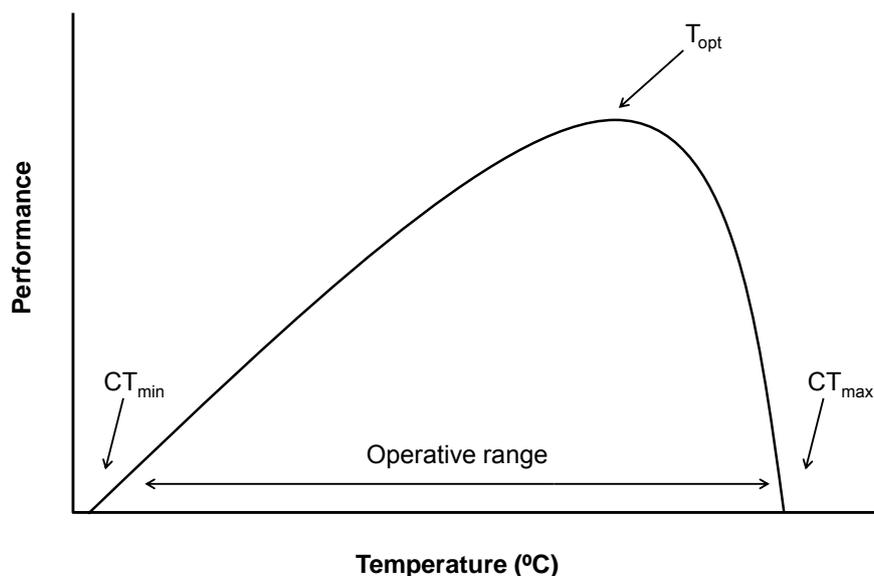
Figure 1.4. The typical thermal performance curve of an insect, indicating the optimum temperature for performance (T_{opt}) as well as maximum and minimum critical thermal limits (CT_{max} and CT_{min}) (redrawn from Angilletta, 2009).

Deutsch et al. (2008) used the TPC to determine two measures of climate change sensitivity: the warming tolerance (WT = CT_{max} - habitat temperature (T_{hab})) and the thermal safety margin (TSM = T_{opt} - T_{hab}), which was then mapped across the planet to estimate likely extinction risks associated with climate change. Given the temperature-dependent performance of insects discussed

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above, the 1.8°C to 4°C increase in average ambient temperatures predicted by the year 2100 (IPCC, 2001) will likely have an effect on thermally-sensitive functions such as locomotor performance, growth and reproduction (Midgley et al., 2003; Tolley et al., 2009). In turn, this might influence fitness, population persistence and dispersal ability (Deutsch et al., 2008), especially if there is limited scope for behavioural thermoregulation (e.g. Kearney et al., 2009). Climate change is furthermore predicted to alter geographic distributions of species (Hannah et al., 2005; Parmesan, 2006). The overall effect of changes in performance and species composition could result in dramatic changes to total ecosystem functioning through changes in food-web networks or the frequency of predator-prey interactions (e.g. O'Gorman and Emmerson, 2009; Vucic-Pestic et al., 2011). Range or abundance variation with climate change may therefore have considerable implications for invasive and pest species management worldwide.

1.3   Fruit flies as a model species

Mediterranean fruit flies, *Ceratitis capitata* (Weidemann) (Diptera: Tephritidae), are one of the most notorious agricultural pests worldwide with more than 150 different host plants in Africa, of which many are economically or agriculturally important (De Meyer et al., 2002). *Ceratitis* is an Afrotropical genus which has, with increased agricultural trade over the past 100 years, spread from its native range in sub-Saharan East Africa across much of the globe and currently also threatens to invade new areas (Malacrida et al., 1998, 2007). In South Africa, *Ceratitis* spp. are especially known for decreasing the net worth of the citrus industry through damage to fruit which makes it unmarketable, export risks and quarantine practises associated with accidental introductions, and, through economic costs associated with attempts to control these pests (Barnes, 2000). Part of the invasion ability of *C. capitata* is based on characteristics such as a broad thermal and diet niche, short generation time and large reproductive output, and perhaps also a greater evolutionary adaptive potential (Malacrida et al., 2007; Nyamukondiwa and Terblanche, 2009).

High levels of gene-flow between populations of *Ceratitis capitata* in South Africa were detected by Karsten et al. (2013) which were attributed to some form of long-distance jump dispersal. On a local scale, *C. capitata* can disperse relatively well on its own accord, with distances of up to 21 km predicted in a flies' lifetime, although the majority of individuals travel only about 200-900 m (Meats and Smallridge, 2007). A study of the dispersal ability of *C. capitata* in relation to environmental variables such as temperature is thus extremely important, not only to predict future spread, but also because it has implications for population control programmes, such as the

release of sexually sterilized individuals (the sterile insect technique, SIT) (Meats et al., 2006; Karsten et al., 2013).

With a changing climate and the possibility of new invasions by pest species (Meynard et al., 2013), it is therefore clear why there is increasing interest in understanding specific environmental effects on physiology, dispersal ability and transport costs of animals in their environment. *C. capitata* is the ideal study organism to address these questions, given its relative ease of rearing, our basic knowledge of its biology, and its history as an invasive species that has had profound economic consequences for agriculture.

1.4   Study aims

The objectives of this study are threefold:

1)   to determine the temperature-dependence of flight performance in *Ceratitis capitata* and examine the effect that within-generation thermal history has on flight performance. Both ambient temperature and developmental acclimation temperature are predicted to influence flight performance (the concept of thermal acclimation is described in Chapter 2);

2)   to determine whether underlying intrinsic factors: morphological (body mass, wing width, wing length, wing area, wing loading, and aspect ratio), physiological (whole-animal resting metabolic rate) or biochemical (cytochrome c oxidase activity) could be correlated with temperature-dependent changes in flight performance, as all these factors have previously been implicated in influencing flight performance in the field or the laboratory, and finally

3)   to place the outcomes of the experimental research into a broader, applied context by exploring the implications of these results on invasive and pest species management.

Chapter 2 :

The effects of within-generation thermal history on the flight performance of *Ceratitis capitata*: Colder is better*

2.1   Introduction

The thermal environment experienced by ectothermic organisms has widespread effects on their physiological performance and survival, with ambient temperatures directly influencing their physical ability to perform functional activities such as locomotion (Kaufmann and Bennett, 1989; Dillon and Frazier, 2006; Clusella-Trullas et al., 2010). Such effects can be caused by temperature-induced changes in energy availability via changes in mitochondrial functioning (e.g. O'Brien et al., 1991; reviewed in Hochachka and Somero, 2002; Seebacher and James, 2008), which, in some cases, directly influences the mechanical power output of muscles (Bennett, 1985; Swoap et al., 1993; Lehmann, 1999; reviewed in James, 2013). Furthermore, several other important physiological processes (e.g. metabolic and development rates) are also strongly influenced by ambient temperature (reviewed in Frederich and Pörtner, 2000; Chown and Nicolson, 2004; Irlich et al., 2009; Dell et al., 2011), and may in turn have indirect effects on locomotor capacity. The resulting changes in performance can affect the short- and long-term dispersal capacity of arthropods with significant implications for ecology and evolution (reviewed in e.g. Feder, 2010; Bonte et al., 2012; Clobert et al., 2012; Gomez and van Dyck, 2012).

Performance varies as a function of ambient temperature and this relationship is dependent on thermal history at several time-scales. Indeed, it is well documented that performance is flexible both within and between generations in ectothermic animals (Hoffmann et al., 2003; Rako and Hoffmann, 2006; Chown and Terblanche, 2007; Kingsolver, 2009). Conditions experienced either over the short term within a single life-stage (hardening responses, e.g. Kellett et al., 2005; Basson et al., 2012), throughout a developmental or adult stage (e.g. Kristensen et al., 2008; Fischer et al., 2010; Waagner et al., 2013), or over evolutionary time-scales (among populations or between species, e.g. Gibert et al., 2001; Kelty and Lee, 2001; Steigenga and Fischer, 2007) can radically alter tolerance and performance under a given set of environmental conditions. Moreover, it is increasingly clear that the different time-scales of thermal exposure may result in different underlying mechanisms and responses (e.g. Colinet and Hoffmann, 2012; Teets and Denlinger, 2013; Waagner et al., 2013).

Within-generation changes in performance phenotypes may reflect responses to environmental conditions, referred to as phenotypic plasticity, and are defined as genotype-by-environment interactions (DeWitt and Scheiner, 2004; Ghalambor et al., 2007; Whitman and Agrawal, 2009). Acclimation is defined by Wilson and Franklin (2002) as "*any facultative modification in a physiological trait in response to changes in an environmental variable in the lab.*

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Changes can be in response to the developmental environment or long-term environmental shifts during the later stages of the life history of an organism." Acclimation has, however, been used to refer to the outcome as well as the treatment of an exposure (see e.g. Bowler and Terblanche, 2008). In this study, the term will specifically be used to define the treatment conditions, which may or may not result in reversible or irreversible phenotypic plasticity (Piersma and Drent, 2003; Terblanche and Chown, 2006).

There are several main hypotheses proposed to describe the form and nature of phenotypic plasticity, in particular, the variation in performance after exposure to differing thermal conditions (Huey and Berrigan, 1996; Huey et al., 1999; Deere and Chown, 2006). Notable among these hypotheses, is the beneficial acclimation hypothesis (BAH), which states that *"acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment"* (Leroi et al., 1994; and see Wilson and Franklin, 2002). The BAH has not however received strong support, largely owing to the inability to refute possible alternative hypotheses (Deere and Chown, 2006). Foremost among these alternatives are the colder is better (CIB) (e.g. Frazier et al., 2008) and the hotter is better (HIB) (e.g. Frazier et al., 2006) hypotheses which have also received some support depending on the traits and taxa examined. These hypotheses propose that organisms will perform best after exposure to either colder or hotter conditions respectively (see review in Huey et al., 1999). Other potential explanations for acclimation responses include the optimal acclimation hypothesis (OAH) (e.g. Zamudio et al., 1995; Terblanche and Kleynhans, 2009) or deleterious acclimation hypothesis (DAH) (e.g. Loeschcke and Hoffmann, 2002; Terblanche and Kleynhans, 2009) which propose that a particular intermediate environment will result in improved performance, or that the acclimation conditions resulted in damage which led to lower performance upon subsequent testing. The null hypothesis for all of these different acclimation responses is that there will be no phenotypic plasticity response under any environmental conditions (Huey et al., 1999).

Even though phenotypic plasticity has been studied extensively in the context of organism performance, the underlying physiological and biochemical mechanisms driving the performance outcomes are not straightforward (e.g. Sørensen et al., 2009). For insects, some studies have shown a strong thermal acclimation response of resting metabolic rate (e.g. Terblanche et al., 2009; Terblanche et al., 2010a) with insects from cool environments having a steeper metabolic rate-temperature reaction norm than insects from warmer environments. Even in such cases, however, the underlying mechanisms of acclimation responses remain unclear (Terblanche et al., 2010a;

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Vorhees et al., 2013). Another potential mechanism for acclimation responses in insects may be the direct impact of temperature on metabolic enzymes, but the effects of acclimation temperature on energy production and efficiency (e.g. activity of cytochrome c oxidase (CCO)) also vary among ectotherms (Dahlhoff and Somero, 1993; Rogers et al., 2004). Metabolic pathway enzymes in insects are generally correlated with increased performance at certain temperatures (e.g. Laurie-Ahlberg et al., 1985; McMullen and Storey, 2008) and CCO activity is argued to potentially be a rate-limiting step in ATP production in mitochondria (Suarez et al., 2000; Hochachka and Somero, 2002). Temperature-related morphological changes, such as variation in wing size and shape (Cavicchi et al., 1991; Zera and Harshman, 2001) and body size (Nunney and Cheung, 1997; Frazier et al., 2001), could likewise be driving the outcome of various performance traits and their responses to temperature.

The immediate effects of ambient temperature on insect flight performance have been well documented (Chown and Nicolson, 2004; Dillon and Frazier, 2006; Samejima and Yoshitaka, 2010), but in contrast, the effects of developmental or rearing temperature on flight performance have been less extensively studied. Two notable exceptions, however, include recent work by Frazier et al. (2008) on *Drosophila melanogaster* and Ferrer et al. (2013) on *Grapholita molesta*. Both of these studies focussed on laboratory responses and showed marked effects of developmental temperature on traits of adult flight performance. These studies are, however, limited in their ability to interpret the acclimation hypotheses, mainly because they do not acclimate and test individuals in conditions that are both above and below the optimal rearing temperature. For example, Frazier et al. (2008) only focused on low temperature flight ability in *Drosophila melanogaster* (14-18°C), meaning that a crucial knowledge gap exists across a wider, more benign range of thermal conditions that insects are likely to encounter in the field. Field studies have also examined the impact of rearing temperature on dispersal, and by implication, flight performance (e.g. Kristensen et al., 2008; Loeschcke and Hoffmann, 2007; Chidawanyika and Terblanche, 2011). As the studies by Frazier et al. (2008) and Ferrer et al. (2013) could not fully assess potential trade-offs between elevated low temperature flight performance and high temperatures, it thus leaves the information from field assessments of acclimation responses by recapture at bait stations (e.g. Kristensen et al., 2008) not especially well linked to laboratory responses (though see discussions in Sørensen et al., 2009; Chidawanyika and Terblanche, 2011).

In this study, the temperature-dependence of flight ability and its response to rearing temperature in *Ceratitis capitata*, a global agricultural pest, was examined in a full-factorial experimental design. This design aimed to cover the thermo-biological range of typical flight

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activity in *C. capitata*. Given that an acclimation response was largely expected based on the aforementioned literature, factors that may have resulted in this change in flight ability and performance were also investigated. To this end, a range of morphological variables (body mass, wing width, wing length, wing area, wing loading, and aspect ratio) were examined, as all have previously been implicated in influencing flight performance and manoeuvrability in the field and in the laboratory (e.g. Bartholomew and Casey, 1978; reviewed in Dudley, 2000; Harrison and Roberts, 2000; Berwaerts et al., 2002; Gomez and van Dyck, 2012). As proximate explanations for variation in flight performance, whole-animal resting metabolic rate and a key aerobic energy pathway enzyme activity (cytochrome c oxidase) were assessed.

2.2   Materials and methods

2.2.1   Study organisms

Individuals of *Ceratitis capitata* were obtained from a large outbred culture reared indoors under variable though buffered temperatures at Citrus Research International (Nelspruit, South Africa). On arrival at Stellenbosch University (Stellenbosch, South Africa), pupae were divided into four developmental acclimation groups (T_{acc}: 15, 20, 25 and 30°C) and maintained in temperature-controlled incubators (MRC LE-509, Holon, Israel) under a 12h:12h L:D photoperiod. Pupae were kept at their respective acclimation temperatures until peak adult eclosion (T_{acc}=30°C for 5 to 6 days, T_{acc}=25°C for 6 to 7 days, T_{acc}=20°C for 7 to 8 days and T_{acc}=15°C for 12 to 13 days), after which the flies were allowed to mature for 7 to 8 days at 25°C with sugar and water available ad libitum to ensure that flight muscles were fully developed (e.g. Skandalis et al., 2011). Flies were kept at 25°C to ensure that developmental effects were in fact due to longer-term alteration of ontogenetic trajectories and not changes associated with morphological re-organization upon eclosion (see Bowler and Terblanche (2008) for similar discussion in terms of thermal tolerance). Flies from the different T_{acc} groups were selected at random for trials and all assays were undertaken on flies at the same adult developmental age, i.e. a week after eclosion.

2.2.2   Flight performance

A full-factorial experimental design was used to determine flight performance of the developmental acclimation groups at four different test temperatures (T_{test}: 15, 20, 25 and 30°C). Flight experiments were performed on a custom-built 0.36 m^2 double-jacketed temperature stage, under which 1:1 water: propylene glycol mix was pumped from a programmable water bath (Huber CC-410wl, Huber, Offenburg, Germany). A thermocouple (type K, 36 SWG) connected to a digital thermometer (Fluke 54 series II, Fluke Cooperation, China) was used to verify the stage surface temperature and insect body temperatures were measured with a handheld infrared thermometer (Fluke 63 IR series, Fluke Cooperation, China; accuracy 0.05°C at 5cm distance) to ensure that this was always at equilibrium with the chamber surface temperature.

A total of n = 480 randomly selected flies (120 per T_{acc}; 15 males and 15 females per T_{test}) were each individually introduced to an inverted transparent plastic container (12cm length x 12cm width x 7cm height) and allowed a 2 minute thermal equilibration period on the surface of the temperature stage. Each fly was encouraged to perform a response by gently prodding them with a

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thermally-equilibrated, thin plastic rod inserted between the plastic container and the thermal stage. Performance in the first minute was either recorded as "flight" (score=2; the ability to stay airborne and travel length or height of the container, indicating sustained flight), "lift" (score=1; temporary lift, but with insufficient distance travelled) or "failure" (score=0; walking or no activity). In the latter case, prodding continued until a maximum of 5 minutes had passed or until flight or lift was achieved. According to the flight scores assigned, a flight score of 2 contains a significant aspect of flight performance. For this reason, flight score outcomes are used to reflect performance and not simply the propensity or willingness to fly. No flies were re-used at another temperature. A fly could only be scored in one behaviour category and was removed once this behaviour category was determined. All flies were weighed to 0.1 mg using a digital microbalance (Mettler Toledo MS104S, Switzerland) before each trial to determine fresh mass.

2.2.3   Wing morphology

For measurement of wing morphology, each fly used in the flight performance trials were euthanized at -80°C. Their wings were removed with a scalpel and the right wings were mounted on a microscope slide with clear nail varnish. Wings for two-dimensional image analysis were photographed using a Leica MZ16A automontage microscope fitted with a Leica DFC 290 fixed digital camera (Leica, Wetzlar, Germany). Fig. 2.1 shows the landmarks (present on wings from all individuals) used to determine wing length, wing width and wing area of each wing, where:

1= anterio-anal corner of cell c; 2= termination of vein R_{4+5}, inner side of cell r_{2+3}; 3= subcostal break (Scb); 4= A_1+Cu_2 termination; and the red lines run between the anterio-costal corner of cell c, Scb, R_{2+3}, R_{4+5}, M, Cu_1 and A_1+Cu_2 termination landmarks on the edge of the wing.

The variables were calculated from the digital images using analysis tools that accompany the Leica software (Leica Application Suite (LAS) v 4.1). From these measurements, aspect ratio and wing loading were calculated with the following equations (Dudley, 2000):

$$AR = \frac{4R^2}{S} \qquad (1),$$

where AR is the aspect ratio, R is the wing length in mm and S is the wing area in mm^2;

$$WL = \frac{M_b}{S} \qquad (2),$$

where WL is the wing loading, M_b is the body mass in mg and S is the wing area in mm^2.

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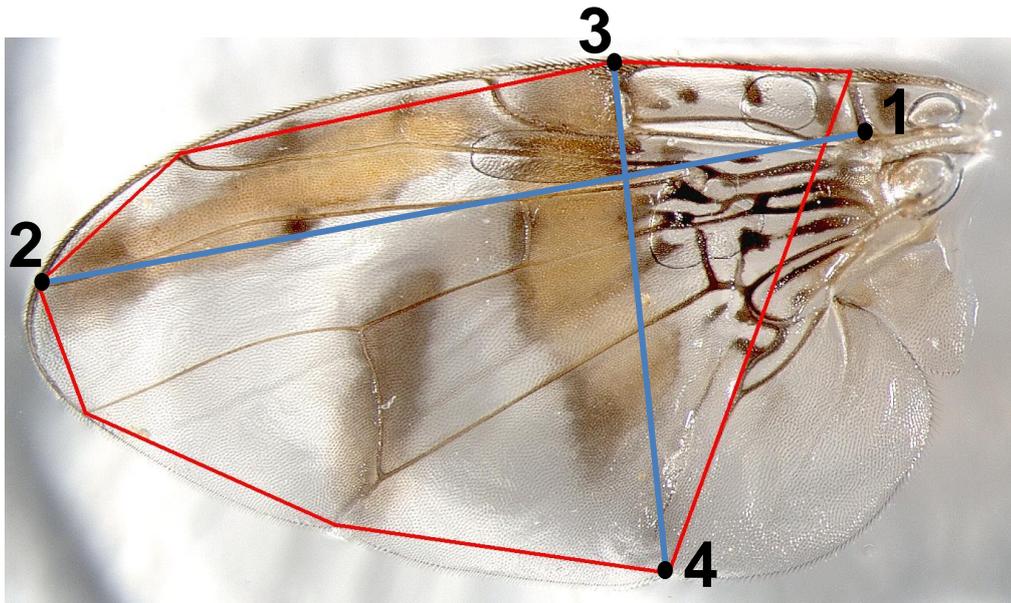
Figure 2.1. Landmarks used for measuring the wing length (1 to 2), wing width (3 to 4) and wing area (red lines) of *Ceratitis capitata*.

2.2.4   Metabolic rate

For this part of the study, a new batch of fly pupae were exposed to developmental acclimation temperatures as stated above. The V_{CO_2} production of individual adult fruit flies was then recorded using a multiplexed flow-through respirometry system as described by Potter and Woods (2012). The airflow was regulated via a mass control valve (Sidetrak, Sierra International, USA) linked to a mass flow control box (Sable Systems, Las Vegas, Nevada, USA). Next, air was pushed through the first channel of an infra-red CO_2/H_2O analyser (LI-7000, LiCor, Lincoln, Nebraska, USA) to obtain a baseline reading, passed through the respirometry cuvette, and returned to the LI-7000 for differential recording of insect V_{CO_2} production. The set-up included an eight-channel multiplexer with the temperature of the respirometry cuvettes being regulated by a programmable, circulating water bath (Huber cc410-wl, Offenburg). The first channel of the multiplexer was used as an empty reference channel to determine baseline readings, whereas the remaining seven were used to record the gas exchange of individual flies. Each of the eight channels of the multiplexer was consecutively recorded for 30 minutes and this was repeated at every temperature: 15, 20, 25 and 30°C. A total of 14 randomly selected flies per T_{acc} (7 males and 7 females) were measured at each T_{test}. Recording traces where V_{CO_2} values were low and stable were used to identify inactive periods, as verified in independent pilot trials with the use of electronic activity detectors. Outlier data points (values greater than 3 times the mean value for the

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specific T_{acc} x T_{test} treatment) were deleted from the dataset, as this indicated that the individuals were probably not at rest, e.g. fanning their wings. V_{CO_2} data recorded in parts per million were converted to ml.h^{-1} at STP. Owing to the short recording period, H_2O data were discarded as readings had not yet stabilized before the respirometry multiplexer channels were switched. All flies were weighed to 0.1 mg using a digital microbalance (Mettler Toledo MS104S, Switzerland) before and after each respirometry trial to determine fresh mass.

2.2.5 CCO activity

For the cytochrome c oxidase (CCO) activity assay, a total of six samples per T_{acc} group were measured. For this assay, a total of six samples per T_{acc} group were measured. Each sample consisted of 60 whole male flies, totalling ca. 300mg as required for sufficient mitochondrial extraction. A mitochondrial fraction was prepared from each sample by means of a mitochondrial isolation kit (MITOISO1; Sigma, Missouri, USA) following the manufacturer's protocol (and see Lachenicht et al., 2010). Enzyme activity of the isolated mitochondrial extraction was then measured using a CCO assay kit (CYTOCOX1; Sigma, Missouri, USA) and a temperature controlled spectrophotometer (PowerWave HT; BioTek, Winooski, USA) at 25°C. All measurements of sample absorbance were made every 5 seconds over the course of 1 minute. First the inactive samples were measured, after which ferrocytochrome c was added to start the reaction. The resulting absorbance changes were then recorded. The average absorbance over a minute (ΔA_{550}/min) of the inactive sample was subtracted from the average absorbance over a minute of the activated samples to calculate the absolute change in absorbance. CCO activity in units per ml was calculated using the difference in extinction coefficients ($\Delta\varepsilon^{mM}$) between reduced and oxidised cytochrome c at 550 nm. One unit is equal to the amount of CCO that will oxidise 1.0 mmole of ferrocytochrome c per minute (pH 7.0, 25°C). The original fresh mass of each sample was used to convert the results into mass specific CCO activity (U/ml/mg).

Of the six samples used per T_{acc}, three were frozen in liquid nitrogen and placed in a -80°C freezer before use and three consisted of fresh flies. A generalized linear model with a normal distribution and a log link function showed that the data could be pooled, seeing as there were no significant differences between frozen and fresh tissue samples (P=0.102). There was also no indication that CCO activity was influenced by an interaction between T_{acc} and the tissue state of the samples (P=0.084).

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2.2.6 Statistical analyses

2.2.6.1 Flight performance

To address the main question of whether the flight scores at different test temperatures were influenced by thermal history, the ordinal logistic regression model method adopted by Frazier et al. (2008) was followed. This method delivers the odds of changing between flight categories "failure" and "lift" or between categories "lift" and "flight", based on the different parameters in the model. Firstly, to determine which factors to include in the ordinal logistic regression, a minimal adequate model was obtained using the approach outlined in Crawley (2007). This consists of initially fitting the maximal model (which included T_{acc}, T_{test}, body mass (M_b), sex and all possible interaction terms) and then simplifying the model, starting with the highest order interactions. The full model specifically included sex (where males and females were assigned 0 or 1, respectively) and M_b as separate factors, because both may independently influence flight performance. The full model also specifically included a test for the interaction between T_{test} and T_{acc} as an indicator of phenotypic plasticity.

2.2.6.2 Morphology

To assess the effects of T_{acc} and fresh M_b on morphological variables, a full generalized linear model (GLZ) with a normal distribution of errors and a log link function was run for each main variable separately (wing length, wing width, wing area, *AR* and *WL*), and included a T_{acc} x M_b interaction term. Male and female flies were investigated separately as sex seemed to be a major factor influencing the phenotypic plasticity at certain T_{acc}. If the T_{acc} x M_b interaction was not significant, this indicated that the slopes of T_{acc} groups were homogeneous. If the interaction term was significant, it was used to interpret the slope variation between groups.

Preliminary analyses suggested that some of the variation in morphology might be sex-related. Therefore, sex was also examined as a factor between T_{acc} groups, and the interaction between T_{acc} and sex was explicitly tested in a separate set of GLZ analyses run for each morphological variable (M_b, wing length, wing width, wing area, *AR* and *WL*).

In order to explore variation in morphological features (e.g. low or high *WL*) within and between flight score categories, within and between T_{acc} groups, an alternative approach to the logistic regression was used. Specifically, morphological variables were treated as the dependent

variable and plotted as a function of flight score for both sexes separately and GLZ were used to test if morphology varied consistently among flight score groups.

2.2.6.3 Metabolic rate and CCO activity

Owing to repeated measures on the same individual at different T_{test} for V_{CO_2} estimates, a repeated measures ANCOVA (Kenward-Rogers method) was used to determine the minimum adequate model (based on lowest number of terms and lowest AIC value). The log-transformed values for body mass and V_{CO_2} measurements were used for this analysis because this better satisfies the assumptions of the mixed model (e.g. homogeneity of variance).

For CCO activity, a GLZ (normal distribution and a log link function) was used owing to violation of the assumptions of ANOVA (variances were heterogeneous). In all cases where GLZ was used, scaled deviances were inspected for potential over-dispersion which was not evident in any analyses.

All GLZ analyses were run in Statistica 11 (Statsoft, Tulsa, Oklahoma, USA) with arithmetic means and error bars indicating 95% confidence intervals unless otherwise stated. Repeated measures models were run in SAS (proc mixed, Version 9.4, SAS Institute, Cary, NC, USA). The ordinal logistic regressions and linear regression analyses were run in R (version 2.15.2; R Foundation for Statistical Computing, Vienna, Austria) and included the use of the *MASS* (Venables and Ripley, 2002) library.

2.3   Results

2.3.1   Flight performance

The odds of changing between flight categories "failure" and "lift" or between categories "lift" and "flight" are hereafter considered equivalent to flight performance. Flight performance was significantly influenced by T_{test} and the interaction term T_{test} x T_{acc}, but not by T_{acc} alone (Table 2.1). T_{acc} was however retained in the model, because of the higher order interaction term.

Table 2.1. Results of the best-fit, ordinal logistic regression assessing the effects of sex (males coded as 0, females coded as 1), body mass (M_b), test temperature (T_{test}), acclimation temperature (T_{acc}) and the interaction between T_{test} x T_{acc} on *Ceratitis capitata* flight performance.

Variable	Coefficient	Std. Error	t-value	*P* value	Odds ratio	2.50%	97.50%
Sex	-0.516	0.243	-2.121	**0.0339**	0.597	0.371	0.962
M_b	0.448	0.134	3.352	**<0.001**	1.565	1.204	2.034
T_{test}	0.474	0.040	11.741	**<0.0001**	1.606	1.484	1.738
T_{acc}	0.012	0.035	0.347	0.7286	1.012	0.946	1.083
T_{test} x T_{acc}	-0.008	0.002	-4.744	**<0.0001**	0.992	0.989	0.995

The variables in this model were selected with the minimal adequate model approach as described in Crawley (2007). T_{acc}, even though it was excluded from the minimum adequate model, was retained because of the presence of a higher order interaction term.

The positive coefficient shows that an increase in T_{test} results in increased flight performance across all T_{acc} groups, e.g. at T_{test}=15°C only 10% of flies had successful flight, whereas at T_{test}=30°C the success rate was 76.5% (Fig. 2.2A). A significant negative interaction between T_{test} x T_{acc} was detected (Table 2.1; *P*<0.0001). When multiple comparisons of flight performance outcomes were done between T_{acc} groups at each T_{test}, it was found that the only significant differences in performance occurred at T_{test}=15°C and 20°C (Table B.1). T_{acc}=15°C and 20°C performed better than T_{acc}=25°C and 30°C at T_{test}=15°C, but T_{acc}=15°C, 20°C and 25°C performed better than T_{acc}=30°C at T_{test}=20°C. No significant differences in flight performance outcomes were detected between the T_{acc} groups at T_{test}=25°C and 30°C (Table B.1; Fig. 2.2A). According to the best-fit model, sex and M_b also had a significant effect on flight performance with males generally performing better than females for a given body size (Fig. 2.2B,C) and a positive relationship between increasing flight score and M_b.
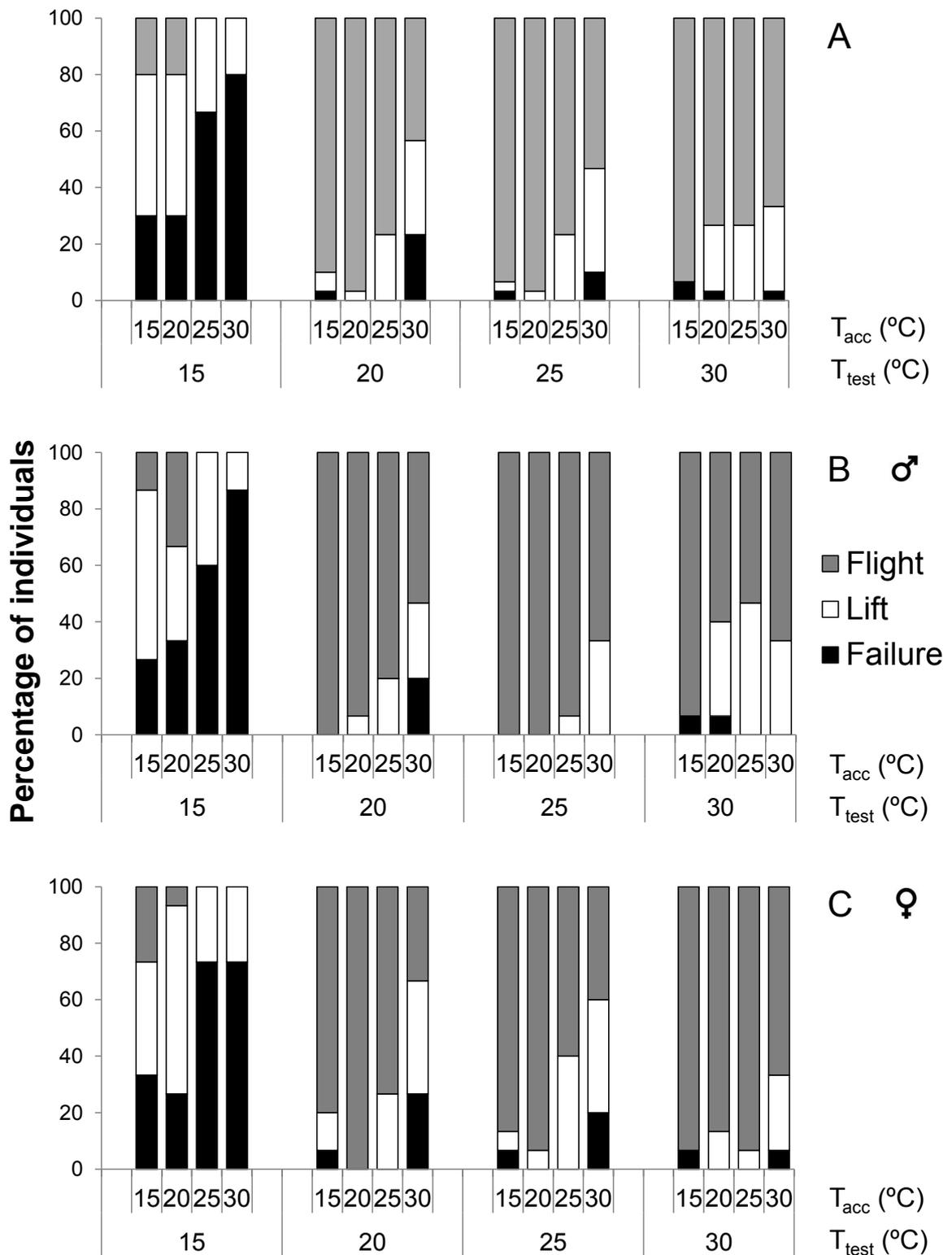
Figure 2.2. The effect of developmental acclimation temperature (T_{acc}) on flight performance of *Ceratitis capitata* flies at different test temperatures (T_{test}). A total of 30 flies (A) (15 males (B), 15 females (C)) from each T_{acc} were tested at each T_{test}. The black bars indicate failure to fly, the white bars indicate the ability to obtain lift (but no flight) and the grey bars show the proportion of individuals that were able to perform successful flight.

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2.3.2 Morphology

The scaling relationship between body mass and wing length, wing width and wing area varied with T_{acc} for males but not for females (Table 2.2; T_{acc} x M_b variables). There were no effects of T_{acc} on *AR* for either sex. Overall, morphological variation (wing length, width, area and *WL*) changed in relation to M_b only in cold-acclimated males (Fig. 2.3). In all four of these morphological variables, the trend was for males reared at T_{acc}=15°C to be significantly steeper (in the case of wing length, width and area) or significantly shallower (in the case of *WL*) than the warmer acclimation groups. Generally, the intermediate acclimation groups did not differ significantly from each other.
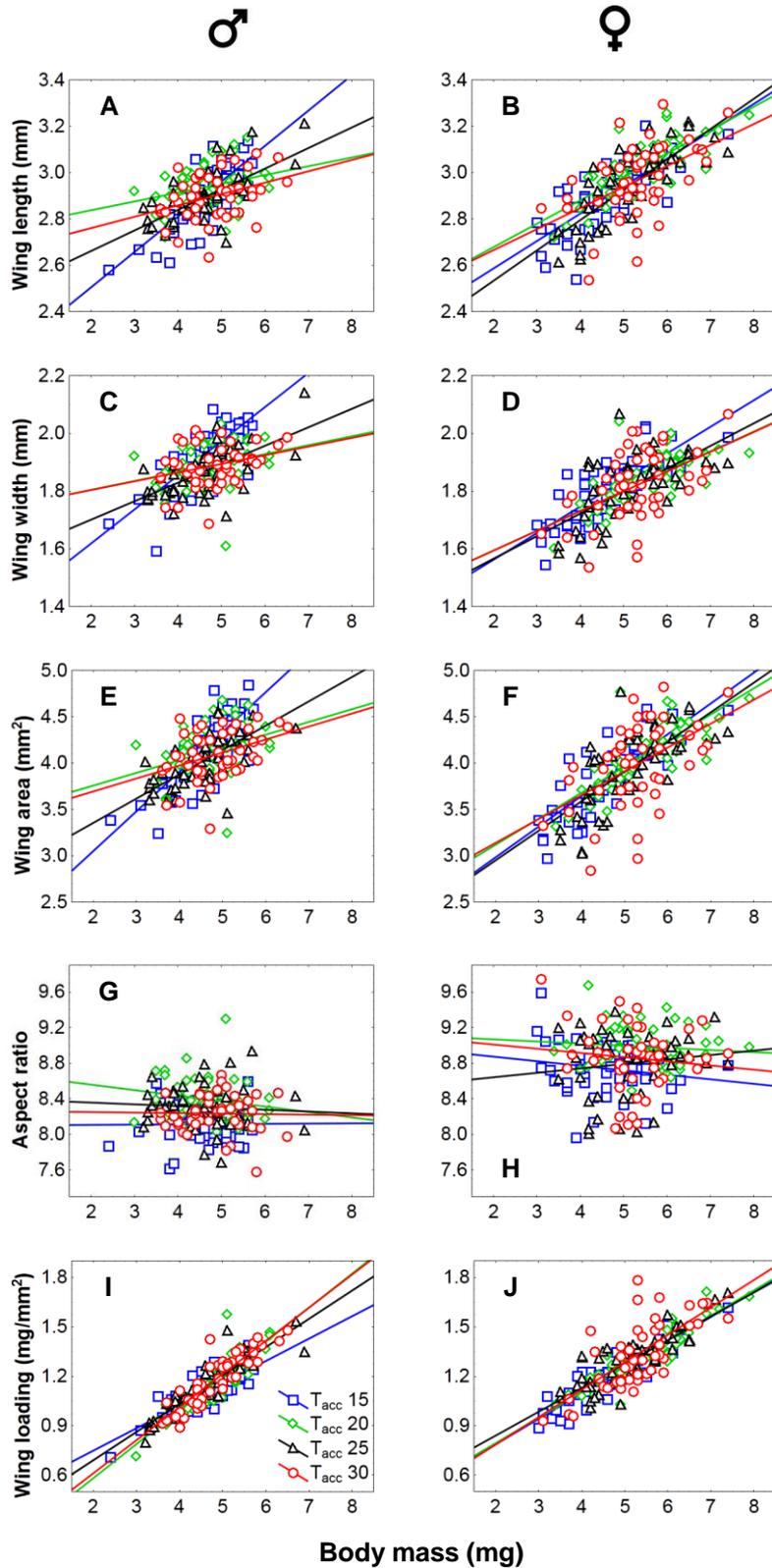
Figure 2.3. The effect of body mass on A-B) wing length, C-D) wing width, E-F) wing area, G-H) aspect ratio and I-J) wing loading in *Ceratitis capitata* males (first row of panels) and females (second row of panels) acclimated at 15°C (blue squares), 20°C (green diamonds), 25°C (black triangles) and 30°C (red circles). Lines indicate linear fit through raw data for each developmental acclimation group (T_{acc}).

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Table 2.2. Generalized linear models showing the effect of acclimation temperature (T_{acc}) and mass (M_b) on A) wing length, B) wing width, C) wing area, D) aspect ratio and E) wing loading in *Ceratitis capitata*.

	Sex	Effect	Degrees of freedom	Wald statistic	*P* value
A. Wing length (mm)	Males	Intercept	1	4527.2	**<0.0001**
		T_{acc}	3	27.1	**<0.0001**
		M_b	1	94.4	**<0.0001**
		T_{acc} x M_b	3	26.3	**<0.0001**
	Females	Intercept	1	4133.3	**<0.0001**
		T_{acc}	3	4.8	0.1892
		M_b	1	195.2	**<0.0001**
		T_{acc} x M_b	3	3.8	0.2888
B. Wing width (mm)	Males	Intercept	1	872.4	**<0.0001**
		T_{acc}	3	20.7	**<0.0001**
		M_b	1	86.4	**<0.0001**
		T_{acc} x M_b	3	26.5	**<0.0001**
	Females	Intercept	1	466.4	**<0.0001**
		T_{acc}	3	1.1	0.7670
		M_b	1	152.2	**<0.0001**
		T_{acc} x M_b	3	2.4	0.4850
C. Wing area (mm^2)	Males	Intercept	1	1707.6	**<0.0001**
		T_{acc}	3	20.4	**<0.0001**
		M_b	1	107.7	**<0.0001**
		T_{acc} x M_b	3	23.4	**<0.0001**
	Females	Intercept	1	1137.1	**<0.0001**
		T_{acc}	3	1.6	0.6648
		M_b	1	169.2	**<0.0001**
		T_{acc} x M_b	3	1.8	0.6059
D. Aspect ratio	Males	Intercept	1	29756.3	**<0.0001**
		T_{acc}	3	4.3	0.2355
		M_b	1	1.0	0.3287
		T_{acc} x M_b	3	1.3	0.7381
	Females	Intercept	1	24484.3	**<0.0001**
		T_{acc}	3	3.6	0.3035
		M_b	1	0.6	0.4421
		T_{acc} x M_b	3	3.1	0.3841
E. Wing loading (mg/mm^2)	Males	Intercept	1	367.8	**<0.0001**
		T_{acc}	3	9.1	**0.0278**
		M_b	1	582.8	**<0.0001**
		T_{acc} x M_b	3	10.5	**0.0149**
	Females	Intercept	1	141.5	**<0.0001**
		T_{acc}	3	1.2	0.7629
		M_b	1	446.6	**<0.0001**
		T_{acc} x M_b	3	0.9	0.8323

T_{acc} and sex significantly influenced all wing traits measured (Table 2.3; Fig. 2.4). The significant T_{acc} x sex interaction results indicate that male and female morphology responded differently to T_{acc} treatments with regards to body mass, wing width and wing area (Table 2.3). However, it is difficult to point out general patterns (*cf.* Fig. 2.4A,E). Females tended to be larger, and have greater wing loading and aspect ratios whereas males tended to have wider wings. Males might have a stronger response to T_{acc} in terms of wing width, but females might have a stronger response to T_{acc} in terms of body mass and wing area. Nonetheless, the main differences appear to be due to the colder T_{acc} treatment.

Table 2.3. Generalized linear models showing the effect of developmental acclimation temperature (T_{acc}) and sex on A) body mass, B) wing length, C) wing width, D) wing area, E) aspect ratio and F) wing loading in *Ceratitis capitata* flies.

	Effect	Degrees of freedom	Wald statistic	*P* value
A. Body mass (mg)	Intercept	1	36815.7	**<0.0001**
	T_{acc}	3	17.9	**<0.001**
	Sex	1	31.5	**<0.0001**
	T_{acc} x Sex	3	9.5	**0.0233**
B. Wing length (mm)	Intercept	1	253130.5	**<0.0001**
	T_{acc}	3	25.3	**<0.0001**
	Sex	1	15.9	**<0.0001**
	T_{acc} x Sex	3	6.9	0.0752
C. Wing width (mm)	Intercept	1	59394.2	**<0.0001**
	T_{acc}	3	5.0	0.1696
	Sex	1	63.3	**<0.0001**
	T_{acc} x Sex	3	7.8	**0.0495**
D. Wing area (mm^2)	Intercept	1	104029.0	**<0.0001**
	T_{acc}	3	6.5	0.0908
	Sex	1	13.9	**<0.001**
	T_{acc} x Sex	3	8.9	**0.0303**
E. Aspect ratio	Intercept	1	1912376.0	**<0.0001**
	T_{acc}	3	53.0	**<0.0001**
	Sex	1	482.0	**<0.0001**
	T_{acc} x Sex	3	4.0	0.2180
F. Wing loading (mg/mm^2)	Intercept	1	856.8	**<0.0001**
	T_{acc}	3	22.5	**<0.0001**
	Sex	1	91.1	**<0.0001**
	T_{acc} x Sex	3	3.9	0.2687
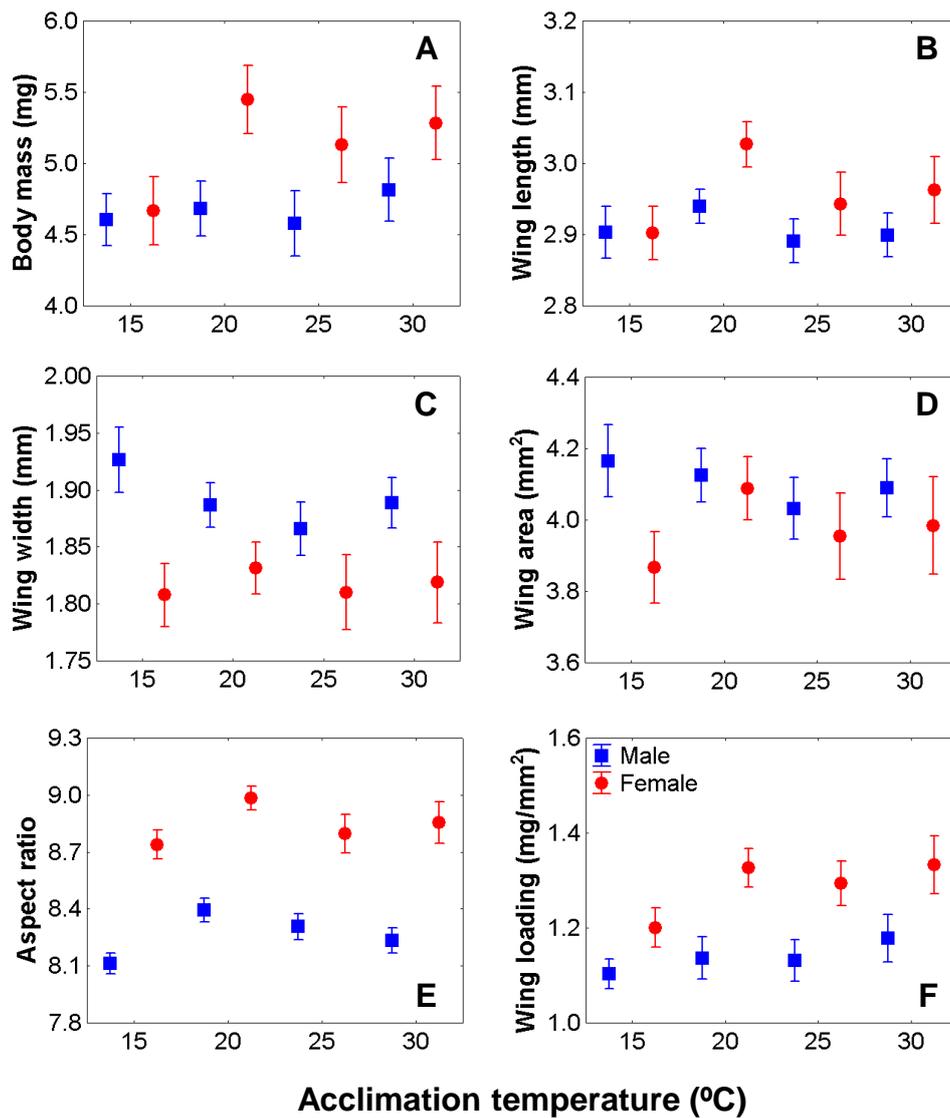
Figure 2.4. The effect of acclimation temperature on A) body mass, B) wing length, C) wing width, D) wing area, E) wing loading and F) aspect ratio in *Ceratitis capitata*, grouped by sex (males=blue squares; females=red circles). Error bars indicate 95% confidence intervals.

Models to predict fly morphology using flight performance suggest that male flight scores may have been influenced by wing length and female flight scores by body mass, wing length, wing width and wing area. However, there was not necessarily a simple relationship between a particular morphological trait and a flight score category. For example, flight failure (score=0) seemed to be associated with low wing width, but high wing width was not necessarily indicative of flight success (flies with high wing width had similar scores of 0, 1 and 2) in females (Fig. 2.5F; and see Addendum A, Fig. A.1 for flight scores pooled across T_{acc}). Overall no interaction was found

between T_{acc} and flight score with regards to any of the morphological traits (Table 2.4; Fig. 2.5). Female morphology was generally more strongly associated with a particular flight score (M_b, wing width, wing area; $P<0.05$) and influenced by T_{acc} (M_b, wing length, *AR*, *WL*; $P<0.05$), whereas in males, only wing length was significantly different among flight performance scores ($P=0.04$) and wing width, wing area and *AR* were influenced by T_{acc} ($P<0.05$).

Table 2.4. Generalized linear models showing the effect of developmental acclimation temperature (T_{acc}) and flight score (0, 1 or 2, referred to in the table as Score) on A) body mass, B) wing length, C) wing width, D) wing area, E) aspect ratio and F) wing loading in *Ceratitis capitata* flies.

	Sex	Effect	Degrees of freedom	Wald statistic	*P* value
A. Body mass (mg)	Males	Intercept	1	12791.7	**<0.0001**
		T_{acc}	3	3.2	0.3641
		Score	2	3.5	0.1731
		T_{acc} x Score	6	5.9	0.4371
	Females	Intercept	1	11440.0	**<0.0001**
		T_{acc}	3	14.6	**0.0022**
		Score	2	6.8	**0.0342**
		T_{acc} x Score	6	2.6	0.8596
B. Wing length (mm)	Males	Intercept	1	112853.2	**<0.0001**
		T_{acc}	3	6.1	0.1056
		Score	2	6.5	**0.0382**
		T_{acc} x Score	6	10.5	0.1058
	Females	Intercept	1	75925.8	**<0.0001**
		T_{acc}	3	13.5	**0.0037**
		Score	2	14.7	**<0.001**
		T_{acc} x Score	6	5.7	0.4581
C. Wing width (mm)	Males	Intercept	1	28486.2	**<0.0001**
		T_{acc}	3	16.4	**<0.001**
		Score	2	2.4	0.3039
		T_{acc} x Score	6	8.7	0.1939
	Females	Intercept	1	15505.7	**<0.0001**
		T_{acc}	3	2.3	0.5087
		Score	2	13.7	**<0.001**
		T_{acc} x Score	6	8.0	0.2357

	Sex	Effect	Degrees of freedom	Wald statistic	*P* value
D. Wing area (mm^2)	Males	Intercept	1	49330.3	**<0.0001**
		T_{acc}	3	8.7	**0.0330**
		Score	2	5.5	0.0644
		T_{acc} x Score	6	10.4	0.1101
	Females	Intercept	1	27591.0	**<0.0001**
		T_{acc}	3	4.3	0.2302
		Score	2	13.6	**0.0011**
		T_{acc} x Score	6	7.1	0.3112
E. Aspect ratio	Males	Intercept	1	814419.9	**<0.0001**
		T_{acc}	3	31.6	**<0.0001**
		Score	2	1.1	0.5762
		T_{acc} x Score	6	3.0	0.8045
	Females	Intercept	1	583978.0	**<0.0001**
		T_{acc}	3	19.7	**0.0002**
		Score	2	0.3	0.8489
		T_{acc} x Score	6	12.4	0.0540
F. Wing loading (mg/mm^2)	Males	Intercept	1	119.0	**<0.0001**
		T_{acc}	3	5.1	0.1649
		Score	2	0.8	0.6556
		T_{acc} x Score	6	9.4	0.1504
	Females	Intercept	1	524.7	**<0.0001**
		T_{acc}	3	18.5	**<0.001**
		Score	2	3.0	0.2185
		T_{acc} x Score	6	5.9	0.4296
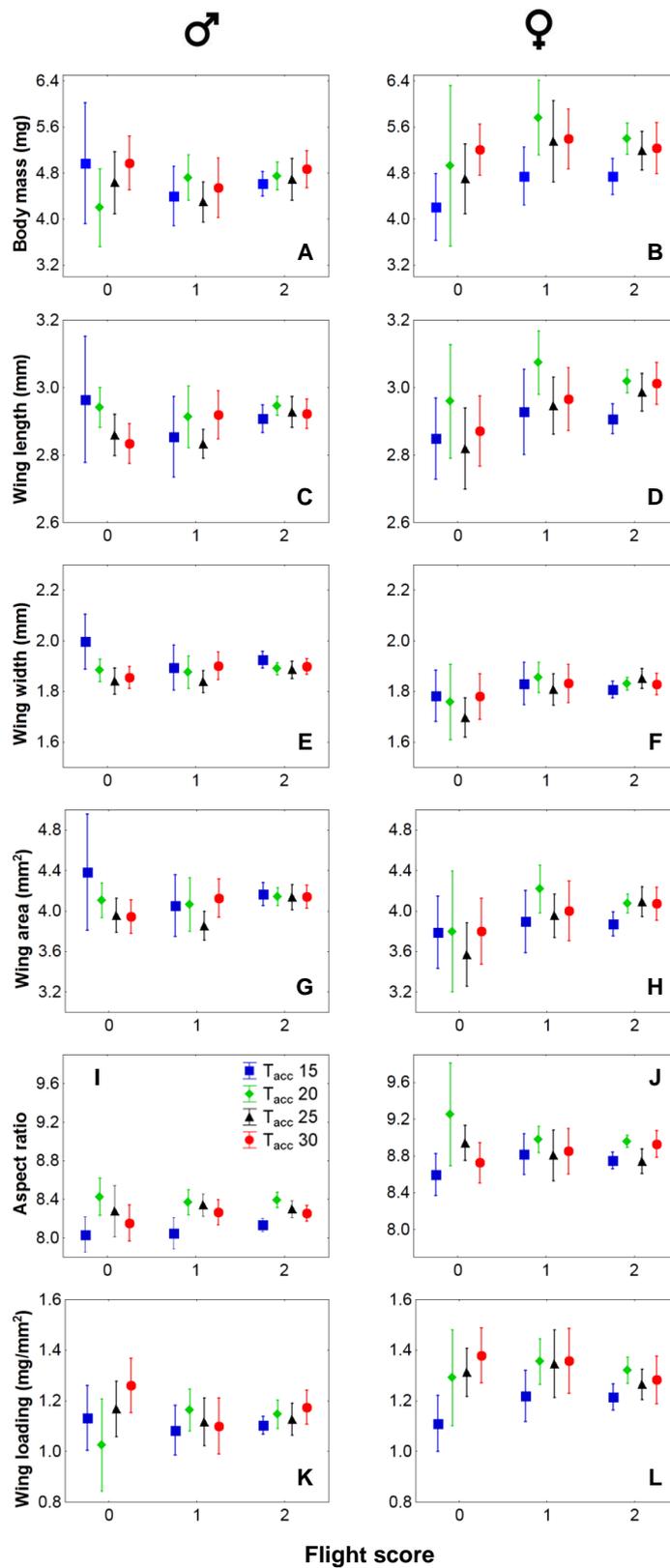
Figure 2.5. Recorded flight scores (0=failure, 1=lift, 2=flight) across the range of test temperatures as a function of A-B) body mass, C-D) wing length, E-F) wing width, G-H) wing area, I-J) aspect ratio and K-L) wing loading in *Ceratitis capitata* males (first row of panels) and females (second row of panels) acclimated at 15°C (blue squares), 20°C (green diamonds), 25°C (black triangles) and 30°C (red circles).

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2.3.3   Metabolic rate and CCO activity

The CCO assay revealed that enzyme activity at 25°C increased with an increase in T_{acc} (P=0.021), however the only significant differences in CCO activity occurred between T_{acc}=15°C and T_{acc}=30°C (indicated by lack of overlap in the 95% confidence intervals; Fig. 2.6A). Log-transformed mass, sex, T_{test} and the interaction term T_{acc} x T_{test} all significantly influenced log V_{CO_2} (P<0.0001), but the effect of T_{acc} on this variable was not significant (P=0.24). In general, metabolic rates increased as T_{test} increased, however, the relationship between V_{CO_2} and test temperature differed across acclimation groups e.g. flies acclimated to 30°C did not respond as strongly to test temperature (i.e., had a shallower slope) (Fig. 2.6B).
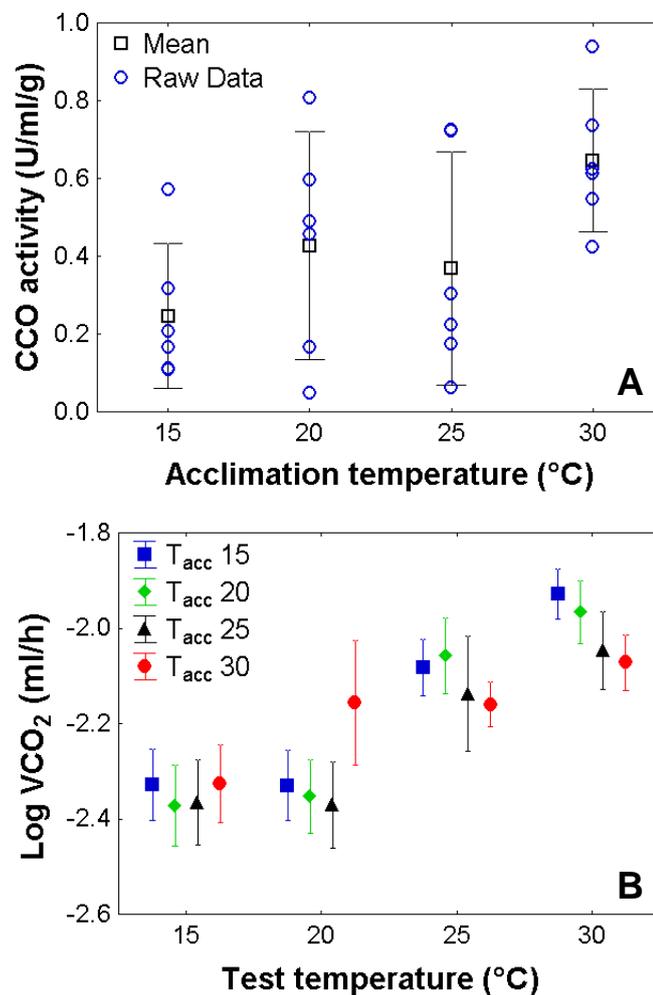
Figure 2.6. A) The effect of test temperature on VCO_2 grouped by acclimation temperature and B) the effect of acclimation temperature on CCO activity in *Ceratitis capitata*. Error bars indicate 95% confidence intervals.

2.4   Discussion

This study shows that flight performance of adult *Ceratitis capitata* is affected not only by the immediate thermal surroundings, but also by thermal history, i.e. the temperatures experienced during developmental stages. Several findings of this work are significant for understanding the evolution of phenotypic plasticity and temperature-dependent performance of ectothermic animals more generally, of which three aspects are perhaps most significant. First, it was found that in *C. capitata* flight performance increased with increasing test temperature, largely as might be expected based on other previous examinations of insect flight (e.g. Frazier et al., 2008; Kristensen et al., 2008; Chidawanyika and Terblanche, 2011, Ferrer et al., 2013). A significant influence of thermal history on adult flight performance was also found, with the major result being that cooler developmental temperatures resulted in improved flight ability at cool test temperatures. This result excludes the "hotter is better", "optimal acclimation" as well as the "deleterious acclimation" from being possible acclimation hypotheses for *Ceratitis capitata* flight performance (see section 2.1). Given that the colder acclimated flies performed better at low temperatures, compared to flies that were not cold acclimated, but hotter acclimated flies did not perform better at higher test temperatures, the "beneficial acclimation" hypothesis can also be ruled out. Thus, by a process of elimination, it was found that these results support the "colder is better" acclimation hypothesis, although this is only the case at lower test temperatures. This CIB result is striking, given that this is a tropical (though cosmopolitan) insect pest, which likely originates from stable, warm environments in East Africa (Gasperi et al., 1991). To date no studies have showed support for CIB in flight ability of insects across a wide range of commonly-encountered temperatures. This hypothesis has, however, been supported in locomotor performance of some terrestrial arthropod species from the sub-Antarctic Marion Island, where it is thought to reflect adaptation to this predominantly low temperature environment (Deere and Chown, 2006).

For *Drosophila melanogaster*, field assessments of dispersal ability support the BAH (Kristensen et al., 2008) while laboratory studies of flight performance in the same species perhaps support a similar conclusion (Frazier et al., 2008) although the latter study focused mainly on low temperature flight performance and not the fuller, more benign range of thermal conditions which would allow for a more comprehensive test of the alternative acclimation hypotheses. Here, this study is unique since it can, using a different model species, assess potential trade-offs in flight performance across a broader range of conditions, although these are still likely representative of field thermal conditions experienced by *C. capitata* (Terblanche et al., 2010b).

The second major finding of this work is that, while thermal development clearly influences morphology (e.g. Berwaerts et al., 2002; Gomez and Van Dyck, 2012; Ferrer et al., 2013), these effects are less straightforward than what might have been expected based on prior work from *Drosophila* species. For example, Frazier et al. (2008) showed that lower developmental temperatures resulted in flies having much larger wing area and smaller changes in body mass (cooler flies became larger) resulting in lower overall *WL*. They concluded that the improved low temperature flight ability observed was largely driven by these morphological changes in wing area and *WL*. The results for *C. capitata* are similar in some respects in that here, morphological outcomes to rearing temperature were also found. Outcomes for *C. capitata* were, however, in the opposite direction to that found in Frazier et al. (2008), resulting generally in lower T_{acc} flies having lower body mass which resulted in lower overall *WL*. The results were also both temperature- and sex-specific, in contrast with Frazier et al. (2008) who generally found similar rearing temperature responses in both sexes, with males and females differing relatively consistently in their morphology (see e.g. Fig. 2 in Frazier et al., 2008). In *C. capitata* examined here, wing morphology changed readily in low-temperature acclimated males compared to higher T_{acc} groups, but to a far lesser extent in females. In some morphological traits measured, there was pronounced sexual dimorphism (e.g. *AR*), but in others less so (e.g. wing area). Under some of the rearing temperature conditions, the sexual dimorphism was abolished (e.g. $T_{acc}= 15°C$, M_b and wing length relative to rearing at optimal conditions), while in other cases such $T_{acc}= 20°C$ (M_b; wing length) and $T_{acc}= 30°C$ (wing width) the dimorphism became more pronounced. The reasons for this variation are unclear at present.

The third main finding of this work was to show how these morphological responses to rearing temperature in turn affect locomotor performance in the adult stage. Clearly, sex of the flies influenced the morphology and this was temperature-dependent, with e.g. female morphology generally more strongly associated with a particular flight score. There was not however, a straightforward relationship between a particular morphological trait and flight ability. Instead, the opposite appears to be more broadly true, such that, e.g. low *WL* or high wing area is not always associated with flight, but having a high *WL* or small wing area may well be associated with the failure to achieve flight. Thus, although flight failure could be explained due to the absence of a particular morphological trait, the presence of that trait does not necessarily confer greater flight performance, and consequently, dispersal potential (*contra* e.g. Berwaerts et al., 2002; Gomez and van Dyck, 2012). Such a result, if it holds more broadly, therefore possibly has far-reaching implications for predictions of climate change impacts, or temperature variation, on dispersal abilities of insects.

The limited association between morphology and flight ability detected here may be due to physiological or biochemical adjustments that are employed to compensate for thermal conditions experienced during development, which may override the impacts of morphological variation on acclimation. This could be particularly true under the highly energy-demanding circumstances of flight (Suarez et al., 1996; Harrison and Roberts, 2000; Skandalis et al., 2011). A proximate mechanism for potentially explaining flight performance in *C. capitata* was however, not forthcoming. Specifically, it was found that CCO activity increased with acclimation temperature, while if physiological or biochemical compensation was the major expectation, one might expect an elevated CCO activity in the lowest acclimation temperature group, which could then perhaps explain the elevated performance of this group across a broader range of test temperatures later in adult life. In the case of resting metabolic rates, although finding a significant acclimation and test temperature interaction, the direction of effects was again not consistent with the elevated physiological rates hypothesis. Instead, higher rates of energy consumption in the 15°C acclimated flies at 30°C test conditions were found, with no pronounced difference among the four acclimation groups at 15°C test conditions.

Overall, this study shows that the temperature at which flies are reared affects their morphology and performance in various ways. These morphological changes have been found to be sex-specific and cannot necessarily be used to explain the flight performance outcomes in a specific individual. It is nevertheless possible that motivational and behavioural factors, along with other key physiological or morphological features not examined here e.g. variation in phosphoglucose isomerase (Rank et al., 2006), flight muscle mass and/or mitochondrial density and fibre type composition (Swank et al., 2006; and see Skandalis et al., 2011) are playing a significant role in the flight performance outcomes. Further work examining changes in wing beat frequencies and muscle fibre composition and performance, how these factors differ between sexes, and their temperature-dependence in *C. capitata* would thus be useful.

One potential implication of this work is that lower female M_b in $T_{acc}=15$°C could mean a trade-off between improved flight (i.e. lower *WL*) and reproduction success, which is usually positively correlated with body size (Nunney and Cheung, 1997; Zera and Denno, 1997; Zera and Harshman, 2001; but see also Zamudio et al., 1995) and serve as broad indicators of population fitness. The implications of this study for the tests of acclimation hypotheses are however especially significant, as support for the Colder Is Better hypothesis was found, but not for other hypotheses that have generally been more widely supported to date (e.g. BAH, *cf.* Kristensen et al., 2008; Chidawanyika and Terblanche, 2011). However, a clear physiological mechanism explaining these

apparent plasticity responses is still lacking. Given the broader interest in *C. capitata* as an agricultural pest, this study's results are also significant from a pest management and invasion perspective (e.g. Karsten et al., 2013) and the information presented here may be useful in aiding predictions of the physiological responses of these flies to thermal exposure during development, and to better forecast flight performance, population connectedness, and geographic range structure in variable thermal environments.

Chapter 3 :

Research implications and future directions

3.1　　The implications of this research in a broader context

Understanding dispersal ability of insects is critical to a range of areas of interest, including invasion biology, the study of disease vectors and pest management (see Chapter 1). Of the many threats to biodiversity posed by human actions, anthropogenic climate change is perhaps the most severe of all. Just as concerning as negative effects however, are the positive effects that climate change may have on some insect species that have negative effects on ecosystems and human activities, i.e. invasive alien species (IAS) and agricultural pests. To forecast potential short and long-term climate change effects and understand responses to abiotic environmental conditions in the field, knowledge of the temperature-dependence and plasticity of dispersal capacity in pest species, is of value. The experimental research presented in the main body of this thesis is directly relevant to understanding flight ability of *Ceratitis capitata*, a notorious global pest of agriculture. This work also has further practical value to understanding field population dynamics in this particular model organism, and in pest species in general.

To illustrate the potential applied value of this knowledge, the main flight performance outcomes (T_{acc} and T_{test} effects on flight performance, see Table 2, Chapter 2) were coupled with a degree-day (thermal development) model to assess whether knowledge of flight ability might improve estimates of predicted population dynamics, based only on field trap capture information and microclimate temperatures. To generate the degree-day model, a study site was used (located near Villiersdorp in the Western Cape province of South Africa) for which good baseline knowledge of *C. capitata* monthly trap capture data, as well as two years of high-resolution microclimate data were available. More specifically, for each hour (from January 2009 until December 2010) a degree-day model, which calculated the required number of hours for pupal development, was run (degree-day threshold values sourced from Grout and Stoltz, 2007), and the average temperature across that developmental period was calculated. This developmental temperature (T_{acc}), along with the ambient temperature (T_{test}) of the current hour, were then incorporated into generalized linear models (with a quasibinoimal distribution, built from data in Chapter 2), to predict the probability of flight during each hour. Three models were generated that included: 1) the T_{test} x T_{acc} interaction 2) T_{acc} and T_{test} as main effects and 3) T_{test} only.

If the predicted probability of flight exceeded 75%, the flies were considered to be flying and the total number of flight hours for each month was calculated. A measure of the relative number of flight hours within each month (number of flight hours/maximum flight hours×100), as well as the relative number of trap captures (number of trap captures/maximum trap captures×100) at

Villiersdorp, were generated and compared to one another. For this, four basic modelling scenarios were run:

1) predicted fly abundance based on degree-days accumulated (but excluding information on physiological thresholds from the model);

2) predicted fly abundance based on degree-days including physiological thresholds which, if exceeded, causes the model to re-set expected abundance to zero (similar to the model run in Nyamukondiwa et al., 2013; see Fig. 3.1);

3) predicted fly abundance based on predicted relative flight activity using flight test temperature information only (T_{test} as in Chapter 2), without any knowledge of degree-days and finally,

4) predicted fly abundance based on predicted relative flight activity, but including an acclimation effect during development (i.e. allowing T_{acc} x T_{test} effects, as in the main results of Chapter 2).
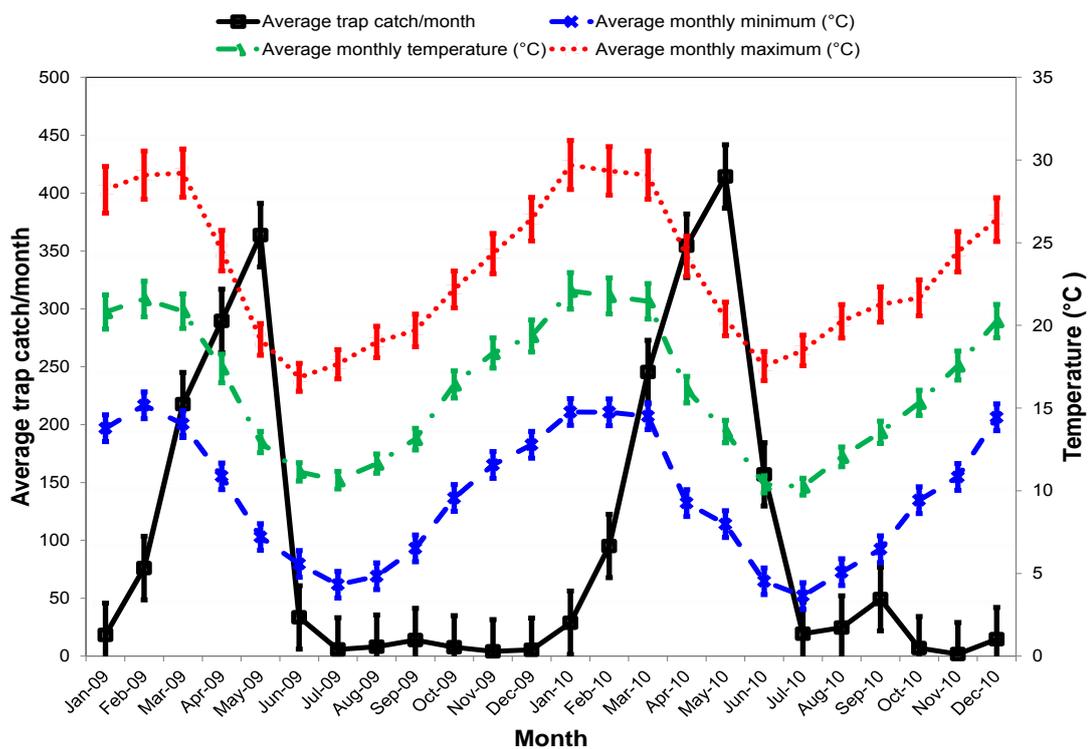
Figure 3.1. Average trap captures of *C. capitata* across four sites in the Western Cape as a function of average monthly, average minimum and average maximum temperature during 2009 and 2010. (from Nyamukondiwa et al. (2013)).

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The predicted relative monthly trap capture and flight activity predictions were compared to one another, and the model of best fit determined based on root mean square deviation (RMSD) values for each of the four scenarios listed above. Scenario 4, developmental temperature thresholds, acclimation temperature and test temperature incorporated, predicted trap data most accurately, as can be seen by the lowest RMSD value (Table 3.1, Fig. 3.2). All model simulations were run in R (version 3.0.1; R Foundation for Statistical Computing, Vienna, Austria). See Addendum C for additional information regarding the methods described in this section.

Table 3.1. Relative accuracy of model simulations based on root mean square deviation (RMSD).

Model	Development parameters	Temperature parameters	RMSD
Relative population abundance			
1	Degree-day model with no thresholds	No additional parameters	385.98
2	Degree-day model with developmental temperature thresholds	No additional parameters	223.37
Relative flight activity			
3	No degree-day model	T_{test}	232.1
4	Degree-day model with developmental temperature thresholds	$T_{test} \times T_{acc}$	208.61

A limitation to this approach which should be considered, however, is the fact that absolute abundance data for the population is not known. The model currently makes the assumption that each new month starts with a new batch of individuals, not taking into account that the surviving individuals from previous months could also be caught in the new month's traps. Another potential shortcoming of using trap capture data to estimate insect abundance, is that lure efficiency (i.e. chemical and physical properties of the lure, e.g. volatility) might be temperature-dependent. If this information were available and included in the model, the T_{test} x T_{acc} predictions would likely be shifted to the right and would thus be better aligned with the trap capture data presented in Fig. 3.2.

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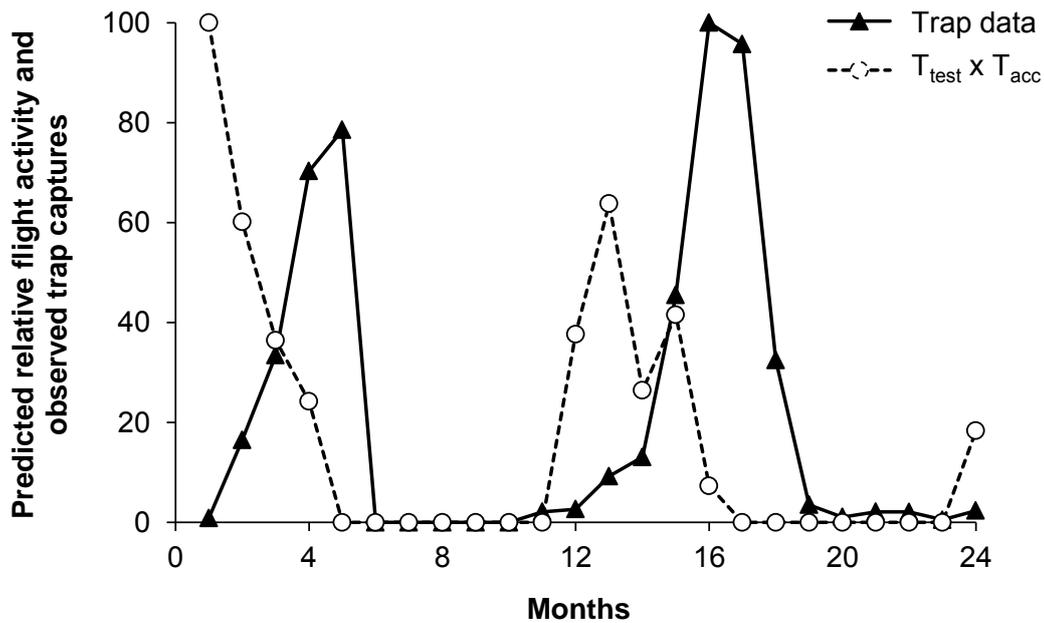
Figure 3.2. Predicted relative flight activity and relative observed trap captures of *C. capitata*. The model simulation with the ambient (test) temperature and developmental (acclimation) temperature interaction (T_{test} x T_{acc}) plotted with relative trap capture data per month for *C. capitata* at Villiersdorp from January 2009 until December 2010.

In South Africa, *C. capitata* populations are currently managed mainly through the use of insecticides, which include full cover sprays and bait methods (Ekesi and Billah, 2007). Besides the economic cost associated with chemical control of these invasive pest species, the method is also harmful to the environment. A solution to this problem comes in the form of the Sterile Insect Technique (SIT) (Hendrichs et al., 2002), which consists of the release of mass-reared sterile males into a wild population. These males then mate with females in the field population, resulting in sterile eggs and a decrease in population numbers up to a point where the population is incapable of recovery (Bonizzoni et al., 2002). The success of the SIT method is, however, greatly dependent on the dispersal ability of individuals and on population connectivity, and therefore also on the flight ability of individual flies (Estoup and Guillemaud, 2010). The information gathered in the present study should prove valuable for quality control of the SIT method, in the sense that rearing and release conditions of sterile males could be optimised (see discussions in Sørensen et al., 2012).

3.2   Future directions

The underlying mechanisms promoting the variation in flight performance between different T_{acc} groups in *C. capitata* could not be explained by the different morphological, physiological or biochemical analyses undertaken in the present study. This could be due to the fact that flies were only acclimated during a single life-stage. A longer period (or more life-stages) may produce a different result (Frazier et al., 2008). It could also be that flight performance is driven by mechanisms not tested here. For this reason, further research could examine other possible drivers, within or among generations. Differences in T_{acc} groups and flight performance scores with regards to wing beat frequencies (e.g. Frazier et al., 2008), flight muscle properties (e.g. Lehmann, 1999), as well as possible variation in other enzyme activity such as phosphoglucose isomerase (e.g. Rank et al., 2006), could be investigated. Since it has previously been found that wing development may be regulated by temperature-dependent changes in gene-expression (e.g. Cavicchi et al., 1985; Carreira et al., 2013), such genetic effects could also be investigated in future to try and explain differences in flight performance between acclimation groups.

A related issue could be intrinsic motivation or behavioural drive for flight, since an external stimulus was used to motivate flight. It would be useful to know if flies left to perform voluntarily would produce a similar performance result. Other aspects of flight performance could also be investigated in future, such as flight time, distance and speed (e.g. Ferrer et al., 2013). Based on the findings presented here, it may also be profitable in future research to analyse males and females separately to try and explain sex-specific differences in flight performance and better comprehend sex-related morphological variation at different rearing temperatures. Furthermore, the reproductive success of individuals from the different T_{acc} treatments could be tested to see whether locomotor performance and reproductive success is indeed a trade-off, as suggested from other work (see Chapter 1). It would also be useful to know how flight performance and its temperature-dependence in this laboratory culture compares with field-collected or recently cultured individuals.

Although ambient temperature significantly influences many physiological functions of ectotherms and can, to some extent, be used to predict dispersal ability in the field, other environmental variables that might have additional effects on flight performance should also be taken into account. With shifting optimum climatic envelopes and species' distributions moving to higher altitudes (Thomas, 2010), air density may also become an environmental factor that should be investigated. The best data on maximal V_{CO_2} for flying insects have already come from experiments where individuals were exposed to manipulated air densities to increase work load

(Harrison and Roberts, 2000; e.g. Vance et al., 2009). It has also been found that the minimal gas density needed to stay aloft decreases with relative increase in thoracic muscles and increases with body mass (Roberts et al., 2004). The same study also reported that metabolic rates significantly increased with decreasing air density. Precipitation and wind are other examples of complex environmental factors that might also hinder flight in individuals (Pasek, 1988), but their specific effects on flight capacity and dispersal potential are yet to be well described (Dickerson et al., 2012), especially in *Ceratitis capitata*. Possible biological factors that could also play a major part in flight motivation are the presence of predators or prey and host plant or mate availability.

It could furthermore be beneficial if studies such as the one presented here on *C. capitata*, i.e. temperature-dependent flight performance assays in the laboratory, could be done on other invasive and pest species in the future. While such studies are time-consuming, they are relatively affordable and easy to execute with limited resources required. Flight ability under variable temperature conditions of species such as *Ceratitis rosa* (another flight-capable agricultural pest) or *Harmonia axyridis*, (the Harlequin ladybird, an IAS species (Stals and Prinsloo, 2007)), could also be investigated in the laboratory to obtain pilot performance data. Climate data from most areas of southern Africa have been documented extensively and are now easily accessible. Once obtained, climate and the species' physiological data could be used to determine the possible spread of the pests or IAS between invaded and pristine sites. Additionally, the potential success of biological control agents such as the gall fly, *Dasineura rubiformis* (control agent for *Acacia mearnsii*, the black wattle (Impson et al., 2008)) could be assessed with this method, since the success of such introductions, as is the case with IAS, relies heavily on the ability of individuals to move between the point of introduction and other sites.

To conclude, the results and insights obtained from this study could be broadly applicable to a variety of insect species and can lead to increased understanding of the functioning of flight-capable arthropods in their environment, as well as potential abiotic constraints on performance. Through this investigation, it has also been made clear that to recognise the impact of environmental factors on locomotor performance and flight energetics is of fundamental importance to understanding evolution and ecology of insects. Although the underlying mechanisms of flight performance are not always straight-forward, addressing the unanswered questions will likely result in the field of insect physiology seeing numerous novel contributions in the near future.

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Addenda

Addendum A
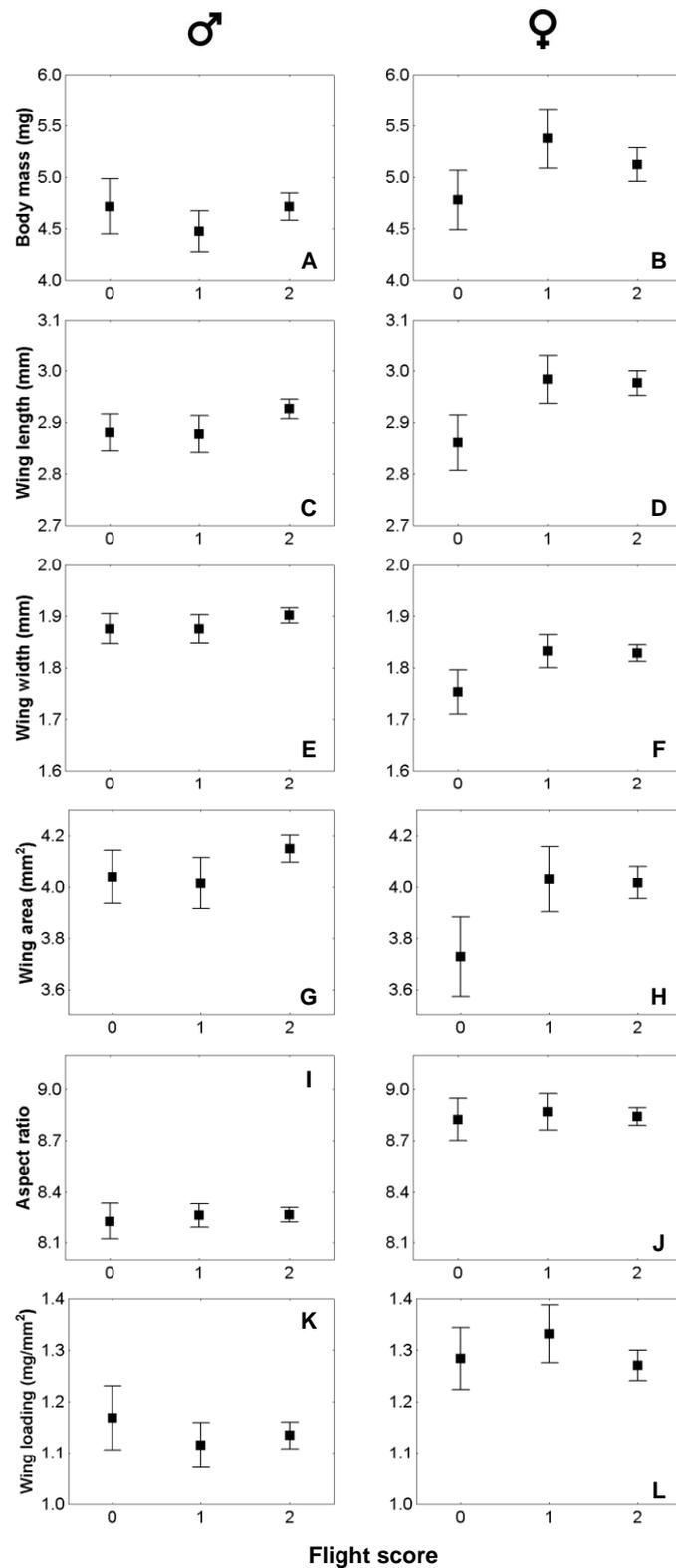
Figure A.1. Recorded flight scores (0=failure, 1=lift, 2=flight) across the range of test temperatures as a function of A-B) body mass, C-D) wing length, E-F) wing width, G-H) wing area, I-J) aspect ratio and K-L) wing loading in *Ceratitis capitata*.

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Addendum B

Table B.1. Multiple comparisons of flight performance outcomes between developmental acclimation groups (T_{acc}) at each test temperature (T_{test}) for all *Ceratitis capitata* flies pooled, as well as males and females separately. The table shows *P* values resulting from a Kruskal-Wallis test with significant flight score differences between T_{acc} groups shown in bold.

Sex	(°C)	T_{acc}	15	20	25	30
All	T_{test}=15	15		1.0000	**0.0321**	**0.0153**
		20			**0.0321**	**0.0153**
		25				1.0000
		30				
	T_{test}=20	15		1.0000	1.0000	**0.0360**
		20			1.0000	**0.0130**
		25				0.1715
		30				
	T_{test}=25	15		1.0000	1.0000	0.2709
		20			1.0000	0.1106
		25				1.0000
		30				
	T_{test}=30	15		1.0000	1.0000	0.5588
		20			1.0000	1.0000
		25				1.0000
		30				
Males	T_{test}=15	15		1.0000	0.5501	0.0544
		20			0.3511	**0.0313**
		25				1.0000
		30				
	T_{test}=20	15		1.0000	1.0000	0.0675
		20			1.0000	0.1693
		25				0.3501
		30				
	T_{test}=25	15		1.0000	1.0000	1.0000
		20			1.0000	1.0000
		25				1.0000
		30				
	T_{test}=30	15		0.9128	0.2896	1.0000
		20			1.0000	1.0000
		25				1.0000
		30				

Sex	(°C)	T_{acc}	15	20	25	30
Females	T_{test}=15	15		1.0000	0.1437	0.6323
		20			0.2474	0.9301
		25				1.0000
		30				
	T_{test}=20	15		1.0000	1.0000	1.0000
		20			1.0000	0.2196
		25				1.0000
		30				
	T_{test}=25	15		1.0000	1.0000	0.2311
		20			1.0000	0.0542
		25				1.0000
		30				
	T_{test}=30	15		1.0000	1.0000	1.0000
		20			1.0000	1.0000
		25				1.0000
		30				

Addendum C

C.1   Additional information regarding the trap catch prediction model

Accumulated thermal stress

1. *If the ambient temperature at hour(x) is less than or equal to 9°C, then stress = 1*

2. *If stress at hour(x) is greater than 0, then accumulated stress is the sum of stress over the previous 8 hours (where the stress period is 8 hours)*

Setting critical limits:

3. *If:*

    A. *Temperature is above critical maximum*

    B. *Temperature is below critical minimum*

    C. *Accumulated stress is greater than the accumulated stress threshold,*

    Then death is equal to 1 (the animal has died)

The degree-day (DegDay) loop run for each hour of the time series data:

If it's the first hour of the simulations: where T_{min} = lower developmental threshold.

    DegDay(hour)= Temperature(hour) - T_{min}

    Degday(accumulated) = 0

For all hours thereafter:

    Degday(accumulated) = DegDay(hour) + SUM(Degday(hour-1), Degday(hour-2), Degday(hour-3),… Degday(hour-n))

Where 'n' is the hour at which the number of degree-day units required for adult eclosion has been attained, at which point development stops and:

    Adult eclosion = 1

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Development time = hour – hour(n)

T$_{acc}$ = average temperature (hour : hour(n))

If death is incurred during this developmental period

Adult eclosion = 0

Development time = 0

T$_{acc}$ = 0

Predicting flight activity from T$_{acc}$ and T$_{test}$:

For each hour:

If adult eclosion = 1

Flight probability = a*T$_{test}$(hour)*T$_{acc}$(hour) + c

Flight probability was greater or equal to 0.75

Then flight activity(hour)= 1

Data for impacts of temperature, development rates and degree-days sourced from Duyck and Quilici (2002) and Grout and Stoltz (2007).