Causes and Consequences of Dispersal in the Mediterranean Fruit fly, *Ceratitis capitata*

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2015

Abstract

The ability of insects to perform under challenging environmental conditions is paramount to their survival, population growth and evolutionary fitness. Understanding why some organisms persist in certain habitats but not others is the first step to comprehending present, past and future species distributions, of particular importance under future global climate change. Key traits that may assist a species to continue to perform under poor conditions and may potentially assist in surviving future variable and warming conditions include enhanced dispersal capabilities, a wide performance breadth and plastic responses. Repeated mark-release-recapture (MRR) experiments were conducted to measure the performance (dispersal) and plastic responses of the Mediterranean fruit fly, *Ceratitis capitata* (a prolific global invader). Dispersive and philopatric individuals were morphometrically assessed (including wing size and shape, body mass, abdomen mass, thorax mass and various ratios thereof) to identify phenotypic traits associated with enhanced dispersal. Thereafter, focussed laboratory experiments were undertaken to determine which aspects of flight performance are enhanced, or associated with, potential dispersal traits. Performance was then compared under various thermal limits (chill coma recovery, CCRT; heat knockdown time, HKDT; critical thermal minimum and maximum, CTmin and CTmax, respectively) to examine the influence of different thermal acclimation regimes and determine the responses of phenotypic plasticity in *C. capitata*. Subsequently, the costs and benefits of dispersal and its plasticity were measured under semi-field (greenhouse) and field conditions to determine how close laboratory predictions are to the real world. These experiments allowed the discovery of the phenotypic trait associated with dispersal (larger thorax mass: body mass). However, contrary to a widely-held expectation, it did not result in enhanced whole-animal flight performance, but was rather related to willingness to disperse (i.e. dispersal propensity). Furthermore, the

integration of the three operational environments (laboratory, semi-field and field) illustrated that *C. capitata*'s performance is influenced by thermal conditions and highlighted the best acclimation treatment (20°C acclimation, especially in warmer conditions) for enhanced performance. A challenge for invasion biology is the development of a predictive understanding of species invasion ability. Clarity on the species dispersal potential and the factors that influence it is an integral part of the problem. From this study, it is shown that dispersal is condition dependent (e.g. phenotypic traits and behaviour) as well as context dependent (e.g. thermal history and environmental temperature). This may benefit predictions of the future invasion risk of *C. capitata* and potentially improve current management strategies.

Opsomming

Die vermoë van insekte om take uit te voer onder uitdagende omgewings-toestande is uiters belangrik vir hul oorlewing, bevolkingsgroei en evolusionêre fiksheid. Deur te verstaan waarom sommige organismes volhard in sekere habitatte, maar nie ander nie is die eerste stap om die teenwoordige, verlede en toekomstige spesie verspreiding te begryp, wat van besondere belang is onder toekomstige globale klimaatsverandering. Sleutel kenmerke wat 'n spesie kan help om voort te gaan en om take uit tevoer onder hierdie toestande en wat potensieel hulle kan help oorleef in die toekomstige veranderlike en warmer toestande sluit in verbeterde verspreiding vermoëns, 'n wye prestasie breedte en plastiese reaksies. Herhaalde merk-loslaat-vang eksperimente is onderneem om die prestasie (verspreiding) en plastiese reaksies van die Mediterreense vrugtevlieg, *Ceratitis capitata* (globale indringer) te meet. Verspreiders en nie-verspreider individue is morfometies beoordeel (insluitend vleuel grootte en vorm, liggaamsmassa, abdomen massa, toraks massa en verskeie verhoudings daarvan) om fenotipiese eienskappe wat verband hou met verbeterde verspreiding te identifiseer. Daarna is gefokusde laboratorium eksperimente onderneem om te bepaal watter aspekte sal die vlug prestasie verbeter, of wat verband hou met, potensiële verspreiding eienskappe. Prestasie is toe vergelyk onder verskillende termiese grense (koue koma herstel tyd; verhitting omval tyd, kritiese termiese minimum en maksimum, CTmin en CTmax onderskeidelik) om die invloed van verskillende termiese akklimasie patrone te ondersoek en om die reaksie van fenotipiese plastisiteit in *C. capitata* te bepaal. Daarna was die koste en voordele van die verspreiding en die plastisiteit in *C. capitata* gemeet onder semi-veld (kweekhuis) en veldtoestande om te bepaal hoe naby laboratorium voorspellings is aan die regte wêreld. Hierdie eksperimente het toegelaat dat die fenotipiese einskap wat geassosieer is met verspreiding (groter toraks massa: liggaamsmassa) ontdek is. Alhoewel, in teenstelling met 'n wyd-gehoude verwagting, het dit nie gelei tot verbeterde heel-dier vlug prestasie nie, maar is eerder verwant aan

bereidwilligheid om te versprei (bv verspreiding geneigdheid). Verder het die integrasie van die drie operasionele omgewings (laboratorium, semi-veld en die veld) geïllustreer dat die prestasie van *C. capitata* beïnvloed word deur termiese omstandighede en die beste akklimasie behandeling uitgelig (20°C Akklimasie, veral in warmer toestande) vir 'n beter prestasie. 'n Uitdaging vir indringerbiologie is die ontwikkeling van 'n voorspellende begrip van spesies inval vermoë. Duidelikheid oor die spesie se verspreiding en die faktore wat dit beinvloed is 'n integrale deel van die probleem. Hierdie studie, toon aan dat die verspreiding is toestand afhanklik (bv. fenotipiese eienskappe en gedrag) sowel as konteks afhanklik (bv. termiese geskiedenis en omgewing temperatuur). Hierdie kan dus voorspellings oor die toekomstige inval risiko van *C. capitata* baat en selfs huidige bestuur strategieë verbeter.

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The emotional support that I received from Leigh Torrance cannot be overstated, without her support and understanding the completion of this thesis would not have been possible. I would also like to thank my fellow APE lab members for technical guidance and friendly greetings.

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Dedication

To my parents Jan and Lisl Steyn,

Whom have always encouraged me to pursue paths that I take pleasure in.

Without your insight the fulfilment I experience every day from my research would not have been possible.

Thank you.

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Chapter 1:

General introduction

1.1. Species distribution limits

Insects are one of the most abundant and diverse groups of life on earth. Their importance to ecosystem functioning has given rise to the saying "Little things run the world", coined by the influential biologist Edward O. Wilson. Winged insects in particular exist on every landmass on earth including Antarctica (Gressit 1965). Climate is an important extrinsic factor that influences development, growth and the persistence of populations (Sinclair *et al*. 2012). As such distributions are dependent on their performance limits, such as the critical thermal maximum or minimum (CTmax and CTmin), thermal boundaries beyond which the individual is no longer able to perform and function (i.e. forage and reproduce; Bozinovic *et al*. 2011). These performance limits and other aspects of an organisms' thermal physiology are generally visualised using a thermal performance curve (Fig 1.1). The thermal breadth can be used to make inferences regarding the species optimal conditions that correlate strongly with the known boundaries of their distribution (reviewed in Addo-Bediako, Chown & Gaston 2000; Chown & Gaston 2015). With changing climate these thermal boundaries may shift, allowing colonization of new areas or reducing current geographic range should conditions become unfavourable. Phenotypic plasticity, or the ability of a genotype to produce more than one phenotype when exposed to different environments, may assist this process by allowing an individual to increase their performance breadth (Fig. 1.1.), potentially facilitating activity or performance during unfavourable conditions, reducing the negative impact of climate change on species (Hoffmann & Sgrò 2011). Ultimately, species may successfully respond to climate change in two key ways; i) through genetic adaptation (i.e. evolution), ii) phenotypic plasticity, or iii) by moving to more favourable conditions (dispersal and/or range shift).

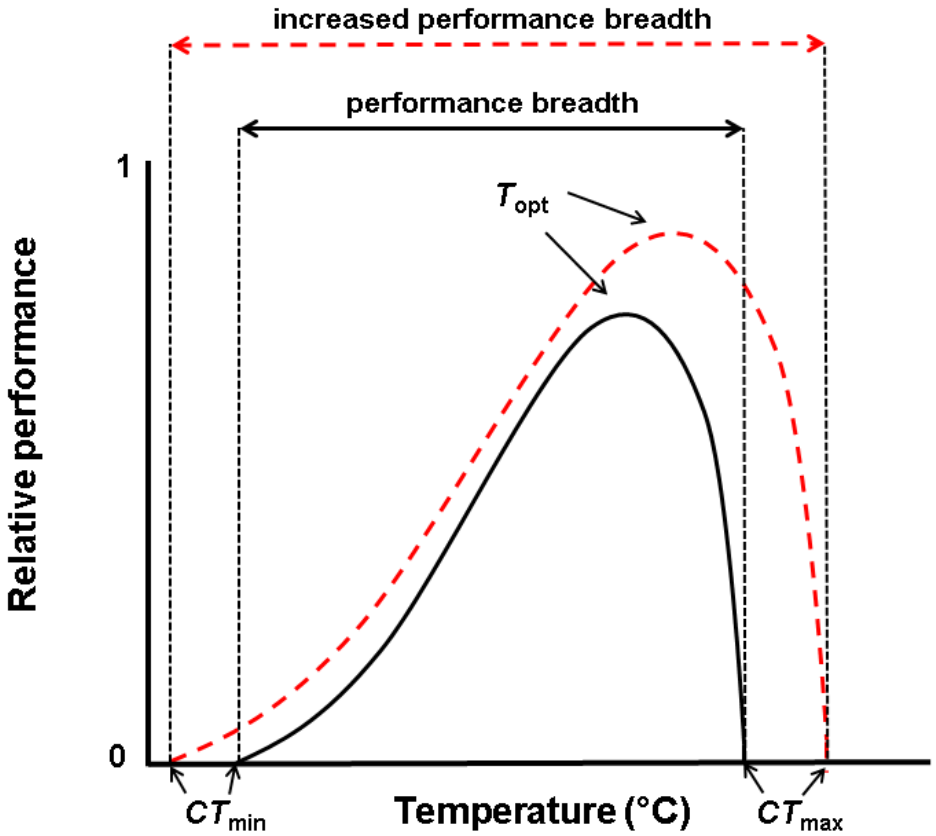

Fig. 1.1. A generalized thermal performance curve of an invertebrate. Relative performance decreases from the thermal optima (Topt) to CTmin and CTmax, the arrows indicate the thermal performance breadth. The red dashed curve indicates how the performance curve may change due to phenotypic plasticity.

1.2. Adaptation under changing environments

The individuals that are able to tolerate these harsh or unfavourable conditions may in turn have higher fitness or performance gains than individuals that cannot, allowing them to leave more progeny and ensure local adaptation under the stable thermal conditions (Savolainen, Lascoux & Merilä 2013). In a more variable environment or under climate change, the critical thermal limits to performance (i.e. CTmin and CTmax) of the individual may be very important as environmental conditions are more likely to extend outside an organisms' tolerance breadth (e.g. Deutsch *et al*. 2008). Plastic phenotypes that allow the extension of these performance limits may have higher overall fitness benefits (Gerken *et al.* 2015). However, both in stable and variable environments, the selection process may be disrupted under certain gene flow scenarios (e.g. Bridle & Vines 2007). When limited adaptive potential exists in the population, immigrants may provide new variation for natural selection to act; however, there are many alternative scenarios in this situation, all with varying impacts (reviewed by Sexton *et al*. 2014).

In studies conducted in natural populations, the gene flow scenario with the greatest support is 'isolation by distance'. This scenario predicts that as the distance between two populations increase, so do the costs associated with dispersal, leaving immigrants competitively inferior (Wright 1943; Slatkin 1993). Isolation by environment suggests that gene flow between similar environments will be higher than predicted under isolation by distance between dissimilar environments (Wang & Bradburd 2014) as immigrants would perform best under the conditions they are adapted to (Nosil *et al*. 2005). Gene flow may then interrupt local adaption by changing the genetic variance at the locus under selection and 'swamping' locally adapted alleles (Lenormand 2002). Therefore dispersal can either inhibit or facilitate local adaption, and is a phenomenon that is particularly important in light of concerns for species' range changes with climate change.

1.3. Dispersal and population dynamics

Dispersal, or the movement and establishment of individuals from a natal patch to a novel environment (Ronce 2007), is one of the most important aspects of population dynamics as it affects both evolution and the ecology of a species (Pennekamp *et al*. 2014). It allows the colonization of new habitats, ensuring that there are more resources (nutrition and space) available for the population to utilize (Bowler & Benton 2005). Dispersal is also crucial for countering genetic isolation due to habitat fragmentation and global climate change which would otherwise decrease the effective population size, increasing susceptibility to stochastic extinction events, Allee effects (positive density dependence of population size and individual fitness; Soulé 1987; Dennis 1989; Courchamp *et al*. 1999) and inbreeding (Ronce 2007). As such, dispersal, and the eventual shift in distribution of a species' range, allows organisms to escape unfavourable conditions. However, as dispersal is a measure of performance, it is subject to significant costs including energetic, risk and opportunistic effects (Bonte *et al*. 2012). These negatives may be exaggerated by a fragmented landscape as the distances between fragments necessitate further resource use (Fahrig 2003; Kokko & Lopez-Sepulcre 2006). It is only when the benefits of relocating to a new environment (e.g. survival and reproduction) outweigh the costs that their genotype will be selected for the next generation.

Dispersal may be affected by one or a number of stimuli that are either biotic (intrinsic or extrinsic) or abiotic in nature. Intrinsic stimuli or traits are those that act on the individual from within its own body (e.g. body condition), whilst extrinsic stimuli are external biotic factors that influence the individual i.e. density, relatedness (Pennekamp *et al*. 2014). A summary of effects observed in previous studies is provided in Table 1.1. Phenotypic traits such large body size, wing size and thorax mass: body mass ratio may increase dispersal

Table 1.1. Summary of biotic and abiotic factors that affect the dispersal of invertebrates.

Abiotic/Biotic	Type of trait	Traits or Factors	Outcome	Species	Reference
Biotic	Intrinsic	*pgi* allele[1]	More dispersive if possess, seen more in newly established populations	*Melitaea cinxia*, Glanville fritillary butterfly (Lepidoptera: Nymphalidae)	Hanski & Saccheri 2006
			Increased flight metabolic rate, flight performance, and fecundity	*Melitaea cinxia*, Glanville fritillary butterfly (Lepidoptera: Nymphalidae)	Saastamoinen & Hanski 2008; Niitepold *et al*. 2009
Biotic	Intrinsic	*for* gene	Larvae were more active and lead to adults more prone to dispersal	*Drosophila melanogaster*, Vinegar fly (Diptera: Drosophilidae)	Edelsparre *et al*. 2014
Biotic	Intrinsic	Wings Size	Larger wings increased dispersal distance	*Phengaris teleius*, Scarce large blue; *Phengaris nausithous*, Dusky large blue (Lepidoptera: Lycaenidae)	Skorka *et al*. 2013
			Long-winged morphs have greater dispersal distance than short-winged morphs	*Gryllus rubens*, Southeastern field cricket (Orthoptera: Gryllidae)	Zerra & Denno 1997
Biotic	Intrinsic	Wings Shape	Female dispersers had different wing shape to non-dispersers	*Melitaea cinxia*, Glanville fritillary butterfly (Lepidoptera: Nymphalidae)	Breuker, Brakefield & Gibbs 2007
Biotic	Intrinsic	Sex	No difference in dispersive patterns	*Melitaea cinxia*, Glanville fritillary butterfly (Lepidoptera: Nymphalidae)	Breuker, Brakefield & Gibbs 2007
			Difference in dispersive patterns, due to flight metabolic rate and gene expression	*Melitaea cinxia*, Glanville fritillary butterfly (Lepidoptera: Nymphalidae)	Niitepold *et al*. 2011
			Males increased flight performance due to higher flight metabolic rate	*Melitaea cinxia*, Glanville fritillary butterfly (Lepidoptera: Nymphalidae)	Kvist *et al*. 2015
			Females dispersed further than males	*Phengaris teleius*, Scarce large blue (Lepidoptera: Lycaenidae)	Skorka *et al*. 2013
Biotic	Intrinsic	Body size	Larger body size decreased dispersal distance	*Phengaris teleius*, Scarce large blue; *Phengaris nausithous*, Dusky large blue (Lepidoptera: Lycaenidae)	Skorka *et al*. 2013

Abiotic/Biotic	Type of trait	Traits or Factors	Outcome	Species	Reference
Biotic	Intrinsic	Thorax mass : body mass ratio	Thought to increase dispersal, marginal populations larger ratio, than core populations	*Pararge aegeria*, Speckled wood butterfly (Lepidoptera: Nymphalidae)	Hughes, Hill & Dytham 2003
Biotic	Intrinsic	Age	Negative association with dispersal distance	*Ceratitis capitata*, Mediterranean fruit fly (Diptera: Tephritidae)	Meats & Smallridge 2007
Biotic	Extrinsic	Competition	Kin competition avoidance promotes dispersal	*Platyscapa awekei*, Pollinating fig wasp (Hymenoptera: Agaonidae)	Moore, Loggenberg & Greeff 2006
			Smaller males likely to disperse first in male rich environment	*Tetraopes tetrophthalmus*, Red milkweed beetle (Coleoptera: Cerambycidae)	Lawrence 1987
Biotic	Extrinsic	Density	Dispersal distance increased with density	*Tetranychus urticae*, Two-spotted spider mite (Trombidiformes: Tetranychidae)	Bitume *et al*. 2013; Bitume *et al*. 2014
Biotic	Extrinsic	Irradiation	Decreases flight ability	*Dacus cucurbitae*, Melon flies *(Diptera: Tephritidae*	Nakamori & Soemori 1981
Abiotic		Temperature	No correlation between temperature and dispersal distance	*Bactrocera tryoni*, Queenslan fruit fly (Diptera: Tephritidae)	Weldon & Meats 2010
			Dispersal distance further under cooler conditions	*Colias* species (Lepidoptera: Pieridae)	Kingsolver 1983
			Higher temperature increases flight performance	*Drosophila melanogaster*, Vinegar fly (Diptera: Drosophilidae)	Lehmann 1999
			Better flight ability when reared at low temperatures	*Grapholita molesta*, Oriental fruit moth (Lepidoptera: Tortricidae)	Ferrer, Dorn & Mazzi 2013
Abiotic		Landscape	Highly fragmented environment increased dispersal distance, but decreased rate of dispersal	*Boloria eunomia*, Bog fritillary butterfly (Lepidoptera: Nymphalidae)	Mennechez, Schtickzelle & Baguette 2003
Abiotic		Human mediated	Range expansion due to new introductions	*Cameraria ohridella*, Horse chestnut leafminer (Lepidoptera: Gracillariidae)	Gilbert *et al*. 2004

[1] (consistently found to affect dispersal in one species, but see Mitikka & Hanski 2010)

distance, whereas age is known to decrease dispersal ability (see Table 1.1). Abiotic variables such as temperature and landscape are consistently shown to affect the dispersal of various species. However, whether the observed factor affects dispersal may be dependent on the organism examined. For example, the *pgi-1* allele (responsible for increasing flight metabolic rate, flight performance and fecundity) has only been found to exist in butterflies (Hanski & Saccheri 2006; Saastamoinen & Hanski 2008; Niitepold *et al*. 2009; Mitikka & Hanski 2010). Dispersal ability, therefore, is not only an important predictor for future responses to changes in climate, but also plays an important role in current population dynamics and adaptive processes. Understanding the factors that influence whether an individual will disperse and successfully locate and propagate a new habitat is vital for our understanding of adaptive processes in natural populations (Clobert *et al*. 2009), from both a conservation and invasive species perspective.

1.4. Dispersal and invasion risk

The spread (dispersal leading to the expansion of present range) of invasive species arises from the combined effect of population growth and dispersal (Liebhold & Tobin 2008). These essential parameters regulate the invasion process and may help with predicting future risk and developing effective management plans (Sakai *et al*. 2001). It is the ability of a species to disperse affectively that encourages their invasive tendencies (South & Kenward 2001). Therefore, the dispersal potential of any invasive species provides vital information on the species invasion risk (Karsten *et al.* 2013). Invasive species are the second largest threat to biodiversity, increasing competition for resources, modifying habitats, causing disease, predation or parasitisation, and hybridization (Norton & Warburton 2015). The species invasion process occurs in three distinct steps; arrival, establishment and spread (Kristensen, De Barro & Schellhorn 2013). All three of these steps are essential in order for an invasive

species to be successful in colonizing a new area (Elton 1958; Clobert *et al*. 2009; Kristensen, De Barro & Schellhorn 2013; Lombaert *et al*. 2014). Arrival is influenced by propagule pressure, defined as the cumulative number of new (invading) individuals that reach the novel habitat (Leung *et al*. 2004). The greater the propagule pressure, the more successful the establishment phase due to reducing Allee effects and increasing the effective population size (Taylor & Hastings 2005; Havel & Medly 2006). The expansion phase is governed by the movement of individuals into new regions, with habitat quality and competition being particularly important for facilitating the dispersal of the first individuals (Clobert *et al.* 2009). Dispersal ability is the key factor for both the first and the last step of the invasion process (Bowler & Benton 2005), and accurate measurement and understanding of the traits involved in dispersal is key to predicting species' risks to both extinction and invasion potential under changing climates.

1.5. Measuring dispersal

Dispersal is made up of two measurable traits; behaviour, or the willingness to disperse, (dispersal propensity), and performance, such as flight distance or endurance. Dispersal propensity has been calculated for populations by releasing individuals and then measuring the number and distance individuals move away from the release point. This is recorded as the dispersal kernel, or the probability that an individual will move a particular distance from the release point, within a specific time (Neubert & Caswell 2000). Using the dispersal kernel on flying organisms indirectly measures flight performance; however, more direct approaches are also possible. Measurement of dispersal distance and/or endurance is possible through the use of flight mills (e.g. Nakamori & Simizu 1983; Berwaerts *et al*. 2002; Lombaert *et al*. 2014; Ferrer, Mazzi & Dorn 2014). The flight mill is a device used under controlled, laboratory conditions to measure dispersal of flying insects (predominantly Lepidoptera). The

animals are tethered to a rotating metal arm and encouraged to fly in circles until they succumb to exhaustion, often recording metabolic rate simultaneously (e.g. Rascón & Harrison 2005). Another more mechanistic laboratory measure of dispersal is the measurement of the physical force produced during tethered flight. An early yet comprehensive assessment of flight force (spanning several orders and more than 100 species) was conducted by Marden (1987). This study involved placing weights of increasing size on the insects until flight was no longer possible. Force transducers have since been used to accurately measure in-flight vertical force production (Rascón & Harrison 2005); however, these laboratory methodologies still cannot take behavioural elements, such as dispersal propensity, into account. Inferences regarding dispersal distance are likely to be imprecise if laboratory studies are viewed in isolation (Bruzzone *et al.* 2008). Thus such laboratory measures should not be used in isolation or as a proxy for dispersal but rather in collaboration with semi-field and field experiments so that aspects of behaviour can be included into estimations (Terblanche 2014). These include mark-release-recapture (MRR) experiments under partially controlled (i.e. greenhouse) environments or under natural (field) conditions. As the name suggests, insects for MRR experiments are marked, released, and re-caught by pre-baited traps. From this data, the effect of environmental complexity on performance (refers here to dispersal/flight activity) may be calculated using the number of recaptures at any given time or temperature. The more controlled semi-field environment removes the low recapture rates that often occur in mark-release-recapture (MRR) studies due to predation and emigration (Manly 1985). It is also especially useful for examining the performance (or dispersal) of invasive species without actively releasing them, allowing more informed predictions about their future distributions to be obtained.

1.6. *Ceratitis capitata* as model organism

The Mediterranean fruit fly, (*Ceratitis capitata;* Wiedemann*)* (Fig. 1.2.) is an agricultural pest that causes severe crop losses worldwide and significantly impacts food security (De Meyer *et al*. 2002; Malacrida *et al*. 2007; Karsten *et al*. 2013), effectively ruining fruit for export. Their hosts include over 200 types of fruits and vegetables such as citrus, peach, pear and apple (De Meyer *et al.* 2008). This causes an economic drain on the producer and the country, threatening food security (Malacrida *et al*. 2007). Because of its host generality, high reproductive potential and its ability to adapt to unfavourable conditions (Klassen, Lindquist & Buyckx 1994*), C. capitata* is considered a prolific global invader (present on all continents except Antarctica) and subject to rigorous control methods. In South Africa, these include a Malathion pesticide and bait mix that attracts and kills flies, cover sprays that are sprayed over entire orchards and Sterile Insect Technique (SIT). The SIT constitutes the release of sterile males that mate with natural females effectively reducing the number offspring in the next generation (Karsten *et al.* 2013). SIT is steadily gaining favour as it is more environmentally friendly and opens export markets due to lower pesticide use. Dispersal contributes to its success, as flight performance allows sterile males to search for mates, food and shelter (Calkins & Parker 2005). *Ceratitis capitata* has marked variation in dispersal abilities amongst individuals from the same population (Meats & Smallridge 2007), as the majority disperse only 400-700m and <1% disperse longer distances (maximum dispersal: 9.5km) in their life time. Invasions are like natural experiments; highlighting the natural processes occurring at an accelerated pace compared to purely native systems (Sakai *et al.* 2001). Therefore the use of *C. capitata* allows the investigation of important questions regarding dispersal whilst contributing to the knowledge on this species' dispersal potential, which is vital on a practical (development of better sterile insects for use in biocontrol) and economic level (less crop damage, reducing its impact on fruit export).

Fig. 1.2. *Ceratitis capitata* male first described by Wiedemann, photograph by Daniel Feliciano. Creative Commons: CC-BY-SA-4.0,3.0,2.5,2.0,1.0.

1.7. Study aims

There are two broad aims for this thesis. First, to measure the performance (dispersal) of *Ceratitis capitata* within the native range of this species and determine phenotypic traits associated with dispersive and philopatric individuals. Second, to examine how environmental conditions (in particular, temperature) influence dispersal performance and contribute to short term costs and benefits of phenotypic plasticity in dispersal-related traits.

Specifically, in chapter 2, I quantify the phenotypic (observable) traits of *C. capitata* that enhance or are associated with dispersal ability following multiple Mark-Release-Recapture (MRR) experiments. Thereafter, I undertake focussed laboratory experiments to determine which aspects of flight performance are enhanced, or associated with, potential dispersal traits. In chapter 3, dispersal performance is compared under various environmental conditions (i.e. temperature and humidity) in the laboratory to determine the responses of phenotypic plasticity in *C. capitata*. Then the costs and benefits of dispersal (increase or decrease in flight performance measured as the number of recaptures) and its plasticity in *C. capitata* are measured under semi-field (greenhouse) and field conditions to determine how close laboratory predictions are to the real world. This work integrates measurements at different levels of environmental control and habitat complexity to comprehensively assess costs of plastic responses in dispersal in this species. The results from this thesis will provide details of the traits and environmental conditions that influence dispersal ability in *Ceratitis capitata* that should inform management policies and suggest future directions for research on this species.

Chapter 2:

Dispersal propensity, but not flight performance, explains variation in dispersal behaviour*

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Steyn, V.M., Mitchell, K.A. & Terblanche, J.S. "Dispersal propensity, but not flight performance, explains variation in dispersal behaviour"

2.1. Introduction

Dispersal, the movement of individuals from a natal patch to a novel environment, has a significant impact on several aspects of population dynamics and therefore animal ecology and evolution (Ronce 2007; Pennekamp *et al*. 2014), allowing the colonization of novel habitats (Le Galliard, Massot & Clobert 2012), facilitating gene flow between populations (Bowler & Benton 2005) and contributing to evolutionary adaptation (Clobert *et al.* 2009). The probability that an individual will move a particular distance from their natal habitat within their lifetime is depicted by the 'dispersal kernel' (Neubert & Caswell 2000; Ronce 2007) and generally follows a specific right-skewed frequency distribution (Murrell, Travis & Dytham 2002). As such, only a small proportion of individuals in a population disperse over long distances, with the majority remaining in closer proximity to their natal patch (e.g. Bitume *et al.* 2013); however, the few individuals with the largest dispersal distance are those most likely to found new populations. This has been well documented in several invertebrate species (e.g. Meats & Smallridge 2007; Bitume *et al.* 2013). For example, Meats & Smallridge (2007) found that the majority of Mediterranean fruit flies *Ceratitis capitata* disperse a short distance, (400-700m) with less than 1% of marked flies dispersing longer distances (maximum dispersal: 9.5km). Recent work on spider mites (*T. urticae*) suggests that the population density experienced by the individual as well as its ancestors (maternal effects) influences their dispersal distance (Bitume *et al.* 2014). As the dispersal kernel examines movement at the population level, it does not provide information regarding trait variation between individuals. As such, using this population-level approach may lead to the conclusion that dispersal is only affected by external environmental factors (Vinatier *et al.* 2011), or that stochasticity determines dispersal behaviours, which is certainly not the case (Lowe & McPeek 2014).

It has long been recognised that individuals within a population may exhibit marked variation in their dispersal ability, thus allowing the characterization of philopatric and dispersive individuals based on phenotypic traits. Typically morphometric traits are the focus, such as wing size in insects (e.g. Berwaerts, Van Dyck & Aerts 2002) and birds (Nilsson 1989) and leg length in vertebrates (e.g. Phillips *et al.* 2006; Perkins *et al.* 2013), assuming that these are linked to functional performance. Differences in dispersal-related phenotypes are vital to understanding adaptive variation in dispersal ability or selection for enhanced locomotor performance or dispersal syndromes (Hanski *et al.* 2004; Lowe & McPeek 2014), and especially how environment- or context-specific demography and ecology affects the individual disperser (Clobert *et al.* 2009). This adaptive variation in phenotypes between individuals has been well documented in, for example, the cane toad (*Rhinella marina*) as it spread throughout Australia. Phillips *et al.* (2006) showed that traits favouring dispersal (e.g. longer legs) were strongly selected for and then greatly exaggerated in the individuals on the periphery of the invasion front, in contrast to the beneficial reproductive traits that were selected for by the individuals from the core of the population. This exaggeration of beneficial dispersal traits may in turn lead to accelerated population expansion due to the enhanced dispersal ability of these individuals (Lombaert *et al*. 2014; Peischl, Kirkpatrick & Excoffier 2015). The frequency of such exaggerated selection for dispersal traits at range margins remains to be examined in other taxa but may be ameliorated when gene flow from the core population is high (Bridle & Vines 2007; Kubisch *et al.* 2014).

Flight is an effective means of directional dispersal as it allows the organism to cover larger distances, typically via more direct routes. Flight has likely also contributed to the evolutionary success of insects by improving food and mate discovery (reviewed in Chapman, Reynolds & Wilson 2015). At the intraspecific level, variation in flight performance is often related to differences in individual phenotypes such as thoracic muscle

mass, wing size (Dudley 2000) or life history trade-offs (Zera & Harshman 2001; Hanski, Saastamoinen & Ovaskainen 2006) and can impact endurance or power parameters via biophysical mechanisms. For example, wing size, via its influence on wing loading (body mass/wing area), is thought to have a pronounced effect on flight performance and dispersal ability, with larger-winged individuals of the same body mass likely to have greater flight ability due to reductions in power requirements (Marden 1987), and should be favoured for dispersal (e.g. small- and large-winged morphs, Steenman, Lehmann & Lehmann 2015).

Take-off ability may be correlated with variation in the thorax mass to body mass ratio, since a larger thorax allows a steeper take-off angle (Berwaerts, Matthysen & Van Dyck 2008). Individuals with a larger thorax mass to body mass ratio likely invest less energy in reproduction and more into dispersal ability (Dudley & Srygley 1994; Hughes, Hill & Dytham 2003; Niitepold *et al.* 2009). Ratiometric based indices are however problematic in describing changes since one or both variables may be varying simultaneously or selected for in opposite directions (e.g. antagonistic selection of thorax and wing size; Hoffmann *et al.* 2007). Regardless, a trade-off between reproduction and dispersal ability is frequently reported among several invertebrate species (e.g. planthoppers, *Prokelisia dolus*, Denno, Olmstead & McCloud 1989; butterflies, *Pararge aegeria*, Hughes, Hill & Dytham 2003; reviewed in Zera & Harshman 2001; Weigang & Kisdi 2015).

The mechanisms underlying a dispersal-reproduction trade-off has generally been well examined in species with distinct dispersal morphs (polymorphisms) (reviewed in Zera & Denno 1997; Zera 2003; Guerra 2011), but seldom tested in species with continuous (unimodal) variation in phenotypes (but see e.g. Berwaerts, Van Dyck & Aerts 2002; Hoffmann *et al.* 2007). The decision to disperse may be influenced by either context- or condition-dependent stimuli (Pennekamp *et al.* 2014). A context-dependent stimulus can

include a change in environmental conditions such as density, resource availability or predation that encourages dispersal (Bowler & Benton 2005; Clobert *et al.* 2009; Bitume *et al.* 2014). However, condition-dependent dispersal occurs in response to changes in intrinsic physical or physiological aspects within the organisms' body such as lipid reserves, muscle mass, hormones and stress (Zera 2003; Guerra 2011). These internal and external stimuli may cause a normally philopatric individual to disperse and *vice versa*, further complicating the search for traits that may be predictive of dispersal ability. Behavioural drivers are therefore thought to be vital components to understanding dispersal ability and have received increasing recent attention (Bowler & Benton 2005; Van Dyck & Baguette 2005; Bitume *et al.* 2013; Edelsparre *et al.* 2014; Palmer, Coulon & Travis 2014; Sinclair *et al.* 2014).

Here I therefore examine the hypothesis that flight performance is linked to traits of morphology, physiology and behaviour. To test this main hypothesis, I first determine the dispersal kernel of *Ceratitis capitata*, based on information of individual dispersal ability, and use this to identify functional traits that may be specific to philopatric or dispersive individuals. The Mediterranean fruit fly, *Ceratitis capitata* is an agricultural pest that causes severe crop losses worldwide and significantly impacts food security (De Meyer *et al.* 2008; Malacrida *et al.* 2007; Karsten *et al.* 2013), effectively restricting fruit for export. Their movement is believed to be limited to favourable conditions and when they have access to suitable hosts, suggesting that their dispersal is context-dependent and strongly influenced by environmental factors such as temperature (e.g. Buyckx 1994; Esterhuizen *et al.* 2014; Weldon, Schutze & Karsten 2014). Furthermore, they have marked variation in dispersal abilities amongst individuals from the same population (Meats & Smallridge 2007), making them an ideal subject to address the main hypothesis. I then aimed to determine which aspects of flight performance are enhanced, or associated with, potential dispersal traits identified previously in other flying insects. I further predicted that either larger wings, or a greater

thorax mass to body mass ratio will lead to increased flight performance due to the increased power-to-weight relationship in both cases, thereby enhancing dispersal, and that these traits would perhaps be coupled with increased flight endurance.

2.2. Materials and methods

STUDY SPECIES

Ceratitis capitata pupae were procured from Citrus Research International (CRI) and, upon arrival in the laboratory in Stellenbosch, were placed in net cages at low density at 25°C with a 12:12 photoperiod in a climate chamber (LE-509, MRC, Holon, Israel). Though protein has been shown to improve reproductive output of flies reared in the laboratory (Teal *et al*. 2013), no studies to my knowledge have shown that flies have access to, or frequently utilise, sources of high protein in the wild. Therefore flies were supplied with water and sugar crystals and were allowed to feed *ad libitum* after emergence. No flies were re-used for more than one experiment.

FIELD RELEASES

Four separate releases were conducted in the summer. To assess if thermal history influenced recapture rates and disperser trait determination, one- or two-day old flies were thermally acclimated at one of three thermal conditions (20°C, 25°C or 30°C) for two days prior to release. Flies from each treatment group were marked with DayGlo® fluorescent powder in three colours (pink, blue and yellow) that were randomly assigned prior to each release. A three-component pheromone attractant (Biolure®, Chempac, Paarl, South Africa) was opened for 10 minutes at one end of a 15x3m rectangular greenhouse before being sealed into an airtight container to create a pheromone gradient. Flies were then released from the opposite end of the greenhouse and allowed to move unobstructed. By creating distance classes (0, 3, 6, 9 and 12 m) and counting how many individuals were in each class every 30 minutes, I determined the proportion as well as the distance flies had moved at each time point. Furthermore the time taken for the first individuals to reach the lure end of the greenhouse was recorded. The first 55 individuals to reach the pheromone lure (typically <20 minutes)

were considered as 'dispersers' and those that did not leave the point of release after 3 h were assigned 'philopatric'. Dispersers and philopatric individuals were placed individually into an Eppendorf tube, placed on crushed ice, and transported back to the laboratory where they were frozen at -80°C prior to being dissected and their wings removed for phenotypic measurements.

MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS

The extracted wings were mounted on microscope slides and imaged using a Leica (MZ16A, Leica, Wetzlar, Germany) microscope fitted with a digital camera. Morphometrics of the wings were conducted using fourteen landmarks on each wing (for instance where two veins meet, Fig. 2.1). Wing size and shape was calculated by comparing the location of these landmarks on the wings of the recaptured individuals in MorphoJ (version 1.06b) (Klingenberg 2011). After the entire fly was weighed, it was dissected using a scalpel to allow the thorax and abdomen to be weighed separately, using an ultramicrobalance (UMX-2, Mettler Toledo Inc., Columbus, OH; to ±1 μg). All traits (relative wing size, thorax mass, abdomen mass, entire body mass) I measured that were not highly correlated ($R \geq 0.8$) with other traits were included in a minimal adequate model (MAM) (following Crawley's (2007) method for model simplification), along with acclimation treatment and sex, to determine if any of these phenotypic traits were associated with dispersal ability. This analysis highlighted that a larger thorax mass to body mass ratio is an important identifier of dispersers relative to philopatric individuals. Therefore the flies used in the force transducer experiment were divided into either dispersers (larger thorax mass to body mass ratio) or philopatric individuals (smaller thorax mass to body mass ratio) using the same criterion identified from the field releases. There is however an inherent significant difference in the thorax mass to body mass ratio between males and females ($n = 358$, $F = 19.77$, $P<0.001$, Fig. 2.2).

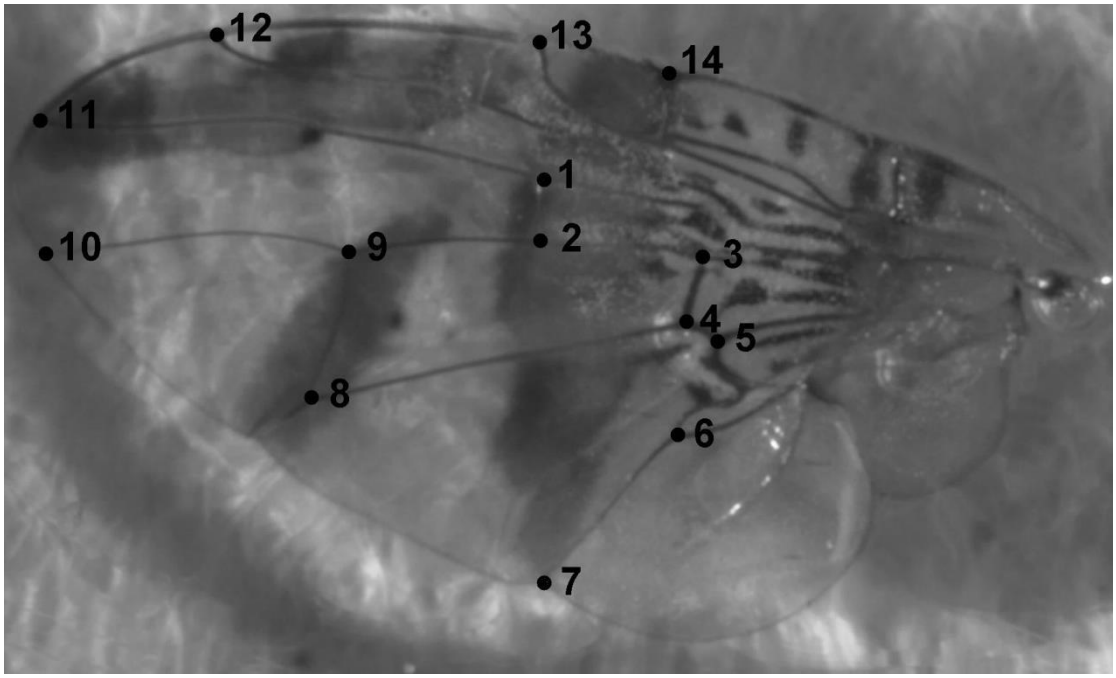

Fig. 2.1. Example image of a *Ceratitis capitata* wing with the 14 superimposed landmarks. The landmarks were used in MorphoJ software to determine differences in wing size or shape between individuals.

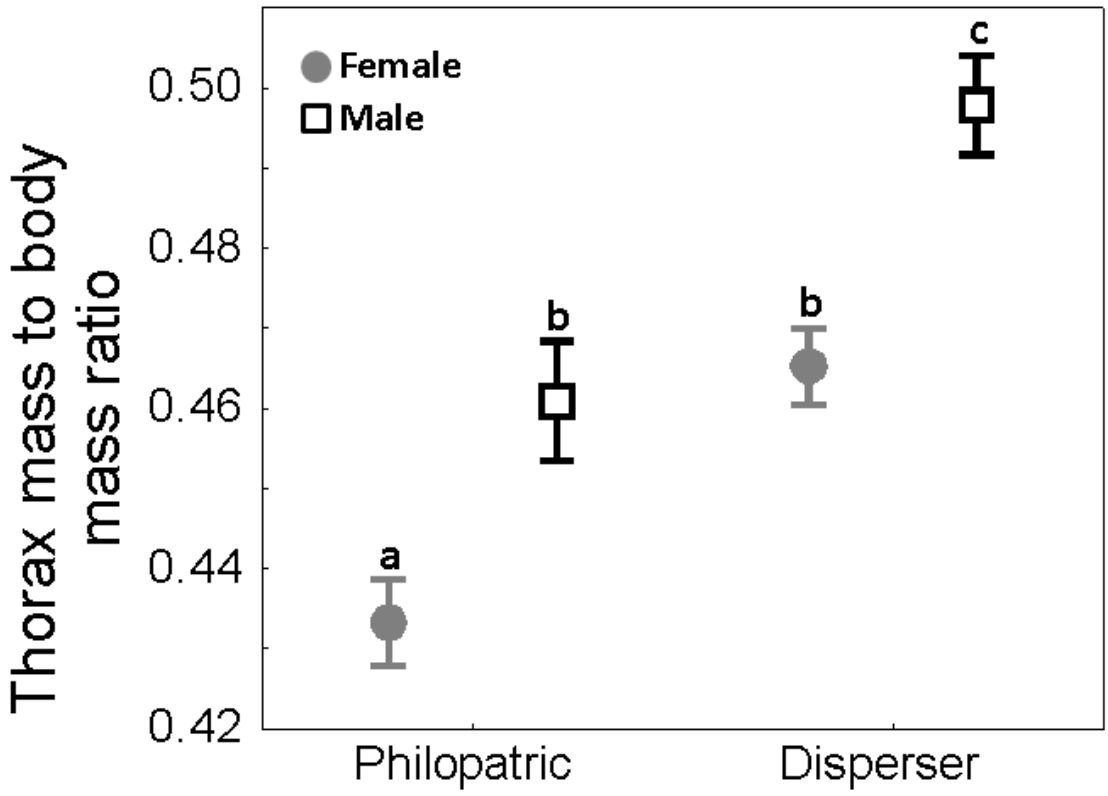

Fig. 2.2. The mean ± SE of the thorax mass to body mass ratio compared between philopatric individuals and dispersers for males and females within a population. Similar lowercase letters indicates non-significance, whereas dissimilar lowercase letters indicates significance between groups. Due to the overlap of thorax mass to body mass ratio between males and females, categories (philopatric or disperser) for further trials were assigned on a sex specific basis.

Therefore the criteria for thorax mass to body mass ratio was assigned on a sex-specific basis. Once the individuals were categorized into dispersers and philopatric individuals, and so long as the sex ratios were equal, all further analyses were conducted independent of sex.

LABORATORY FLIGHT EXPERIMENTS

As in the release-recapture experiments, 1-2 day old flies were reared at 25 °C for two days prior to the flight test. The flies were attached to a FORT10 force transducer (World Precision Instruments, Sarasota, FL, USA) by gluing a #2 insect pin to the thorax with hand-warmed leg wax. The pin was snugly inserted into a custom-designed mount that was then slid firmly onto the leaf of the force transducer (Fig. 3A). The experiment took place in a temperature-controlled room (25 ± 1 °C) where the fly was allowed to fly unobstructed. In the few cases an individual did not fly on its own, in which case the insect was stimulated by blowing air over antenna and head gently so that the force produced during flight could be measured and compared to other flies. If flight was not achieved or if the flight was shorter than 5 s it was regarded as a failed attempt. To obtain estimates of repeatability, seven replicate flight force trials were conducted on each fly, and individuals were allowed at least two minutes of rest between replicates.

The force transducer records the change of force in volts, so in order to convert the voltage reading to the force (in Newton), a calibration was conducted to obtain a correction coefficient. The coefficient was calculated by placing a wide range of masses (and spanning the mass of a typical fly, from 0.0001 g to 1 g) on the force transducer, with the recorded voltage then plotted against the known masses of the weights. The least-squares regression equation ($y = 0.056x - 0.0019$) describes the linear ($R^2 = 0.9959$, $P<0.01$) relationship between the voltage output and the known mass (Fig. 2.3). This ensured conversion from

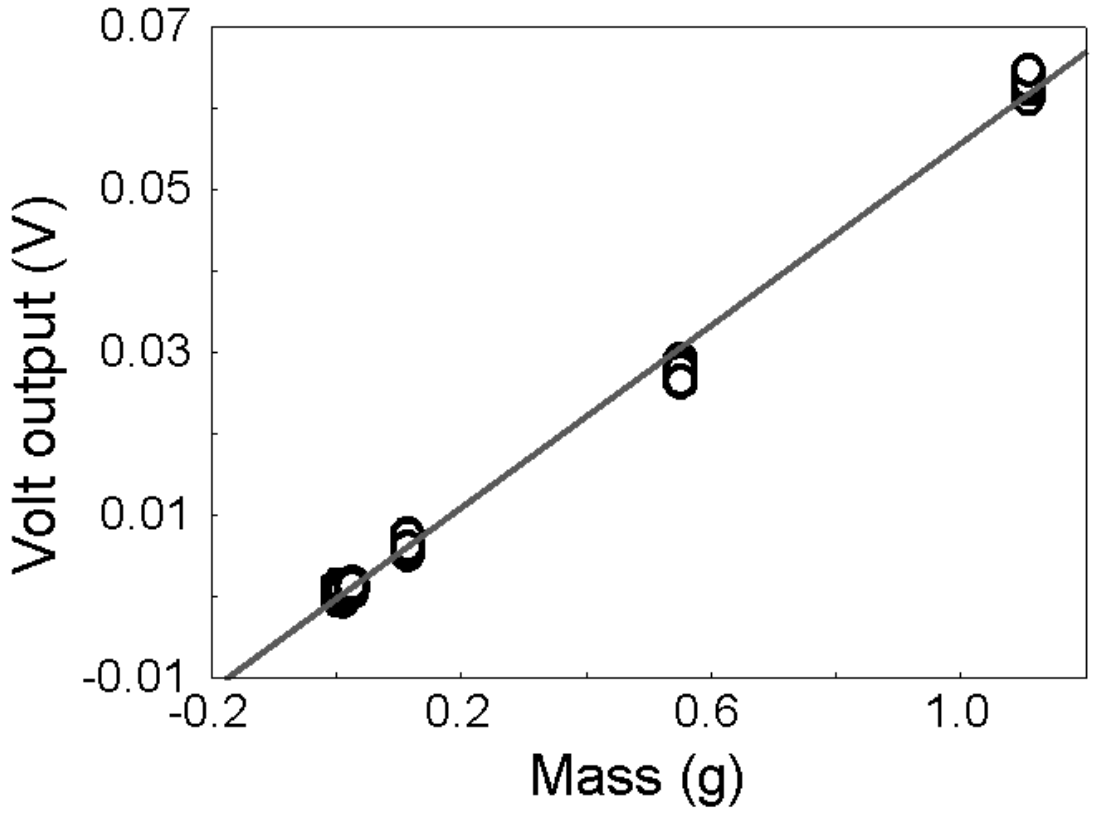

Fig. 2.3. The linear relationship used to determine the calibration coefficient that was used to convert the voltage output measured with the force transducer into a force. The scatterplot plots the voltage output of an object measured by the force transducer against the known mass of the object. The linear model fitted to the data is y = 0.056x -0.0019 and describes 99.59% of the variation in the system.

voltage to grams or Newton were accurate. The vertical force produced by an individual fly was recorded every 0.01 s and data captured and processed using Expedata version 1.8.0.2 (Sable Systems, Las Vegas, NV, USA). Peak forces were determined by calculating the difference between the highest (or strongest) consecutive 5 s of force output within a flight window and a 30 s steady-state when the insect was at rest. Mean force was determined by calculating the difference between the force output of the entire flight window and the 30 s steady-state.

A repeated measures test was conducted, and repeatability (*r*) was calculated from the ANOVA's *F*-statistic (Falconer & Mackay 1996) for the peak force, mean force, total flight length, maximum flight length and total flights (Table 2.1). There were no consistent time (or trial number) effects for all parameters (peak force, mean force, total flight length and maximum flight length) except total flights (Table 2.2). This suggests there was a training effect on total number of flights in a trial, as the number of successful flights increased with trial number, but not for any of the other flight performance variables.

The flight parameters, flight success rate, flight ratio, total flight length, maximum flight length, mean flight length, peak force and mean force, were measured using the high precision force transducer setup. Flight success rate was scored as the number of successful flights (> 5 s) divided by the number of attempts. Flight ratio, whether an individual flew or not, was also compared between philopatric and dispersive individuals.

STATISTICAL ANALYSES

TableCurve 2D (version 5.01.02) (SYSTAT Inc, San Jose, California, USA) was used to fit multiple linear and non-linear equations to the dispersal kernel data.

Table 2.1. Results of analyses of variance to calculate repeatability (r) of peak force, mean force, total flights, total flight length and maximum (max.) flight length in *Ceratitis capitata*, measured in a temperature controlled room using a force transducer. The low, but significant repeatability of force production indicates that there is lots of variability in the assessment, it is not because force cannot be assessed accurately, but rather that there is a lot of natural variability built into these estimates. Factors in **bold** indicate significance at $P = 0.05$.

Trait measured	Source of variation	d.f.	MS	*F* value	*P*	Repeat-ability (r, %)	$LCL_{0.95}$	$UCL_{0.95}$
Peak force (mN)	***Among***	**33**	**0.25**	**4.09**	**<0.001**	**34.1**	**16.5**	**52.8**
	Within	7	0.06					
Mean force (mN)	*Among*	33	1.5×10^{-4}	1.44	0.072	6.9	-4.1	22.2
	Within	7	1.1×10^{-4}					
Total flights (N)	***Among***	**33**	**2.35**	**9.43**	**<0.001**	**58.5**	**40.2**	**73.7**
	Within	7	0.25					
Total flight length (s)	***Among***	**33**	**7.00**	**7.17**	**<0.001**	**50.8**	**32.0**	**67.6**
	Within	7	0.98					
Max. flight length (s)	***Among***	**33**	**10.27**	**9.64**	**<0.001**	**59.1**	**40.8**	**74.1**
	Within	7	1.07					

Table 2.2. Summary results of factorial ANOVA testing whether there were consistent time (or trial number) effects, which would have suggested fatigue or training effects on peak force, mean force, total flights, total flight length and maximum (max.) flight length in *Ceratitis capitata*, measured in a temperature controlled room using a force transducer. Factors in **bold** indicate significance at $P = 0.05$.

Trait measured	Source of variation	d.f.	MS	*F* value	*P*
Peak force (mN)	Individual	33	0.002	0.020	0.892
	Trial number	7	0.164	1.830	0.178
	Individual x Trial number	155	0.005	0.060	0.814
Mean force (mN)	Individual	33	3.4×10^{-5}	0.300	0.586
	Trial number	7	2.7×10^{-5}	0.240	0.625
	Individual x Trial number	155	2.3×10^{-5}	0.200	0.656
Total flights (N)	Individual	33	0.364	0.620	0.433
	Trial number	**7**	**2.820**	**4.780**	**0.030**
	Individual x Trial number	155	1.669	2.830	0.094
Total flight length (s)	Individual	33	7.369	3.840	0.052
	Trial number	7	0.044	0.020	0.880
	Individual x Trial number	155	0.655	0.340	0.560
Max. flight length (s)	Individual	33	5.370	2.110	0.148
	Trial number	7	0.356	0.140	0.708
	Individual x Trial number	155	0.087	0.030	0.854

For each function the parameter count (K), AIC and the delta AIC (Δi), calculated as the difference between that function's AIC and the lowest AIC. The Akaike weight (wi) is the normalized likelihood that the function is the best function in the set and was used to determine the curve that best described the data. The equations were considered to be significantly different if their ΔAIC values were greater than 2. If multiple models were inseparable (ΔAIC<2) the best model was chosen according to the principle of parsimony and therefore the simplest of the models were chosen. To determine whether the dispersal kernel was purely a result of density or if other factors may be playing a role, a simple correlation was performed. This was achieved by testing whether releases with higher release densities caused a larger proportion of the flies to move the maximum (12m) distance the test allowed. All further statistical analyses were undertaken in RStudio (version 0.98.1087; RStudio Inc., Boston, USA). The data were tested for normality using the Shapiro Wilk's test. If the data were not normally distributed they were log transformed prior to further analysis. A generalized linear model, with a Gaussian distribution and an identity link function, was used to determine the effect of sex and dispersal category (i.e. disperser *vs.* philopatric) and their interaction on the morphological characters that included wing size, thorax mass, abdomen mass, body mass and ratios of these in *C. capitata* individuals released in the field. To test the prediction that larger wing size increases flight endurance simple regressions were conducted, total flight length was regarded as the dependent variable and wing area or wing loading regarded as the explanatory variable. To determine whether there was antagonistic selection between thorax mass and wing size (e.g. Hoffmann *et al*. 2007), logistic regressions were conducted where the distance moved (0 m or 12 m) was used as the dependent variable. To quantify the association of thorax mass and wings size on the distance the individuals moved multiple logistic regressions were conducted. The MAM was used to determine the most important phenotypic traits for dispersal in *C. capitata*. The data included into the linear

model were all the phenotypic trait data collected (thorax mass, abdomen mass, body mass, thorax-to-body mass ratio, wing size) as well as categorical variables (e.g. sex and thermal history) (for full starting model Appendix 1). The model was simplified following Crawley's (2007) method, in which the non-significant terms with the highest order interactions are removed from the model, and the models compared after the removal via an ANOVA. If the models were significantly improved, the term or interaction was left out of the model. This process was repeated until the removal of a factor did not improve the model or if the highest order interaction had a significant interaction. Generalized linear models were used to determine the effect of sex and dispersal category on flight parameters measured. The interaction effect was not included in the model as the categories were allocated on a sex-specific basis, therefore sex is already accounted for in the comparisons between the categories. A generalized linear model, with a Gaussian distribution and an identity link function, was used to determine the effect of sex and category on flight success rate for all the tested individuals. Furthermore a generalized linear model, with a binomial distribution and a logit link function, was used to determine the effect of sex and category on flight ratio for all the tested individuals. All further analyses were conducted on only the individuals that achieved flight, therefore a generalized linear model, with a Gaussian distribution and an identity link function, was used to determine the effect of sex and dispersal category on peak force, mean force, total flight length, maximum flight length and mean flight length.

2.3. Results

FIELD RELEASES

Only 3.6 ± 0.5% (mean ± SE) of the individuals released (N = 812.0 ± 131.0 per release) over four releases reached the pheromone side of the greenhouse with the majority of individuals moving <3 m in 3 h (Fig. 2.4). The dispersal kernel for *Ceratitis capitata* is best described by a decaying exponential curve (detailed curve fitting comparisons are shown in Table 2.3) and was consistent between releases. To test for a possible dependence of this relationship on density, we predicted that a positive association would exist between fly release density (or abundance) and the proportion of flies that moved the maximum distance (12 m) across releases that varied in fly abundance. This analysis however suggests that the dispersal kernel was not merely a result of density in a given release, as the correlation between the proportion of flies that moved the maximum distance and density was not significant (R = -0.18, p = 0.72, Fig. 2.5).

Philopatric individuals (flies that moved < 3 m) and dispersers (flies that moved the maximum distance the test allowed, > 12 m) differed according to thorax mass, abdomen mass, body mass, thorax to body mass ratio and abdomen to body mass ratio (Table 2.4). These phenotypic traits also showed a significant difference between the sexes, however there was no sex by dispersal category interaction. This indicates that sex does not influence whether or not individuals will disperse, but rather that there is an inherent difference in body and wing size between the sexes. The mean body mass (pooled for the sexes) of dispersers [6.1 ± 1.2 mg (mean ± SE)] was significantly greater than that of philopatric individuals (5.6 ± 1.2 mg; Table 2.4), with thorax and abdomen mass being significantly larger in dispersers than philopatric individuals (disperser abdomen 2.4 ± 0.7 mg, thorax 2.9 ± 0.6 mg *versus* philopatric abdomen 2.1 ± 0.6 mg, thorax 2.5 ± 0.6 mg; Fig. 2.6B, C).

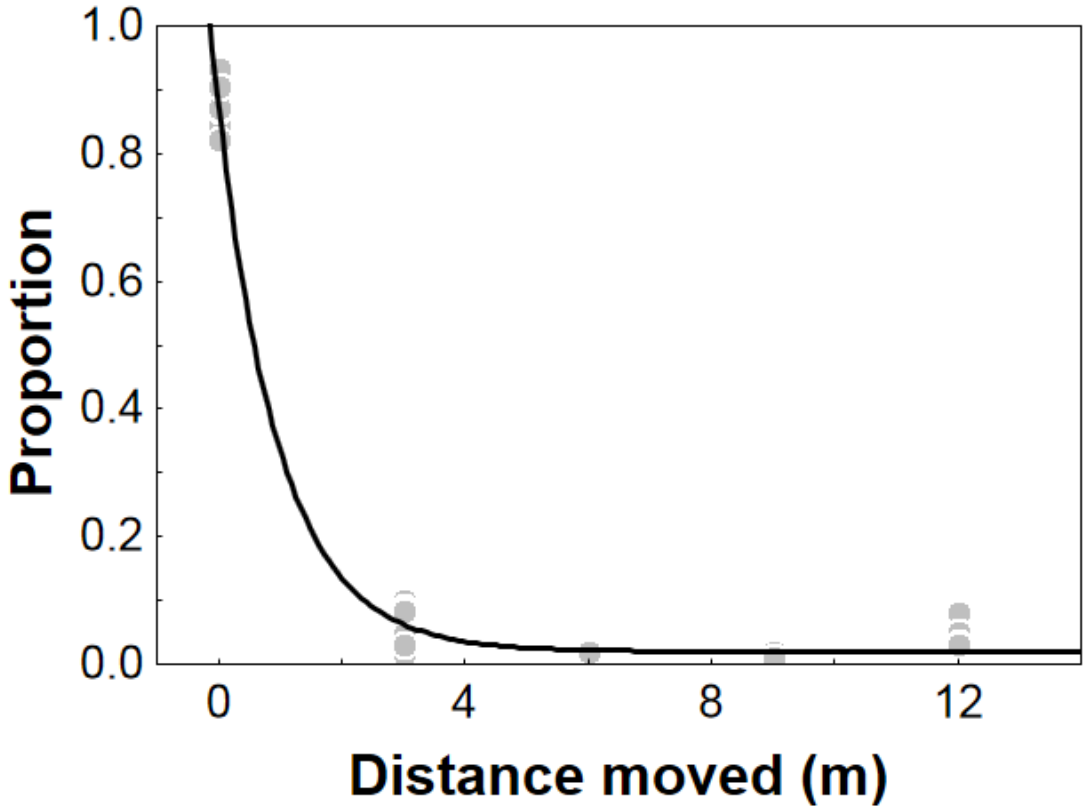

Fig. 2.4. The proportion of *Ceratitis capitata* individuals found at a given distance from the release point (0 m) at one side of a greenhouse to a lure attractant at the opposite end (12 m) of the greenhouse when allowed to move unobstructed for 3 hours. The distances the flies moved were placed into distance classes. The scatterplot includes data from four releases conducted during the summer. The data is best described by a decaying exponential curve (Table 2.3).

Table 2.3. A comparison of linear and non-linear functions to describe the relationship between proportion and the distance of *Ceratitis capitata* individuals in the field. For each function the parameter count (*K*), AIC and the delta AIC (Δ_i), calculated as the difference between that function's AIC and the lowest AIC. The Akaike weight (w_i) is the normalized likelihood that the function is the best function in the set. However because the delta AIC is not >2 for the first seven functions any of these models are all plausible and statistically indistinguishable (functions above line). The best model was thus chosen according to the principle of parsimony and therefore the simplest of these was chosen. Therefore the function in **bold** is regarded as the function that best describes the release dispersal data.

Function	*K*	AIC	Δ_i	w_i	R^2
$y=a+be^{x}+ce^{-x}$	4	-190.140	0.000	0.165	0.996
$y=a+bx^{2.5}+cx^{3}+de^{-x}$	5	-189.772	0.368	0.138	0.996
$y=a+bx^{2}+cx^{3}+de^{-x}$	5	-189.760	0.379	0.137	0.996
$y=a+bx+cx^{3}+de^{-x}$	5	-189.681	0.459	0.132	0.996
$y=a+bx^{3}+ce^{x}+de^{-x}$	5	-189.374	0.766	0.113	0.996
$y=a+be^{-x}$	**3**	**-188.821**	**1.318**	**0.086**	**0.995**
$y=a+bx^{3}+ce^{-x}$	4	-188.572	1.567	0.076	0.995
$y=a+bx+cx^{1.5}+dx^{2}+ex^{3}$	6	-187.772	2.368	0.051	0.996
$y=a+bx+cx^{1.5}+dx^{2}+ex^{2.5}$	6	-187.772	2.368	0.051	0.996
$y=a+bx+cx^{2}+dx^{3}+ex^{4}$	6	-187.772	2.368	0.051	0.996
$y=a+b\ln x$	3	-181.066	9.073	1.8×10^{-3}	0.993
$y=a+b/x^{0.05}$	3	-176.979	13.160	2.3×10^{-4}	0.992
$y=a+b/x$	3	-176.979	13.160	2.3×10^{-4}	0.992
$y=a+b(\ln x)^{2}$	3	-176.697	13.443	2.0×10^{-4}	0.992
$y^{0.05}=a+be^{-x}$	3	-176.552	13.587	1.9×10^{-4}	0.992
$\ln y=a+be^{-x}$	3	-163.747	26.393	3.1×10^{-7}	0.987
$y^{-1}=a+bx^{0.05}$	3	-161.209	28.930	8.6×10^{-8}	0.986
$\ln y=a+bx^{0.05}$	3	-158.776	31.364	2.6×10^{-8}	0.984
$y^{-1}=a+be^{-x}$	3	-150.534	39.606	4.2×10^{-10}	0.979
$y^{-1}=a+bx$	3	-147.397	42.742	8.7×10^{-11}	0.976

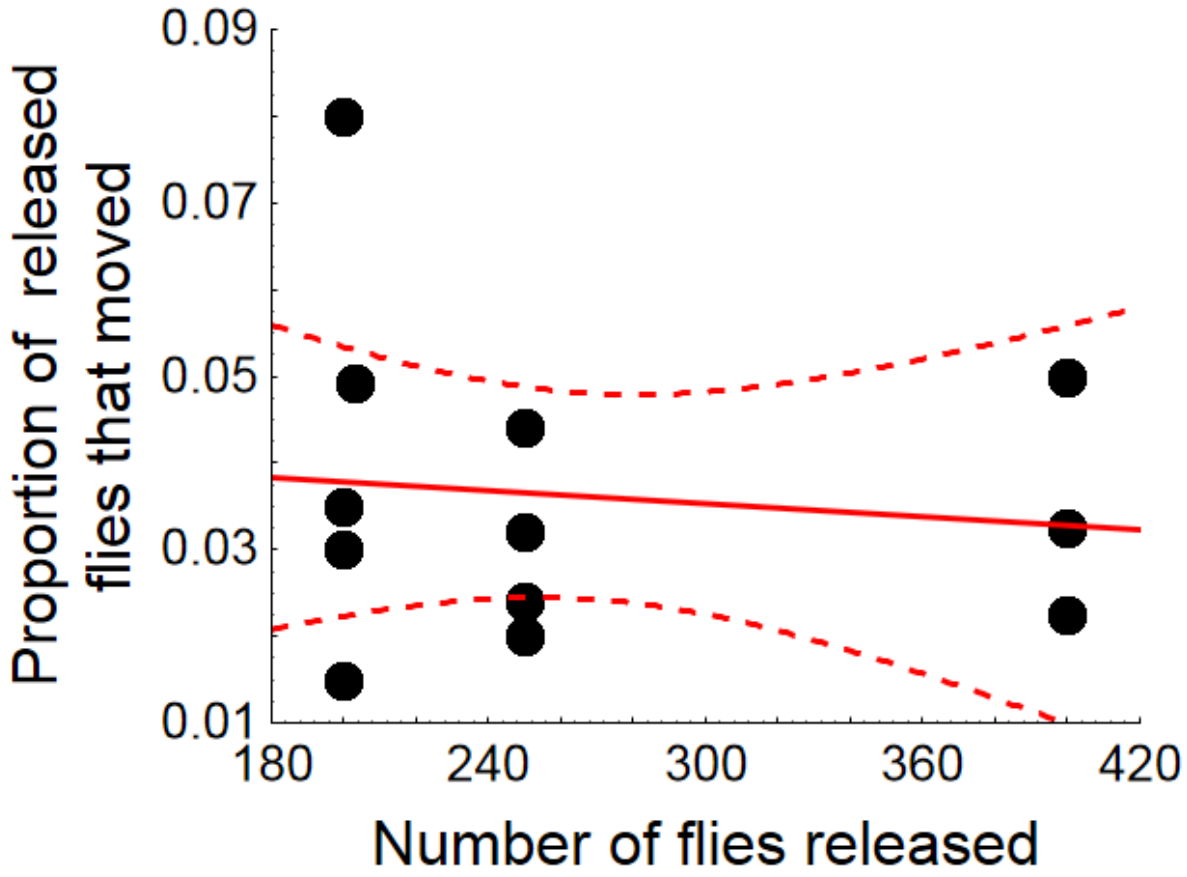

Fig. 2.5. To determine whether there was a correlation between the density of flies and the distance they moved in the semi-field releases a correlation was run. The correlation tested the relationship between the number of *Ceratitis capitata* flies released for a given experiment and the proportion of released flies that moved the maximum distance the test allowed. The line fitted to the data is y = -0.00025x+ 0.0429.

Table 2.4. Summary results of generalized linear models (Gaussian distribution, identity function) of sex and category (i.e. disperser vs philopatric) and their interaction on the wing size, thorax mass, abdomen mass, body mass and ratios of these in *Ceratitis capitata* individuals released in the field. Factors in **bold** indicate significance at $P = 0.05$. AIC = Akaike information criterion.

Trait	AIC	Factor	Wald's χ^2	SEM	*T* value	*P*
Wing size		**Intercept**	**-0.022**	**0.004**	**-5.028**	**< 0.001**
	619.12	**Sex**	**0.043**	**0.006**	**7.228**	**< 0.001**
		Category	0.001	0.001	0.624	0.534
		Sex*Category	$-0.2x10^{-3}$	0.002	-0.155	0.877
Thorax mass		**Intercept**	**2.464**	**0.072**	**34.180**	**< 0.001**
	632.43	Sex	-0.123	0.112	-1.102	0.271
		Category	**0.088**	**0.020**	**4.493**	**< 0.001**
		Sex*Category	0.006	0.032	0.182	0.855
Abdomen mass		**Intercept**	**2.182**	**0.079**	**27.633**	**< 0.001**
	697.54	**Sex**	**-0.504**	**0.123**	**-4.107**	**< 0.001**
		Category	**0.071**	**0.022**	**3.324**	**< 0.001**
		Sex*Category	0.007	0.035	0.193	0.847
Body mass		**Intercept**	**1.735**	**0.023**	**74.443**	**< 0.001**
	-175.97	**Sex**	**-0.102**	**0.036**	**-2.816**	**< 0.01**
		Category	**0.017**	**0.006**	**2.620**	**< 0.01**
		Sex*Category	0.002	0.010	0.156	0.876
Thorax mass to		**Intercept**	**0.425**	**0.007**	**62.083**	**< 0.001**
body mass ratio	-1052.8	**Sex**	**0.026**	**0.011**	**2.482**	**< 0.05**
		Category	**0.008**	**0.002**	**4.303**	**< 0.001**
		Sex*Category	0.001	0.003	0.412	0.681
Abdomen mass		**Intercept**	**0.377**	**0.008**	**45.327**	**< 0.001**
to body mass	-913.62	**Sex**	**-0.058**	**0.013**	**-4.452**	**< 0.001**
ratio		**Category**	**0.005**	**0.002**	**2.141**	**< 0.05**
		Sex*Category	0.004	0.004	1.04	0.299

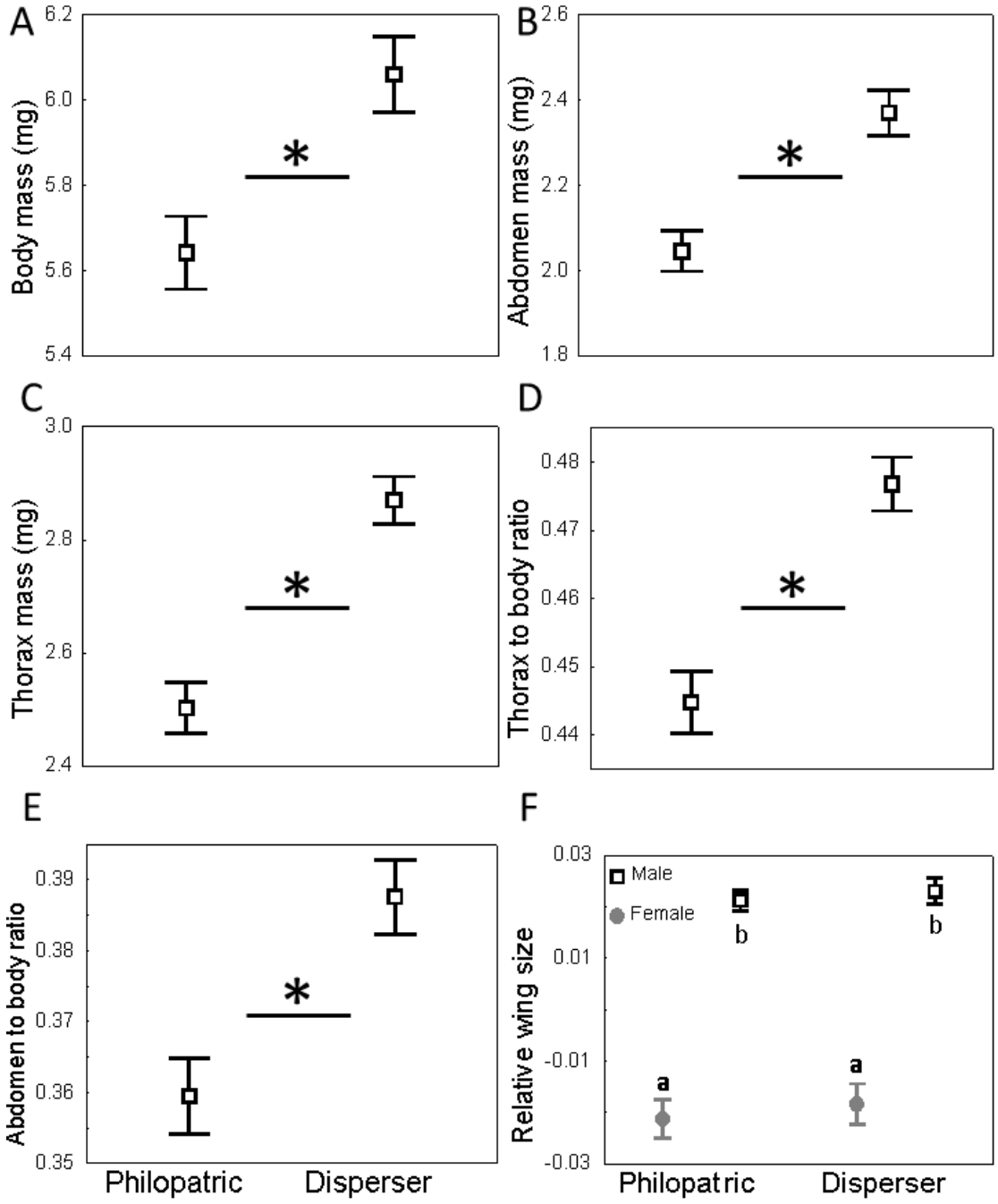

Fig. 2.6. The mean ± SE for each of the phenotypic traits measured on the flies identified through the flied release experiments. Traits include body mass (A), abdomen mass (B), thorax mass (C), thorax mass to body mass ratio (D), abdomen mass to body mass ratio (E) and relative wing size from geometric morphometrics PCA scores (F). The * indicates significance for panels (A-E). In panel (F) similar lowercase letters indicates non-significance, whereas dissimilar lowercase letters indicates significance.

Furthermore the relative mass of the thorax for a constant body mass (i.e. body mass to thorax mass ratio) was significantly different between the two disperser groups (Fig. 2.6D). The thorax mass of the dispersers accounted for 47.7 ± 0.4% (mean ± SE) of the total body mass, whereas the thorax of the philopatric individuals only accounted for a significantly lower proportion (44.5 ± 0.5%) of the total body mass (Fig. 2.6A). The wing size did not differ between dispersers and philopatric individuals, however wing size did differ between the sexes (Fig. 2.6F). The regression between total flight length and wing area was not significant (N = 34; R^2 = 0.02; F = 0.54; P = 0.47), even after correcting for body size (wing loading) (N = 34; R^2 = 0.04; F = 1.30; P = 0.26). There was no evidence of antagonistic selection between thorax and wing size, as relationship was not significant (N = 120; χ^2 = 2.29; P = 0.13). There was a difference in wing shape between the sexes (Fig. 2.7), however there were no differences in wing shape between philopatric and dispersive individuals for either of the sexes (Fig. 2.8).

The MAM was used to determine the most important phenotypic traits contributing to dispersal. The final model included only abdomen mass, thorax mass and body mass as explanatory factors. Therefore wing size, thermal history and sex were excluded as potential explanatory factors from the final model. Also retained in the MAM was a significant interaction between the abdomen and thorax mass as well as abdomen and body mass between dispersers and philopatric individuals. Therefore a larger thorax to body mass ratio is indicative of a disperser in this model selection approach. All further statistical tests reported here include only the variables retained in the MAM (Table 2.5).

LABORATORY FLIGHT ASSAYS

The flight parameters were measured and compared between dispersers (larger thorax mass: body mass ratio) and philopatric (small thorax mass: body mass ratio) individuals assigned according to the thorax mass: body mass ratio seen in the field.

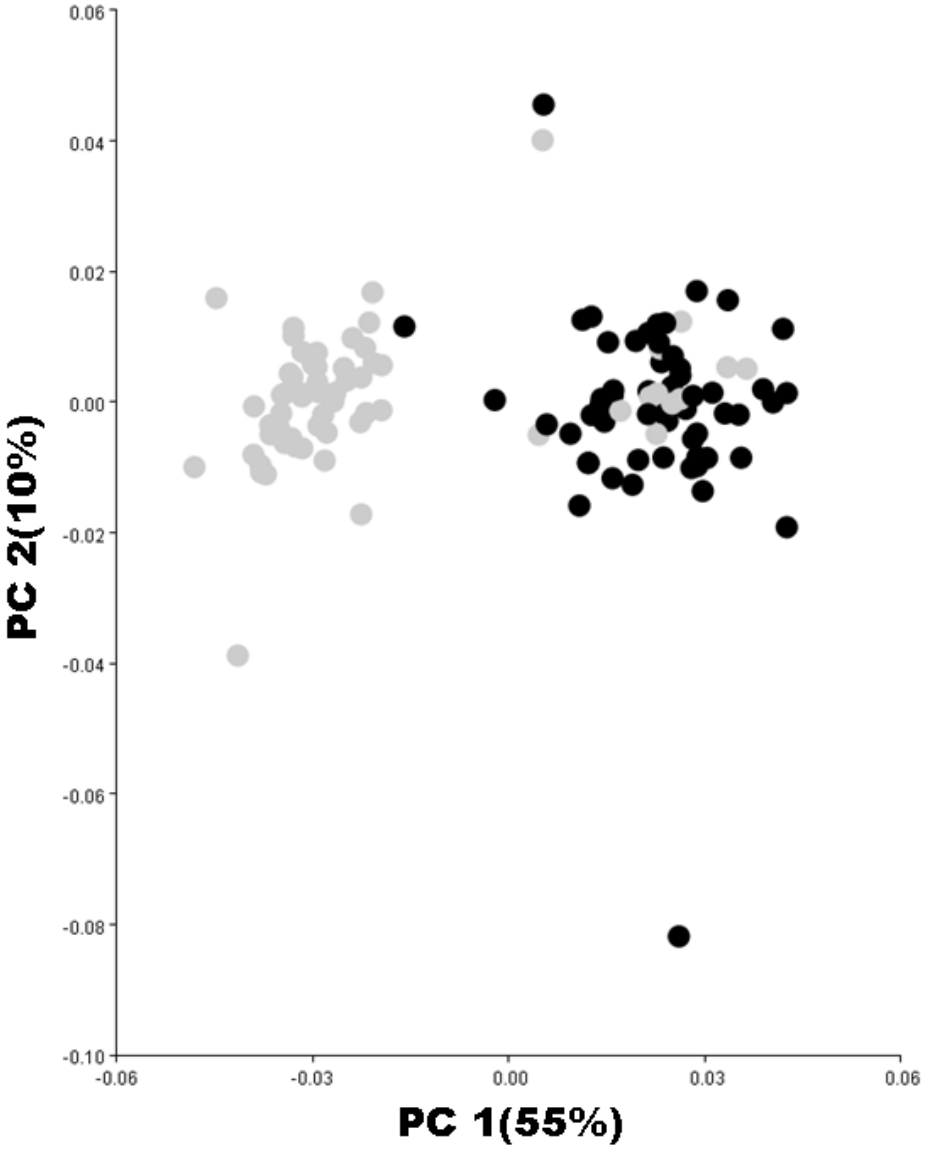

Fig. 2.7. The wing shape of female (grey markers) and male (black markers) *Ceratitis capitata* individuals released in the field. The overlap between the two groups is not due incorrect identification of the sexes, but rather due to the outliers from the female distribution falling within the normal distribution of the male distribution.

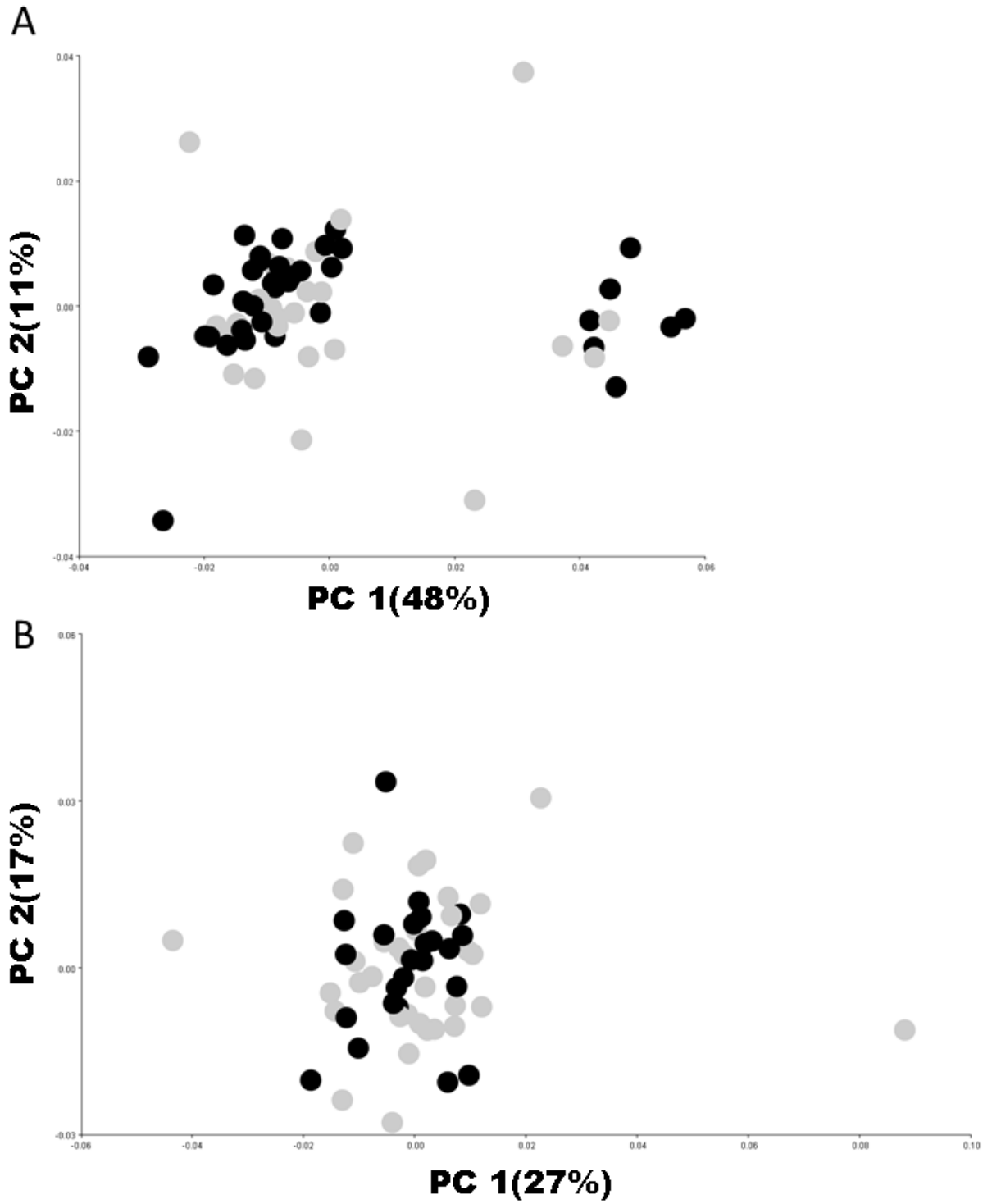

Fig. 2.8. Comparison of the wing shape between philopatric (grey markers) and dispersive (black markers) *Ceratitis capitata* within females (A) and males (B) released in the field. The overlap of the markers shows that wing shape did not differ between the two groups.

Table 2.5. Final linear model from the minimal adequate model (MAM) analysis determining the traits significantly contributing to the phenotypic differences between dispersive and philopatric individuals as determined from the field releases. The full starting model was a general linear model including abdomen mass, body mass, thorax mass, wing size, sex, acclimation treatment and their interaction on dispersal category (philopatric or disperser). Non-significant terms were excluded from the model in accordance with Crawley (2007) method for model simplification. Factors in **bold** indicate significance at $P = 0.05$.

Coefficients	Estimate	SEM	*T* value	*P*
(Intercept)	-0.427	0.509	-0.837	0.405
Abdomen mass	**0.535**	**0.239**	**2.233**	**< 0.050**
Body mass	0.162	0.212	0.764	0.446
Thorax mass	-0.267	0.441	-0.607	0.545
Abdomen mass x Thorax mass	**0.449**	**0.211**	**2.133**	**< 0.050**
Abdomen mass x Body mass	**-0.241**	**0.095**	**-2.534**	**< 0.050**

The flight parameters (Fig. 2.9) did not differ significantly between the sexes (Table 2.6). The flights success rate (Fig. 2.9B) and flight ratio (Fig. 2.C) differed significantly between dispersers and philopatric individuals (Table 2.6). The dispersers successfully achieved and maintained flight 42.7 ± 7.0% (mean ± SE) of the time whereas only 22.7 ± 7.4% of the philopatric individual's attempts to fly were successful. The peak force (Fig. 2.9D) and mean force (Fig. 2.9E) did not differ significantly between dispersers and philopatric individuals. The total, maximum and mean flight length did not differ between dispersers and philopatric individuals (Table 2.6). The maximum flight length of dispersers was 240.7 ± 79.0 s (mean ± SE) and 334.8 ± 125.3 s for philopatric individuals (Fig. 2.9G).

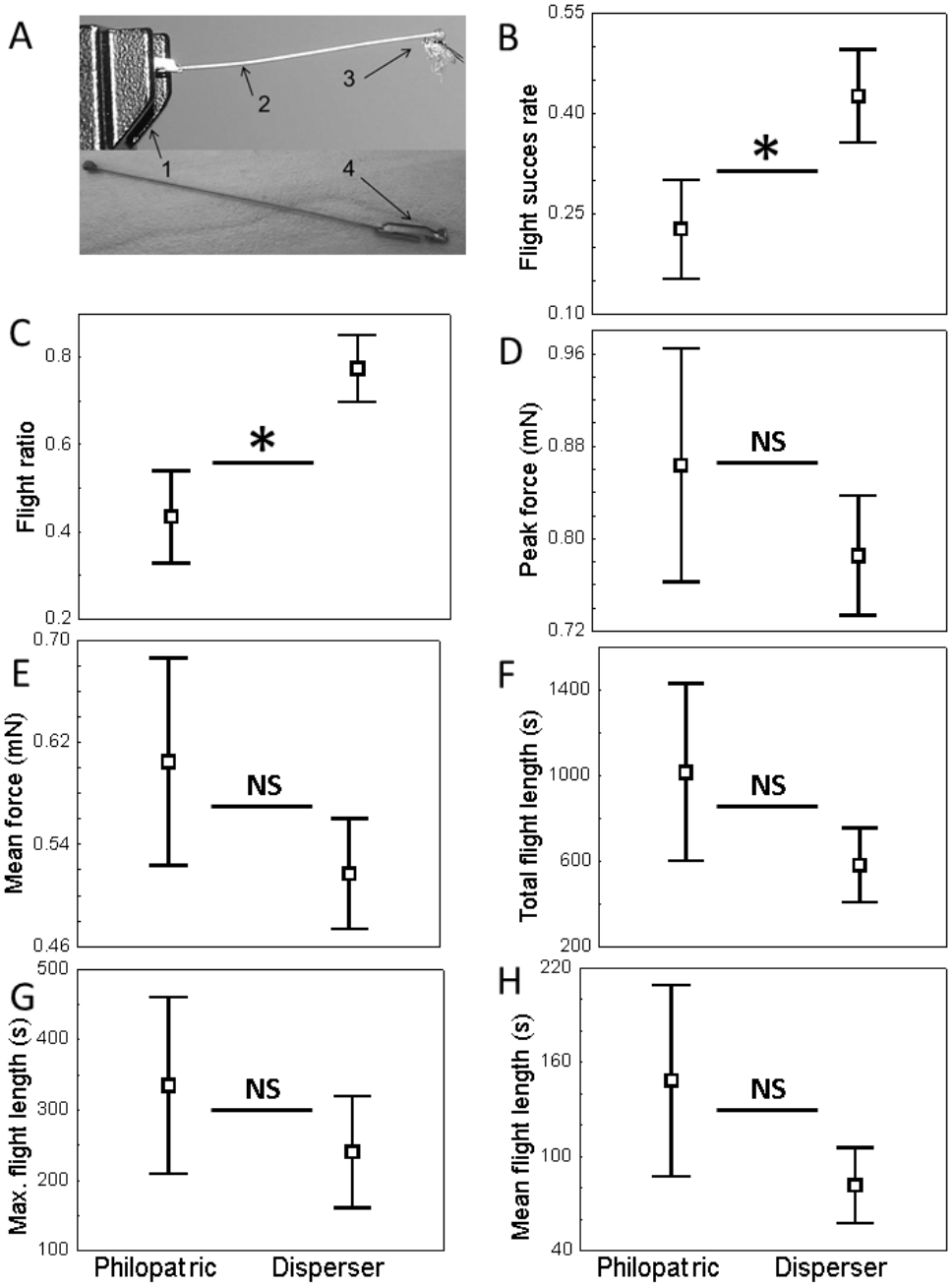

Fig. 2.9. The mean ± SE of the flight parameters measured using the force transducer compared between philopatric and dispersive individuals recorded in a temperature (25.0 ± 1.0 °C) controlled room. Panel (A) the force transducer (1), the number 2 insect pin (2), a *Ceratitis capitata* individual (3) attached to the thorax in a slightly inclined position and the custom designed mount (4). Flight success rate (B), flight ratio (C), peak vertical flight force (D), mean vertical flight force (E) total flight length (F), maximum (Max.) flight length (G) and mean flight length (H) of *Ceratitis capitata* individuals (Table 2.6 for full results).

Table 2.6. Results of generalized linear models of sex and dispersal category (i.e. disperser vs. philopatric) on various flight parameters scored using the force transducer in the laboratory for *Ceratitis capitata.* The flight success rate and flight ratio of all individuals tested and peak force (mN), mean force (mN), total flight length, maximum (max.) flight length and mean flight length of individuals that achieved flight in *Ceratitis capitata* measured in a temperature controlled room with a force transducer. Factors in **bold** indicate significance at $P = 0.05$. AIC = Akaike information criterion. SEM = standard error of the mean. (results shown below solid line are only the individuals that achieved flight).

Trait	AIC	Factor	Wald's χ^2	SEM	*T* value	*P*
Flight success rate	15.76	**Intercept**	**0.279**	**0.048**	**5.853**	**< 0.001**
		Sex	-0.054	0.075	-0.728	0.470
	11.99	**Intercept**	**0.321**	**0.047**	**6.851**	**< 0.001**
		Category	**-0.149**	**0.072**	**-2.082**	**< 0.050**
Flight ratio	74.07	**Intercept**	**0.789**	**0.381**	**2.067**	**< 0.050**
		Sex	-0.606	0.573	-1.057	0.291
	68.61	**Intercept**	**1.232**	**0.430**	**2.868**	**< 0.010**
		Category	**-1.495**	**0.601**	**-2.486**	**< 0.050**
Peak force (mN)	55.61	**Intercept**	**-0.305**	**0.110**	**-2.761**	**< 0.010**
		Sex	0.002	0.186	0.009	0.993
	55.38	**Intercept**	**-0.331**	**0.105**	**-3.141**	**< 0.010**
		Category	0.091	0.194	0.467	0.644
Mean force (mN)	64.04	**Intercept**	**-0.746**	**0.125**	**-5.977**	**< 0.001**
		Sex	0.030	0.210	0.142	0.888
	0.22	**Intercept**	**-0.770**	**0.119**	**-6.468**	**< 0.001**
		Category	0.117	0.220	0.532	0.598
Total flight length (s)	71.32	**Intercept**	**2.517**	**0.139**	**18.112**	**< 0.001**
		Sex	-0.182	0.234	-0.779	0.442
	69.68	**Intercept**	**2.348**	**0.130**	**18.076**	**< 0.001**
		Category	0.357	0.240	1.489	0.146
Max. flight length (s)	81.30	**Intercept**	**1.953**	**0.161**	**12.136**	**< 0.001**
		Sex	-0.001	0.271	-0.002	0.998
	79.58	**Intercept**	**1.848**	**0.150**	**12.300**	**< 0.001**
		Category	0.357	0.277	1.288	0.207
Mean flight length (s)	80.16	**Intercept**	**1.584**	**0.158**	**10.012**	**< 0.001**
		Sex	-0.127	0.266	-0.477	0.637
	78.17	**Intercept**	**1.422**	**0.147**	**9.664**	**< 0.001**
		Category	0.400	0.271	1.474	0.150

2.4. Discussion

The morphological or functional traits associated with dispersal are rarely identified in species with unimodal variation in phenotypes. However, the evidence from the rapidly evolving cane toad populations in Australia suggests these differences, despite of the resulting reproductive trade-off, played a strong role in the success and spread of this introduced species (Phillips *et al*. 2006). Larger/ heavier individuals may also enjoy enhanced dispersing capabilities (mole rats, Spinks, Jarvis & Bennet 2000; lizards, Cote, Clobert & Fitze 2007). Understanding the natural variation in these phenotypic traits within the geographic range of a species is clearly paramount to understanding whether necessary adaptive variation is a pre-requisite or acquired trait in invasive species (Richardson *et al*. 2014). Here, when including all morphological phenotypes previously identified for dispersive individuals in flying organisms, a heavier thorax mass:body mass ratio showed the closest association with dispersal in *C. capitata*¸ although such a relationship is difficult to disentangle completely from effects of body size variation alone. This ratio has been previously identified as indicative of dispersal variation and a reproduction-dispersal trade-off, and can vary markedly between central and range margin populations in butterflies (Hughes, Hill & Dytham 2003). In contrast to previous studies (e.g. Berwaerts, Van Dyck & Aerts 2002), the phenotypic differences between dispersers and philopatric individuals in *C. capitata* did not result in measurable differences in flight performance, but instead revealed a greater willingness to move. Moreover, the flight performance parameters measured on the force transducer under controlled conditions did not differ between philopatric and dispersive individuals for the individuals that achieved flight. This indicates that the physiology of the philopatric individuals was not compromised and that they are as capable of flight as dispersive individuals but did not fly by choice. Therefore I suggest that morphology may be

a predictor of willingness to move and therefore that the dispersal propensity of populations may differ according to their phenotypic traits.

The dispersal kernel of *C. capitata*, measured here under semi-controlled greenhouse conditions, followed a decaying exponential curve, suggesting that at a population level only a few individuals are willing to move extended distances. This is in accordance with previous studies conducted on other invertebrates (e.g. Bitume *et al.* 2013) and other populations of *C. capitata* (Meats & Smallridge 2007). The existence of similarly small proportions of dispersive individuals together with relative kernel shapes with other field studies of *C. capitata* suggests my measures likely reflect natural dispersal variation. The fact that this pattern has persisted in an outbred laboratory-reared colony of this species also suggests an evolutionary advantage exists to maintaining these traits, and that a genetic basis underlying this variation is likely. The main phenotypic difference between philopatric and dispersive individuals is associated with condition dependent behaviour (e.g. relative muscle mass and dispersal propensity); however, this is in contrast to field observations linking dispersal to context-dependent behaviour (e.g. searching for food) in introduced populations elsewhere (California; Pierre 2007). While several studies have examined the effect of landscape and environmental conditions on dispersal ability of diverse species (e.g. Van Dyck & Baguette 2005; Clobert *et al*. 2009, Vinatier *et al*. 2011), few have focused on condition dependent behaviour affecting dispersal. As the physical flight characteristics measured in this study (i.e. vertical flight forces and flight length), showed little variation in dispersing and philopatric individuals, greater consideration needs to be given to plastic responses, both condition- and context-dependent, and potential interactions among these.

Indeed, the various force parameters and flight length measured in dispersing and philopatric individuals here showed no measurable differences, which runs counter to expectations if the larger thorax mass:body mass ratio is indicative of greater power be due to larger allocation

of flight muscle. In fact, although there were no significant differences between these groups, the dispersive individuals tended to produce consistently *lower* estimates of flight force and shorter flight length compared to the philopatric individuals (Fig. 2.9D-H). This is not altogether surprising, as work conducted on *D. melanogaster* has shown that there are larger costs in overcoming drag in flight rather than in producing lift (Lehmann & Dickinson 1997). As dispersal is more likely to be achieved by several, smaller inter-patch movements as opposed to one sustained flight (e.g. Bowler & Benton 2005), the tendency for lower (albeit non-significant) flight length in dispersive individuals measured here is still quite plausible. So, although the dispersive individuals also had a larger body mass, the lift generated (peak force output) by *C. capitata* overall was independent of body mass and wing loading (Fig. 2.10). This supports previous findings across other insect orders (Marden 1987), although in my estimates, the force output measured for *C. capitata* is high overall relative to the other insect orders and falls above the 95% prediction interval (Fig. 2.10A). This relatively high vertical force production for *C. capitata* may be explained by methodological variation. By attaching flies directly to the force transducer, the force needed to obtain flight was no longer an obstacle, as the flies were already suspended in the air, and for this same reason were not subject to ground effects (known to underestimate force measurements; Dudley 2000; Dillon & Dudley 2004). In all likelihood, therefore, the force generated per body mass by *C. capitata* is not dissimilar to that of other Dipterans. However, the flight force of tested insects orders weakly (R^2 = 0.0739) correlates with their wing loading (Fig. 2.10B) in contrast to Marden (1987)'s model that predicts that individuals with relatively larger wings produce more lift for the same power output.

Behavioural characteristics associated with activity have long been known to be both heritable and adaptive (e.g. Swallow, Carter & Garland 1998). Sinclair *et al*. (2014) found

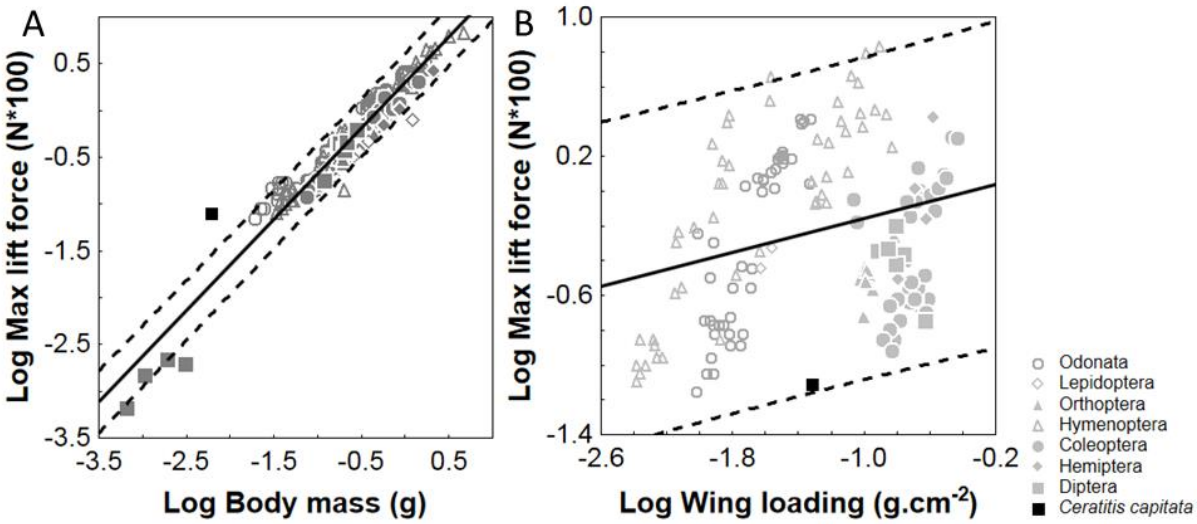

Fig. 2.10. (A) Scatter plot showing the linear relationship between maximum vertical (lift) force production against body mass for several insect orders. (B) Scatter plot showing the linear relationship between maximum vertical (lift) force against wing loading for several insect orders. Data adapted from Marden (1987), Lehmann & Dickinson (1997) and Rascón & Harrison (2005) except for the black marker for *Ceratitis capitata*, measured in this study. Solid markers represent insects with asynchronous flight muscle and open markers represent insects with synchronous flight muscles. The broken line is a 95% prediction interval and the solid line is the linear regression between maximum lift force and body mass.

that exercised fish, exposed to four weeks of chronic exercise, had better endurance compared to a control group that did not receive any exercise.This positive feedback situation in which the individual engages in a behaviour (e.g. dispersal), which in turn increases the individuals' ability to engage in that behaviour in future is termed "self-induced adaptive plasticity" (Swallow, Rhodes & Garland 2005). In this study, it is possible that an individual with a larger thorax may have exercised (flown) more, which caused more muscle growth ensuring the larger thorax and greater willingness to move. However, as all individuals were maintained under the same environmental conditions prior to testing, each had the same, if somewhat limited, ability to exercise within their mesh insect cages. That some individuals may have still possessed a greater inclination to move is still of great import. Whether this is influenced by the sex of the disperser has shown mixed results (sex-specific, Merckx & Van Dyck 2006; Berwaerts, Matthysen & Van Dyck 2008; Esterhuizen *et al*. 2014; *cf.* Breuker, Brakefield & Gibbs 2007). In this study I found clear inherent phenotypic differences (body mass, thorax mass, abdomen mass, wing size) between males and females; however, this did not translate into differences in dispersive patterns or whole animal flight performance between the sexes. Therefore the larger thorax mass to body mass ratio was equally effective in predicting dispersive individuals within each of the sexes. Whether this ratio may be evidence of a dispersal-reproduction trade-off is presently unclear. The conundrum of high fitness costs often associated with dispersal, together with the increased likelihood of these individuals founding new populations, requires further examination in order to understand the natural selection and evolution of dispersive behaviour. Another difference in resource allocation may arise from favouring cognitive development over body development, potentially changing the way in which the organism responds to stimuli (condition-dependent behaviour). For example, *Pieris rapae* experience developmental plasticity in resource allocation, such that individuals with greater cognitive power tend to allocate fewer resources

to body development (Snell-Rood & Steck 2015). Therefore, the different allocation of resources by dispersers in this study (larger thorax mass) may arise from a cognitive trade-off, influencing their willingness to explore and ultimately their dispersal ability. This has been studied in rats and mice, where willingness to exercise has been successfully been selected for (Garland & Rose 2009), with less willing individuals displaying fear or anxiety associated with the exercise, rather than being more sedentary in nature. Further work examining the candidate genes influencing voluntary flight behaviour (willingness to move) and behavioural syndromes will likely enhance understanding of movement ecology and the impacts of climate variability on this and other pterygote insect species.

Chapter 3:

Understanding costs and benefits of plastic physiological performance: insights from the integration of laboratory, semi-field and field assessments*

The contribution of the author of this thesis, Steyn, V.M., to this chapter includes:
All semi-field data collection, as well as the majority of field data collection
Performed all analysis and subsequent formatting of figs, tables and text
Wrote manuscript together with co-supervisor (Mitchell, K.A.)
Terblanche, J.S. and Mitchell, K.A. provided conception and experimental design

*Article in preparation for publication in the journal *Functional Ecology* as: Mitchell, K.A., Steyn, V.M., Nyamukondiwa, C. & Terblanche, J.S. "Understanding costs and benefits of plastic physiological performance: insights from the integration of laboratory, semi-field and field assessments"

3.1. Introduction

The ability of insect species to perform and reproduce under a variety of, often stressful, environmental conditions is paramount to their long-term survival and evolutionary success. During less extreme conditions, insects are generally able to move between microhabitats and behaviourally manage their daily activities (e.g. Barton & Terblanche 2014). However, under more extreme conditions, these species require physiological tolerance in order to survive poor abiotic conditions until the ideal environment for reproduction and foraging returns. As such, emphasis has been placed on understanding performance limits, geographic range boundaries and potential for adaptive responses. As insect species' geographic distributions and performance limits are closely linked to - and potentially defined by - their thermal tolerance (Addo-Bediako *et al*. 2000; Kimura 2004; Bozinovic *et al*. 2011), the majority of studies concerned about the impact of climate change in this group focus on obtaining accurate, field-representative estimates of heat and cold tolerance (e.g. Deutsch *et al*. 2008; Huey *et al*. 2009; Sinervo *et al.* 2010; Overgaard, Kearney & Hoffmann 2014; Castañeda, Rezende & Santos 2015; Frishkoff, Hadly & Daily 2015). Fewer studies have considered and incorporated the effects of environmentally induced trait variation, or phenotypic plasticity, on climate change impacts (e.g. Gerken *et al*. 2015), although such effects are widely considered to potentially modify or buffer climate change impacts (Charmantier *et al.* 2008; Valladares *et al*. 2014; Williams, Chick & Sinclair 2015; but see Chown *et al*. 2007; reviewed in Chown *et al*. 2010 and Hoffmann & Sgrò 2011). Plastic responses may be critical for allowing activity to continue during unfavourable conditions, i.e. despite a mismatch between thermal tolerance and the environment, and potentially provide an opportunity for adaptation of tolerance to occur over longer time scales (reviewed in Chown & Terblanche 2006; Ghalambor *et al*. 2007; Hoffmann & Sgrò 2011). Despite the apparent benefits of plastic responses, not all conditions are ideal for the evolution of plastic responses

and may result in plasticity being actively selected against (reviewed in Ghalambor *et al*. 2007).

Thermal plasticity, such as acclimation, is predicted to work optimally in variable environments when induced responses predict the future environment that the organism will experience (Levins 1969; Hoffmann 1995; Padilla & Adolph 1996; Kristensen *et al*. 2008). There are scenarios where it is likely costly to acclimate (Gilchrist & Huey 2001) that have been previously observed, mostly in field estimates (Loeschcke & Hoffmann 2007; Kristensen *et al*. 2008; Hoffmann 2010; Schou, Loeschcke & Kristensen 2015). Direct costs may be incurred through maintaining and producing plastic responses, as well as indirectly through information acquisition and genetic costs, i.e. where the genes promoting plasticity may either have a negative effect on other traits (pleiotropy), or modify other genes (epistasis; DeWitt, Sih & Wilson 1998).

There are many hypotheses for how acclimation may influence performance (reviewed in Huey & Berrigan 1996) that can be distinguished experimentally (e.g. Clusella-Trullas, Terblanche & Chown 2010). These include the beneficial acclimation hypothesis, which predicts that individuals given the opportunity to acclimate will perform better than those that were not (Leroi, Bennett & Lenski 1994; Huey & Berrigan 1996). Colder/hotter-is-better are environment-specific hypotheses that predict that acclimation to either colder or warmer conditions respectively will consistently improve performance relative to other environmental conditions, irrespective of the conditions of the target environment (Zamudio, Huey & Crill 1995; Huey & Berrigan 1996). Understanding the situations where costs associated with plastic responses mismatch the environmental conditions and of being plastic in stable environments is currently not well studied (but see Kleynhans, Clusella‐Trullas &

Terblanche 2014) and is paramount to understanding species' potential to respond to global climate change.

In order to adequately control all environmental variables, thermal tolerance and plasticity assays are generally conducted using well established laboratory methods that are later extrapolated into "real world" or field situations (e.g. Deutsch *et al*. 2008; reviewed by Terblanche *et al*. 2011). However, there are a handful of studies that have directly examined the correlation between laboratory and field conditions by including an assessment of field fitness, often examined as the recapture rate of acclimated individuals following marking and release in a resource-poor or novel natural habitat (e.g. Kristensen *et al*. 2008; Chidawanyika & Terblanche 2011) or predation/parasitism rate for biocontrol agents (*Trichogramma carverae*, Thomson, Robinson & Hoffmann 2001; *Adalia bipunctata*, Sørensen, Toft & Kristensen 2013). These have generally shown a strong association in field estimates, with individuals acclimated to the conditions most similar to those experienced during field assays typically performing better (e.g. Kristensen *et al*. 2008; Chidawanyika & Terblanche 2011; Sørensen *et al*. 2013; reviewed in Terblanche 2014). The costs of not being acclimated or of being acclimated to the wrong conditions, may not always be observed when estimated under laboratory conditions (e.g. survival assays, Kristensen *et al*. 2008), or evident in field releases under benign conditions (e.g. Thomson *et al*. 2001).

It is not possible to determine if the mismatch or absence of observed acclimation costs in studies of laboratory and field performance is evidence of a lack of correlation between these estimates or the absence of sufficiently stressful conditions in either the lab (e.g. Kristensen *et al*. 2008) or the field (Thomson *et al*. 2001). One potential method to tease out such problems and to disentangle the mismatch between laboratory and field estimates of acclimation costs is to conduct experiments in three operational environments simultaneously; field, semi-field

and the laboratory (Terblanche 2014). The specific inclusion of the semi-field environment for releases, conducted within an enclosed space but still exposed to natural environmental conditions, removes the low recapture rates that often occur in mark-release-recapture (MRR) studies due to predation and emigration (Manly 1985). It also provides a suitable alternative for examining the performance (flight activity/dispersal) of invasive species under novel environmental conditions without actively releasing them, allowing more informed predictions about their future distributions to be obtained.

In order to examine the costs and benefits of acclimation on thermal tolerance and performance in an insect pest species, laboratory assays of common thermal tolerance estimates (heat knockdown time, chill coma recovery and critical thermal limits, CTLs) were performed together with MRR under semi-field and field conditions in *Ceratitis capitata*. While other studies have used semi-field performance estimates to assay the potential costs of acclimation, this is the first study to my knowledge to take a multifaceted approach. I conducted acclimation regimes at three different thermal conditions (20, 25 and 30°C) and across different life stages in order to utilise the most effective method for comparison. I also incorporated a variety of different environmental descriptors (mean, maximum, minimum temperature and season) into the MRR analyses to determine if there are particular variables that differ between semi- and field assays or may best describe performance in this species. I predict that thermal tolerance assays will show similar patterns of benefits and costs of acclimation to field assays, whilst the semi-field MMR will provide a more simplified setting in order to quantify the costs of thermal history on performance.

3.2. Materials and methods

Ceratitis capitata (Weidemann; Diptera: Tephritidae) pupae were obtained from a stock culture maintained at Citrus Research International (CRI) in Nelspruit and allowed to complete development at 25°C on a 12:12 light: dark cycle. Adults were maintained in square Bugdorm® cages with access to food and water *ad libitum*. All pre-treatments used one of three environmental conditions, (20, 25 and 30°C). These conditions were imposed on different life stages and examined using laboratory thermal tolerance assays in order to determine the most appropriate conditions for further testing. The three protocols were; i) adult acclimation – rearing larvae under standard laboratory conditions (25°C) before placing 2 day-old adults at one of the three thermal environments for 5 days prior to testing; ii) developmental acclimation – rearing larvae at one of the three temperatures before returning adults back to the control temperature (25°C) following eclosion, and; iii) combination treatment – rearing larvae and maintaining adults under the same thermal environment until testing. 7 day-old adult flies were then used for assessing thermal tolerance. Larvae for the developmental and combination acclimation treatments were obtained as eggs from CRI on moist tissue paper before being placed onto a wheat bran based diet medium described by Barnes (1979). Newly hatched first instar larvae were then subjected to one of the three acclimation treatments, as outlined above.

CRITICAL THERMAL LIMITS

The methodology employed to assess critical thermal (CTL) limits, notably the starting temperature and rate of increase/decrease employed, has been repeatedly shown to significantly influence estimates (e.g. Terblanche *et al*. 2007; Chown *et al*. 2009; reviewed in Terblanche *et al*. 2011). As the influence of these conditions on estimates of thermal limits has already been well documented in this species (e.g. Nyamukondiwa & Terblanche 2010),

standard protocol for CTL estimates were chosen in this study. Flies were placed singly into a double jacketed chamber, or 'organ pipes' connected to a programmable, circulating fluid bath (cw410-wl, Huber, Germany) containing 50% propylene glycol: water mixture. A thermocouple attached to a digital thermometer was placed into the central chamber to monitor the real time change in temperature during each experiment. Both critical thermal maxima (CT_{max}) and critical thermal minima (CT_{min}) were started from a set point of 25°C before temperature was increased/decreased at a rate of 0.25°C/min until the physiological end point was reached. This was determined as the temperature at which the individual flies lost coordinated muscle function and could no longer respond to gently prodding using a fine plastic rod. 20 individuals of mixed sex from each temperature and acclimation type were assessed per trait. Sex was not identified for each individual as this has been shown to have little effect of CTLs in *C. capitata* (Nyamukondiwa & Terblanche 2009).

HEAT KNOCKDOWN AND CHILL COMA RECOVERY TIME

Following protocols outlined by Weldon, Terblanche & Chown (2011), flies were placed singly into vented 7ml plastic vials before being placed into each assay. For heat knockdown time (HKDT), flies were placed onto a heated thermal stage attached to a circulating fluid bath (as above). This stage is constructed of an eclosed Perspex® box seated atop an aluminium base through which a 50:50 propylene glycol: water mixture is circulated. In order to achieve a target stage temperature of approx. 43°C, the fluid bath was set at 54°C. HKDT was recorded as the time following placement on the thermal stage that each fly became incapacitated i.e. lost the ability to right itself.

Chill coma recovery time (CCRT) was determined by placing flies into a zip lock bag and submerged into a programmable fluid bath containing 100% ethanol at 0°C for one hour. The flies were then returned to 25°C and the time taken for each to regain consciousness and

mobility i.e. to stand erect, following removal was recorded. 20 individuals of mixed sex from each temperature and acclimation type were assessed per trait.

SEMI-FIELD PERFORMANCE

In order to assess field performance under controlled conditions, adult flies were released inside a 15 x 3m enclosed greenhouse located at the Stellenbosch University experimental farm at Welgevallen in Stellenbosch, South Africa. Following the results of the laboratory trials, adult acclimation treatments were employed. Briefly, a maximum of 1000 adult flies (obtained as pupae from the stock population) were acclimated for two days at the three environmental temperatures (20, 25 and 30°C; approx. 330 flies each) before being placed into plastic rearing jars, dusted with different colours of DayGlo® fluorescent powder (in order to identify each treatment) and transported to the greenhouse for release. Colours were randomised between the three acclimation treatments in order to eliminate any inherent bias that may exist with a particular colour/texture of a powder. A three-component pheromone lure (BioLure®; ammonium acetate, trimethylamine hydrochloride and 1,4-diaminobutane; Chempac Pvt. Ltd., Paarl, South Africa) that attracts both sexes of *C. capitata* (Grout *et al*. 2011) was placed at the opposite end of the greenhouse from the release point for 10 minutes before being removed to a vacuum-sealed container so as to not fill the greenhouse with excess pheromone. A perceptible gradient in pheromone could be detected when walking the length of the greenhouse. The greenhouse was divided into 5 equal sized sections and monitored continuously following the initial release until the first flies were found in the furthermost (5^{th}) section i.e. the source of the pheromone lure. Movement was monitored for a maximum of 5 hours and the number of individuals recorded in each section before all individuals within the greenhouse were manually removed to avoid any individuals carrying over to subsequent releases. Only the counts of individuals recorded in the furthest section

from the release point were used for subsequent analysis. A Thermochron iButton® (Dallas Semiconductors, Model DS1920; 0.5°C accuracy) data logger was suspended from the ceiling in each section to record ambient temperature and relative humidity throughout the trial. Care was taken to avoid releasing on wet and overcast days so as to avoid effects of moisture and light intensity.

FIELD PERFORMANCE

Four field releases were conducted, two during summer (February) and the other two in autumn (May) at Stellenbosch within the Western Cape region of South Africa. For all field releases, adult flies were acclimated for 5 days, 3 days longer than for the semi-field releases. The longer acclimation period was chosen as, although the time course for inducing an acclimation response for thermal tolerance reaches its peak following 24 hours of acclimation in *C. capitata*, the reversal time for heat tolerance in particular is somewhat proportional to the acclimation period (Weldon *et al*. 2011). Therefore, in order for the flies to remain acclimated throughout the recapture period of 5 days, the longer exposure time was necessary. Approximately 3000 flies per site were acclimated as adults, dusted with fluorescent powder (as above), transported and released from a central location at the Stellenbosch University experimental farm at Welgevallen [33°56' S, 18°52' E]. Two types of *Ceratitis* traps (bucket and delta) were baited with BioLure® (as above) and a small block of Dichlorvos DDVP was placed at the bottom of each trap to kill the attracted flies. Ambient microclimate temperature and humidity was recorded throughout the course of each release-recapture experiment using three shaded iButtons® with a 5 min sampling frequency for 5 days located on the ground, mid-tree height and the upper canopy at the central release point. Traps were hung in a rectangular pattern around a single central release point with three traps (15m apart) in each external row and two traps in the middle row (30m apart) with the single

release point in the middle (following e.g. Chidawanyika & Terblanche 2011). All traps were hung at ~1.5-2m (see Martinez-Ferrer *et al*. 2010) oriented along the rows and secured to reduce wind-related movement. Traps were checked daily for 5 days for new individuals; however as there were no new recaptures 3 days after release, only the data from the first 3 days were included in the study. Captured individuals that had no obvious or clearly identifiable coloured powder were determined as resident and not included in the analysis.

STATISTICAL ANALYSES

In order to compare between different estimates and days (particularly for the semi- and field releases), data were normalised relative to the 25°C groups which act as handling controls and aimed to eliminate potential cohort effects. Performance refers to flight activity/ dispersal measured as the number of individuals recaptured. This relative performance was calculated separately for each release day by dividing the values for the 20°C and 30°C groups by the values obtained by those acclimated at 25°C for a given day. This allowed the variation (i.e. different cohorts or possible differences in rearing or handling) between the different release days to be controlled effectively. All results are presented as relative tolerance or relative number of recaptures (unless otherwise stated) and 25°C was set as the control for comparisons in the subsequent analyses. All analyses were performed using full factorial generalized linear models with Gaussian distribution and identity link function in SAS (Enterprise guide 5.1, SAS 9.1, SAS Institute, Inc., North Carolina). The relative effect of acclimation temperature and acclimation type (adult, developmental and combined acclimations) on laboratory thermal tolerance estimates was conducted following $\log_{10}$ transformation to account for overdispersion in R (ver. 3.1.0; R Development Core Team 2014). The appropriate environmental variables to include for further analysis were determined using a principle components analysis in R using the *prcomp* function (Fig. 3.1),

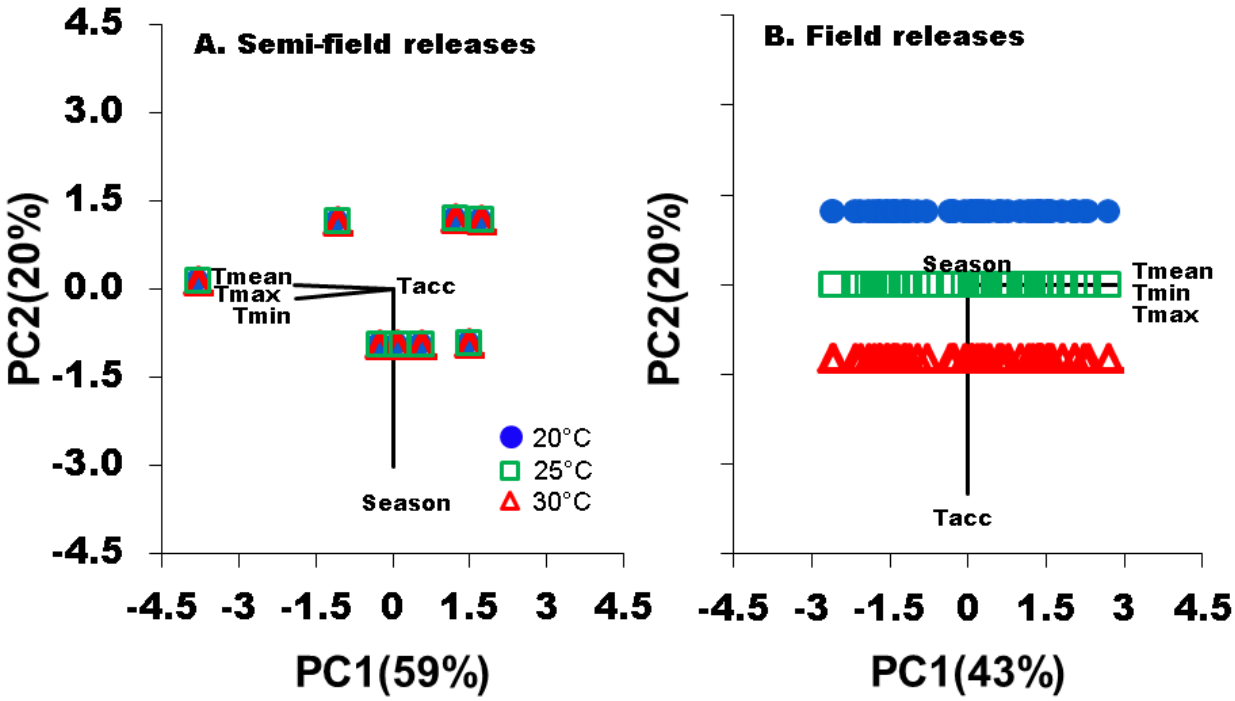

Fig. 3.1. Principle component analysis examining the interaction of environmental descriptor variables of *Ceratitis capitata* following mark-release-recapture in either semi-field (greenhouse, panel A) or field (farm, panel B). Environmental descriptors are; Tmin, Tmax and Tmean, minimum, maximum and mean recorded temperature during recapture period; Tacc, adult acclimation treatment experienced prior to release (thermal history); season, time of year (i.e. summer or winter) when the release was conducted; hours, time of day.

these analyses were done separately for the two release methods. In order to extract and graph PC scores the component axes were rotated. The effect of the identified environmental descriptor variables (Season, Tmean and Tacc) on the relative recapture rate (recapture ratio; i.e. proportion of flies caught in an acclimation group relative to the control group) was examined using a principle components analysis in R using the *prcomp* function. Due to the high number of zeros in the recapture data (most notably in the field releases) the data was transformed using $\log_{10}$ (n+1). Using generalized linear models (Gaussian distribution, identity link function) in SAS and scaling data by deviance to account for overdispersion, the effect of mean temperature (within the day/hour of recapture) and acclimation temperature on relative field and semi-field recapture numbers were examined. Models were also fitted separately for each acclimation treatment and method in order to gain a better understanding of the relationship between environmental temperatures and recapture rate. In the semi-field the data seemed to be non-linear, thus TableCurve 2D (version 5.01.02) (SYSTAT Inc, San Jose, California, USA) was used to fit multiple linear and non-linear equations. The model was chosen by in short (detailed in Chapter 2) using the Akaike weight (wi), which is the normalized likelihood that the function is the best fit compared to the other options tested.

3.3. Results

LABORATORY ASSAYS

The magnitude of the effect of acclimation temperature on CTLs was lower than for HKDT and CCRT, particularly for CTmax (Fig. 3.2A). However, in general the colder acclimation temperature resulted in a better estimate for the cold tolerance traits (CTmin and CCRT) and vice versa for heat (CTmax and HKDT). The two CTLs shared the same significant factors and interactions in the analyses, apart from a non-significant difference between the 25°C and 30°C acclimation treatments in CTmin (Table 3.1). This is likely because the increase in resistance in the 30°C acclimation groups for the developmental and combined treatments are evened out by the limited effect of the adult acclimation group (Fig. 3.2B), confirmed by the significant Adult x Development and Adult x Combination treatment effects (Table 3.1; Adult acclimation is set as the base for comparisons).

HKDT showed high levels of significance in all factors and interactions except there was no significant difference between the Adult and Developmental acclimation types or between the controls and 20°C x Combination treatment interaction, indicating the direction of the response was similar for these treatments and acclimation types (Table 3.1, Fig. 3.2C). Only the main effects were significant for CCRT, with both acclimation temperatures being significantly different from the controls and with the Developmental being significantly different from the Adult only acclimation (Table 3.1, Fig. 3.2D).

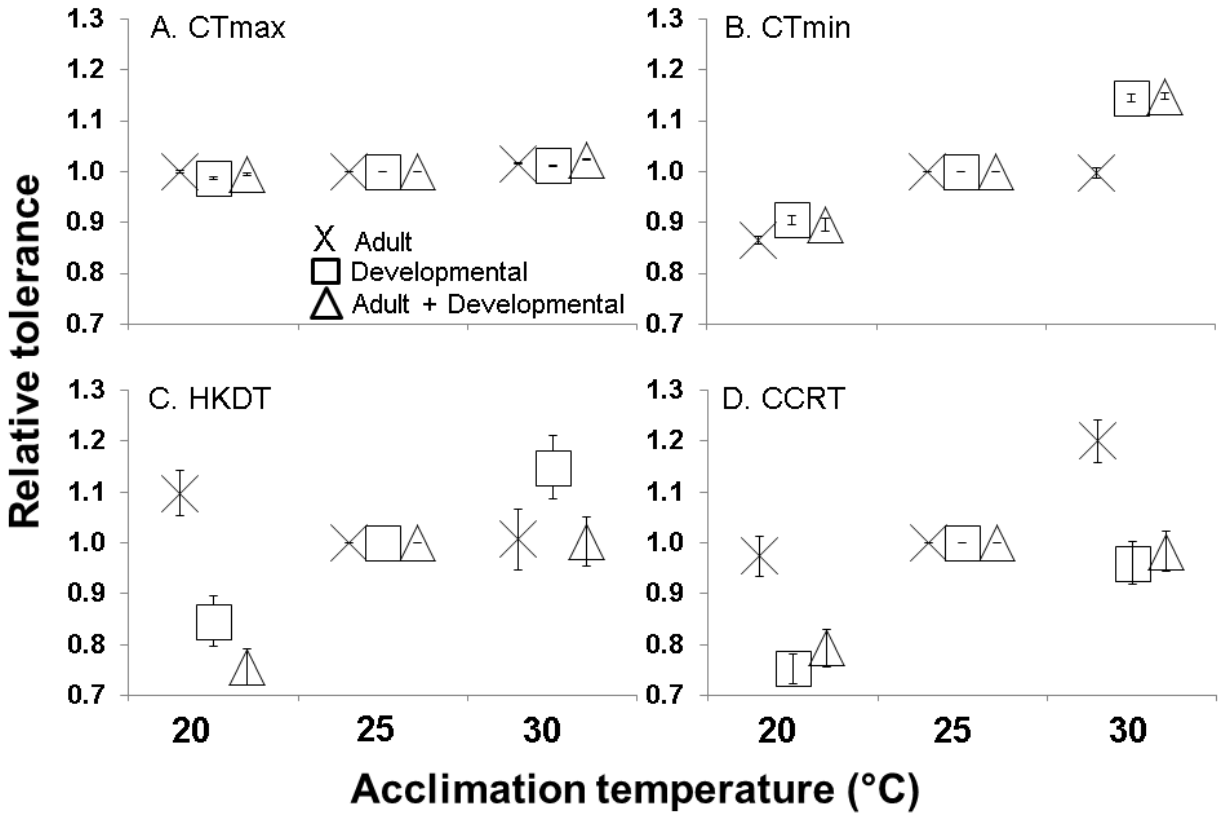

Fig. 3.2. Laboratory thermal tolerance estimates of *Ceratitis capitata* following acclimation to three environmental temperatures at either the developmental (squares), adult (crosses) or both (developmental + adult, open triangle) life-stages. Estimates for the critical thermal maxima (CTmax, A), minima (CTmin, B), heat knockdown time (HKDT, C) and chill coma recovery time (CCRT, D) are expressed relative to the control temperature group which remained at a constant 25°C for each estimate. Symbols above 1.0 indicate acclimated flies had a higher relative tolerance than non-acclimated flies for a particular treatment; symbols below 1.0 indicate lower tolerance.

Table 3.1. Generalized linear models (Gaussian distribution, identity link function) of the effect of acclimation temperature (20, 25 – control, and 30°C; Tacc), acclimation type (adult, developmental or combination) on thermal tolerance estimates, critical thermal maxima, (CTmax), minima, (CTmin), heat knockdown time (HKDT) and chill coma recovery time (CCRT) on *Ceratitis capitata*. The 25°C (control) temperature and adult acclimation treatments were set as the base level for the analysis. SEM= standard error of the mean. Factors in **bold** indicate significant effects at $p<0.05$.

Trait	Factor	Wald's $\chi 2$	SEM	*T value*	*P*
CTmax	**Intercept**	**3.727**	**0.001**	**4142.037**	**<0.001**
	20°C Tacc	**0.013**	**0.001**	**11.611**	**<0.001**
	30°C Tacc	**0.006**	**0.002**	**3.703**	**<0.001**
	Development	**0.003**	**0.001**	**2.705**	**0.008**
	Combination	**0.003**	**0.001**	**2.631**	**0.009**
	20°C Tacc x Development	**-0.005**	**0.002**	**-3.362**	**0.001**
	30°C Tacc x Development	**-0.005**	**0.002**	**-2.234**	**0.027**
	20°C Tacc x Combination	0.002	0.002	1.023	0.308
	30°C Tacc x Combination	0.002	0.002	0.921	0.358
CTmin	**Intercept**	**2.014**	**0.005**	**384.123**	**<0.001**
	20°C Tacc	**0.118**	**0.006**	**18.332**	**<0.001**
	30°C Tacc	-0.008	0.009	-0.920	0.359
	Development	**-0.052**	**0.007**	**-6.970**	**<0.000**
	Combination	**0.021**	**0.007**	**2.856**	**0.005**
	20°C Tacc x Development	**-0.046**	**0.009**	**-5.087**	**<0.001**
	30°C Tacc x Development	**-0.029**	**0.013**	**-2.252**	**0.026**
	20°C Tacc x Combination	0.008	0.009	0.859	0.392
	30°C Tacc x Combination	0.019	0.013	1.511	0.133
HKDT	**20°C Tacc**	**1.392**	**0.032**	**44.139**	**<0.001**
	30°C Tacc	**0.160**	**0.039**	**4.146**	**<0.001**
	Development	**0.108**	**0.055**	**1.971**	**0.050**
	Combination	-0.011	0.045	-0.243	0.808
	20°C Tacc x Development	**-0.153**	**0.045**	**-3.439**	**0.001**
	30°C Tacc x Development	**-0.212**	**0.055**	**-3.877**	**<0.001**
	20°C Tacc x Combination	**-0.192**	**0.077**	**-2.488**	**0.014**

	30°C Tacc x Combination	-0.020	0.055	-0.371	0.711
	Development	**-0.213**	**0.077**	**-2.763**	**0.006**
CCRT	**Intercept**	**1.512**	**0.025**	**59.615**	**<0.001**
	20°C Tacc	**0.121**	**0.031**	**3.900**	**<0.001**
	30°C Tacc	**-0.091**	**0.044**	**-2.073**	**0.040**
	Development	**0.139**	**0.036**	**3.864**	**<0.001**
	Combination	0.015	0.036	0.430	0.668
	20°C Tacc x Development	-0.015	0.044	-0.334	0.738
	30°C Tacc x Development	0.117	0.062	1.884	0.061
	20°C Tacc x Combination	-0.010	0.044	-0.220	0.826
	30°C Tacc x Combination	-0.003	0.062	-0.047	0.962

SEMI-FIELD AND FIELD ASSAYS

The influence of identified environmental variables (season, mean-, minimum-, maximum temperature and acclimation temperature), were assessed for their influence on the relative recapture rate using a principle components analysis (Fig. 3.3). There was a marked difference in the influence of the different predictors on the variance structure between the two estimates. For the semi-field releases, recapture ratio was the largest contributor to the variance on the first axis, accounting for approx. 37% of the total variance. Mean temperature (Tmean) and season (i.e. summer/winter) contributed to axis 2, explaining a further 25% of the total variance (Fig. 3.3A). For the field releases, however, the environmental variables contributed to both axes one (30%) and two (27%), with Tmean and season contributing most to axis 2 and Tacc contributing most to PC1 (axis 1) (Fig. 3.3B). Within the semi-field and field the mean temperature was an important predictor for the recapture ratio. However, in the field releases (Fig. 3.3B) the cold acclimated group is less clustered suggesting that mean temperature is less important for their recapture success.

Under semi-field conditions, there was no significant predictor of fly recapture numbers, even after the recapture number was standardised by the control (recapture ratio). Acclimation, therefore, had no effect under semi-field conditions (Table 3.2). There appeared to be a negative association between recapture ratio of both the cold (20°C) and hot (30°C) acclimation treatments and the mean temperature during the recapture period in the semi-field (Fig. 3.4B), but likely non-linear. After performing the TableCurve 2D analysis, the best-fit relationship between the recapture ratio and Tmean was identified as a decaying exponential curve (Fig. 3.4B) for 20°C (R^2 = 0.650, P = 0.016) and 30°C (R^2 = 0.630, P = 0.018) acclimation treatments.

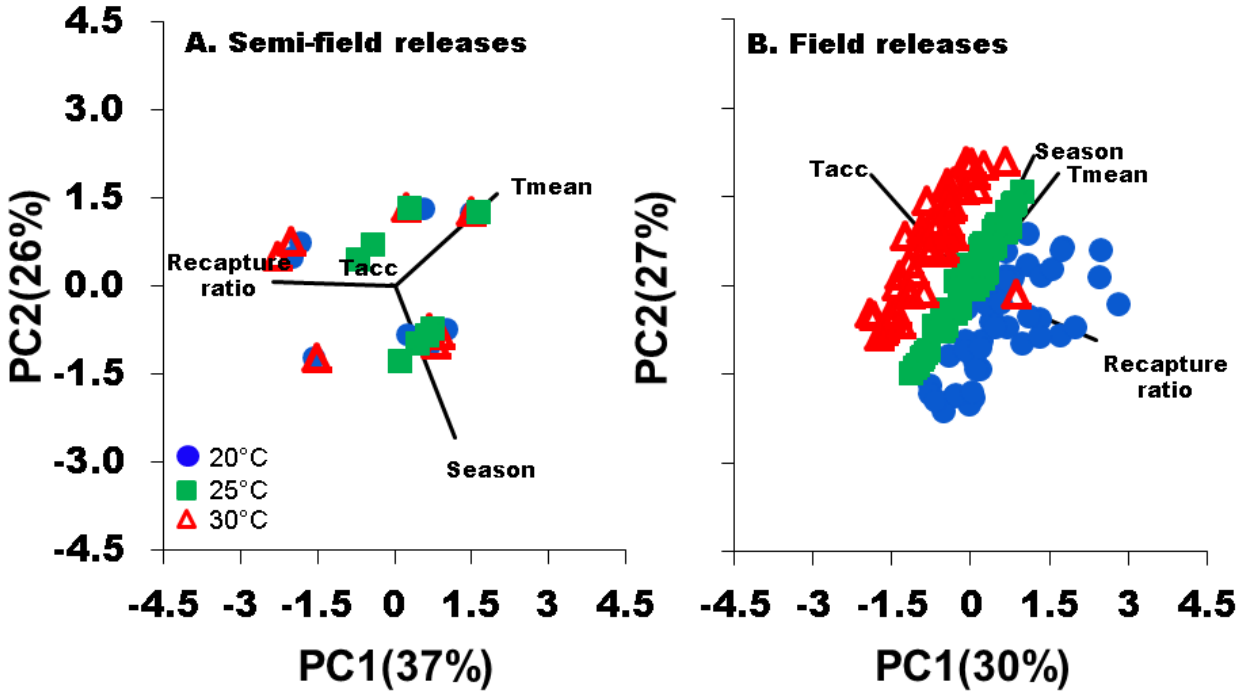

Fig. 3.3. Principle component analysis examining the effect of environmental descriptor variables on the relative recapture rate (recapture ratio) of *Ceratitis capitata* following mark-release-recapture in either semi-field (greenhouse, panel A) or field (farm, panel B). Environmental descriptors are; Tmean, mean recorded temperature during recapture period; Tacc, adult acclimation treatment experienced prior to release (thermal history); Season, time of year (i.e. summer or winter) when the release was conducted.

despite the generalized linear model analysis showing an absence of improvement in acclimation treatment relative to the control group (Table 3.3), both acclimations were recovered more frequently at temperatures below 25°C.

Under full field conditions there was a significant effect of field temperature and its interaction with acclimation (Table 3.2). When the relationship between mean temperature and recapture ratio was calculated for each acclimation treatment independently, the model fitted to the cold acclimation treatment was significantly positive (slope = 0.06), whereas the hot acclimated treatment was not significant (Table 3.3). This may indicate a cost of warm acclimation and potential benefit for cold under the measured conditions.

The overall analysis of both semi-field and field methods simultaneously found there was a significant difference between the two (Table 3.3). Thus there was no universal significant effect of the acclimation on the recapture ratio between the methods utilised.

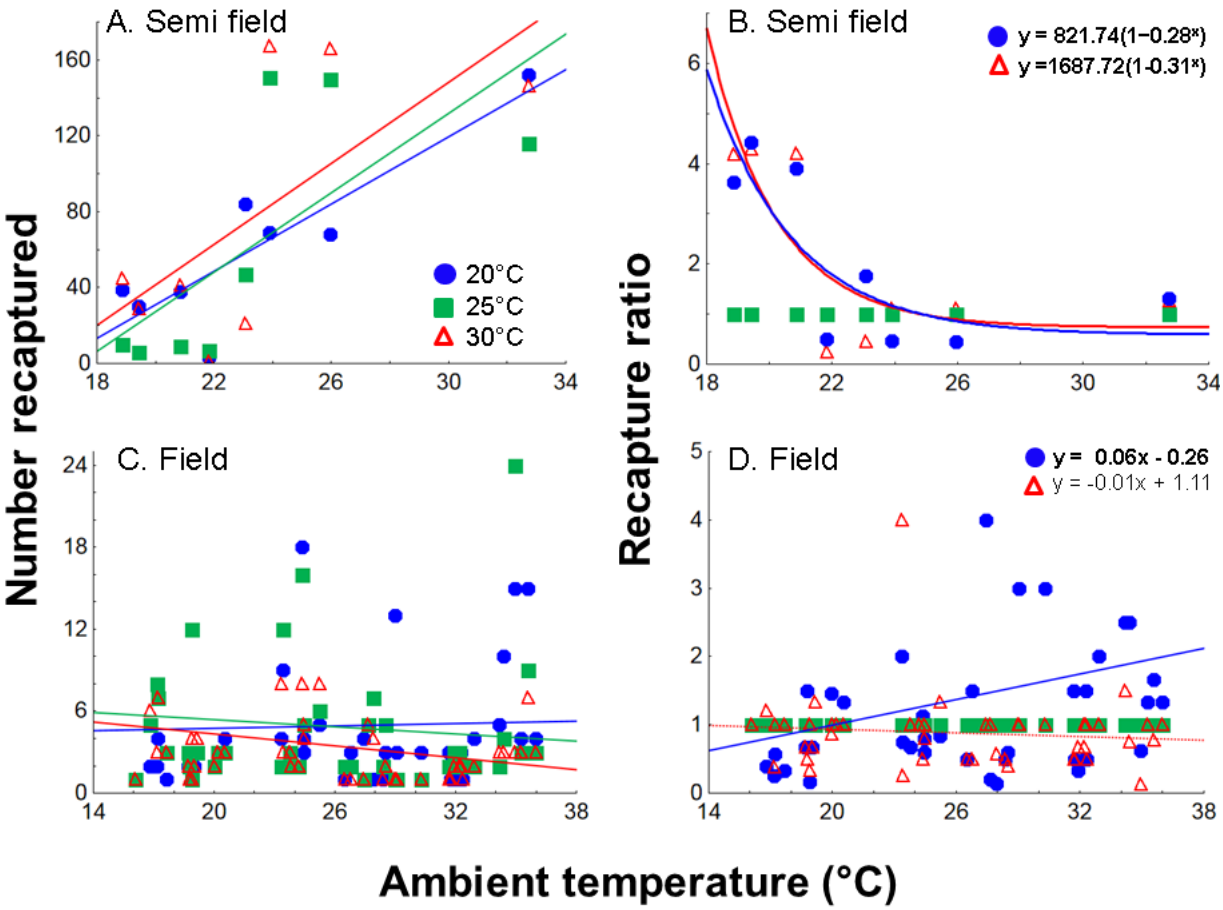

Fig. 3.4. Relationship between mean ambient temperatures recorded during recapture period and the recapture ratio of *Ceratitis capitata* following marked-release-recapture after 2- or 5-days acclimation for semi- (A, B) and field (C, D) conditions, respectively. Data were transformed log(n+1) in order to account for zero recapture, and recapture ratios determined relative to the 25°C control group. Each acclimation group were fitted separately (i.e 20 and 30°C), with solid lines indicating significant responses and dashed, non-significant. Fitted model equations for the recapture ratio treatment groups (B, D) with **bold** values are indicative of significant effects.

Table 3.2. Summary of results of generalized linear model (GLM) analyses (Gaussian distribution, identity link function) investigating the effects of acclimation temperature (20, 25, 30°C; 'Acclimation') on the absolute numbers of recaptured flies and the proportion of recaptured flies relative to the intermediate control group (25°C; recapture ratio). In all cases investigated, the effect of acclimation temperature (Tacc) and field temperature (Tmean) were the continuous variables and recapture ratios or absolute number captured as the dependent variable. The Wald's $\chi 2$ was used to test for significant differences between acclimation groups. Significant effects are highlighted in **bold** font.

Method	Effect	Estimate	SEM	Wald's $\chi 2$	*P*
Semi-field	Number recaptured				
(greenhouse)	Tacc	-0.024	0.137	0.030	0.861
	Tmean	0.051	0.146	0.120	0.725
	Tacc x Tmean	0.001	0.006	0.030	0.864
	Recapture ratio				
	Tacc	-0.024	0.103	0.050	0.817
	Tmean	-0.051	0.110	0.210	0.646
	Tacc x Tmean	0.001	0.004	0.050	0.820
Field	Number recaptured				
	Tacc	0.048	0.032	2.220	0.136
	Tmean	0.051	0.031	2.810	0.094
	Tacc x Tmean	-0.002	0.001	3.120	0.080
	Recapture ratio				
	Tacc	**0.048**	**0.022**	**4.670**	**0.031**
	Tmean	**0.058**	**0.021**	**7.390**	**0.007**
	Tacc x Tmean	**-0.002**	**0.001**	**6.550**	**0.011**

Table 3.3. Summary results of generalized linear models (Gaussian distribution, identity link function) of the effect of mean temperature recorded during recapture period (Tmean) and acclimation temperature (20, 25 – control, and 30°C; Tacc) on recapture ratio of *Ceratitis capitata* following release under semi-field and field conditions. The 25°C (control) temperature was set as the base level for the analysis. Data from the semi-field and field were analysed separately before being compared in the 'comparison' analysis. Factors in **bold** indicate significant effects at p = 0.05. AIC = Akaike information criterion.

Trait	AIC	Factor	Estimate	SEM	Wald's $\chi 2$	*P*
Semi-field	25.564	Intercept	0.000	0.730	0.000	1.000
(greenhouse)		20°C Tacc	1.169	1.032	1.280	0.258
		30°C Tacc	0.930	1.032	0.81	0.368
		Tmean	-0.000	0.031	0.000	1.000
		20°C x Tmean	-0.044	0.044	1.010	0.315
		30°C x Tmean	-0.034	0.044	0.600	0.437
Field	9.326	Intercept	-0.000	0.156	0.000	1.000
		20°C Tacc	**-0.476**	**0.221**	**4.640**	**0.031**
		30°C Tacc	0.005	0.221	0.000	0.983
		Tmean	0.000	0.006	0.000	1.000
		20°C x Tmean	**0.017**	**0.008**	**4.110**	**0.043**
		30°C x Tmean	-0.005	0.008	0.300	0.583
Comparison	42.914	Intercept	0.083	0.104	0.630	0.428
		20°C Tacc	-0.013	0.051	0.070	0.797
		30°C Tacc	-0.077	0.051	2.31	0.129
		Tmean	0.002	0.004	0.270	0.604
		Method	**-0.152**	**0.060**	**6.410**	**0.011**

3.4. Discussion

The importance of linking the environmental variation of performance estimates made in the laboratory to responses seen in the field, particularly in light of global environmental change cannot be understated. Many studies have made strong assertions or directly implied that performance and tolerance estimates measured in the laboratory are adaptive and would provide an advantage under field conditions, yet very few have actively tested and reported this (but see Kristensen *et al*. 2008; Chidawanyika & Terblanche 2011; Sørensen *et al*. 2013). Few studies have examined the costs and benefits of plastic responses and those that have typically found results to be equivocal (e.g. costs, Kristensen *et al*. 2008 *cf* no costs, Thomson *et al.* 2001). Whilst gaining an understanding of the costs of acclimation using natural systems would be optimal, this is not always feasible; however, the effect that using laboratory estimates has on our overall conclusions remains to be understood.

In accordance with the results of the laboratory assays, this study detected significant effects of acclimation and release temperature on MRR estimates under field conditions. There were very little similar trends between the field and semi-field releases; however, the conditions were far less controlled in the field (as one would expect), with many other factors interacting to increase the variance and environmental influence on the recapture rates. This reduced variance in the semi-field proved to provide a valuable null model against which the field data could be compared and highlighted that there is a lot of nuance in environmental effects on performance estimates that is lost when associations are made using only one variable. This can be particularly seen from the contribution of the many different environmental descriptors assessed during the field MMR assays in the principle components analysis (Figs 3.1 and 3.3). In contrast to the field, the semi-field assays showed very little but similar effects of all environmental variables measured, and this was reflected in the absence of

discernible costs of acclimation below 25ºC. Despite both temperature treatments performing similarly in the semi-field releases, these effects were substantially altered in the field estimates, with an absence of a significant difference in the warm-acclimated group and shift to positive association of recapture ratio with temperature in the cold-acclimated group. This highlights that acclamatory costs and benefits may only be realised under specific conditions and that fundamental impact of temperature on performance should always be assessed under stressful temperatures, even when other environmental variables are controlled. These costs may be induced by increased metabolic rate requiring higher resource use for flight (Rascón & Harrison 2005). Considering that a failure to recapture acclimated flies relative to controls occurred at temperatures below 20ºC under field conditions (Figure 3.4C), what may be considered 'stressful conditions' is remarkably relative. This indicates that the semi-field (null model) experiments may be useful in detecting the importance of the different responses seen in the field.

Under semi-field conditions, both acclimation treatments were recaptured more often than the 25ºC control flies up until the release temperature reached approximately 25ºC. After this point, there were little discernible differences between acclimation groups, indicating that there are two acclimation patterns at work under semi-field conditions. Below 25ºC, a more broad beneficial acclimation effect is seen (i.e. individuals given the chance to acclimate perform better than those that were not; Leroi *et al*. 1994), whilst above 25ºC, the optimal acclimation hypothesis (OAH; Clusella-Trullas *et al*. 2010) is apparent, with 25ºC flies being recaptured as frequently, if not more so, than the acclimated flies, resulting in an apparent 'reduction' in recapture rate of acclimated flies. This would indicate that there is a threshold for acclimation benefits in this species, with no discernible costs to acclimation below 25ºC but marked costs above. The cost of acclimation at temperatures above 25°C was not observed for the 20°C acclimation treatment in the field assays, ensuring better performance

overall for this group (relative to the control and 30°C treatment), especially under warmer conditions. This pattern, whilst odd in comparison to the semi-field results, can also be observed in the laboratory estimates. For example, in the HKDT assays, the 20°C adult acclimation group had better heat tolerance than all other acclimation groups except the developmental 30°C acclimation, indicating some cross-tolerance effects may be occurring under warmer temperatures. It is possible that the prior exposure to a different stressor, such as cold for short time periods at the adult life stage, may be inducing the expression of heat shock proteins (HSPs), as has been found in cross-tolerance studies in other species (e.g. *Belgica antarctica*, Benoit *et al*. 2009); however, it was outside the scope of this thesis to examine this hypothesis. If HSPs, which are known to improve heat tolerance in almost all organisms examined (reviewed in Feder & Hofmann 1999), were involved in this instance, then the effect should have been more obvious in the warm-acclimated group. This is somewhat unexpected and should be investigated in more detail in this particular species.

The overall response of recapture rate to temperature was different for both acclimations and release method, i.e. negative, non-linear correlation with Tmean for semi-field but positive for cold-acclimated flies in field MRR. This indicates that, while beneficial acclimation (supported by Ferrer, Mazzi & Dorn 2014) may be the best-fitting hypothesis for performance in *C. capitata* during laboratory and semi-field estimates (when there are no costs), under natural conditions colder acclimated flies generally outperformed. Based on this information, the "colder is better" hypothesis seems the best fit under field conditions, though different to the laboratory and semi-field findings. This suggestion of colder-is-better is in keeping with previous thermal work conducted on laboratory performance estimates in this species (flight performance under varying thermal conditions; Esterhuizen *et al*. 2014), indicating that the laboratory thermal tolerance estimates heavily relied upon for our understanding of performance limits and acclimation costs in most species may be quite

removed from 'real world' situations, as previous studies have also suggested (e.g. Kristensen *et al*. 2008). However, some similarities do still persist between the laboratory and field estimates (e.g. the potential cross-tolerance effect of cold acclimation during warm temperatures). More effort to undertake the field and semi-field MRR assays need to be made in other species to further understand the source of these differences.

There are several key methodological differences that need to be considered when interpreting the results obtained from the semi-field and field MRR assays regarding the cost of acclimation. Firstly, it was not possible to conduct as many semi-field releases under warmer (+27°C) conditions as were assessed for the field releases. This is due to a very simple reason; there were few very warm (+27°C) days that fell during the semi-field testing period. The ideal design would have allocated an equal number of the warmer release days to the semi-field releases; however, due to the difference in acclimation time between the two MRR assays, it was not possible to change the release method of the flies. Perhaps using a more standardised acclimation regime length may overcome this effect in the future.

Another key factor relating to the acclimation length differences is the potential additional stress imposed by the three extra days exposure for the field released flies, particularly in the 30°C acclimation group. Although it is possible that the observed change in response to temperature between the semi-field and field releases may be due to the more varied environmental conditions the flies were exposed to under natural conditions, it is equally likely that the greater stress of the longer acclimation significantly altered the fitness of these flies. It is not possible to differentiate between these possibilities using the current dataset but future comparisons of this kind will need to consider these factors in the experimental design. Using only adult acclimation for the releases may have impacted the results and changing the type of acclimation may provide different results.

Despite these caveats, this study was able to find a beneficial effect of adult acclimation on performance under all three methods and this is likely due to the use of the recapture ratio, where data were standardized by the control group (25°C), allowing relative comparisons to be made. Further studies of the potential costs/benefits of acclimation would be necessary using the multifaceted approach highlighted in this study in order to better understand the costs and benefits of acclimation in natural conditions. Obtaining accurate estimates of phenotypic plasticity and its associated costs is vital to improving our prediction of species' responses to global climate change. Thermal tolerance traits estimated in the laboratory setting can provide an accurate estimate of field costs to acclimation; however, the severity and range of thermal environments the organisms are exposed to during field assays plays a significant role in the outcome.

Chapter 4:

General discussion

Understanding why organisms persist in certain habitats but not others is the first step to comprehending present, past and future species distributions, of particular importance under future global change (Hoffmann & Sgrò 2011). There are several key traits that may assist a species to continue to perform under unfavourable conditions and potentially to survive future variable and warming conditions. These include a wide performance breadth, enhanced dispersal capabilities and plastic responses, as highlighted in Chapter 1 (Clobert *et al.* 2009; Savolainen, Lascoux & Merilä 2013; Gerken *et al*. 2015). These traits are generally found in abundance in invasive species, likely contributing to their success in novel environments and potentially also under changing climates (Sakai *et al*. 2001; South & Kenward 2001).

Dispersal ability has a two-fold impact during adaptation in natural populations, facilitating gene flow and providing more genetic variation for selection to act on but at the same time potentially 'swamping' locally adaptive alleles, disrupting the process (Lenormand 2002; Bridle & Vines 2007; Ronce 2007). Understanding the factors that influence whether an individual will disperse and successfully propagate a new population is vital for our understanding of adaptation to changing climates (Clobert *et al*. 2009). It is also vital for predicting the invasion potential of pest species as dispersal ability is the key factor for both the first and the last step of the invasion process (arrival and establishment; Bowler & Benton 2005). In general, measurements of dispersal potential made using laboratory methodologies fail to simultaneously account for the behavioural (willingness) and performance (endurance) elements of the dispersal trait. By combining laboratory estimates with some measure of field fitness, either in the more stable, greenhouse environment or directly in the natural habitat, a broad understanding of the costs and benefits of particular phenotypes and thermal history on flight performance can be gained.

To examine this in the global invader *Ceratitis capitata*, individual performance was tested by conducting laboratory (thermal limits and flight force), semi-field and field (measuring dispersal) experiments under various environmental conditions. This allowed the discovery of the phenotypic driver for dispersal (Chapter 2) and highlighted the best acclimation conditions and associated costs (Chapter 3) for enhanced performance in *C. capitata*. These results suggest that performance of *Ceratitis capitata* is both condition (phenotype and behaviour) and context (environment) dependent.

4.1. Phenotypic traits of dispersive individuals

The variation in flight ability between *Ceratitis capitata* individuals from the same population was successfully explained by morphological differences. Though various traits differed between philopatric and dispersive individuals (Fig. 2.5; Chapter 2), I identified thorax mass relative to overall body mass to be a reliable predictor of dispersal ability for both sexes. Contrary to my expectations, wing size and shape did not affect dispersive patterns in a predictable manner, suggesting that differences in wing structure between individuals of the same species may be of little consequence. The observed phenotypic differences between philopatric and dispersive individuals were not associated with measurable differences in whole-animal flight performance (e.g. mean and peak vertical force, total, mean or maximum flight length), contrary to expectation (see Chapter 2). Rather, performance differences in dispersers were characterised by their willingness to disperse (dispersal propensity). Therefore voluntary behaviour may give rise to dispersal differences that are not associated with large physiological differences (here shown in non-polymorphic dispersal systems), but that nevertheless result in significant alterations of movement behaviour and realized dispersal in the field.

The further examination of flight force production of *C. capitata* relative to orders previously measured indicated that this species was capable of producing slightly higher force than other Dipterans (Fig. 2.9A). However, when vertical force (max. lift produced) was examined relative to the wing loading, distinct clusters form for each of the flight muscle types (synchronous and asynchronous) (Chapter 2, Fig. 2.9B). This suggests that flight muscle type may determine lift generated, with taxa with asynchronous flight muscle able to become airborne with lower power output for the same wing loading. This finding influences the importance of vertical force in the calculation of dispersal distances as it may differ according to the flight muscle type present in the insect family. *Ceratitis capitata* makes use of asynchronous flight muscle, therefore the applicability of these results to other insect taxa without asynchronous muscles is not recommended.

The force transducer proved a reliable and precise tool for measuring flight forces, and provided a platform in which other flight parameters could be measured (see Chapter 2). The accuracy of the instrument was successful in measuring small (<0.1mN) differences in flight force between individuals of the same population (Fig. 2.8). In addition, it proved to be a relatively inexpensive method in both time (as performance could be scored directly) and resources (no costs other than equipment). Therefore I suggest that the force transducer be used as a new methodology for assessing dispersal potential and to provide basal information of flight capabilities for different populations and species of even the smallest winged insects.

4.2. Costs and benefits of plastic responses in dispersal traits

External stimuli may impact performance of individuals (summarised in Table 1.1), with their ability to perform under challenging environmental conditions being paramount to their survival, population growth and evolutionary fitness (Bozinovic *et al*. 2011; Sinclair *et al*. 2012; Richardson *et al*. 2014). Phenotypic plasticity is likely to influence survival by

providing a rapid tolerance response in variable environments (Charmantier *et al.* 2008; Hoffmann & Sgrò 2011). By integrating three operational environments (laboratory, semi-field and field) in this study I illustrated that the performance of *C. capitata* is influenced by thermal conditions (See Figs 3.2 and 3.4; Chapter 3). Furthermore, successful recaptures (See Fig. 3.4D) at high ambient temperatures (>25°C) under field conditions showed that they are still able to perform under these conditions; however, this was most pronounced in flies pre-acclimated to cold conditions (20°C). By pre-exposure to various thermal conditions (adult acclimation) I was able to examine the extent to which their phenotypic plasticity aided their performance under different environmental conditions (see Chapter 3.4). Overall cold acclimation proved most successful in enhancing performance under the various experimental environments, particularly under warm conditions, indicating a cross-tolerance effect in this response. As thermal environments warm, the absence of phenotypic plasticity following pre-exposure to warm conditions, together with an apparent tolerance threshold above 25°C under controlled conditions (semi-field results, Fig. 3.4B), may impede their response to climate change using phenotypic plasticity (Gerken *et al*. 2015). This may inhibit *C. capitata* from inhabiting warmer climates; however, significant thermal fluctuations and exposure to colder environments may still enable this response. As such, this species may still become a more prolific invader in future. Further work focusing on basal and plastic responses across all life stages is required to understand the full consequences under changing climates.

4.3. Future directions

The dispersal information acquired from this study may enhance the efficiency of the control practices currently employed to manage this pest species. One control method currently being implemented that may derive benefit from this study is the Sterile Insect Technique (SIT). This is a bio-control strategy in which laboratory reared males are sterilised and then mass

released into the natural population to mate with natural females in an attempt to reduce reproductive output of that population (Dyck, Hendrichs & Robinson 2005). Currently in the Western Cape of South Africa, the facilities producing these sterile males have been very successful in rearing large quantities, with Fruit Fly Africa producing on average 15 million *Ceratitis capitata* every week (Barnes & Venter 2006). Despite the large amount of sterile males being released, *C. capitata* is still a major threat to fruit producers in the region (de Villiers *et al.* 2013), indicating the effectiveness of this technique could be improved and made more economically sustainable at controlling this species in the long term. The quality (i.e. ability to compete with natural males for mates) of the sterile males being released is likely to be more important than the quantity produced. Recent studies have focused on how to make SIT more efficient through different release strategies (Gavriel *et al*. 2012) and quality control before release (Liedo *et al.* 2005); however, few have looked at how to improve the quality of the actual sterile males after they are released (see Calkins & Parker 2005). Strong flight is an essential trait for the sterile males to possess for the SIT program to be successful as it allows the insect to more effectively search for shelter, food and, importantly, mates (Calkins & Parker 2005).

My study identified various intrinsic and extrinsic traits that may enhance dispersal or flight ability in *C. capitata*. For instance individuals that had a relatively larger thorax mass were more prone to disperse, and cold acclimated flies may benefit from enhanced post-release performance. However, as this study was conducted on normal flies (unsterilized) and radiation exposure may cause decreased fitness, I suggest that these questions be re-examined using sterile flies to ensure the robustness of these findings. If the results (larger relative thorax = disperser) do hold true for sterile flies, then more competitive sterile males can be selectively bred, leading to more efficient control of this and other agricultural pests.

The thorax: body ratio is linked to willingness to disperse and it has persisted in an outbred laboratory-reared colony of this species. This suggests an evolutionary advantage exists to maintaining these traits long after selection has been removed and that there may be a genetic basis underlying this variation. Should this be the case, it may provide a genetic test for quantifying or predicting the invasion risk present in a particular population to then monitor over time. One method to examine this would be to conduct a quantitative genetic study in which several replicate lines are selected to increase the number of dispersers (large thorax: body ratio), and/or sedentary individuals (small thorax: body ratio) relative to an unselected control line. The dispersive capabilities (flight force, duration and willingness) can then be compared between the different lines and within different generations using the MRR methods outlined in this thesis. This will allow one to determine whether the selection enhances or has no effect on dispersal ability and subsequent genetic crosses could be used to determine the genetic architecture underlying dispersal behaviour in *Ceratitis capitata*.

4.4. Concluding remarks

A challenge for invasion biology is the development of a predictive understanding of species invasion ability. Determining the species dispersal ability and the factors that influence it is an integral part of the problem. From this study, valuable knowledge on the dispersal ability of *C. capitata* has been gained and, (in line with my predictions), showed that dispersal in *C. capitata* is condition dependent (e.g. phenotypic traits and behaviour, see Chapter 2) as well as context dependent (e.g. thermal history and environmental temperature, see Chapter 3). This may benefit predictions of the future invasion risk of *C. capitata* that could potentially improve current management strategies (i.e. SIT), should similar phenotypic markers of dispersal exist in irradiated flies.

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Appendix 1

What characteristics describe a dispersal prone fruit fly?

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Introduction

- Dispersal is the movement of individuals from a natal patch to a novel environment[1]
- Dispersal ability is critical for colonization and invasion, yet only a small number of individuals disperse a significant distance from their natal patch[2]
- Polymorphic species may have long- and short-winged forms, that trade-off dispersal ability for reproductive potential[3]
- However, in species without distinct morphs, it is unclear what traits characterize dispersive vs philopatric (sedentary) individuals

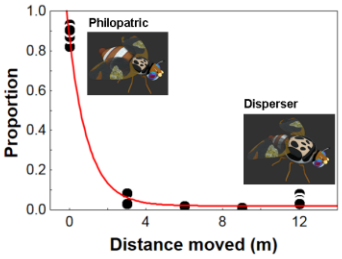
Aims

- **Determine the phenotypic traits that influence dispersal ability in *Ceratitis capitata***
- **Determine differences in flight performance between philopatric and dispersive individuals**

Materials and methods

- The phenotypic traits that enhance or are associated with dispersal ability were determined by comparing philopatric and dispersive individuals (Figs 1 & 2)

Fig. 1. The proportion of *Ceratitis capitata* individuals from four releases that moved from the release point (0m) to a pheromone attractant (12m).



- Force transducer used to measure flight parameters (e.g. force, length and success rate) of the two morphologically distinct groups (Fig. 3)

Results

Fig. 2. Phenotypic traits estimated in philopatric and disperser flies.

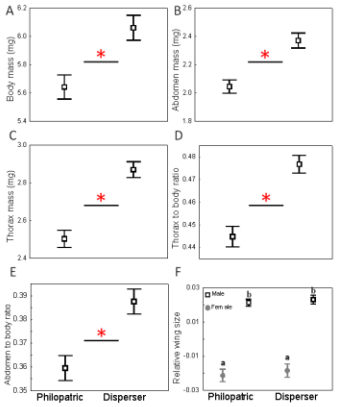


Table 2. Repeatability of flight parameters.

Trait measured	Source of variation	d.f.	F-value	P	Repeatability (r, %)
Peak force (mN)	**Among**	**33**	**4.09**	**<0.001**	**34.1**
	Within	7			
Mean force (mN)	Among	33	1.44	0.072	6.9
	Within	7			
Total flights (N)	**Among**	**33**	**9.43**	**<0.001**	**58.5**
	Within	7			
Total flight length (s)	**Among**	**33**	**7.17**	**<0.001**	**50.8**
	Within	7			
Max. flight length (s)	**Among**	**33**	**9.64**	**<0.001**	**59.1**
	Within	7			

Fig. 3. Flight parameters measured using the force transducer in philopatric and disperser flies.

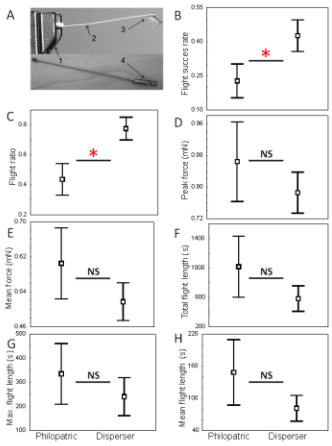


Table 1. Summary results of the Minimal Adequate Model used to identify key traits associated with dispersal ability.

Coefficients	Estimate	SEM	T value	P
(Intercept)	-0.427	0.509	-0.837	0.405
Abdomen mass	**0.535**	**0.239**	**2.233**	**< 0.05**
Body mass	0.182	0.212	0.764	0.446
Thorax mass	-0.267	0.441	-0.607	0.545
Abdomen mass x Thorax mass	**0.449**	**0.211**	**2.133**	**< 0.05**
Abdomen mass x Body mass	**-0.241**	**0.096**	**-2.534**	**< 0.05**

CONCLUSIONS

- There are morphological differences between philopatric and dispersive individuals
- However, whole-animal flight performance did not differ between philopatric and dispersive individuals
- Philopatric individuals physically capable of flight, but choose not to
- **Therefore flight propensity (willingness) separates philopatric and dispersive individuals**

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