THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Selecting for invasive tendencies: the evolution of morphological, physiological, and movement behaviour traits associated with dispersal

Pieter Andrew Arnold
Bachelor of Science (Honours Class 1)

A thesis submitted for the degree of Doctor of Philosophy at
The University of Queensland in 2016
School of Biological Sciences

Abstract

Dispersal, defined as any movement of an individual over various spatial scales that may contribute to gene flow, is an essential component of species ecology. It provides a mechanism that allows organisms to track optimal environmental conditions, regulate population density and interactions with conspecifics, and colonise new areas. Dispersal ability varies widely among individuals, and this variation has been strongly linked to a suite of physiological, morphological, and behavioural traits that together constitute a dispersal syndrome. The evolution of dispersal-related traits can occur not only by natural selection, but also by spatial sorting, where individuals that have traits facilitating their dispersal accumulate at range edges and are limited proximally to mating with other dispersive individuals. The relationships among traits constituting the dispersal syndrome, their dynamics with age, and evolution under spatial selection on dispersal have not been comprehensively explored. Furthermore, integrating evolution into the study of dispersal is imperative to understand the mechanisms that select or constrain the evolution of dispersal-related traits. The overall aim of this thesis was to investigate a suite of physiological, morphological, and movement behaviour traits associated with the dispersal syndrome using a model system: laboratory dispersal apparatuses and *Tribolium castaneum* (red flour beetle).

The research presented in this thesis focused on the following traits: body size, locomotor apparatus size, metabolic rate, spontaneous activity, and movement behaviour in a maze (including speed, path length, displacement distance, and behavioural intermittence). The first specific aim was to determine how the onset of sexual maturity and age throughout early life affects dispersal-related traits (Chapter 2). I found that prior to sexual maturity, *T. castaneum* have low metabolic rate and moved significantly less than mature ones. The low energy expenditure was attributable to reduced energy demand and inactivity, which was hypothesised to be a protective mechanism while the cuticle is undergoing sclerotisation.

The second specific aim was to determine the relationships among metabolic rate, body size, relative leg length and different movement behaviour traits (Chapter 3). A dominant axis of movement ability described variation in several movement traits and was positively related to relative leg length, but unrelated to body size or metabolic rate. A mechanistic relationship between stride length and movement ability is therefore likely. The data suggested that the dispersal syndrome may be more strongly tied to morphology rather than physiology.

The third specific aim was to investigate the dispersal rate of *T. castaneum* through three-patch dispersal apparatuses to determine which design would be most effective for artificial selection on the basis of dispersal success (Chapter 4). The distance and slope of the tubing that connected between patches significantly affected dispersal rate, therefore the apparatus design that yielded the most consistent dispersal rate was chosen for subsequent experiments.

The fourth specific aim was to investigate the evolutionary changes of a suite of traits under artificial selection for and against dispersal via spatial sorting (Chapter 5). Body size responded rapidly to selection; non-dispersers increased in size while dispersers decreased in size over seven generations. Once mass differences had been accounted for, limb length and movement behaviour did not clearly diverge between dispersers and non-dispersers, but metabolic rate was slightly higher in non-dispersers. Interestingly, despite imposing strict selection, the dispersal rate of the selected lines did not strongly diverge. The association between traits and movement behaviour in a maze was different to that of the association between traits and the dispersal apparatus, suggesting that selection for dispersal by climbing does not correlate well with movement along a flat surface. Variance in dispersal rate and movement was maintained even under intensive selection, indicating that individuals may maximise their fitness by producing offspring that exhibit a variety of dispersal behaviours.

The fifth aim was to determine whether a trade-off between dispersal and reproduction occurs in *T. castaneum* by cross-breeding the selected lines (Chapter 6). Dispersal and reproduction are both energetically expensive behaviours that may compete for resources. However, the selected dispersers, non-dispersers, and crossed lines did not differ in reproductive output, which suggests that dispersal and reproduction do not necessarily trade off in this system.

Overall, the findings presented in this thesis demonstrate that many phenotypic traits are important for movement behaviour and dispersal, particularly body size, and metabolic and locomotor efficiency, providing empirical support for spatial sorting contributing to phenotypic evolution. Taken together, these results highlight the importance of experimental approaches to gain a more comprehensive understanding of dispersal ecology and evolution.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the policy and procedures of The University of Queensland, the thesis be made available for research and study in accordance with the Copyright Act 1968 unless a period of embargo has been approved by the Dean of the Graduate School.

I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material. Where appropriate I have obtained copyright permission from the copyright holder to reproduce material in this thesis.

Publications during candidature

Peer-reviewed publications

Arnold, P.A., Cassey, P., White, C.R. (2016). Maturity matters for movement and metabolic rate: traits dynamics across the early adult life of red flour beetles. *Animal Behaviour*, **111,** 181-188. DOI: 10.1016/j.anbehav.2015.10.023

Arnold, P.A., Cassey, P., White, C.R. (2016) Functional traits in red flour beetles: the dispersal phenotype is associated with leg length but not body size or metabolic rate. *Functional Ecology*, DOI: 10.1111/1365-2435.12772.

Arnold, P.A., Cassey, P., Rafter, M.A., Malekpour, R., Walter, G.H., White, C.R. (accepted) Investigating movement in the laboratory: dispersal apparatus designs and the red flour beetles, *Tribolium castaneum*. *Entomologia Experimentalis et Applicata*

Conference abstracts – oral presentations

Arnold, P.A., Cassey, P., White, C.R. (2014). Physiological trait dynamics during the life of adult red flour beetles, *Tribolium castaneum*. *In*: Main Meeting of the Society of Experimental Biology, The University of Manchester, Manchester, UK.

Arnold, P.A., Cassey, P., White, C.R. (2014). Physiology, behaviour and movement: trait correlations and dynamics during the life of adult red flour beetles, *Tribolium castaneum*. *In*: 31st Meeting of the Australian and New Zealand Society of Comparative Physiology and Biochemistry, The University of New England, Armidale, Australia.

Arnold, P.A., Cassey, P., White, C.R. (2016). Experimental evolution of dispersal-related traits in a model insect: morphological, physiological, and behavioural responses to spatial selection. *In*: 33rd Meeting of the Australian and New Zealand Society of Comparative Physiology and Biochemistry, Western Sydney University, Hawksbury, NSW, Australia.

Publications included in this thesis

Arnold, P.A., Cassey, P., White, C.R. (2016). Maturity matters for movement and metabolic rate: traits dynamics across the early adult life of red flour beetles. *Animal Behaviour*, **111,** 181-188. – Incorporated as Chapter 2 with minor changes.

Contributor	**Statement of contribution**
Pieter A. Arnold (Candidate)	Experimental design (85%), data collection (100%), analysis (80%), drafting & writing (90%)
Phillip Cassey	Experimental design (5%), analysis (10%), drafting & writing (5%)
Craig R. White	Experimental design (10%) analysis (10%), drafting & writing (5%)

Arnold, P.A., Cassey, P., White, C.R. (2016) Functional traits in red flour beetles: the dispersal phenotype is associated with leg length but not body size or metabolic rate. *Functional Ecology*. – Incorporated as Chapter 3 with minor changes.

Contributor	**Statement of contribution**
Pieter A. Arnold (Candidate)	Experimental design (85%), data collection (100%), analysis (80%), drafting & writing (90%)
Phillip Cassey	Experimental design (5%), analysis (10%), drafting & writing (5%)
Craig R. White	Experimental design (10%) analysis (10%), drafting & writing (5%)

Arnold, P.A., Cassey, P., Rafter, M.A., Malekpour, R., Walter, G.H., White, C.R. (accepted) Investigating movement in the laboratory: dispersal apparatus designs and the red flour beetles, *Tribolium castaneum*. *Entomologia Experimentalis et Applicata.* – Incorporated as Chapter 4 with minor changes.

Contributor	**Statement of contribution**
Pieter A. Arnold (Candidate)	Experimental design (75%), data collection (100%), analysis (80%), drafting & writing (85%)
Michelle A. Rafter	Experimental design (5%)
Rokhsareh Malekpour	Experimental design (5%)
Gimme H. Walter	Experimental design (5%), drafting & writing (5%)
Phillip Cassey	Experimental design (5%), analysis (5%), drafting & writing (5%)
Craig R. White	Experimental design (5%), analysis (15%), drafting & writing (5%)

Contributions by others to the thesis

Craig White, Phillip Cassey, and Gimme Walter, though discussions with myself, had significant input to the conception and development of the theoretical and experimental components of this thesis. Rokhsareh Malekpour and Michelle Rafter provided important experimental design suggestions for Chapter 4. Candice Bywater provided assistance with data collection for Chapter 6. Assistance with data analyses, and critical reviewing of thesis chapters were provided by Craig White, Phillip Cassey, and Gimme Walter.

Statement of parts of the thesis submitted to qualify for the award of another degree

None.

Acknowledgements

Craig White, I think you've earned first acknowledgement – for many very good reasons! Sometime during my Honours year, I recall a certain conversation on the roof where I asked whether there might be a potential opening for a PhD. Indeed there was, and I'm incredibly grateful that I took that opportunity. Over the last (nearly) four years you have taught me so much, and have instilled in me an enthusiasm for asking interesting questions, thinking critically, problem-solving, and for conducting good research. You encouraged me to get stuck into R, make contraptions in the lab, supported me for conferences, and pushed me to be an independent researcher – especially when you left UQ to become your alter ego 'Prof. White' and were rarely seen again! Thanks for beers, coffees, opportunities, travel, for your mentorship and generosity with time and advice, even during busy or chaotic times, and most importantly for introducing me to research. I'm looking forward to our next project!

To Phill Cassey – thanks for adding your brilliant scientific mind to this project, for helping solve experimental and statistical problems, improving my R coding and writing, providing timely feedback, and for the opportunities to visit your lab and visit Adelaide. To Gimme Walter – thank you for your support throughout, for jumping on board as my primary supervisor in the final year, for opening up collaborative opportunities, and for imparting your wisdom about science, academia, and life in general.

To Robbie Wilson and Daniel Ortiz-Barrientos, thanks for the advice and encouragement when it was needed, and particularly for pushing me to better understand and better present my work at each of the milestones! Thanks to the Ecosciences Precinct scientists: Andrew Ridley for introducing me to and providing the beetles at the start of this project, and also Andrew Hayes, Manon Griffiths, and Helen Nahrung for their support and collaboration. Thanks to all of the inspiring researchers I met at conferences, particularly Vincent Careau for advice about control lines, and Lewis Halsey and Mike Kearney for insightful and encouraging discussions. Thanks to Chris Clemente, Phil Matthews, and Ross Dwyer for guidance on tracking animal movement and for technical help when lab equipment failed.

To my fellow White Lab survivors, Jules and Hugh, thanks for keeping the lab spirit alive and for your comradery and friendship through the highs and lows that we've each faced along the way. To the lab and office mates past and present – Taryn, Dan, Lesley, Nat, Candice, Skye, Bec, Chopper, Carmen, Gwen, Billy, and many Honours students – thanks for all the great times and for sharing this journey. The chats in the office and over coffee that were either completely absurd or deeply intellectual were equally valuable, and the support and encouragement from everyone was amazing. Together with friends from undergrad that stuck around to do a PhD and the other wonderful people I met on the way – Pip, Essie, Mel, Maddie, Andrew, Cara, Owen, Niky, Beau, Roxy, Michelle, Aleksej, Sheree, Verna, and Rhys – you all you made this experience the best it could have been. Outside of UQ, Ellen, Byron, Dylan, and Paul were great friends that made getting away from work easy and always showed interest in what I was doing, even though it probably seemed a bit crazy.

I am truly indebted to Mum and Dad, for teaching me to think scientifically from a very young age, to providing the education and encouragement that allowed me to achieve the best possible outcome, and inspiring me to undertake research. Your insight into the scientific world, limitless advice and encouragement, interest in my studies, constructive feedback on my writing, and the continuous love and support has allowed me to achieve what I have.

Finally, and most importantly, thanks to Monica. You were my constant companion throughout this journey; inspiring, supportive, encouraging, and patient through experiments that lasted weeks on end and the times I was travelling or overly involved in writing. You were always able to bring me back to reality and gave me the chance to share amazing life experiences with you. I am so appreciative of everything you’ve done for me and I can only hope to support you as well in your future endeavours and in our life together.

Keywords

dispersal, movement, experimental evolution, behaviour, physiology, morphology, metabolic rate, body size, spatial sorting, beetles

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 060399 Evolutionary Biology not elsewhere classified, 40%
ANZSRC code: 060699 Physiology not elsewhere classified, 40%
ANZSRC code: 060801 Animal Behaviour, 20%

Fields of Research (FoR) Classification

FoR code: 0603, Evolutionary Biology, 40%
FoR code: 0606, Physiology, 40%
FoR code: 0606, Zoology, 20%

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AIC – Akaike’s information criterion
ANOVA – analysis of variance
C – control selected group
CI – 95% confidence intervals
CO_2 – carbon dioxide
d – Cohen’s *d* (effect size)
D – disperser selected group
d.f. – degrees of freedom
for – *foraging* gene
fps – frames per second
GLMER – generalised linear mixed effects regression
H_2O – water
LRS – lifetime reproductive success
Hz – hertz
LMER – linear mixed effects regression
MR – metabolic rate
MS – mean squares
n – sample size
PC – principal component
PCA – principal components analysis
PC1 – first principal component
PC2 – second principal component
pgi – phosphoglucose isomerase
PKG – cyclic guanosine monophosphate protein kinase
QTL – quantitative trait locus
R – resident selected group
RMR – resting metabolic rate
SE – standard error
SS – sum of squares
µL – microlitre
°C – degrees centigrade

Chapter 1

General introduction

Overview of dispersal

Dispersal is defined as the movement of an individual that has the potential to contribute to gene flow, typically between their natal and breeding sites (Ronce 2007; Benton & Bowler 2012a; Matthysen 2012). Dispersal is a fundamental mechanism for gene flow among and within populations (Ronce 2007). It allows organisms to track optimal conditions, regulate population density, reduce resource competition with conspecifics and kin, and reduce inbreeding potential while increasing mating opportunities (Roderick & Caldwell 1992; Gandon 1999; Lambin *et al.* 2001; Leturque & Rousset 2002; Bonte *et al.* 2012). Organisms vary greatly in their propensity and capacity to disperse (Figure 1.1), as well as in their dispersal response to the interactions between internal and external cues (Benard & McCauley 2008; Starrfelt & Kokko 2012). The variation within, and the interaction among internal factors (e.g. age, sex, life stage, competitive ability, body condition, morphology, and behaviour) with external factors (e.g. habitat quality, population density, and environmental conditions), determines how and when dispersal occurs (Clobert *et al.* 2009; Matthysen 2012; Duputié & Massol 2013). Understanding the mechanistic and evolutionary causes for variation in dispersal, and dispersal-related traits, is important for predicting the evolution of these traits and the responses of species to environmental and climatic change.

Causes and consequences of movement

Dispersal has multiple proximate causes that are expressed under particular conditions, dependent on the interaction between an organism and its environment (Matthysen 2012). Dispersal may reduce the number of potential interactions among kin, which has the clear benefits of avoiding both inbreeding and kin competition (Lambin *et al.* 2001; Rousset 2012). In habitats that have high conspecific density or crowding, dispersal is likely to be beneficial for the dispersing individual and also for the kin that it leaves behind by reducing resource and mate competition, and inbreeding potential (Gandon 1999). Conversely, in habitats with low conspecific density, Allee effects, the lack of mate availability, or social structure can increase dispersal of some species (Kuussaari *et al.* 1996; 1998). Therefore, dispersal behaviour may often be density-dependent. Dispersal most often increases with density (Matthysen 2005), but can also decrease with density (Baguette *et al.* 2011), or be both positively and negatively density-dependent simultaneously (Kim *et al.* 2009). Dispersal may also be a beneficial response to deteriorating local conditions, for example, if a stressor has decreased survival probability or resource availability in the local habitat (Bell & Gonzalez 2011; Hui *et al.* 2012). Individuals that disperse throughout their reproductive lifetime may receive a fitness benefit, by distributing their offspring in multiple patches and across different environmental conditions (Lakovic *et al.* 2015). This strategy ensures that a large proportion of individuals will survive if, for example, predator density is high or conditions are favourable in some habitats but not in others.

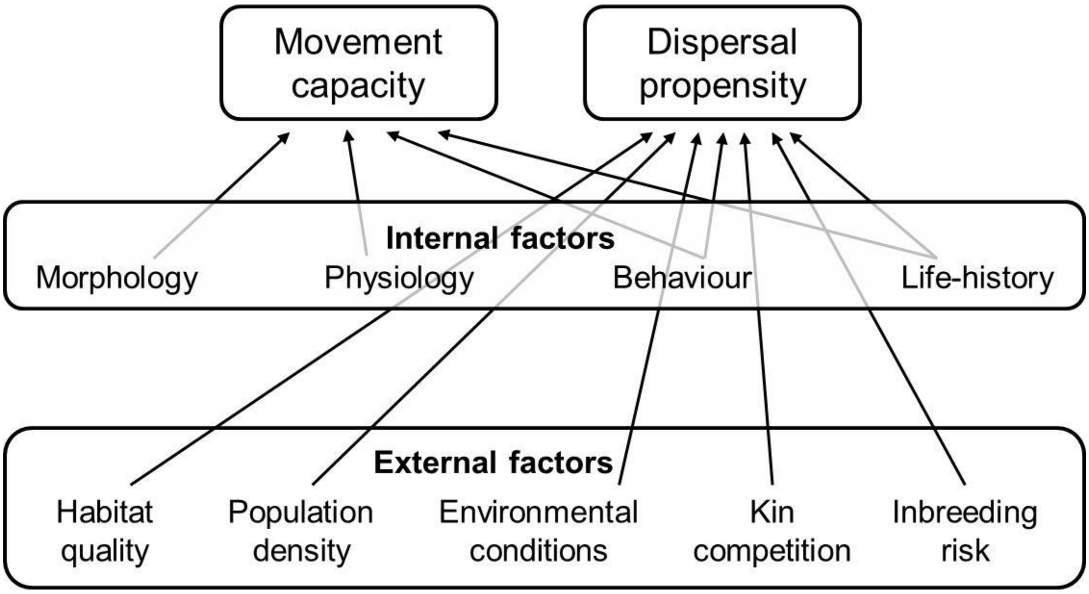


Figure 1.1 Schematic of the relationships between internal and external factors on movement capacity and dispersal propensity. Partially adapted from Clobert *et al.* (2009).

While dispersal is ultimately beneficial for individuals and populations via gene flow, it has many associated costs that can be categorised as opportunity, time, risk, and energetic costs (reviewed in Bonte *et al.* 2012). These costs vary in impact on the organism, from potentially minor loss of time available for other activities, to ultimate fitness costs of failure to reproduce or survive (Figure 1.2). Costs of dispersal may also be distinct across the different phases of dispersal: emigration (also ‘departure’), transfer (also ‘transience’), and immigration (also ‘settlement’), and across life-history (Bowler & Benton 2005; Bonte *et al.* 2012; Matthysen 2012). Opportunity costs may be incurred throughout the dispersal process, as losses of local adaptation, familiarity, kin nepotism, or social rank. Time costs refer to the time spent dispersing or selecting an optimum habitat, rather than conducting other activities that would contribute to fitness. Risk costs refer to the chances of mortality through predation, wounding, adverse environmental conditions, and the possibility of not locating an appropriate habitat or mates. Energetic costs refer to the metabolic energy invested into locomotive structures (e.g. wings and associated muscles) and energy required to initiate and maintain dispersal, which diminish investments into reproduction and other activities. In spite of these costs, even if the dispersal landscape is heterogeneous with respect to fitness, nearly all individuals will disperse at least once in their lifetime. The benefits of dispersing include the accrual of spatial knowledge of resources, conspecifics and predators, avoidance of inbreeding and kin competition, and potentially access to unexploited resources (Bonte *et al.* 2012; Rousset 2012). It is therefore necessary to gain a greater understanding of the relative importance of traits, behaviours, and factors that influence dispersal with respect to fitness costs and benefits (Stevens *et al.* 2012; Burgess *et al.* 2016).

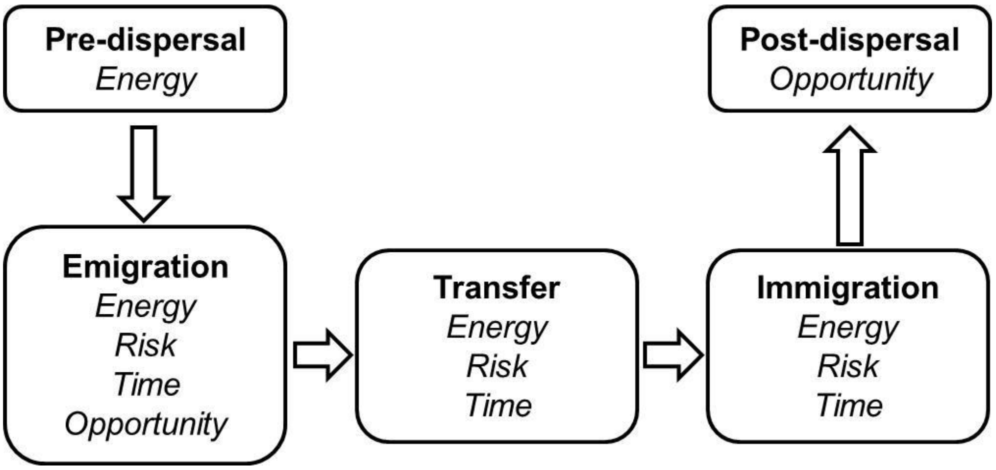


Figure 1.2 Costs associated with dispersal (italics) across the different phases of dispersal (bold). Partially adapted from Bonte *et al.* (2012).

Traits associated with dispersal

While conspecific density, availability of mates and resources, and environmental conditions are strong influences on dispersal, a significant proportion of the variance in dispersal can be ascribed to traits that determine dispersal propensity and movement capacity (Bowler & Benton 2005). Dispersal propensity describes the ‘willingness’ of an individual to disperse; it is dependent on life-history, behaviour, internal state, and the external environment (Bowler & Benton 2009). Movement capacity describes the physiological ability of an organism to move, and therefore disperse; it is strongly dependent on morphology, particularly locomotive structures and underlying physiological traits including muscle architecture and metabolic rate (Ducatez *et al.* 2012; Hillman *et al.* 2014). Both dispersal propensity and movement capacity have been used to categorise individuals broadly as either dispersers or residents (Cote *et al.* 2010a). Dispersers (also 'emigrants' or 'immigrants') are individuals that have a high propensity and capacity to disperse. Residents (also 'non-dispersers' or 'philopatrics') are individuals that have a strong affinity to stay near their natal site.

An individual's biological purpose for dispersal is influenced by life-history (Bowler & Benton 2005; Stevens *et al.* 2012). In insects, dispersal often only occurs in the adult stage, as there is little reason for larvae to move if resources in their natal site are usually abundant. Locomotive structures for dispersal may also only develop or become functional in particular life stages, usually an adult, motile stage. Even within the adult stage, age and sex have a profound influence on dispersal, as immature (pre-reproductive) males and females may have to move to find a suitable mate and reproductive site, and thus have a high dispersal propensity (Bowler & Benton 2009). When mature, however, some females are less inclined to disperse while they are producing eggs, as both movement and ovulation are energetically expensive. Moreover, dispersal may be risky during reproduction, whereas males may continue to disperse in order to find mates (Benton & Bowler 2012a). The body condition (body mass relative to body size) of an individual can affect their ability to disperse and be competitive. Individuals with high body condition may more easily abide the costs of dispersal, and thus disperse earlier or farther than lower condition individuals (Bonte 2009; Delgado *et al.* 2010).

Morphology significantly affects dispersal ability. Body size scales positively with locomotor apparatus size and energy storage capacity, and is usually associated with high competitive ability, therefore larger individuals generally have a great dispersal capacity (e.g. Hill *et al.* 1999; Hughes *et al.* 2007; Brown *et al.* 2013; Laparie *et al.* 2013). However, in certain contexts, small individuals (which are typically less competitive than large ones) may disperse away from a competitive environment (Bowler & Benton 2005), or be more able to afford the costs of dispersal than larger individuals (Léna *et al.* 1998). Relative locomotor apparatus size is also strongly associated with dispersal. For example, in comparison to residents, dispersive cane toads (*Rhinella marina*) had longer legs relative to their body size (Phillips *et al.* 2006) and dispersive field voles (*Microtus agrestis*) had relatively longer feet (Forsman *et al.* 2010) than their respective resident conspecifics.

Physiological traits also affect dispersal. For example, the metabolic rate (MR) of Glanville fritillary butterflies (*Melitaea cinxia*) during flight was higher in individuals that had a greater movement capacity, which is more costly from an energy budget perspective (Niitepõld *et al.* 2009). However, the higher energy cost to fuel increased flight may be subsidised by the fitness benefits gained by increasing flight mobility (Niitepõld *et al.* 2009). A similar pattern emerges with resting metabolic rate (RMR). Dispersive round gobies (*Neogobius melanostomus*) have a higher RMR than residents (Myles-Gonzalez *et al.* 2015). RMR represents the minimal energy required for an ectotherm to self-maintain (Speakman *et al.* 2004), and will be higher when tissues require more energy to be maintained. If dispersing individuals have exaggerated locomotor structures and supporting musculature, or have other metabolically-demanding tissues, then their RMR will be higher than residents. Furthermore, behavioural characteristics such as increased activity, boldness, and exploration, which are associated with high dispersal, are also strongly linked to high RMR (Biro & Stamps 2010).

Behavioural and personality traits are frequently associated with movement (Cote *et al.* 2010a; Canestrelli *et al.* 2016). Dispersive individuals are often more aggressive (Aguillon & Duckworth 2015), more exploratory (Haughland & Larsen 2004), less social (Cote *et al.* 2010b), more inclined to take risks (Cote & Clobert 2010), and more bold (Fraser *et al.* 2001) compared to residents. In particular, boldness correlates strongly with dispersal, and is associated with continuous, directed movement behaviour (Dingemanse *et al.* 2003; Delgado & Penteriani 2008). As dispersal is the result of movement behaviours, specific characteristics of movement are undoubtedly related to dispersal (Hawkes 2009). Individuals vary in their patterns of movement; strong proximate measures of complex movement are traits such as activity, speed, intermittency, path straightness, and distance travelled (Kramer & McLaughlin 2001; Bartoń *et al.* 2009; Bazazi *et al.* 2012). Both dispersal propensity and movement capacity, which together define the ability to disperse, can only be described proximately by studying the combination of the underlying traits that shape them, within contextual bounds. Identifying combinations of underlying traits that explain the variance in dispersal propensity and movement capacity among individuals and populations has recently become a field of study in its own right, namely that of ‘dispersal syndromes’.

Dispersal syndromes

Dispersal syndromes, also called ‘dispersal phenotypes’, describe suites of covarying phenotypic traits that are associated with dispersal (Ronce & Clobert 2012). The accurate identification of patterns of covariation among dispersal and other morphological, physiological, behavioural, and life-history traits can facilitate understanding of the proximate and ultimate causes of dispersal across diverse taxa (Stevens *et al.* 2014). Essentially, dispersal syndromes attempt to attribute intra-specific variance in dispersal to multiple traits which might be correlated. Considering the relationships between the aforementioned traits and dispersal, a ‘good disperser’ might be expected to have a large body size, a high RMR, large locomotor muscles and structures, and follow straight paths while moving quickly across long distances. Traits such as these, which are very likely correlated due to their underlying physiological mechanisms and evolutionary history, may explain significantly more variance in dispersal patterns when taken into consideration together (Ronce & Clobert 2012). Thus far, dispersal syndromes have been described in numerous taxa, with notable examples found in insects, lizards, toads, and fishes (e.g. Rehage & Sih 2004; Hanski *et al.* 2006; Sinervo *et al.* 2006; Clobert *et al.* 2009; Cote *et al.* 2010b; Laparie *et al.* 2013; Lindström *et al.* 2013; Stevens *et al.* 2013; 2014).

Recently, the large white butterfly (*Pieris brassicae*) has been used to explore the drivers of dispersal (Legrand *et al.* 2015). This species exhibits a dispersal syndrome where dispersers and residents differ phenotypically. Flight performance, which was measured as time spent flying and maximum flight distance, was correlated with the decision to disperse (Ducatez *et al.* 2012). Further, the size and shape of *P. brassicae* wings were strongly correlated with both time in flight and flight distance (Ducatez *et al.* 2012). Dispersers that had better flight performance and large, broad wings also had a tendency to orient and maintain flight direction, i.e. fly more continuously in the same direction (Larranaga *et al.* 2013). This behaviour, which is likely heritable, would thus increase the efficiency of movement between patches and reduce the time- and risk-associated costs of dispersal (Bonte *et al.* 2012; Larranaga *et al.* 2013). Dispersers are therefore not a random fraction of the population, but are rather individuals with covarying morphological and behavioural traits that describe variation in dispersal in *P. brassicae* (Legrand *et al.* 2015).

Dispersal syndromes are clearly important when considering the causes of dispersal and understanding variation in dispersal. Many phenotypic traits, alone or as part of a syndrome, correlate with dispersal and are important to its evolution. Therefore, it is also imperative to understand the mechanisms that select for or constrain the evolution of traits that are associated with dispersal.

Evolution of dispersal

The evolution of dispersal traits can most easily be seen at the range edge of an expanding population, where dispersing individuals accumulate when moving into new territory (Therry *et al.* 2014a; Chuang & Peterson 2016). Two evolutionary mechanisms have been proposed to drive change in dispersal: natural selection and spatial assortative mating. Natural selection proposes that organisms with traits better suited to their local environment will have a survival or reproductive advantage, and therefore higher fitness (Darwin 1859). Highly dispersive individuals may arise in a population by natural selection for dispersal ability, provided that dispersing also confers an increase to fitness. Spatial assortative mating (also called ‘spatial sorting’) proposes that individuals with traits facilitating dispersal will accumulate and persist at range edges through proximity-limited mating between highly dispersive individuals, which may be non-adaptive (Travis & Dytham 2002; Shine *et al.* 2011). On expanding range edges where population density is lower, fitness advantages may be conferred through reduced competition and greater resource availability (Perkins *et al.* 2013). Therefore, when spatial sorting interacts with natural selection such that dispersive individuals have heritable and adaptive traits, this is termed spatial selection, a recently proposed alternative evolutionary mechanism to describe phenotypic evolution (Figure 1.3; Shine *et al*. 2011; Perkins *et al*. 2013).

A well-documented example of spatial sorting is found in the introduced cane toad (*Rhinella marina*) and its invasion of northern Australia. Since their introduction in 1935, cane toads have increased their rate of spread from approximately 10 km/year to at least 55 km/year (Phillips *et al.* 2007). Throughout this range advancement, toads at the invasion front have evolved numerous traits that facilitate their dispersal ability. These individuals have longer legs relative to their body size, which significantly affects the distance they move (Phillips *et al.* 2006). Dispersers also exhibit different movement behaviour to resident toads from long-established populations: they move more often, move farther in a given time while following straighter paths, and have greater endurance (Alford *et al.* 2009; Llewelyn *et al.* 2010; Brown *et al.* 2014). Further, dispersive toads have increased growth rate and exhibit a shorter time to maturity (Phillips 2009). All of these observed traits indicate a dispersal syndrome exists and is evolving in this species. These phenotypic traits are heritable, thus the rate of range expansion has potential to continue accelerating through time as offspring become better dispersers (Phillips *et al.* 2010a). Distinguishing between natural selection and spatial sorting is possible on the grounds of fitness. That is, if spatial sorting were driving phenotypic change, reproductive success would not be different between dispersers and residents. On the other hand, natural selection may be intertwined with spatial sorting processes if an advantage is conferred to the individuals that disperse (Shine *et al.* 2011). In these toads, dispersers appear to be less reproductively fit than residents, therefore the spatial sorting hypothesis is currently supported (Hudson *et al.* 2015).

Phenotypic variation in dispersal-related traits is strongly associated with the range expansion of invasive species (Chuang & Peterson 2016), therefore understanding contemporary evolution dynamics of morphological, physiological, and behavioural traits is crucial for effective distribution modelling (Travis & Dytham 2002; Bocedi & Travis 2016) and invasive species management (Lennox *et al.* 2015). Many invasive species (including amphibians, birds, fish, and insects) have been found to exhibit certain traits that assist in their dispersal ability, and rapid phenotypic change occurs at expanding range edges (Llewelyn *et al.* 2010; Berthouly-Salazar *et al.* 2012; Laparie *et al.* 2013; Myles-Gonzalez *et al.* 2015; Davenport & Lowe 2016). As climate change and anthropogenic disturbance of natural systems continues, the importance of understanding biological invasions grows, therefore elucidating the impact of evolutionary processes in the spread and ecological impact of invasive species is a vital component of this understanding (Colautti & Lau 2015). Crucially, the evolution of dispersal syndromes is not easily disentangled from environmental variation and stochastic processes that are found at natural range edges; therefore our understanding of selective processes on dispersal is constrained. To address this, laboratory experiments that study dispersal syndromes and their evolution over multiple generations, while controlling environmental factors, are important stepping stones between theoretical predictions and field observations.

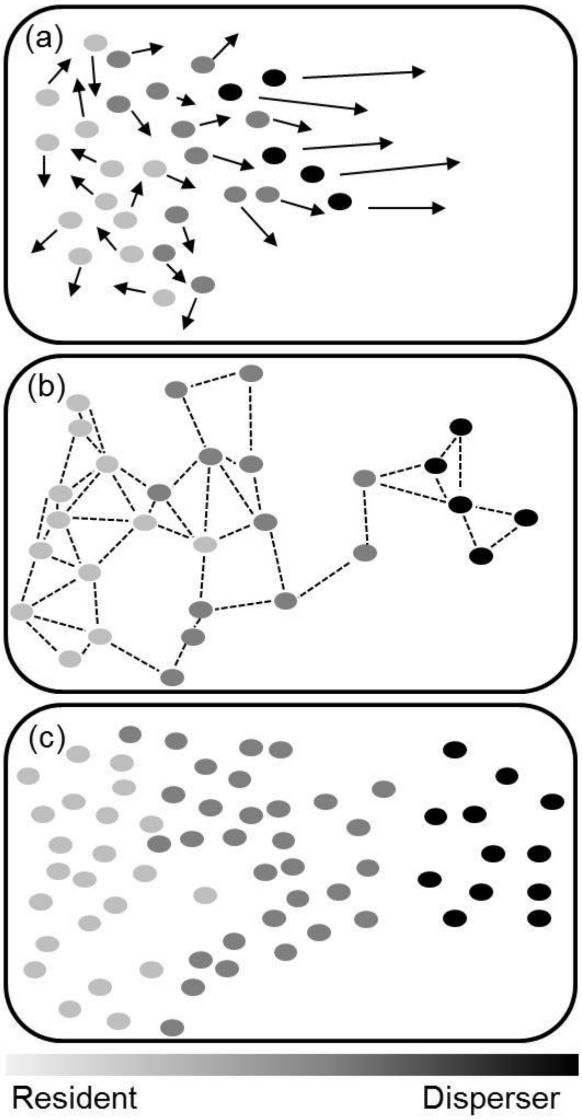


Figure 1.3 Example of spatial assortative mating. (a) Individuals with traits facilitating dispersal accumulate at range edges, then (b) because these highly dispersive individuals are limited to mating with other dispersers by proximity, and (c) provided that the dispersal-related traits are heritable, the resulting offspring will maintain the dispersal phenotype in the population and range expansion may accelerate. Partially adapted from Chuang and Peterson (2016).

Aims of the research presented in this thesis

A continuum of dispersal strategies and dispersal-related traits is maintained in a population, yet our understanding of how these important traits change over time, relate to others, and evolve through space and time is limited. Laboratory models provide a mechanism by which patterns observed in the field or predicted by theory can be explicitly tested through experimental manipulations and under controlled conditions, thus bridging the knowledge gap between theory and field. The overall aim of the research presented in this thesis was to gain a better understanding of the evolution of phenotypic traits that are related to dispersal, using a laboratory model species that can be artificially selected for differential dispersal strategies.

The first specific aim was to determine how age and sex affects physiological, behavioural, and movement traits in adult *Tribolium castaneum* (Chapter 2). At the onset of sexual maturity, organisms undergo significant physiological and biochemical change, and undertake costly reproductive and dispersal behaviours. Therefore, sexually mature individuals were predicted to have higher energy expenditure, and move farther, faster, and more continuously than immature individuals that do not undertake these costly behaviours.

The second specific aim was to determine the relationships among metabolic rate, body size, relative leg length, and three different movement behaviour traits, which have been identified as constituents of the dispersal syndrome, using *T. castaneum* (Chapter 3). Although the correlations among these traits are well-studied, the strength, variance, and even direction of these relationships can vary widely, depending on species and context.

The third specific aim was to investigate the dispersal rate of *T. castaneum* through three-patch dispersal apparatuses based on previous designs, to determine which of these was most effective for artificial selection on the basis of dispersal success (Chapter 4). The distance and slope of paths between patches can significantly affect dispersal, but the effects of manipulating these apparatus design aspects have not been previously quantified.

The fourth specific aim was to investigate the evolutionary changes of a suite of traits in *T. castaneum* that are thought to comprise the dispersal syndrome, under artificial selection for and against dispersal via spatial assortative mating (Chapter 5). Significant trait differences have been commonly observed among populations, but to understand the changes leading to these differences, a manipulative and multigenerational approach must be taken.

The fifth specific aim was to determine whether a trade-off between dispersal and reproduction occurs in *T. castaneum*, by cross-breeding selected lines (Chapter 6). Dispersal and reproduction are energetically expensive behaviours that compete for resources, but dispersers are also expected to be colonisers that may have high reproductive output. These opposing hypotheses were tested.

Study species

The red flour beetle (*Tribolium castaneum*, Coleoptera: Tenebrionidae, Herbst 1797) is a cosmopolitan pest species that originates from the Indo-Australian region (Smith & Whitman 1992). Its exact origin is not known, presumably because this prolific invader was rapidly distributed across the world through natural colonisation (Ziegler 1976) and anthropogenic transport networks such as the trade of processed grain (Hernandez Nopsa *et al.* 2015). It is a generalist pest that feeds on a variety of grain crops, including sorghum, rice, and maize, but is most often associated with milled wheat and flour products (Figure 1.4). It is a remarkable survivalist that can withstand high temperatures under prolonged desiccating conditions (Mahroof *et al.* 2003), resists starvation for several weeks (Daglish 2006), and has developed strong resistance to the fumigant phosphine (Jagadeesan *et al.* 2012). The strain of *T. castaneum* that was used for all experiments presented in this thesis was the QTC4 strain, sourced from the Postharvest Grain Protection Team (Department of Agriculture, Fisheries and Forestry; Brisbane, QLD, Australia). The QTC4 strain originated from a storage facility in Brisbane (QLD, Australia) in 1965. It has been cultured ever since in the absence of selective pressures from insecticides (Jagadeesan *et al.* 2012).

Figure 1.4 Adult red flour beetle (*Tribolium castaneum*) moving through wheat flour. Photo: Pieter Arnold.

These beetles are typical holometabolous insects. At 30 °C their development cycle is as follows: egg (3–4 days), through eight larval instars (16–18 days), to a sedentary pupal stage (4–6 days), metamorphosing to the adult stage that is sexually immature for 2–4 days postemergence (Sokoloff 1977). They are small (1.5–3.5 mg), but relatively long-lived, with reports of some individuals reaching over 300 days of age as adults (Park *et al.* 1961). Female *T. castaneum* have high reproductive potential; they may mate multiple times and are prolific egg producers. At 29 °C, a single female can oviposit up to 15 eggs per day, for more than 100 days, after which oviposition rate declines but does not cease until closer to 200 days of age (Park & Frank 1948; Soliman 1987).

As a laboratory model, *T. castaneum* has been used since the 1930s (Park 1937; Leslie & Park 1949; Willis & Roth 1950). The ease of culturing and maintaining stock populations ensured its continued use for studying ecology phenomena throughout the 20th century, and it remains a constant presence in the fields of ecology, behaviour, evolution, genetics, resistance, development and pest management (Brown *et al.* 2009; Melbourne & Hastings 2009; Ridley *et al.* 2011; Ahmad *et al.* 2012; Schlipalius *et al.* 2012; Daglish *et al.* 2015; Drury *et al.* 2016). *Tribolium castaneum* also has a long history as a model organism for the study of animal dispersal (Naylor 1961; Prus 1963).

As cosmopolitan pests, *T. castaneum* infrequently have to disperse long distances to find resources. They are highly active animals that use different methods of locomotion to move around their local environment (Figure 1.5), including walking (Campbell 2012), climbing (Cline & Highland 1976), tunnelling (Hagstrum & Smittle 1980), and flying (Ridley *et al.* 2011). Although *T. castaneum* can fly, it is typically a small percentage of individuals that do so (Díez & López-Fanjul 1979). The primary modes of locomotion that are used within grain storage warehouses are walking and climbing, thus most studies to date have investigated dispersal via these modes. Previous studies have investigated the dispersal of *T. castaneum* and a close relative *T. confusum*, across different ages (Ziegler 1976), and in response to food type and quality (Ogden 1970b; Ziegler 1977; Campbell & Runnion 2003), and different population densities (Naylor 1961; Zyromska-Rudzka 1966a; Łomnicki 2006). Other studies have investigated the heritability of dispersal behaviour (Ogden 1970a; Ritte & Lavie 1977; Riddle & Dawson 1983; Korona 1991), fitness consequences of dispersal (Ziegler 1976; Lavie & Ritte 1978), and the response of life-history traits to dispersal (Lavie & Ritte 1978; Zirkle *et al.* 1988; Ben-Shlomo *et al.* 1991; Łomnicki 2006) using artificial selection experiments.

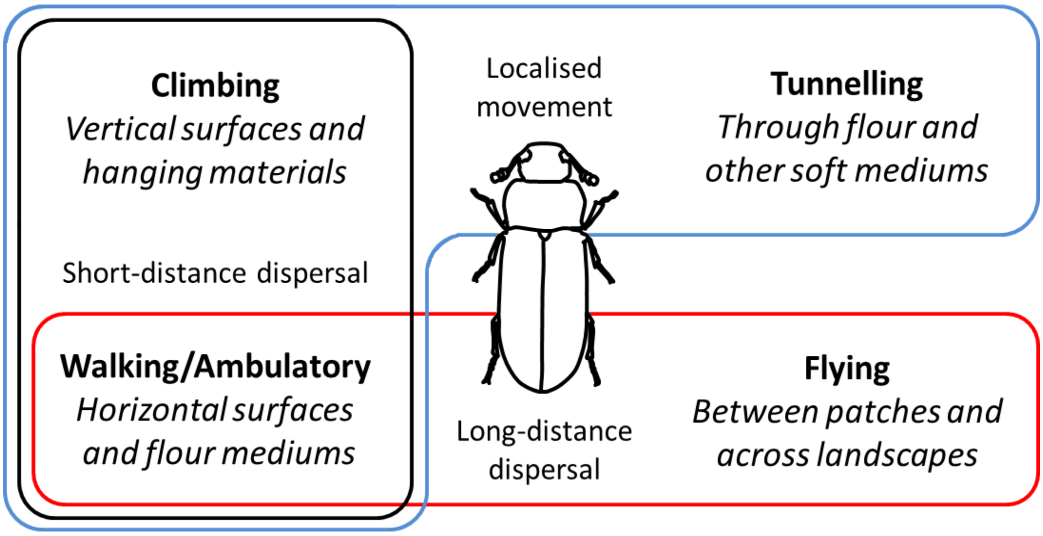


Figure 1.5 Scheme of different movement types used by *T. castaneum*. Movement types are categorised as localised movement (blue), short-distance dispersal (black), and long-distance dispersal (red).

Owing to their short reproductive cycle, ease of culture, and quantifiable dispersal behaviour, *T. castaneum* represents an ideal species with which to test theories pertaining to dispersal syndromes and the spatial sorting hypothesis. Individual *T. castaneum* vary in their dispersal behaviour, and this variation appears to be heritable (Ritte & Lavie 1977; Díez & López-Fanjul 1979), therefore individuals may naturally fall along a continuous gradient from residents to dispersers. Such different dispersal strategies may be indicative of different reproductive strategies. For example, Lavie and Ritte (1978) found that dispersing *T. castaneum* had high reproductive potential, but predicted that these individuals have a high probability of death during dispersal, and therefore may not fulfil their reproductive potential. Residents had low reproductive potential, but were predicted to have a low probability of death by dispersing less often or later in life (Lavie & Ritte 1978). Thus, both of these dispersal strategies can simultaneously persist within a population.

Given the variation in dispersal behaviour within *T. castaneum* populations, it is likely that phenotypic traits constituting dispersal syndromes in other species (e.g. body size, metabolic rate, and movement characteristics) may also be present in this species. Studying the dynamics of important phenotypic traits across age, the functional relationships among these traits, and the responses of these traits to selection for dispersal strategy, will provide a more complete understanding of traits that affect dispersal in this important model species. Further, this species provides a strong model with which to test the theory that spatial sorting drives change in phenotypic traits that constitute the dispersal syndrome.

Structure of thesis

This thesis comprises five experimental chapters (Chapters 2–6) that report the result of laboratory investigations of the dynamics, correlations, and evolution of physiological, morphological, and movement behaviour traits that are related to dispersal. Chapter 2 comprises a published paper, Chapter 3 has been accepted for publication, Chapter 4 is a manuscript under review, and Chapters 5 and 6 will be submitted to scientific journals in due course. Therefore, each chapter is presented as a complete scientific manuscript with an abstract, introduction, methods, results, and discussion. The final chapter of this thesis (Chapter 7) presents a synthesis of all the studies, and discusses directions and considerations for future research.

Chapter 2

Maturity matters for movement and metabolic rate: trait dynamics across the early adult life of red flour beetles

Abstract

Transitioning between life stages involves significant changes to the physiology, structural morphology, biochemistry, and behaviour of an organism. Eclosion, metamorphosis, and the onset of sexual maturity have consequences for the life-history evolution of an organism by initiating reproductive and dispersal-related behaviours that are both energetically costly and directly related to fitness. Animal movement, particularly dispersal when sexually mature, is critical for mate location, regulating population density, and promoting gene flow. Here I examine changes in dispersal-related and physiological traits during a significant transitional period in red flour beetles, *Tribolium castaneum* (Coleoptera: Tenebrionidae). I measured the metabolic rate, spontaneous activity, body size, and movement behaviour traits of individuals of known age and sex. Traits were compared between immature and sexually mature adults, as well as across early adult life where there is a strong tendency to disperse and reproduce. Spontaneous activity, movement speed, and metabolic rate were distinctly reduced in immature adults prior to the onset of sexual maturity, and immature individuals moved more intermittently than mature ones. I found that the mean value of these traits increased with age following eclosion, up to a relatively stable mean once sexual maturity was attained. The reduced metabolic expenditure found in immature individuals was attributable to a reduced energy demand due to relative inactivity, which I hypothesise to be a protective mechanism from conspecifics while the cuticle is undergoing sclerotisation. Understanding the precise developmental trajectories of behavioural and physiological traits allows us to interpret the trait syndromes that underlie dispersal and their evolution.

Introduction

For organisms with complex life cycles, specific age and life stages are specialised to perform differently. In many insects, development involves several larval instars designed for intense growth, followed by a distinct metamorphic pupa stage and then finally an adult stage specialised for dispersal and reproduction (Wilbur 1980). While the pre-adult stages are significant to the transition and resulting fitness of adults (De Block & Stoks 2005), it is only in the adult stage that this fitness potential can be realised through the processes of dispersal and reproduction.

Many adult holometabolous insect species demonstrate a short sexually immature and non-reproductive adult transitional period following emergence and before the onset of sexual maturity. This transitional period, here defined as maturation, occurs over variable lengths of time between species, from several hours to many days following imaginal eclosion (e.g. Norris 1954; Happ 1970; Pitnick *et al.* 1995; Teal & Gomez-Simuta 2002). Maturation induces behaviours associated with reproduction and dispersal, which have potential risks including predation, adverse environmental conditions, unsuitable resources or lack of mates, and failure to reproduce or survive. During the transition to maturity, selection is expected to be strong, because this critical life history transition is when individuals are more active and therefore exposed to predation and risks associated with conspecific interaction through dispersive and reproductive behaviours (Werner & Anholt 1993; De Block & Stoks 2005).

Locomotor activity is involved in nearly all behavioural activities from basic locomotion to complex courtship behaviours (Martin 2003) and is tightly linked with energy expenditure and metabolism. The locomotor musculature that an organism uses to conduct its daily foraging and activities is a substantial contributor to whole-animal metabolic rate (MR) during activity (White & Kearney 2013). Whole-animal MR is a fundamental physiological trait that provides an estimate of the essential costs of living for an organism (Kleiber 1961; Chown & Gaston 1999). While size, activity, phylogeny, and temperature are all considered to be causes of variation in metabolic rate (Konarzewski & Książek 2013; White & Kearney 2013), the influence of other intrinsic factors such as maturity, age, and sex on MR have not been studied over a wide range of insect species.

Metabolic rate, energy dynamics, and the individual’s physiological state strongly influence the resources available for animal movement and the nature of the movement path itself, which is critical for dispersal, reproduction, and fitness (Bell 1990). Characteristics of movement such as speed, step length, path length, and tortuosity are dependent on the biological requirements for an individual, which vary with life stage, age, and body size (With 1994; Mancinelli 2010; Potenza & Mancinelli 2010). Behavioural intermittence of an animal’s movement is the combination of forces acting to intersperse otherwise continuous movement, such as pauses to change orientation or take sharp turning angles, adjustment of behaviour to sensory inputs and the dynamics of acceleration (Bartumeus 2009). The discontinuous aspects of movement paths taken by animals, particularly turning, can have significant costs for that animal's net energy gain (Wilson *et al.* 2013) and also alters their exposure to potential predation (Anholt & Werner 1995; Cuddington & Yodzis 2002; Yoder *et al.* 2004). Direct measurement of complex behaviours and traits in animals (such as dispersal ability, reproductive fitness, or personality) is often difficult, and therefore the use of more simplified proximal traits is required (e.g. Heidinger *et al.* 2010; Sekar 2012; Stevens *et al.* 2013). Integrating multiple movement behaviour traits with physiology and activity provides a relevant and useful proxy for more complex behaviours.

Here, I aimed to determine behavioural and physiological trait changes during the transition from immature adults to sexually mature adults, which occurs at approximately three days post-eclosion (Sokoloff 1974), and across early mature life of adult red flour beetles, *Tribolium castaneum*. Complex behaviours, including movement, dispersal, and reproduction are associated with the onset of sexual maturity in *Tribolium* species (e.g. Ziegler 1976; Soliman 1987; Arnaud & Haubruge 1999; Perez-Mendoza *et al.* 2011). I studied the dynamics of traits (movement characteristics, activity, and MR) that fundamentally underlie animal movement, dispersal, and reproduction. Specifically, I tested the hypothesis that immature beetles would be less active than mature ones, and as sexual maturity is reached, movement characteristics would change to reflect the onset of reproductive behaviour and increased dispersal tendencies. I also predicted that males would move more actively than same-aged females, at least around the onset of maturity, because males tend to disperse and attempt to mate earlier than females (Prus 1966; Ogden 1970b; Arnaud & Haubruge 1999). Finally, I predicted that MR would be positively correlated with size, activity, and movement speed, due to the strong association between energy expenditure and locomotive structures.

Material and methods

Animals and housing

A stock population of *T. castaneum* sourced from the Post-harvest Grain Protection Team (Department of Agriculture, Fisheries and Forestry; Brisbane, QLD, Australia) was used to establish stocks for a series of experiments. This stock was a wild-type susceptible line (QTC4) which was maintained in 1 L cylindrical containers containing 200 g of medium

(95% wholemeal stone-ground wheat flour supplemented with 5% torula yeast) under laboratory conditions of 29.5 ± 1 °C and 40–60% relative humidity. The original stock was bulked up, divided, and maintained as two sub-stocks which were cultured fortnightly.

Beetles were collected as pupae and sexed by examining the external dimorphic genitalia (Halstead 1963) under a stereomicroscope (Olympus SZ61; Olympus Australia Pty. Ltd., Notting Hill, VIC, Australia). Pupae were separated into groups of five by sex and relative day of eclosion in 70 mL containers with approximately 5 g of flour medium. The specific age of beetles was controlled to ± 12 h, and the age range used for this study was from 0 to 21 days post-eclosion. Maturity was defined as the age at which mating was first commonly observed and the cuticle was entirely melanised; approximately three days post-eclosion under the aforementioned laboratory conditions (Sokoloff 1974). At least 24 h prior to trait measurements, beetles were separated and individually placed in containers to fast before respirometry, with the exception of age 0 beetles which were not provided with flour before measurement. Trait measurement proceeded in the following order: metabolic rate and spontaneous activity, movement behaviour, mass, and then morphometrics. Total sample size was 220 across 22 ages, such that five males and five females were measured for each age.

Movement behaviour in a complex maze environment

Movement behaviour of individuals was assessed by placing individual beetles in a complex artificial maze environment. The custom maze (Cottage Plastics, Sumner Park, QLD, Australia) was constructed from 5 mm thick acrylic, based on a design of 12 square passageways radiating from the central point (Figure 2.1). The minimum distance between each passageway was approximately equal such that progression through each passageway was linear. The base was constructed from white acrylic (390 × 390 × 4 mm), covered by a layer of white paper (60 g/m^2) to allow adult beetles to maintain traction on the surface and also right themselves efficiently if required. The walls were white acrylic (6 mm high) melded to a clear acrylic cover in a complex symmetrical pattern, and a single 8 mm diameter hole was drilled in the centre of the cover to initially introduce beetles into the maze.

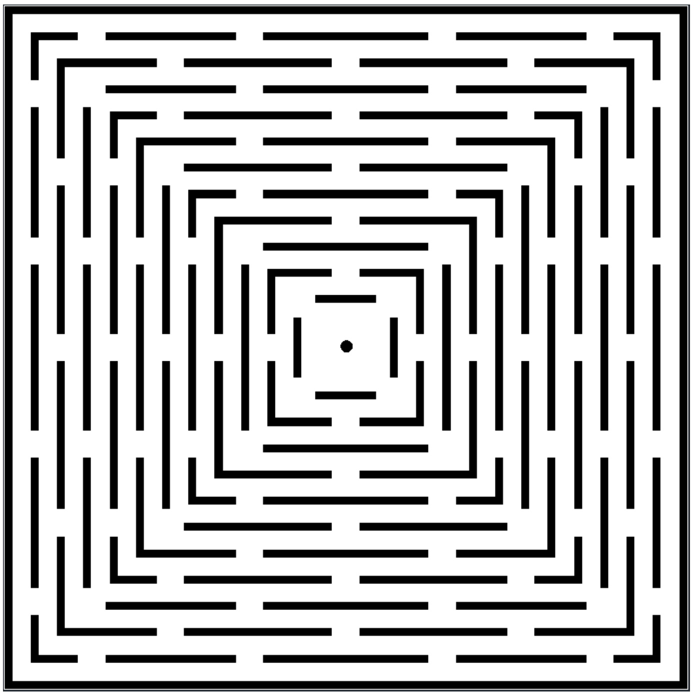


Figure 2.1 Symmetrical acrylic maze (390 × 390 × 6 mm) used to examine movement behaviour of individual *T. castaneum* beetles. The design is based on 12 evenly spaced square passageways radiating from the centre where the minimum distance between each passageway through the maze was equivalent. The maze walls are covered by a layer of clear acrylic and beetles are introduced into the maze centre through a small hole.

This maze represents a spatially complex environment with barriers to movement and multiple junctions. In an open arena, *T. castaneum* will actively move towards and remain along the arena edge (Arnold PA, pers. obs.), whereas in a maze, movement is typically more varied among individuals and multiple movement attributes can be easily measured. Within the maze environment *T. castaneum* exhibit exploration behaviour similar to that observed in laboratory stock populations, warehouses, and natural populations (Arnold PA, pers. obs.).

For each trial, a single adult beetle was introduced into the centre of the maze, then released and recorded for approximately four minutes using a high definition (1280 × 720 pixels) webcam at 10 fps (Microsoft LifeCam Studio; Microsoft Corporation, Redmond, WA, USA). Filming conditions were under ambient and non-direct lighting in a controlled temperature room at 29.5 ± 1 °C. The resulting video recordings of each beetle’s run were frame cropped from the first distinctive movement away from the centre (frame 1) to three minutes after introduction (frame 1801). The tracking of beetle movement was conducted in MATLAB software (MATLAB R2013A; The Mathworks, Inc., Natick, MA, USA), running a digitizing tracking script (DLTdv5; Hedrick 2008). The tracking script utilised an extended Kalman filter to precisely track the approximate path taken by a beetle automatically (frame-by-frame to produce *x* and *y* Cartesian coordinates) within a user-defined residual threshold. Movement characteristics calculated from the tracking analysis were: average speed, minimum speed (lower 5th percentile of speed), maximum speed (upper 5th percentile of speed), total path length and behavioural intermittence (stopping frequency; which is defined as less than 0.2 mm of movement between frames at 10 fps). The time for an individual to complete the maze was calculated as the number of frames to reach the final maze passageway (maximum time: 1800 frames). If an individual did not complete the maze, they were assigned a censored value of 1801+ for the Cox proportional hazards model of time to maze completion. For linear model analyses that required discrete values, these individuals were assigned the whole number value of 2000 frames to represent that they did not complete the maze.

Metabolic rate and spontaneous activity

The metabolic rate of individual beetles was measured to determine whether maturity status, age, and sex affect the routine energy usage in these animals. Flow-through respirometry was used to measure the rate of CO_2 production of individual adult beetles as a proxy for metabolic rate (MR) (Lighton 2008). Air drawn into the system was first chemically scrubbed using columns containing soda lime (Ajax Finechem Pty. Ltd., Taren Point, NSW, Australia) and Drierite (Sigma-Aldrich Co., St. Louis, MO, USA) to remove CO_2 and water vapour, respectively. The flow rate of the incurrent air was regulated by mass flow controllers for four channels (GFC17; Aalborg Instruments & Controls, Inc., Orangeburg, NY, USA) set to a constant flow rate of approximately 25 mL/min (standard temperature and pressure, dry). Air was then passed through four custom metabolic chambers housing individual beetles before being passed through a pair of two-channel LI-7000 CO_2/H_2O analysers (Li-COR Inc., Lincoln, NE, USA). Li-COR software was used to plot and record the CO_2 produced through respiration over a 1 h period, with a resolution of 0.1 ppm at a recording frequency of 1 Hz.

Chambers were approximately 2 mL airtight glass cylinders contained in a multi-channel locomotion activity detector (LAM10H; TriKinetics Inc., Waltham, MA, USA) which used nine infrared LED emitter-detector pairs to detect motion and count each instance that any infrared beam was interrupted by movement of the animal. This allowed spontaneous activity (as counts/h) to be measured synchronously with MR, so that activity could be included as a covariate in the analysis of MR. The respirometry chambers were housed in a controlled temperature incubator at 30 ± 1 °C in darkness to ensure temperature stability and remove light stimulus. Adult beetles were fasted for approximately 24 h prior to MR measurement and placed in similar conditions prior to measurement to acclimate, then measured continuously for 1 h. All basic assumptions of resting metabolic rate (standardised thermal and dark environment to reduce activity, and animals were in a post-absorptive and non-reproductive state) were met by experimental conditions. Activity during MR measurement was monitored and included as a covariate of MR in data analyses, however MR measured in this study is defined as routine metabolic rate (routine MR) due to the presence of activity (Mathot & Dingemanse 2015).

Mass and morphometrics

Individual beetles were weighed using a precision microbalance (XS3DU; Mettler-Toledo, Columbus, OH, USA) to measure fresh body mass (to 0.01 mg), and morphometrics were taken as alternate measurements of body size. Morphological measurements were obtained using a microscope-mounted camera (PL-B686; PixeLINK, Ottawa, ON, Canada) to capture dorsal and ventral images of each beetle. The following metrics were extracted using ImageJ software version 1.46r (National Institutes of Health, Bethesda, MD, USA): length of the elytron (from apex to base), width of elytron (at the widest point), length and width of the pronotum (at the widest point), and the length of the femur on the hind leg.

Statistical analyses

Data were analysed using the R software environment for statistical and graphical computing version 2.15.3 (R Development Core Team 2015) utilising the R packages *lme4* (Bates *et al.* 2014) and *survival* (Therneau 2014). The analyses investigated multiple quantitative traits over the age range of 0 to 21 days post-eclosion at one day intervals. Principal Components Analysis (PCA) was used to derive the measures for movement speed and body size from multiple correlated predictor variables. The first principal component (PC1) of movement speed accounted for approximately 94.9% of variance among speed traits (the average, lower and upper 5th percentiles of movement speed). PC1 of body size accounted for approximately 85.2% of the variance among the size traits (elytron length, elytron width, protonum length, protonum width, femur length, and body mass). Linear mixed effects regression (LMER) models where maturity status, age, and sex were the principal predictor variables of interest were used to analyse movement speed, behavioural intermittence, spontaneous activity, and routine MR as response variables. Each model tested the effect of principal predictors on a response variable, while accounting for all other covariate measurements. A Cox proportional hazards model was used to analyse the time taken to complete the maze as the response variable. Random effects of respirometry channel and stock were included in all models. Full models including interaction terms were fitted initially, with non-significant interaction terms removed to simplify models.

Results

Speed increases and intermittence decreases during maturation

Consistent patterns of increasing movement characteristics with age, particularly during maturation are observable in the lower 5th percentile of speed (Figure 2.2a), average speed (Figure 2.2b) and upper 5th percentile of speed (Figure 2.2c). Age was significant positively correlated with movement speed; however maturity status, routine MR, spontaneous activity, and body size were not (Table 2.1). The increased spontaneous activity found in males was not expressed in their movement behaviour, as sex was not significantly correlated with movement speed. Behavioural intermittence and maze completion were also significantly related to movement speed, where individuals that moved more frequently were able to move further with greater speed to successfully complete the maze (Table 2.1).

Table 2.1 LMER model of the response of movement speed (PC1) to maturity status, age, sex, log-routine MR, activity, body size, behavioural intermittence, and maze completion of beetles aged from eclosion to 21 days post-eclosion.

Coefficients	Estimate ± SE	*t*	*P*
(Intercept)	25.794 ± 5.305	4.863	<0.001
Maturity status (mature)	0.602 ± 0.821	0.733	0.463
Age	0.187 ± 0.035	5.297	<0.001
Sex (male)	-0.168 ± 0.384	-0.437	0.514
log-routine MR	1.591 ± 2.305	0.690	0.829
Spontaneous activity	0.0001 ± 0.001	0.112	0.571
Body size (PC1)	0.431 ± 1.615	0.267	0.789
Behavioural intermittence	-0.023 ± 0.002	-11.338	<0.001
Maze completion	-0.003 ± 0.0004	-6.915	<0.001

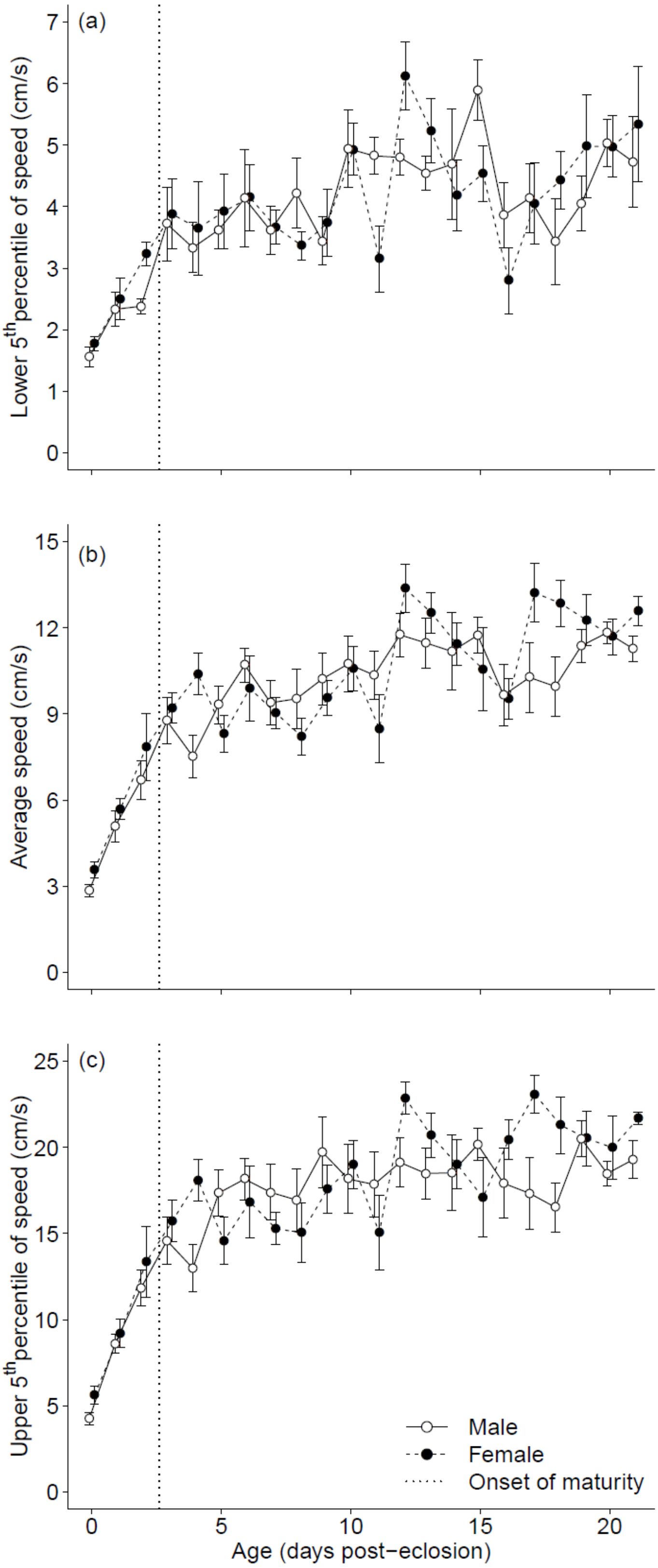


Figure 2.2 Mean movement speed (cm/s) of individual adult *T. castaneum* aged from eclosion (age 0) to 21 days post-eclosion. (a) Lower 5th percentile of movement speed in response to age, (b) average speed in aged beetles, and (c) upper 5th percentile of movement speed in response to age. Each age had *n* = 5 males and *n* = 5 females, for a total of *n* = 220 data points. Data presented are means ± SE.

Behavioural intermittence was significantly correlated with age, maturity status, and their interaction (Table 2.2), where individuals aged three days post-eclosion and older moved significantly more frequently than younger immature individuals of either sex (Figure 2.3). Behavioural intermittence was also significantly correlated with movement speed (Table 2.2), as expected by the intuitive relationship between the time an individual spends immobile and its average speed, which would consequently reduce the distance able to be travelled.

Table 2.2 LMER model of the response of behavioural intermittence to maturity status, age, sex, log-routine MR, spontaneous activity, body size, movement speed, and maze completion of beetles aged from eclosion to 21 days post-eclosion.

Coefficients	Estimate ± SE	*t*	*P*
(Intercept)	935.436 ± 120.191	7.783	<0.001
Maturity status (mature)	-211.116 ± 25.159	-8.391	<0.001
Age	104.905 ± 14.162	7.408	<0.001
Maturity × Age	105.89 ± 14.076	7.523	<0.001
Sex (male)	5.003 ± 8.939	0.56	0.576
log-routine MR	-28.014 ± 50.534	-0.554	0.579
Spontaneous activity	-0.003 ± 0.028	-0.107	0.915
Body size (PC1)	-45.219 ± 37.497	-1.206	0.228
Speed (PC1)	-13.733 ± 1.263	-10.869	<0.001
Maze completion	0.063 ± 0.011	0.595	0.552

The proportion of individuals that completed the maze was significantly greater in mature individuals, and these also completed the maze in a shorter time compared to immature individuals (Figure 2.4). Both maturity state and age were significantly related to the time taken to complete the maze (Table 2.3), such that a lower proportion of immature individuals (males: 0.4, females: 0.27) were able to complete the maze within the set time compared to mature individuals (males: 0.86, females: 0.87).

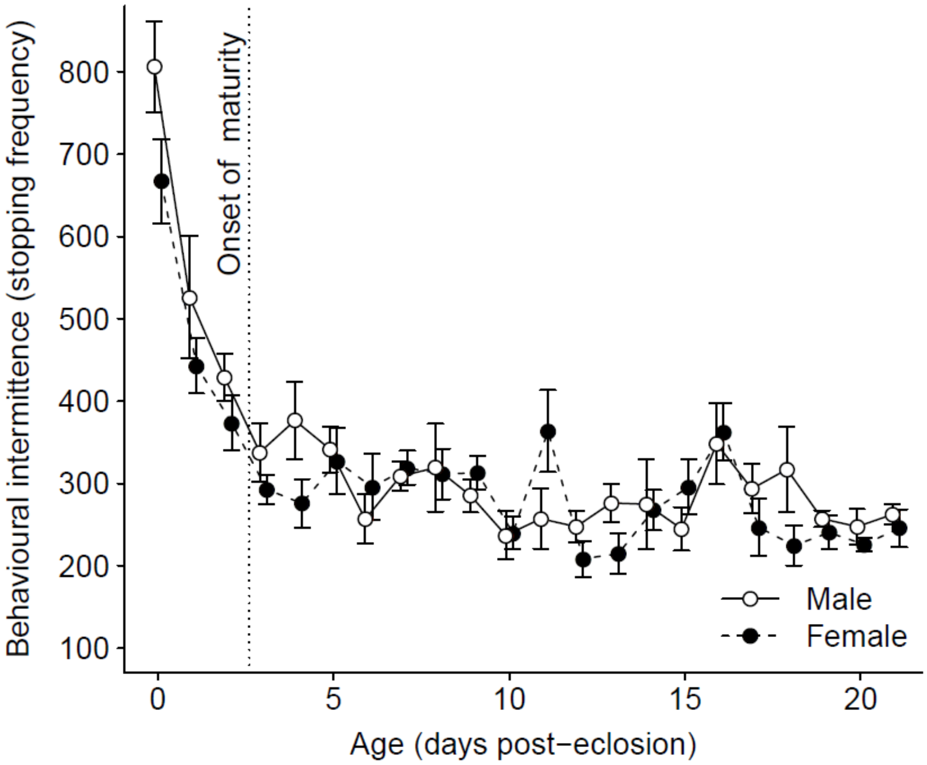


Figure 2.3 Mean behavioural intermittence count (stopping frequency; the number of frames where the individual was not detected moving) during movement of individual adult *T. castaneum* aged from eclosion (age 0) to 21 days post-eclosion. Each age had n = 5 males and n = 5 females, for a total of n = 220 data points. Data presented are means ± SE.

Table 2.3 Cox proportional hazards model of the response of time to maze completion to maturity status, age, sex, log-routine MR, spontaneous activity, and body size of beetles aged from eclosion to 21 days post-eclosion.

Coefficients	Estimate ± SE	Hazard Ratio (95% CI)	*Z*	*P*
Maturity status (mature)	1.403 ± 0.399	4.067 (1.860 – 8.893)	3.515	<0.001
Age	0.028 ± 0.014	1.028 (1.001 – 1.057)	2.019	0.043
Sex (male)	-0.078 ± 0.163	0.925 (0.672 – 1.274)	-0.476	0.634
log-routine MR	0.218 ± 1.243	1.243 (0.214 – 7.217)	0.243	0.808
Activity	-0.0006 ± 0.999	0.999 (0.998 – 1.000)	-1.135	0.256
Body size (PC1)	-0.207 ± 0.813	0.813 (0.208 – 3.181)	-0.298	0.766

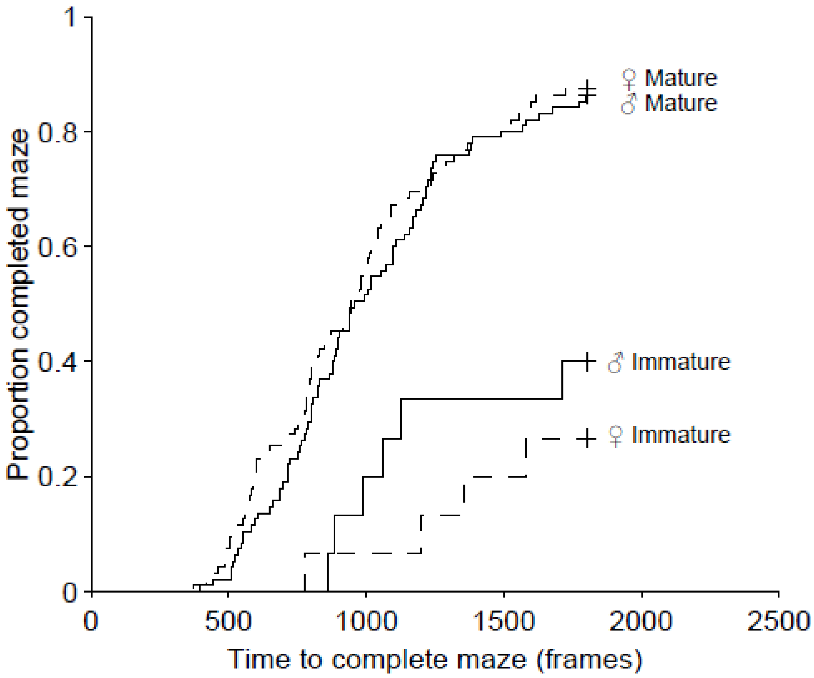


Figure 2.4 Mean proportion and total time taken by individuals to reach the final passageway of the complex maze environment (successful movement through the maze). Immature (age 0 to 3 days post-eclosion) and mature (age 4 to 21 days post-eclosion) life stages are indicated on the figure, and divided into male and female groups. Each age had *n* = 5 males and *n* = 5 females, for a total of *n* = 220 data points.

Spontaneous activity and metabolic rate ramps up when maturity is reached

Spontaneous activity as a response variable was found to be significantly correlated with both sex and maturity status (Table 2.4). Generally, male beetles were more active than females during respirometry; however there was substantial variation in activity counts across ages and particularly within males (Figure 2.5). Spontaneous activity was considerably reduced in immature individuals, thereafter increasing from the onset of sexual maturity (Figure 2.5) in a pattern similar to that of routine MR (Figure 2.6). As expected, the reciprocal relationship between spontaneous activity and routine MR was positive; however the effect was not significant, likely due to the large variability of spontaneous activity (Table 2.4).

Routine MR was significantly different between immature and mature adults (Figure 2.6; Table 2.5). Routine MR was neither significantly correlated with age nor was it significantly different between males and females. Routine MR scaled positively with body size, which was a significant covariate (Table 2.5), however spontaneous activity was not significantly correlated with routine MR (Table 2.5).

Table 2.4 LMER model of the response of spontaneous activity to maturity status, age, sex, body size, log-routine MR, movement speed, behavioural intermittence, and maze completion of beetles aged from eclosion to 21 days post-eclosion.

Coefficients	Estimate ± SE	*t*	*P*
(Intercept)	-371.129 ± 314.12	-1.181	0.237
Maturity status (mature)	127.243 ± 45.527	2.795	0.005
Age	0.601 ± 2.120	0.283	0.777
Sex (male)	46.316 ± 21.413	2.163	0.031
Body size (PC1)	69.222 ± 91.014	0.761	0.447
log-routine MR	191.483 ± 128.555	1.490	0.136
Speed (PC1)	0.555 ± 3.849	0.144	0.885
Behavioural intermittence	-0.036 ± 0.148	-0.246	0.806
Maze completion	0.024 ± 0.026	0.941	0.347

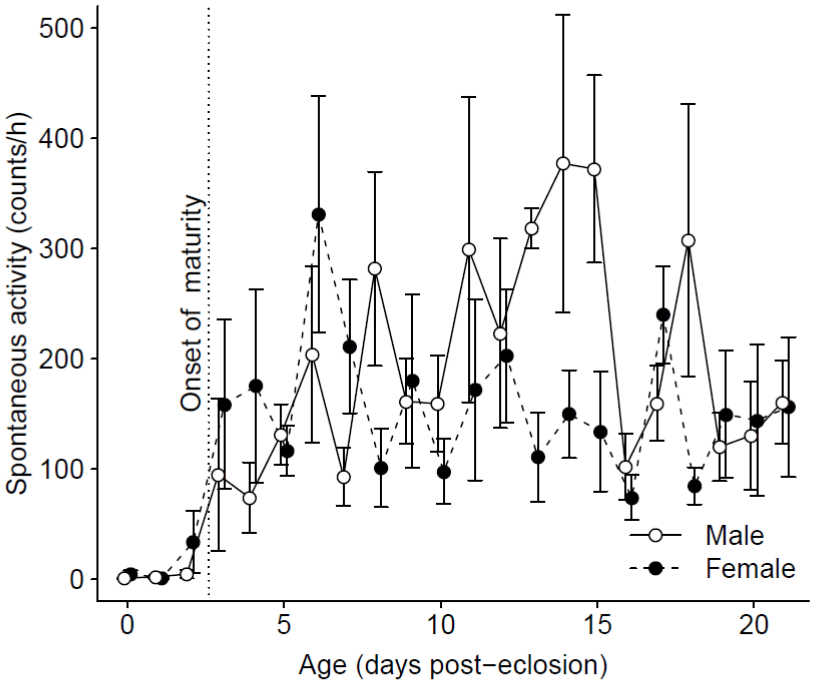


Figure 2.5 Mean spontaneous locomotor activity (counts/h) during respirometry of individual adult *T. castaneum* aged from eclosion (age 0) to 21 days post-eclosion. Each day of age had *n* = 5 males and *n* = 5 females, for a total of *n* = 220 data points. Data presented are means ± SE.

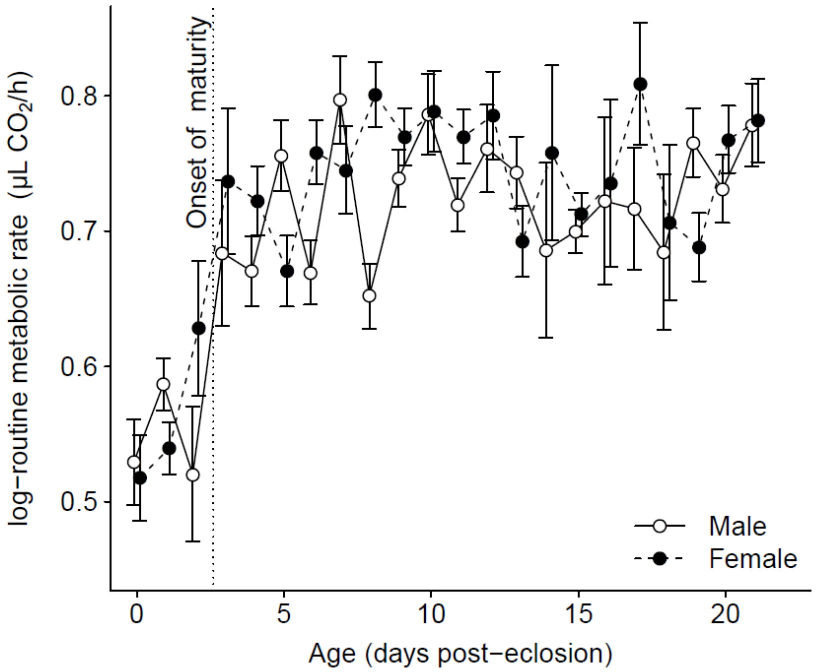


Figure 2.6 Mean log-transformed routine metabolic rate (routine MR; measured as the rate of CO_2 production, μL CO_2/h) of individual adult *T. castaneum* measured from eclosion (age 0) to 21 days post-eclosion. Each day of age had *n* = 5 males and *n* = 5 females, for a total of *n* = 220 data points. Data presented are means ± SE.

Table 2.5 LMER model of the response of log-routine MR to maturity status, age, sex, body size, spontaneous activity, movement speed, behavioural intermittence, and maze completion of beetles aged from eclosion to 21 days post-eclosion.

Coefficients	Estimate ± SE	*t*	*P*
(Intercept)	0.0510 ± 0.1600	0.310	0.756
Maturity status (mature)	0.1310 ± 0.0220	5.884	<0.001
Age	-0.0004 ± 0.001	-0.354	0.724
Sex (male)	-0.0100 ± 0.0110	-0.912	0.362
Body size (PC1)	0.1710 ± 0.045	3.766	<0.001
Activity	$5.9\times10^{-5} \pm 3.5\times10^{-5}$	1.684	0.092
Speed (PC1)	-0.0007 ± 0.0020	0.361	0.718
Behavioural intermittence	$-7.8\times10^{-5} \pm 7.6\times10^{-5}$	-1.027	0.305
Maze completion	$6\times10^{-6} \pm 1.3\times10^{-5}$	0.147	0.883

Discussion

The results of this study have demonstrated that the process of maturing has a significant effect on organisms in terms of their behaviour and physiology. Most aspects of movement behaviour, spontaneous activity, and metabolic rate are significantly reduced in younger individuals prior to the onset of sexual maturity. Movement speed was predicted to be related to spontaneous activity, routine MR, and body size due to the link between locomotive structures and energy expenditure; however, surprisingly these traits were not strongly correlated with speed. Patterns of increasing movement speed from eclosion through to attaining sexual maturity emerged, particularly in average speed and upper 5th percentile of speed (Figure 2.2). These results support the non-exclusive hypotheses that *T. castaneum* that are not yet sexually mature either limit their energy expenditure by remaining relatively inactive or have a limited physiological ability to move prior to reaching maturity.

By introducing an individual beetle into the maze used to assess their movement characteristics they are exposed to a novel unfamiliar environment. The behavioural response of an individual in this novel environment may be partially determined by their physiology, but also their relative personality trait of exploration or boldness (Biro & Stamps 2008). Given that individuals, prior to reaching sexual maturity, do not have a strong drive to seek mates, conspecifics or even to find food, it seems logical that their only motivation to move around would be to find refuge from a potential threat (Bell 1990). This hypothesis is supported by the strong declining response of behavioural intermittence with age, where *T. castaneum* prior to sexual maturity have significantly more bouts of inactivity during movement. The elevated behavioural intermittence indicates that these individuals may be moving discontinuously to assess their immediate environment and adjust their position accordingly by pausing to undertake a sharp turn or gather new sensory input (Bartumeus 2009). After attaining sexual maturity, the motivation to seek mates, but also to seek food as metabolic fuel for increased activity presumably drives the increase in movement and successful completion of the maze.

In terms of energetics, the findings demonstrate that routine MR increases from the point of eclosion over a period of three days until the onset of sexual maturity (Figure 2.6). Studies by Sohal (1982) and Terblanche and colleagues (2004) found that MR increased with age from eclosion through to reproductive maturity in two species of Dipterans, and our results support these findings. Major systemic changes occur during metamorphosis from the pupal stage through to sexual maturity; such changes include the transformation and rearrangement of morphological structures, sclerotisation and melanisation of the cuticle, production and development of gametes and the alteration of neural structures to allow new behaviours such as dispersing and reproducing (Bishop *et al.* 2006).

Despite such drastic physiological changes, the relatively low energy expenditure of individuals prior to the onset of sexual maturity supports the hypothesis that this is likely to be mainly a consequence of inactivity. Spontaneous activity was found to be considerably lower (near zero) in individuals prior to the onset of sexual maturity (Figure 2.5), following a similar pattern to that of routine MR with age and maturity. Being inactive would markedly reduce energy expenditure for these individuals as the energetic cost of transport is high, particularly for the relatively inefficient transport modes of walking and running (Tucker 1975; Reinhold 1999). Immature individuals remain inactive for several days post-eclosion firstly as protection from predation and injury during movement while their cuticle continues to sclerotise (Thompson *et al.* 2002), and secondly the energy reserves accumulated from larval feeding prior to pupation reduces their energy requirements as a callow (Ziegler 1985).

There was also an effect of sex on spontaneous activity, where males tended to have a higher activity level throughout the observed age period. Consistent with our findings, male *T. castaneum* exhibit increased mate-seeking and dispersive behaviour compared to females of equivalent age (Prus 1966; Ogden 1970b). The considerable amount of individual variation among activity, MR, and movement traits is a key finding. Spontaneous activity is a simplified behavioural measurement of the complex decision-making process or motivation of that individual (Martin 2003), therefore individual variation is expected to be present. Metabolic rate is related to the generation of energy, which in turn is correlated with behavioural output (see Biro & Stamps 2010 and references within). I found non-significant relationships between routine MR and all measured movement traits, including spontaneous activity. Such a result suggests that there is not a strong mechanistic link between energy expenditure and movement behaviour, at least within the context of the present study.

Dispersive behaviours have been previously found to occur earlier in males than in females in *Tribolium* species (Prus 1966; Ogden 1970b), therefore I expected that movement behaviour would potentially differ between the sexes. Dispersal of both sexes in *T. castaneum* occurs shortly after sexual maturity is attained (Ziegler 1976). Unmated male *T. castaneum* are generally more emigratory and exploratory than unmated females (Prus 1966; Ogden 1970b), but not ubiquitously (Ziegler 1976). In the present study, movement behaviour did not differ between sexes, with the exception of higher spontaneous activity in males. The general consensus that unmated males (as in the present study) would be more actively exploring to seek females is supported by the finding that around 85% of male-female contacts are initiated by the male (Arnaud & Haubruge 1999). The higher spontaneous activity in males that I observed adheres to this prediction that males would be more active, however movement behaviour, which may be a suitable proxy for dispersal behaviour, does not. The findings of the present study suggest that both sexes respond relatively equally (with substantial individual variation) in terms of exploratory behaviour within the maze once mature. This response could be a result of both sexes responding to stimulus from the novel environment, either perceiving it as a hostile environment or seeking out food, conspecifics, or shelter. To improve our understanding of the exploratory response in *T. castaneum*, it would be useful to compare the movement or dispersal of mated and unmated mature individuals kept in mixed or single-sex cohorts prior to measurements. Furthermore, providing alternate environments with and without a food source, conspecifics, and suitable shelter could improve the ecological relevance of the exploratory behaviour assessment.

The process of maturation has significant effects on many behavioural and physiological traits of individuals. The reduced energy expenditure by immature individuals is likely a result of the combination of lower energy requirements until the onset of sexual maturity, as well as maintaining a relatively inactive state. This inactivity is also likely to provide protection from conspecifics during the cuticle sclerotisation and maturation process. Certainly, from eclosion to the onset of sexual maturity there are significant biological changes occurring within the individual, many of which I have demonstrated through their movement behaviour, physiology, and morphology. For behavioural, ecological, evolutionary, and dispersal-based studies, taking into account the timing and effect of maturity status and age when applying experimental treatments or selection is clearly important. The relative stability of the mean and variance of traits after the onset of maturity meant that only beetles aged at least 4 days post-eclosion were used in Chapter 3–5. In order to understand behavioural syndromes and their underlying physiological mechanisms, it is essential to be informed about the trajectories of the traits of interest throughout the portion of adult life where dispersal and reproduction occurs.

Chapter 3

Functional traits in red flour beetles: the dispersal phenotype is associated with leg length but not body size nor metabolic rate

Abstract

Individuals vary in their ability to disperse. Much of this variation can be described by covarying phenotypic traits that are related to dispersal (constituting the ‘dispersal phenotype’ or ‘dispersal syndrome’), but the nature of the associations among these traits are not well understood. Unravelling the associations among traits that potentially constitute the dispersal phenotype provides a foundation for understanding evolutionary trade-offs due to variation in dispersal. Here I tested five predictions pertaining to the relationships among physiological, morphological, and movement traits that are associated with dispersal, using a species with a long history as a laboratory model for studying ecological phenomena, red flour beetles (*Tribolium castaneum*). I identified a dominant axis of movement ability that describes variation in dispersal-related movement traits. Individuals that scored positively on this axis moved at higher speed, travelled longer distances, had lower movement intermittency, and dispersed quicker to a specified area. Relative leg length, but not body size nor routine metabolic rate related positively with movement ability, indicating a likely mechanistic relationship between increased stride length and movement ability. These data suggest that the dispersal phenotype may be more strongly linked to morphological traits than physiological ones. I demonstrate that associations among many functional traits do not necessarily conform to *a priori* expectations, and predict that the substantial intra-specific variation in trait values may be important for selection. Movement is a complex behavioural trait, but it has a mechanistic basis in locomotor morphology that warrants further exploration.

Introduction

The dispersal phenotype, or dispersal syndrome, is the specific expression of multiple phenotypic (physiological, morphological, and behavioural) traits that facilitate efficient movement and effective dispersal (Ronce & Clobert 2012). The genetic and demographic structure of a population is significantly affected by the patterns of covariation among traits constituting the dispersal phenotype (Ronce & Clobert 2012), and by the variation in dispersal phenotypes among individuals (Bowler & Benton 2005, 2009; Shaw & Kokko 2014).

An animal's size is often related to a range of physiological and fitness-related traits, including metabolic rate (White & Kearney 2013), predation success (Blanckenhorn 2000), mate choice (Jennions & Petrie 1997), fecundity (Honěk 1993), and movement propensity (Stevens *et al.* 2014). Across a range of taxa, body size correlates positively with movement and dispersal ability such that larger individuals have a stronger propensity to move, or move a greater distance (e.g. Anholt 1990; Benard & McCauley 2008; Sekar 2012; Stevens *et al.* 2012; Whitmee & Orme 2013). Explanations for a positive correlation are often directed towards the larger size or enhanced development of locomotor appendages that facilitate movement (Phillips *et al.* 2006; Laparie *et al.* 2013; Stevens *et al.* 2014). However, there are also examples where body size correlates negatively with dispersal (e.g. Hanski *et al.* 1991), or intermediate sized individuals move more (e.g. McDevitt *et al.* 2013) due to complex interactions between the phenotype, population structure, and the environment. In invasive cane toads (*Rhinella marina*), dispersive individuals at the edge of their range that had a stronger propensity to move had longer bodies and greater endurance (Llewelyn *et al.* 2010), and relatively longer legs (Phillips *et al.* 2006). Functional locomotor morphology, such as leg length, wing shape, or supporting muscle architecture for limbs tends to relate positively with different movement characteristics and also has consequences for energy expenditure (Roff & Fairbairn 1991; Choi *et al.* 2003; Ducatez *et al.* 2012; Lowe & McPeek 2012).

Rates of energy expenditure under different contexts are a particularly well-studied area of animal physiology with considerable attention devoted to the inter- and intra-specific variation in metabolic rate (Glazier 2005; Burton *et al.* 2011; White & Kearney 2013). Metabolic rate (MR), basally or at rest, is a proxy for maintenance energy expenditure and is strongly associated with temperature (Clarke & Fraser 2004), body size (White 2011), and physical activity (Speakman & Selman 2003). While there is certainly an association between resting MR and levels of voluntary activity, the mechanism and direction of this association is generally not well understood. These relationships can however, be divided into two general models: performance and allocation (Careau *et al.* 2008). The performance model suggests that individuals with a higher MR at rest are able to attain and invest greater amounts of energy into activity (e.g. salmonids that have a higher standard MR are more aggressive (Cutts *et al.* 1998)). Alternatively, the allocation model suggests that the total energy available for an individual is partitioned between resting MR and activity, such that individuals with a lower resting MR have more energy left to allocate to activity (e.g. mice forced to run more to receive a food reward reduced their resting MR (Vaanholt *et al.* 2007)).

If the partitioning of energy into dispersal is a trade-off with energy available for other traits, then actively dispersing individuals would be expected to have a different phenotype to those that are less exploratory or active (Clobert *et al.* 2009). Even within a species, the morphology of locomotor structures can affect MR. For example, large wing morphs of sand field crickets (*Gryllus firmus*) have a higher MR than smaller wing morphs due to the higher cost of maintaining the larger flight muscle tissues (Crnokrak & Roff 2002). MR appears to be related with dispersal, such that individuals from invasion front populations generally have higher MR than individuals that are less effective at moving (Haag *et al.* 2005; Niitepõld *et al.* 2009; Myles-Gonzalez *et al.* 2015).

Behaviours associated with dispersal are diverse and often challenging to measure. Proximate measurements that have been found to correlate with dispersal include distance travelled (Ducatez *et al.* 2012), movement speed (Phillips *et al.* 2006; Delgado *et al.* 2010), boldness and exploratory behaviours (Rehage & Sih 2004; Cote *et al.* 2010b), and measures of locomotor activity (Socha & Zemek 2003). Intuitively, many movement traits will be fundamentally linked, and therefore strongly associated (e.g. speed and distance travelled). As animal movement and ecology can be altered by behavioural patterns such as travelling along straight or tortuous paths (Brown *et al.* 2014), or moving intermittently (Bazazi *et al.* 2012), examining trait associations remains crucial to understanding drivers of movement. Simple measures of locomotor activity can, in some species, be broadly representative of complex movement behaviours. For example, spontaneous activity; the observable activity of an individual when not specifically externally stimulated (Ewing 1963), is used as a proxy for exploratory behaviour in *Drosophila* species (Martin 2003). The application of spontaneous activity as a simple movement metric in species other than *Drosophila* is rare. Hence, examining the associations between spontaneous activity and more complex measurements would be useful to identify whether a simple measurement may be a suitable alternative to more complex movement metrics.

In this study, I investigated dispersal phenotypes by examining the associations among body size, relative leg length, energy expenditure, and movement behaviour in the red flour beetle, *Tribolium castaneum* (Herbst 1797). This species and its close relative *T. confusum* have long been used as models for laboratory-based microcosm and demographic studies of dispersal (e.g. Naylor 1961; Ziegler 1976; Zirkle *et al.* 1988; Campbell & Hagstrum 2002; Łomnicki 2006; Melbourne & Hastings 2009), however morphology, physiology, and movement behaviour have not been studied concurrently with this study system. I aimed to test the following predictions. First, that the morphological and physiological traits (body size, leg length, and metabolic rate) are all associated. Second, that body size is positively associated with movement, such that larger individuals move farther and faster. Third, that leg length is positively associated with movement, such that individuals with longer legs relative to body size would have a greater capacity to move quickly and efficiently. Fourth, that routine energy expenditure has an association with movement, supporting either the performance or the allocation model of energy expenditure. And finally, that spontaneous activity may be an appropriate proximal measurement for more complex movement traits.

Material and methods

Study species and housing

A laboratory population of *Tribolium castaneum* (Figure 3.1a) established from a wild-type line (QTC4) that was sourced from the Postharvest Grain Protection Team (Department of Agriculture, Fisheries and Forestry; Brisbane, QLD, Australia) was used throughout experiments. Stocks were maintained on 200 g of flour medium (95% wholemeal stoneground wheat flour: 5% torula yeast) in 1 L cylindrical containers under controlled conditions of 29.5 ± 1 °C and cultured fortnightly to separate cohorts and refresh the medium. Pupae were collected from the stock containers and sexed by examining the external genitalia (Halstead 1963) under an Olympus SZ61 stereomicroscope (Olympus Australia Pty. Ltd., Notting Hill, VIC, Australia). Groups of five pupae were separated by the date of eclosion and by sex, and then placed in 70 mL containers with 5 g of flour that was replaced fortnightly. Age in this study ranged from 5–105 days posteclosion, where age was known within ± 12 h. At least 24 h prior to taking measurements, beetles were placed in empty 70 mL containers to fast before respirometry. A total of 290 individuals (n = 145 males and n = 145 females) were each measured once only for multiple traits in the following order: metabolic rate and spontaneous activity, movement behaviour, mass, and morphometrics.

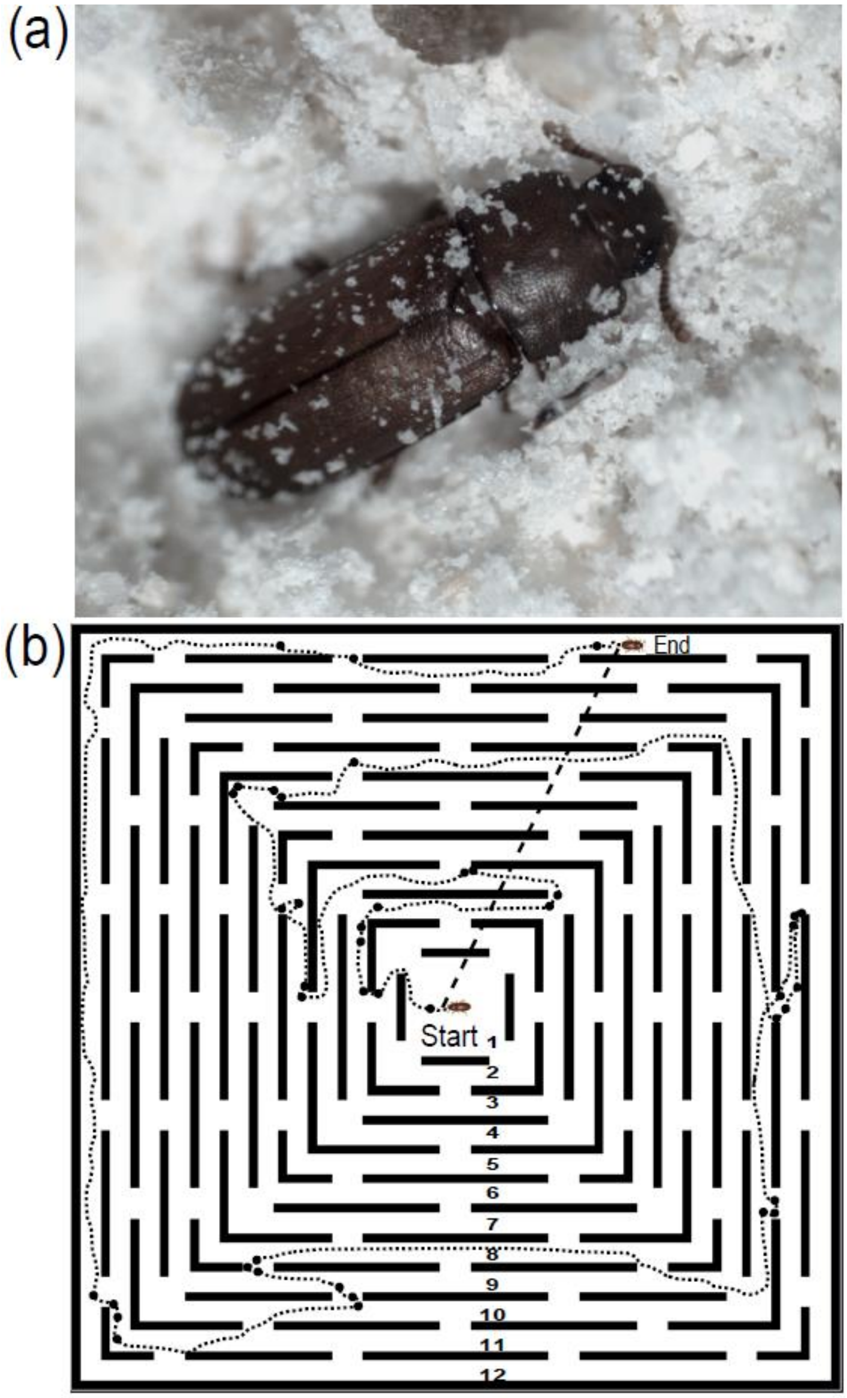


Figure 3.1 (a) Adult red flour beetle *Tribolium castaneum* (Herbst, 1797) moving through wheat flour. Photograph by Pieter Arnold. (b) Complex maze environment based on 12 evenly-spaced square radial passageways used to quantify movement. A typical movement path taken by an individual beetle is shown where the dotted line represents total path length and dashed line represents linear distance travelled from start to end of the trial. Points of no movement during the trial (behavioural intermittence) are shown as solid circles.

Trait measurement

All trait measurements were conducted as described in detail in Chapter 2. Briefly, four beetles were measured at once, where each individual was placed into one of four 2 mL chambers in-line with a flow-through respirometry system utilising two LI-7000 CO_2/H_2O analysers (Li-COR Inc., Lincoln, NE, USA) to measure CO_2 production through respiration over a 1 h period. CO_2 production was used as a proxy for metabolic rate (Lighton 2008), which as measured in this study, was defined as routine metabolic rate (routine MR) due to the presence of spontaneous activity during measurement (Mathot & Dingemanse 2015). Spontaneous activity was measured synchronously with routine MR by using an infrared activity detector (LAM10H; TriKinetics Inc., Waltham, MA, USA). For each discrete interruption of the infrared beam a value of 1 was recorded, and the total number of counts over the measurement period was converted to counts/h.

Following spontaneous activity measurements, individuals were placed in a maze (Figure 3.1b), which represents a spatially complex environment with barriers to movement and multiple junctions. In an open arena, *T. castaneum* will actively move towards and remain along the arena edge (Arnold PA, pers. obs.), whereas in a maze, movement is typically more varied among individuals and multiple movement attributes can be easily measured. Movement through the maze was video recorded (1280 × 720 pixels; 10 fps) for 3 mins, and then individual movement paths were digitized and tracked in MATLAB software (The MathWorks, Inc., Natick, MA, USA) using an extended Kalman filter (Hedrick 2008). Minimum and maximum movement speed (calculated as the lower and upper 5th percentiles of speed during the trial), behavioural intermittence (as frequency of stops during the trial), path length (total length travelled during the trial), linear distance travelled (straight line distance between their positions at time 0 and time 180 s), and time to maze edge (reach the outer passageway) were calculated. Fresh mass (mg) was measured, and then individuals were photographed dorsally and ventrally to measure elytron (modified hardened forewing; dorsal) length and width, pronotum (thorax; dorsal) length and width, and hind femur length using ImageJ software (v1.46r, National Institutes of Health, Bethesda, MD, USA).

Statistical analyses

Data were tested for normality, homogeneity of variance, and the presence of interactions. Spontaneous activity was centred and scaled around zero according to the *Z*-distribution for analyses. The morphometric measurements of elytron length and width, pronotum length and width, and mass were partially collinear; therefore a Principal Components Analysis (PCA) was conducted to distil these body size traits into a single trait (the 1st Principal Component, PC1, which explained 89.4% of the variance among the five traits; Table 3.1), hereafter called 'body size'. A second PCA was conducted to condense path length, linear distance, minimum speed, maximum speed, behavioural intermittence, and time to maze edge into two traits (Table 3.1). PC1 explained 62.3% of the total variance among the movement traits, and was loaded most strongly by path length and both measures of speed (positively), and behavioural intermittence (negatively), and is therefore called 'movement ability' hereafter. PC2 explained a further 17.7% of the variance among movement traits, and was loaded most strongly by linear distance travelled (positively) and time to maze edge (negatively), and is therefore called 'movement displacement' hereafter. Leg length and routine MR are both presented as body size corrected residuals. Data analyses were conducted in the R software environment version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria), utilising the linear mixed-effects model *lme4* v1.0.4 package (Bates *et al.* 2014), the multi-model interference *MuMIn* v1.15.6 package (Bartoń 2012). Linear mixed-effects regression (LMER) models were fitted, and then models were simplified using conditional model averages based on Aikaike weights of > 0.004 to subset the model, then removal of near-zero importance models by fitting a cumulative sum of Aikaike weights to ≤ 0.995. No interactions were significant after the model simplification process.

Table 3.1 Principal Components Analysis and relative loading of morphological and movement traits onto Principal Components that describe body size, movement ability (PC1), and movement displacement (PC2).

Loadings	Body size		Movement	
Body size traits	PC1	Movement traits	PC1	PC2
Elytron length	0.302	Path length	0.492	-0.153
Elytron width	0.154	Linear distance travelled	0.221	0.753
Pronotum length	0.098	Minimum speed	0.384	-0.409
Pronotum width	0.153	Maximum speed	0.463	-0.012
Body mass	0.923	Behavioural intermittence	-0.471	0.192
–	–	Time to maze edge	-0.355	-0.454
Eigenvalue	0.0644		3.737	1.063
Proportion variance	89.45%		62.29%	17.72%

Results

Associations among body size, leg length, and MR

Body size is typically a strong predictor of MR, and here I found that routine MR was significantly positively associated with body size (Figure 3.2a; *Z* = 2.17, *P* = 0.03), but had no relationship with relative leg length (Figure 3.2b; *Z* = 0.68, *P* = 0.496). Unsurprisingly, leg length was significantly positively associated with body size (Figure 3.2c; *Z* = 5.81, *P* < 0.001) as larger individuals typically also had longer legs. Given the scaling of routine MR and leg length with body size, these traits were corrected for body size in further data presentation. Descriptive statistics for all traits are shown in Table 3.2.

Table 3.2 Descriptive statistics (means ± 95% CIs) for all traits that were measured and calculated in this study.

	Male	Female
Trait	Mean (± 95% CI)	Mean (± 95% CI)
Age (days post-eclosion)	35.14 (30.1 – 40.2)	35.14 (30.1 – 40.2)
Routine MR (µL CO_2/h)	5.23 (5.03 – 5.42)	5.49 (5.29 – 5.69)
Spontaneous activity (counts/h)	182.4 (156.1 – 208.7)	134.1 (114.5 – 153.6)
Mass (mg)	2.392 (2.354 – 2.430)	2.504 (2.467 – 2.540)
Elytron length (mm)	2.526 (2.509 – 2.542)	2.590 (2.576 – 2.605)
Elytron width (mm)	1.290 (1.281 – 1.298)	1.313 (1.301 – 1.320)
Pronotum length (mm)	0.808 (0.801 – 0.814)	0.824 (0.817 – 0.831)
Pronotum width (mm)	1.181 (1.173 – 1.188)	1.206 (1.199 – 1.213)
Femur length (mm)	0.705 (0.695 – 0.714)	0.715 (0.705 – 0.724)
Path length (mm)	1769 (1701 – 1839)	1814 (1737 – 1891)
Linear distance travelled (mm)	192.9 (184.7 – 200.0)	197.4 (189.4 – 205.4)
Average speed (mm/s)	9.83 (9.45 – 10.22)	10.08 (9.65 – 10.50)
Minimum speed (mm/s)	4.13 (3.91 – 4.35)	4.21 (3.97 – 4.45)
Maximum speed (mm/s)	16.86 (16.21 – 17.51)	17.27 (16.53 – 18.00)
Behavioural intermittence (freq.)	310.0 (291.3 – 328.7)	297.3 (282.2 – 312.4)
Time to maze edge (s)	115.9 (108.0 – 123.9)	112.3 (104.4 – 120.2)
Body size (PC1)	-0.066 (-0.107 – -0.025)	0.066 (0.027 – 0.105)
Movement ability (PC1)	-0.110 (-0.430 – 0.211)	0.110 (-0.199 – 0.419)
Movement displacement (PC2)	-0.020 (-0.172 – 0.132)	0.020 (-0.163 – 0.203)

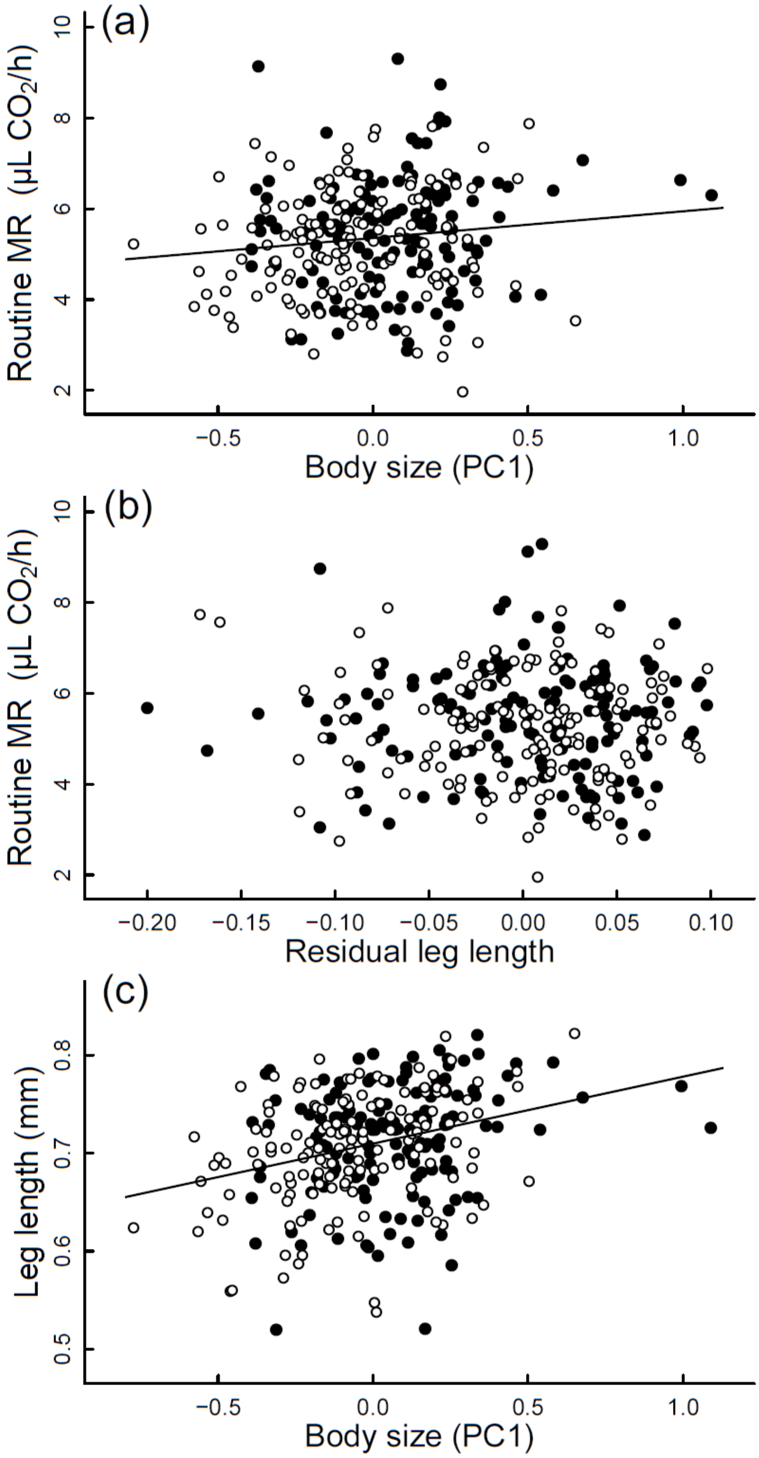


Figure 3.2 Associations among physiological and morphological traits: (a) body size and routine MR, (b) residual leg length (adjusted for body size) and routine MR, and (c) body size and leg length. Regression lines represent significant associations only. Males (*n* = 145) are represented by unfilled circles (○) and females (*n* = 145) are represented by filled circles (●).

Body size and movement

Body size was not positively associated with any measurement of movement, contrary to the prediction that larger individuals should be able to move farther and faster than smaller individuals. Body size was not a significant predictor of movement ability ($Z = 1.39$, $P = 0.166$; Figure 3.3a), or movement displacement ($Z = 0.46$, $P = 0.643$; Figure 3.3d). However, there was a significant negative relationship between body size and spontaneous activity ($Z = 2.04$, $P = 0.04$; Figure 3.3g), where larger individuals were typically less active than smaller ones.

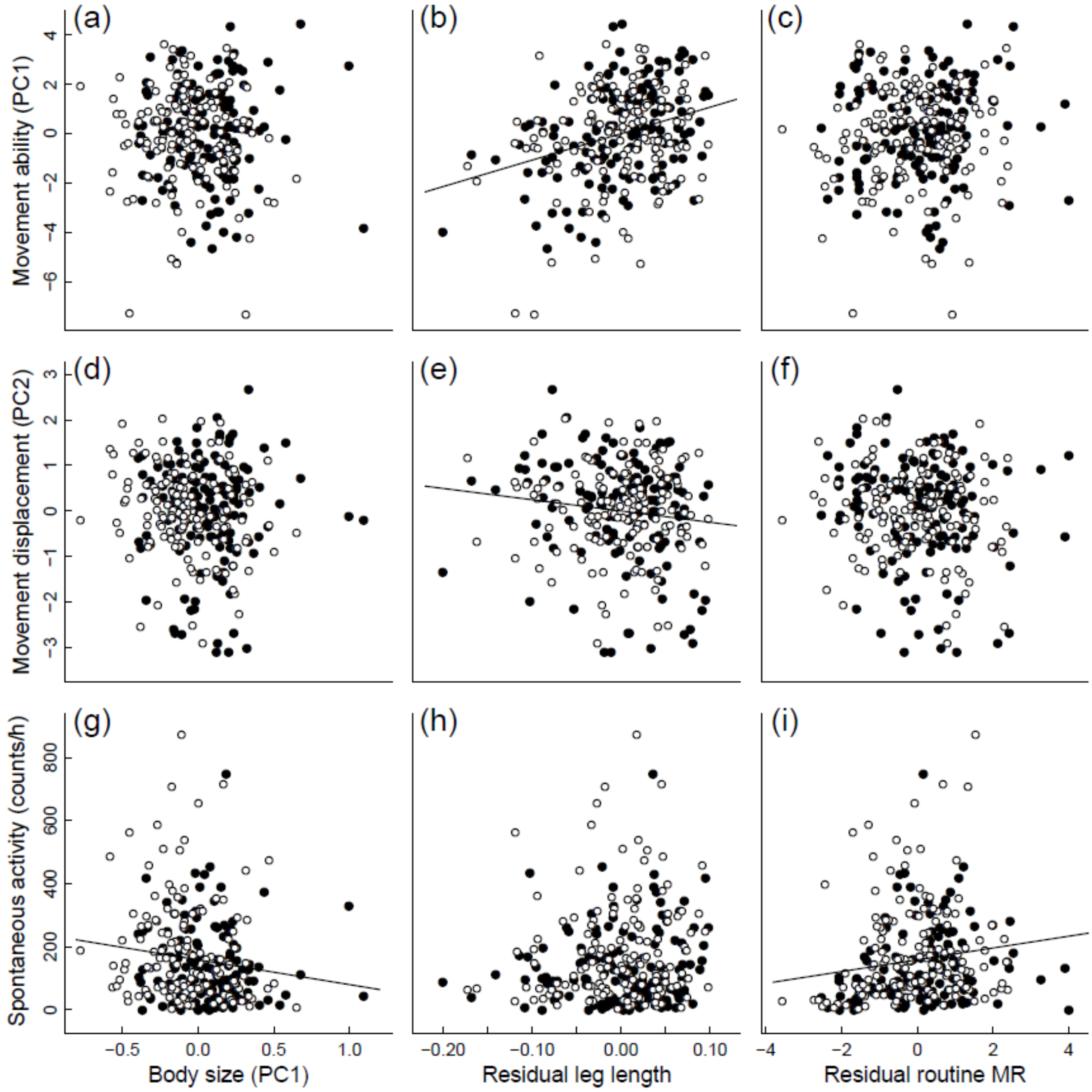


Figure 3.3 Associations among six dispersal-related traits. Body size, residual leg length (adjusted for body size), and residual routine MR (adjusted for body size) were predictor variables for three movement traits: (a–c) movement ability (PC1), (d–f) movement displacement (PC2), and (g–i) spontaneous activity. Regression lines represent significant associations only. Males (*n* = 145) are represented by unfilled circles (○) and females (*n* = 145) are represented by filled circles (●).

Relative leg length and movement

Leg length relative to body size was significantly associated with two measurement of movement. Residual leg length was positively related to movement ability ($Z = 5.97$, $P < 0.001$; Figure 3.3b), which is consistent with the prediction that individuals with longer legs relative to their body size would move farther and faster. However, residual leg length was also significantly negatively associated with movement displacement ($Z = 2.42$, $P = 0.015$; Figure 3.3e); individuals with relatively longer legs ultimately moved less distance away from their starting position. Relative leg length was not significantly related to spontaneous activity ($Z = 1.13$, $P = 0.257$; Figure 3.3h).

Routine MR and movement

Routine MR corrected for body size was only significantly associated with one measurement of movement. Routine MR was not significantly related to movement ability ($Z = 1.18$, $P = 0.236$; Figure 3.3c), or to movement displacement ($Z = 1.95$, $P = 0.051$; Figure 3.3f), however this association was marginally significant and trends negatively. Routine MR was significantly positively related to spontaneous activity ($Z = 4.02$, $P < 0.001$; Figure 3.3i), which is unsurprising given that the two traits were measured concurrently, and that increased activity increases MR.

Spontaneous activity as a proxy for movement

Spontaneous activity has been suggested to be a simple measure for complex movement behaviour. But here spontaneous activity did not relate significantly to movement ability ($Z = 1.55$, $P = 0.120$; Figure 3.4a) or movement displacement ($Z = 0.84$, $P = 0.402$; Figure 3.4b). Full tables of averaged LMER models with the movement traits as response variables, including covariates are presented in the Appendix (Table A3.1 and Table A3.2).

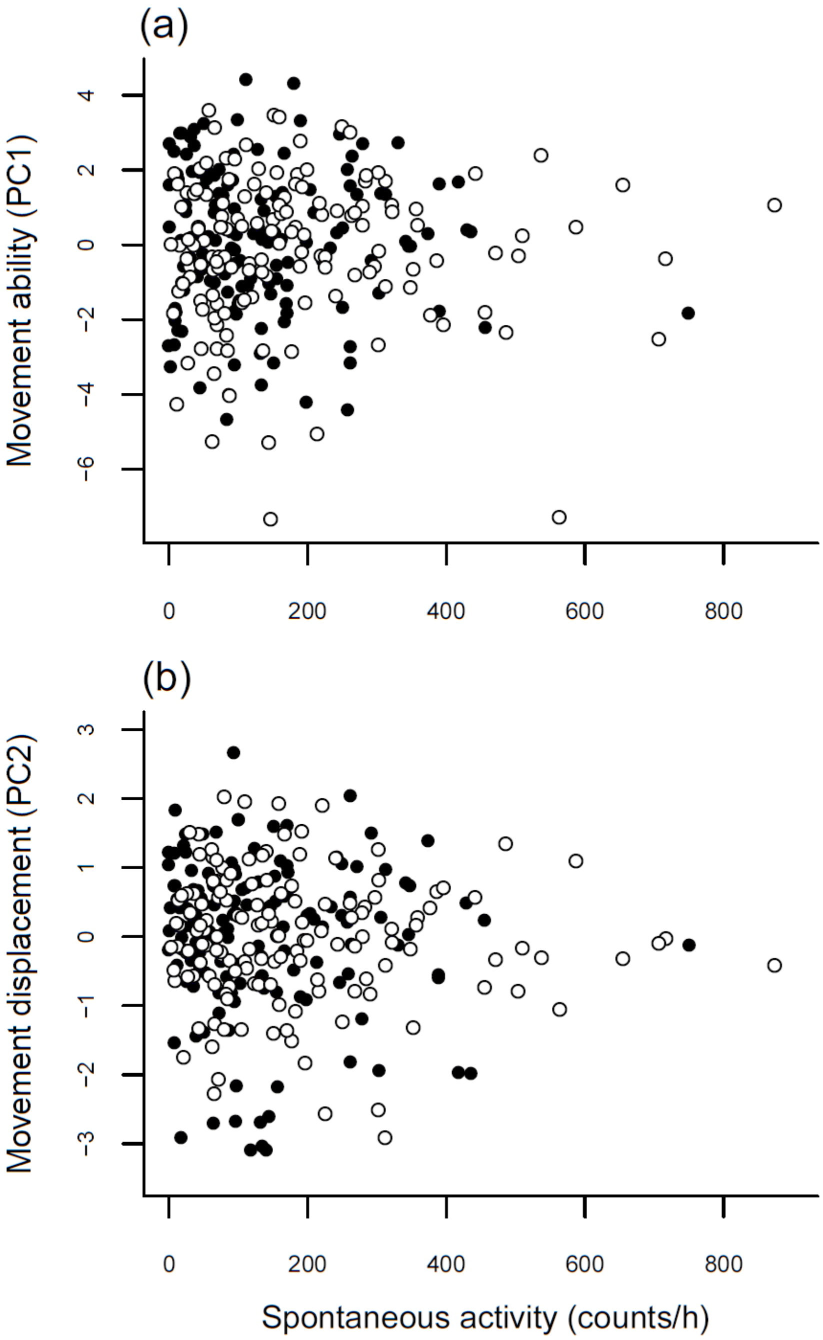


Figure 3.4 Associations among three movement traits. Movement ability (PC1) and movement displacement (PC2) are intrinsically uncorrelated due to the nature of Principal Components Analysis, therefore not shown. Associations between: (a) movement ability and spontaneous activity, and (b) movement displacement and spontaneous activity. Neither relationship is significant. Males (*n* = 145) are represented by unfilled circles (○) and females (*n* = 145) are represented by filled circles (●).

Discussion

The present study demonstrates that while the associations among physiological, morphological, and movement traits associated with the dispersal phenotype may seem relatively intuitive; the strength, variance, and even the direction of relationships may not be. Certainly, the well-established relationships of routine MR and leg length scaling with body size were significantly positively related as expected (Kleiber 1947; White & Kearney 2014). However, contrary to some predictions, I found that only relative leg length, but not body size nor metabolic rate appear to relate positively to movement ability. Given the substantial variance found in most traits measured in this study, the possibility of small effects of body size and metabolic rate on movement remains possible.

Larger individuals were predicted to have a greater movement ability and displacement; however the data presented here do not support this hypothesis. A positive size-dispersal relationship has been found in some species (e.g. Anholt 1990; Stevens *et al.* 2012), but in others, small or intermediate sized individuals may be the ones to disperse (Hanski *et al.* 1991; McDevitt *et al.* 2013). The size-dispersal relationship frequently depends on the context and environment that triggers dispersal events (Benard & McCauley 2008), and the results presented here suggest that short ground-based movement may not strongly relate to within-species variation in body size. Large body size has potential to handicap movement in some species (Benton & Bowler 2012a), and smaller, less competitive individuals may disperse before larger individuals to reach and exploit resources earlier (Bowler & Benton 2005). Larger individuals were less active in the metabolic chambers during routine MR measurement. If the animals can perceive that the metabolic chamber is an energy-limited environment, then larger individuals may opt to conserve energy by reducing activity, as the absolute cost of movement is greater for larger individuals relative to smaller ones (Halsey 2016).

Movement ability was the most representative measure of variance among all dispersal-related traits. Individuals that scored positively along the axis of movement ability had a high minimum and maximum speed, a greater path length, less behavioural intermittence, and reached the maze edge quickly. Highly dispersive individuals have been suggested to share these movement characteristics, and this movement axis may be useful to determine dispersal or diffusion rates. Similarly related movement traits have been identified in dispersive cane toads from the edge of their range, which travelled farther and faster for longer periods without pausing compared to non-dispersive toads (Phillips *et al.* 2006; Alford *et al.* 2009; Llewelyn *et al.* 2010).

I found that individuals with relatively longer legs had greater movement ability. This relationship could be explained mechanistically by longer legs allowing an increased stride length, in turn allowing the animal to move a greater distance with each step taken, therefore also achieving a greater speed (Zollikofer 1994). A long-term study of stream salamanders (*Gyrinophilus porphyriticus*) found that individuals with long forelimbs relative to their hindlimbs dispersed greater distance, indicating that locomotor morphology has a major role in long-distance dispersal and fitness (Lowe & McPeek 2012). Evolutionary mechanisms can select for individuals that have phenotypic traits that allow them to move farther, which may indicate the presence of a dispersal phenotype (e.g. Phillips *et al.* 2006; Laparie *et al.* 2013). Whether selection for greater movement or dispersal increases the length of locomotor structures independent of body size can only be directly determined through experimental evolution. The finding of a significant negative relationship between relative leg length and movement displacement may be a continued searching behaviour. That is, individuals with relatively longer legs have a greater path length and reach the maze edge quickly, but upon reaching the edge, these individuals may back-track and return towards the maze centre, reducing their overall displacement. This suggests that these individuals may have more tortuous movement paths and it is plausible that the PC2 of movement may also be analogous to path straightness.

I predicted that routine MR would be associated with movement, supporting either the performance or allocation hypotheses. Routine MR was not significantly related to either movement ability or displacement, but was with spontaneous activity. This relationship is intuitive; the more an individual moves during measurement of its energy expenditure, the greater its energy expenditure will be (Mathot & Dingemanse 2015), and thus may not be representative of movement or dispersal. Under the performance model of energy expenditure, individuals with greater performance (movement, in the present case) should have a higher MR (Careau *et al.* 2008). Under the allocation model, individuals with a higher resting MR have less energy remaining to allocate to activity (Careau *et al.* 2008). The marginally significant negative relationship between movement displacement and routine MR suggests that individuals with a high routine MR potentially travelled less distance overall.

The data describing this relationship was highly variable, and the allocation model cannot be assessed directly in this study as both maintenance energy and activity are confounded in the measure of routine MR. Resting MR has been previously found to be higher in individuals with greater movement propensity (Niitepõld *et al.* 2009; Myles-Gonzalez *et al.* 2015) and a recent study found that genes underlying metabolic processes are upregulated in dispersive cane toads (Rollins *et al.* 2015). Given the potential involvement of genetic and epigenetic mechanisms, and potential metabolome differences between dispersive and non-dispersive individuals (Rollins *et al.* 2015; Van Petegem *et al.* 2015; 2016b), it may be worth exploring the association between MR and movement in other systems.

These data presented here do not support either the performance model or the allocation model of energy expenditure, and adds to a collection of studies demonstrating that MR does not always correlate with components of performance or fitness (e.g. lifespan: Hulbert *et al.* 2004; growth and survival: Burton *et al.* 2011; reproductive performance: Schimpf *et al.* 2012; locomotor performance: Le Galliard *et al.* 2013; aerobic performance: Merritt *et al.* 2013; scaling: White & Kearney 2013; behaviour: Royauté *et al.* 2015). Under different environmental contexts, the relationship between MR and other traits, or between MR at rest and during activity (i.e. maximum or flight MR) can change and must be cautiously interpreted (e.g. Burton *et al.* 2011; Killen *et al.* 2012; Killen *et al.* 2013; Lebeau *et al.* 2016; Metcalfe *et al.* 2016). Variation in MR is maintained in most natural populations allowing for different strategies for growth, behaviours, and reproduction in specific contexts (Burton *et al.* 2011). Additionally, historical findings that established distinct paradigms of trait associations, including with MR, tend to be based on small sample sizes and therefore low statistical power, which may undermine identification of the true relationship (Button *et al.* 2013).

The final prediction considered that spontaneous activity may be a useful approximate measure of complex movement traits. Spontaneous locomotor activity is thought to represent the activity response of an animal when not specifically stimulated (Ewing 1963) and is the basis of many complex behaviours (Martin 2003). In the present study, neither movement ability nor displacement had any discernible association with spontaneous activity, and the relationship between morphological and physiological traits and movement were not similar to those observed with spontaneous activity. Therefore, at least for *T. castaneum*, spontaneous activity was not a useful proximate trait for complex movement traits.

The results presented here demonstrate that associations among traits are not always intuitive, and do not always conform to expectations from literature paradigms. I found that much of the variance among different movement traits can be described along a dominant axis of variation; movement ability. I have shown here that movement ability (which potentially represents dispersal) is more strongly linked with locomotor morphology than body size or MR. These findings support the hypotheses that locomotor morphology could be a considerable source of variation in distributing individuals through a landscape by spatial sorting or natural selection, thereby accelerating rates of range expansion in invasive species (Shine *et al.* 2011). The movement ability axis and the significant trait associations identified here can be used as a foundation for studying variation in movement and dispersal. *Tribolium castaneum* represents an ideal model species to test this hypothesis using experimental evolution in a laboratory setting (Chapter 5).

Chapter 4

Investigating movement in the laboratory: dispersal apparatus designs and the red flour beetle, *Tribolium castaneum*

Abstract

The natural dispersal of *Tribolium* beetles (Coleoptera: Tenebrionidae) has been emulated in the laboratory for more than 50 years, using a simple dispersal apparatus. This has typically comprised of a starting container (initial resource or patch) connected by tubing, which contains thread for the animals to climb into the tube and subsequently to an end container. That is, beetles move to a new viable resource or patch from an inter-patch zone or non-viable habitat. I modified this basic apparatus design to test the effect of tubing length and tubing insertion angle on the dispersal rate and proportion of successful dispersers. It was expected that the proportion of successful dispersers would be repeatable within each apparatus design, and that increasing tubing length and steepness of the insertion angle would reduce dispersal rate and success across apparatus designs. I found that dispersal success increased linearly through time, at a similar rate for both males and females. The design with the most vertical tubing insertion angle had a lower proportion of successful dispersers, allowing dispersers and non-dispersers to be clearly distinguished. Tubing length also had a negative relationship with dispersal success (as judged by insects reaching the end container), but a significant reduction in dispersal success was only apparent between the shortest and longest tubing between containers. I suggest that locating and climbing the vertical section of string before they can enter the tubing between containers restricts dispersal and that, at higher densities, insects have greater inclination to climb. This type of apparatus has further potential to study the dispersal of *T. castaneum*, as well as other small insect species that primarily use pedestrian locomotion.

Introduction

Flour beetles of the genus *Tribolium* (Coleoptera: Tenebrionidae), particularly *T. castaneum* and *T. confusum*, are major pests of a wide range of grain species and processed stored products globally, and are known to have a high rate of movement among resource patches (Campbell & Hagstrum 2002; Ahmad *et al.* 2012). A diverse array of approaches have been used to study the movement of *Tribolium* beetles including laboratory apparatuses (e.g. Prus 1963; Łomnicki 2006), warehouse scale patch exploitation arenas (e.g. Campbell & Hagstrum 2002; Campbell & Arbogast 2004), and landscape scale sampling (e.g. Ridley *et al.* 2011). Here I focus on movement in apparatuses. Most apparatus designs consist of connected containers that adult beetles can move between, allowing dispersers and non-dispersers to be separated over time. The main phases of successful dispersal can be observed with this design; the inclination to move away from a resource patch (initial container), survival through an unsuitable inter-patch zone (tubing between patches, or in intermediate containers of various suitabilities), and establishment into a new patch (final container) (Bowler & Benton 2009). While this has been the most widely used approach, little consensus has emerged on certain apparatus design attributes.

Prus (1963) described an apparatus to study the tendency for beetles to emigrate from a vial containing flour (*A*) to an empty vial (*B*) by climbing cotton thread into a tube. The tubing was inserted through the vial lids (bridging the two vials) and the thread, which dangled onto the surface in *A* but not in *B*, permitted only one-way movement from *A* to *B*. Emigration rate of beetles from *A* to *B* was repeatable over 10 days and multiple experimental replicates (Prus 1963). Despite inexplicit design specifications (with the exception of tubing internal diameter; 4.5 mm, and initial density: 32 beetles per 8 g of flour), the repeatability of the recorded behaviour and design simplicity ensured its longevity in subsequent studies of *Tribolium* dispersal. Modifications to the Prus design such as that of Ogden (1970a, b) included flour medium in the second vial, and also defining the length, shape and material of tubing (Tygon tubing, U shaped and 150 mm in length), container size (29.5 mL), and population density (either 32 or 50 individuals per 8 g of flour). Ziegler (1976) utilised pipe cleaner, in place of thread, and glass tubing, but described no other design parameters.

The Prus design was used as a platform for investigating *Tribolium* emigration in response to numerous factors, including population density, age, artificial selection for activity or emigration, and also fecundity and life-history responses to selection for emigration (Prus 1966; Zyromska-Rudzka 1966a, b; Ogden 1970a, b; Ziegler 1976; Ritte & Lavie 1977; Ziegler 1977; Lavie & Ritte 1978, 1980; Riddle & Dawson 1983; Zirkle *et al.* 1988; Goodnight 1989; Ben-Shlomo *et al.* 1991). Ritte and Lavie (1977) induced divergent selection on dispersal in just one generation by selecting for beetles that moved from vial *A* to *B* (via a 30 cm long polyvinyl tube), twice in two opportunities, as high dispersers and those that did not move as low dispersers. Łomnicki (2006) used a one-way dispersal apparatus including five beakers of various size (*A*–*E*), three of which contained flour (*A*, *C*, *E*) and two of which were unsuitable habitats (i.e. empty: *B*, *D*), all connected by glass tubing containing string. Dispersal through this apparatus was far slower than previous designs, suggesting that the extra patches limited the successful dispersal rate.

Despite the differences among designs, many of these studies reached similar conclusions. Emigration tendency was low in immature individuals, peaked around sexual maturity, and declined later in life. Males emigrated more rapidly than females when the sexes were kept separately, and keeping the sexes mixed resulted in an overall emigration rate that was intermediate between those of the sexes separately (Prus 1963; Ogden 1970b; Ziegler 1976; Riddle & Dawson 1983). Dispersal was dependent on density and age of infestation; this was thought to be a response of repulsion to flour that was ‘conditioned’ by chemical secretions and frass accumulation over time (Zyromska-Rudzka 1966a; Ogden 1970b). A discernible difference across the emigration rates of lines selected for dispersive and non-dispersive behaviour was identified after five generations of selection (Ogden 1970a) and dispersal behaviour has an underlying genetic component (Ritte & Lavie 1977; Lavie & Ritte 1978; Riddle & Dawson 1983).

Studies on *Tribolium* dispersal in the laboratory have almost ubiquitously used the apparatus design of Prus (1963) with modifications to the container size, tubing length and material, container arrangement, population density, and time period. However, many studies have not provided detailed specifications of apparatus components. The length of tubing between containers (which represents the inter-patch dispersal component), the angle at which the tubing is inserted into container lids (which may increase dispersal difficulty) are unstated, or appear to vary significantly among studies. The importance of these factors to dispersal success has not been investigated previously. In the present study, I have implemented and tested design aspects derived from apparatus revisions by addressing the following questions: 1) does increasing tubing length affect the proportion of successful dispersers and the dispersal rate; 2) does tubing insertion angle affect the proportion of successful dispersers?; and 3) is dispersal rate repeatable within an apparatus design? I predicted that longer tubing and more vertical tubing insertion angle would reduce the proportion of successful dispersers. I addressed these questions using *T. castaneum* and five dispersal apparatus designs, while manipulating tubing insertion angle and length.

Materials and methods

Animals and housing

A wild-type population of *T. castaneum* (QTC4) was sourced from the Postharvest Grain Protection Team (Department of Agriculture, Fisheries and Forestry; Brisbane, QLD, Australia) and used to establish experimental stocks. The QTC4 strain originated from a storage facility in Brisbane (QLD, Australia) in 1965. It has been cultured ever since in the absence of selective pressures from the fumigant phosphine, and therefore these insects exhibit natural susceptibility to phosphine. Stocks were maintained on 210 g of flour medium containing wholemeal stoneground wheat flour (Kialla Purefoods; Greenmount, QLD, Australia) and torula yeast (Lotus Foods Pty. Ltd., Cheltenham, VIC, Australia) at a ratio of 19:1, in 1 L cylindrical containers at 29.5 ± 1 °C and 40–60% relative humidity. Stocks were cultured fortnightly to maintain clean housing and separate cohorts. Beetles used in experiments were collected from stocks as pupae, and sex was determined by examining the external genitalia (Halstead 1963) under an Olympus SZ61 stereomicroscope (Olympus Australia Pty. Ltd.; Notting Hill, VIC, Australia). After sorting by sex, pupae were randomly added to 70 mL containers that held 15 g of flour medium in groups of 50, such that each experimental replicate had five containers (i.e. 250 male and 250 female pupae, and a total of 3000 individuals over six experimental replicates). They were held for six days to allow the resultant adults to reach three days of age post-eclosion before the 70 mL containers were attached as container *A* in the dispersal apparatus, for the dispersal experiments to commence.

Dispersal apparatus designs

Five designs were chosen to test the dispersal capacity of *T. castaneum*, based mostly on the designs of Prus (1963), Ogden (1970a), and Łomnicki (2006). Variables that I manipulated were the length of the tubing and the angle of the tubing as it left and entered containers. Each design used three 70 mL containers (57 × 44 mm, labelled *A*, *B*, and *C*, respectively; Sarstedt Australia Pty. Ltd., Mawson Lakes, SA, Australia) connected through the lid of each container via silicone tubing (4 mm internal diameter), containing a single looped strand of cotton twine that permitted only one-way movement from container *A* to *B* to *C*.

As the angle of tubing was negatively associated with the distance between containers, only Designs 1 and 2 were compared to determine the effect of tubing insertion angle independently of length. Tubing length in Design 1 was 140 mm, with a distance of 70 mm between the tube insertions, yielding a relatively steep insertion angle (55°) for the tubing (Figure 4.1a) to represent the extended vertical climbing distance employed in the design of Prus (1963). Design 2 used the same tubing length as Design 1 (140 mm) over a greater distance between the tube insertions (120 mm), creating a shallower slope (24°) in the tubing (Figure 4.1b). Designs 3, 4, and 5 all had relatively shallow tubing insertion angles (4–16°); these designs were included to test if increasing tubing length reduced dispersal success. Design 3 used 185 mm long tubing over 165 mm (Figure 4.1c), Design 4 used 335 mm long tubing over 310 mm (Figure 4.1d), and Design 5 employed 640 mm long tubing over a distance of 620 mm between tubing insertions (Figure 4.1e). The distances between insertion points in containers in the different dispersal apparatuses were structurally maintained using plywood housing to fix the containers a set distance apart, level with each other and aligned linearly (Figure 4.2).

Dispersal assessment

Each design used two apparatuses, one for males and one for females, to assess dispersal for each sex separately but concurrently. Sexes were not mixed for two reasons: 1) to eliminate the potential for breeding to occur during the dispersal process, which has been shown to slow the rate of dispersal (Ziegler 1976) and 2) to emulate the conditions required for a subsequent experiment that controlled breeding after the dispersal assessment (Chapter 5). The containers with 50 adult beetles of known sex, labelled container *A*, were randomly assigned to an apparatus design and attached to each apparatus, representing the starting point of dispersal (Figure 4.2). Both *A* and *C* contained 15 g of flour and container *B* had a covering of paper to provide grip, but was otherwise an unsuitable habitat for the beetles. Container *B* was included to represent a patch that would not be a suitable resource to establish in, but that had to be passed through as part of the dispersal process (Łomnicki 2006).

Dispersal apparatuses were placed in a controlled temperature room with identical conditions to the stock populations and apparatus position was randomised at the beginning of each experimental replicate. Once container *A* was connected to each of the apparatuses, dispersal assessment commenced and counts were made during 0830–0930 h and 1630–1730 h daily for 96 h (nine counts). Counts of beetles in the connecting tubes (*A*–*B* and *B*–*C*) and in containers *B* and *C* were recorded. Both sets of tubing and container *B* could be counted visually without disturbing the apparatus, however container *C* required detachment. Flour was carefully tipped into a container and gently swept with a paintbrush to draw beetles to the flour surface for counting. Flour and beetles were then funnelled back into container *C* and reattached to the apparatus. Container *A* was left undisturbed throughout the experiment to facilitate natural dispersal through the apparatuses. The number of beetles in container *A* was calculated at each time point by subtracting the total number of beetles in all other containers and tubing from the starting total of 50. Mortality was assessed at the conclusion of each experiment, but was negligible (two adults at most in any given replicate). The assessment was replicated six times, each with a different cohort of beetles.

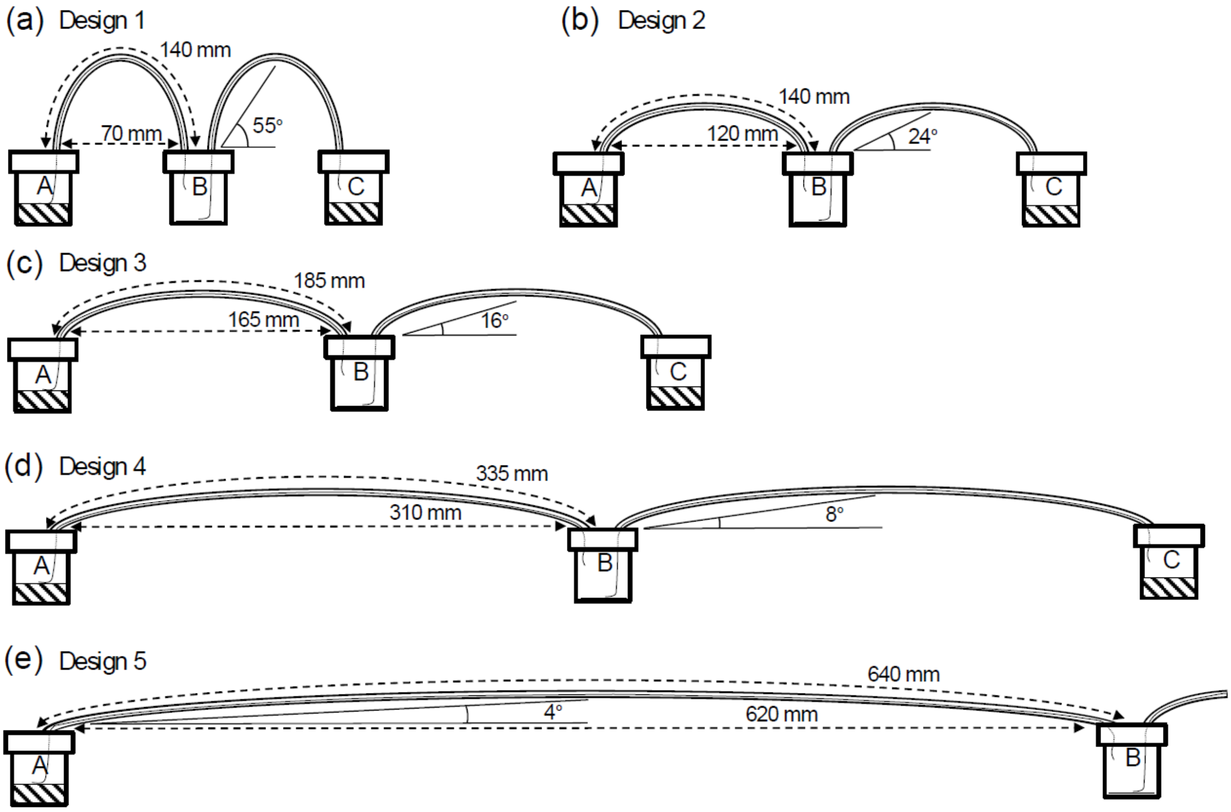


Figure 4.1 Dispersal apparatus designs used to investigate the effects of tubing angle and length on the dispersal success of adult *T. castaneum*. Each design used three containers (*A*, *B*, and *C*) connected by tubing containing string for beetles to climb, allowing one-way movement from *A*–*B*–*C*. (a) Design 1; 55° tubing insertion angle between containers over 140 mm tubing length, (b) Design 2; 24° tubing insertion angle over 140 mm tubing length, (c) Design 3; 185 mm tubing length, (d) Design 4; 335 mm tubing length, and (e) Design 5; 640 mm tubing length. Design 5 *B*–*C* is identical to *A*–*B* but not shown due to its size. Curved dashed arrows show tubing length; straight dashed arrows show distance between insertion points, and angles show the approximate angle from the point of insertion to the maximum height of the tubing over the distance between insertion points.

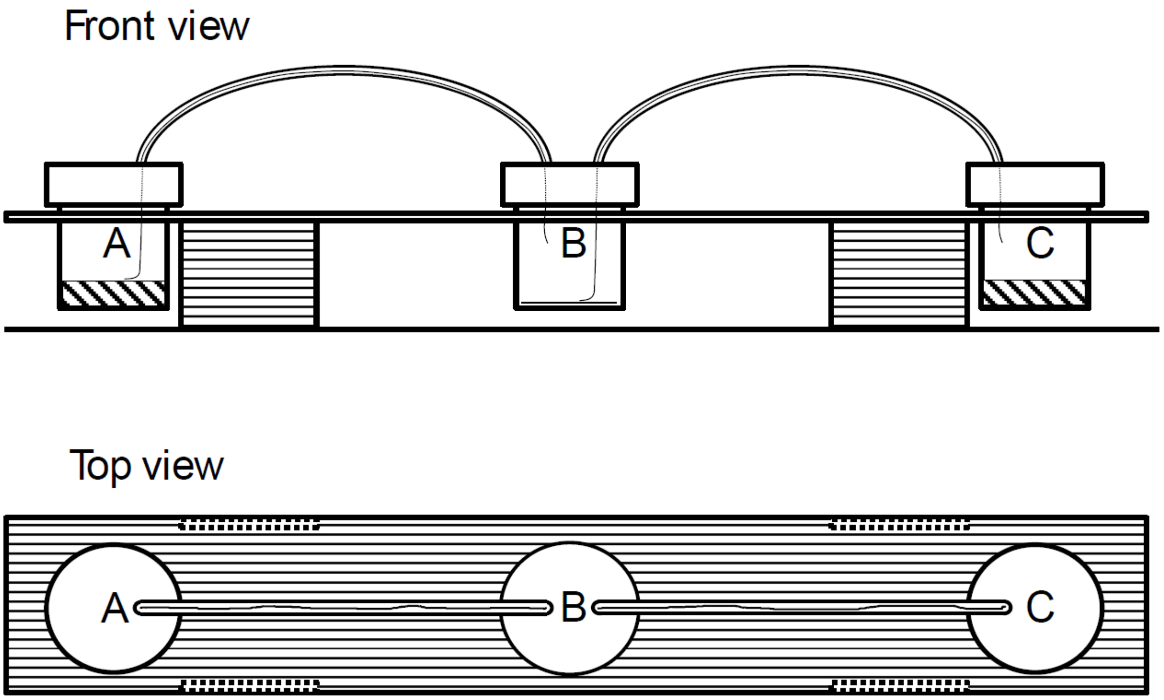


Figure 4.2 Example schematic of Design 3. Front and top views of the plywood housing for maintaining structure and consistency of the tubing insertion angle and tubing length for the dispersal apparatuses.

Statistical analyses

Data were treated as proportional data, where the principle variable of interest was proportion of beetles reaching container *C* (successful dispersers). Fixed factors were apparatus design, tubing length, insertion angle of tubing, sex, and time. Replicate (cohort) was treated as a random factor in all analyses. A full model was fitted using a generalised linear mixed effects regression model with a binomial error structure and logit link function from the *lme4* package (Bates *et al.* 2014) in R 3.2.3 (R Development Core Team 2015). Models were simplified by removing non-significant interaction terms and using ANOVA and Akaike information criterion (AIC) to compare the resulting simplified models. Data presented are mean proportions of beetles reaching container *C* ± SE, and α was set at 0.05.

Results

Dispersal rates and success

The proportion of beetles that reached container *C* at any given time was relatively consistent across all designs and between sexes, where males are presented separately to females

(Figure 4.3). The proportion of successful dispersers increased significantly with time and was consistent across cohorts, following a near-linear trajectory to about 60–80% successful dispersal after 96 h (Figure 4.3). Time was positively correlated with the proportion of successful dispersers and was highly significant for all apparatus designs, but no difference between male and female beetles was detected (Table 4.1). Design 2 was chosen as the reference apparatus against which the other designs were compared as it had the shortest tubing with a shallow insertion angle (Table 4.1). This design was predicted to, and did, yield the fastest dispersal rates in both sexes. Only Design 5 had a significantly lower proportion of successful dispersers than Design 2, whereas all other designs were not significantly different from this design or from each other (Table 4.1).

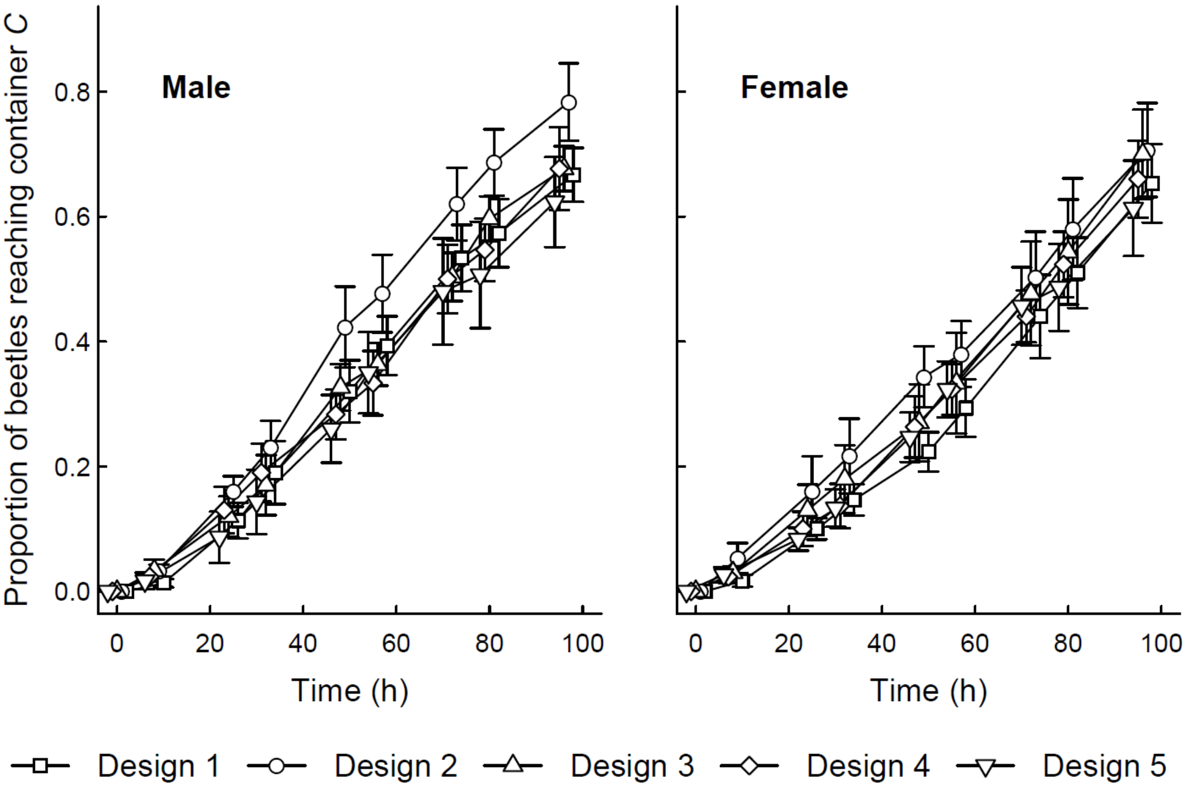


Figure 4.3 Mean proportion of male and female *T. castaneum* adults reaching the final container *C* over time across the five apparatus designs. Data are presented as means ± SE from six independent replicate cohorts.

Table 4.1 Generalised linear mixed effects regression model (GLMER) of the effect of time, design, and sex on the proportion of beetles successfully dispersing from *A* to *C*. Designs are all compared to reference Design 2 (short tubing length and shallow insertion angle).

Coefficient	Estimate ± SE	*Z*	*P*
Intercept	-3.788 ± 0.187	-20.3	<0.001
Time (hours)	0.555 ± 0.001	72.11	<0.001
Design 1	-0.412 ± 0.233	-1.76	0.078
Design 3	-0.321 ± 0.233	-1.38	0.168
Design 4	-0.42 ± 0.233	-1.8	0.072
Design 5	-0.565 ± 0.234	-2.42	0.016
Sex	0.206 ± 0.148	1.39	0.164

Tube length

Increasing tubing length between containers had a negative relationship with the proportion of successful dispersers (i.e. as tubing length increased from 140 mm in Design 1 to 640 mm in Design 5, the regression slope decreased; Table 4.1). The proportion of successful dispersers was lowest in Design 5, where tubing length was greatest between containers, and this was significantly different to Design 2 (Table 4.1). Effectively, the greater the distance between containers, the longer it takes the beetles to move between them. Therefore the overall proportion of beetles in container *C* at the end of the experiment was lower than the designs with shorter tubing between containers (Figure 4.3).

Tubing insertion angle

The angle that tubing projected from the lids of each apparatus container was predicted to have a significant effect on the proportion of successful dispersers, by increasing the difficulty of dispersal thereby reducing the attainable dispersal rate. Designs 1 and 2 were directly comparable with respect to determining the effect of insertion angle independent to tubing length, therefore these were the only designs included in the model (Table 4.2). Time had a significant positive effect on the proportion of successful dispersers and males and females were not significantly different from one another, consistent with the previous model. There was a significant negative effect of tubing angle on the proportion of successful dispersers suggesting that the more vertical tubing angle in Design 1 reduced dispersal success compared to Design 2, which had more horizontal tubing (Table 4.2).

Table 4.2 Generalised linear mixed effects regression model (GLMER) of the effect of time, sex, and tubing insertion angle on the proportion of successful dispersers from container *A* to *C* between Designs 1 and 2.

Coefficient	Estimate ± SE	*Z*	*P*
Intercept	-3.339 ± 0.388	-8.61	<0.001
Time (hours)	0.559 ± 0.012	46.72	<0.001
Sex	0.338 ± 0.223	1.52	0.129
Tubing angle	-0.642 ± 0.299	-2.15	0.032

Discussion

Apparatuses to simulate long-distance dispersal in the laboratory using *Tribolium* beetles have been used for more than 50 years, however many of these studies have not specified apparatus design attributes. In particular, the slope and length of tubing between containers were expected to be critical to the movement of the individual beetles, as even shallow increases in the slope of a gradient can affect the distribution and orientation of *Tribolium* beetles (Graham & Waterhouse 1964). This is consistent with our finding that increasing the steepness of the tubing angle between containers reduces dispersal success. The energy required for insects to climb vertically is much greater than that required to move laterally (Full & Tullis 1990), and as distance and time spent moving along a steep incline increases, the frequency of potential climbing errors increases. The slope of the tubing limited dispersal across designs with identical tubing length, but between designs with different tubing lengths, the difference in number of successful dispersers was small (e.g. Designs 1 and 4). While the effect of tubing insertion angle is significant, if tubing length is increased (i.e. to about 30 cm as in Ritte and Lavie (1977)), the effect of the steep climb may be offset.

The significant effect of tubing angle suggests that the near-vertical section of string that must be climbed prior to reaching the tubing also reduces dispersal rate. The string climbing ability of *T. castaneum* has not been explicitly tested, but this species can climb paper materials (Cline & Highland 1976), readily climbs up string within an apparatus (Ogden 1970b), attempts to climb the walls of housing (Ghent 1963; Surtees 1963), and is frequently seen climbing bag stacks and walls in storages (GH Walter, pers. obs.). Across all of the designs tested in the present study, a near-vertical portion of string (about 30 mm long) came immediately before the section of tubing where the angle could then be engaged by the climbing beetle. This section requires an ability to climb successfully upwards into the tubing, which plateaus, and then descends towards the next container. It seems likely that the near-vertical climb prior to the tubing would also constrain dispersal rate, and this may partially explain the exceptionally slow dispersal rate of *T. confusum* in the study by Łomnicki (2006), as the beakers used there had a vertical string section greater than 40 mm between the flour surface and the tubing. Therefore, the care needs to be taken to ensure that the near-vertical section of string is consistent in length, as it has a similar or perhaps stronger effect on dispersal rate than the tubing insertion angle. As *Tribolium* beetles are highly mobile animals, this design component is essential to constrain movement to sort dispersers from non-dispersers over a practical period of time.

While we found an overall significant decrease in the proportion of successful dispersers with increased tubing length, this effect was only statistically significant when comparing the shortest (140 mm) and the longest (640 mm) tubing lengths between containers. The long tubing used in Design 5 reduced the proportion of successful dispersers but was only significantly lower than Design 2. Additionally, the extreme length between containers of Design 5 made it impractical due to size. For the remaining tubing lengths between the containers (140–335 mm) dispersal rates were similar, which indicates that at least within a practical range, time spent within the apparatus tubing does not strongly limit dispersal.

In *Tribolium* beetles, dispersal is dependent on habitat deterioration, where increasing population density or reducing flour volume increases dispersal rate due to conditioning of the flour by frass accumulation, nutrient depletion and release of quinones by adults (Zyromska-Rudzka 1966a; Ogden 1969). Adult *T. castaneum* are strongly repelled by the smell of same-sex conspecifics, and this repulsion is enhanced when flour becomes ‘conditioned’ (Naylor 1961; Ghent 1963; Ogden 1970b). Thus, unmated individuals in a container with conspecifics will readily disperse as the flour becomes increasingly conditioned, and as finding mates becomes a priority. Therefore, the essentially linear increase in successful dispersers over time may reflect the decreasing population density as individuals emigrate and the continuous but ever-decreasing rate of flour conditioning as population density decreases.

We did not identify a significant difference in dispersal success between the sexes overall, but a greater proportion of males dispersed successfully in Design 2. Males were predicted to disperse faster than females, as previous studies have found males are more active or exploratory in dispersal apparatuses (Prus 1966; Ogden 1970b). However, Ziegler (1976) found that dispersal rates were similar across males and females, as in the present study. We suggest that the absence of potential mates, and repulsion by the scent of same-sex individuals and conditioned flour drove dispersal at a similar rate in both sexes.

The present study demonstrates that tubing length and tubing insertion angle, which have been inconsistent among previous studies, can alter dispersal success for this species but not to the extent that dispersers cannot be effectively sorted from non-dispersers. This general apparatus appears to have relatively flexible design tolerances, and can achieve consistent, controlled dispersal over replicate experiments. For the logistics of assessing dispersal ability of *T. castaneum*, which is highly active, restricting dispersal rate is important. We suggest that in addition to tubing length and insertion angle, the process of locating and climbing the vertical string section, and the inclusion of an intermediate container, reduces dispersal to a practical rate. In the present study, the time taken for more than 50% of individuals to successfully disperse across apparatus designs (about 70–85 h) would be feasible to experimentally separate dispersers from non-dispersers. Design 3 was chosen for use in the experiments presented in Chapter 5. Apparatus designs with a manageable tubing length and more horizontal tubing angle (i.e. Design 2 or 3) could be used to assess dispersal of other small insects that use pedestrian locomotion, including potential and current pest species. More than 50 years after its conception, the laboratory dispersal apparatus remains useful for assessing dispersal and addressing questions in microcosm-based species ecology.

Chapter 5

Experimental evolution of phenotypic traits associated with dispersal through artificial spatial sorting: divergence in body size, but not behaviour

Abstract

Dispersal ability varies significantly among individuals. Dispersal syndromes are suites of covarying morphological, physiological, behavioural, and life-history traits that describe much of the observed variation in dispersal ability. While dispersal syndromes have been well studied within and among populations, our understanding of the evolutionary trajectories of traits associated with dispersal under selection is limited. Here, I used the red flour beetle (*Tribolium castaneum*) in a laboratory dispersal system to assess how selection via spatial assortative mating for and against dispersal, affected dispersal syndrome traits. Body size rapidly diverged over seven generations; non-dispersers increased in size while dispersers decreased in size. Once mass differences had been accounted for, limb length and movement behaviour were not different between dispersers and non-dispersers, but metabolic rate was lower in dispersers. Small individuals were more energetically and biomechanically efficient at climbing, and were therefore the ones that dispersed more. Dispersal rate and movement did not differ among dispersers and non-dispersers; the variance in dispersal rate and movement was maintained even under intensive selection, indicating that individuals may maximise their fitness by producing offspring that exhibit a variety of dispersal behaviours. Investigating dispersal syndromes in species that exhibit multiple modes of locomotion will improve our understanding of the evolution of complex movement strategies as a whole.

Introduction

Dispersal is any movement of an individual that has the potential to contribute to gene flow (Ronce 2007). Dispersal encompasses multiple complex biological processes at various spatial and temporal scales: from individual decision-making to population dynamics (Benton & Bowler 2012a; Matthysen 2012). The traits associated with dispersal vary greatly among individuals and populations and can have significant consequences for fitness (Benard & McCauley 2008; Bonte *et al.* 2012). The morphological, physiological, and behavioural traits that inherently underlie movement capacity and decisions, and therefore dispersal, are identified as a dispersal syndrome (a suite of correlated traits, also known as the 'dispersal phenotype') (Ronce & Clobert 2012).

Understanding the mechanisms, costs, trade-offs, and stability of dispersal syndromes, rather than traits individually, is paramount to a more complete understanding of dispersal ecology and evolution (Ronce & Clobert 2012). Dispersal phenotypes can be as extreme as complete polymorphisms within a population (Roff 1984, 1986; Zera & Denno 1997), or result from continuous variation in a suite of correlated traits resulting in a continuum of movement ability from residents (non-dispersers) to dispersers (Ducatez *et al.* 2012; Ronce & Clobert 2012). Morphological, physiological, behavioural, personality, or life-history traits generally display covariation in a dispersal context due to mechanistic links among traits or pleiotropic effects of underlying genes. For example, body size scales with locomotor apparatus size and thus individuals that are larger are typically expected to have a greater dispersal capacity (Stevens *et al.* 2014; Chapter 3).

In Glanville fritillary butterflies (*Melitaea cinxia*), flight metabolic rate and dispersal ability are positively correlated and both mechanistically and genetically linked to the *pgi* gene that encodes phosphoglucose isomerase, a glycolic enzyme involved in central metabolism (Haag *et al.* 2005; Hanski *et al.* 2006; Niitepõld *et al.* 2009). Another example of correlated traits contributing to a dispersal syndrome is the cane toad (*Rhinella marina*) invasion in Australia. Invasion-front individuals that are highly dispersive have larger locomotor structures relative to body size (Phillips *et al.* 2006), a faster growth rate (Phillips 2009), higher endurance capacity (Llewelyn *et al.* 2010), and move greater distances per movement event and over time, while following straighter paths (Alford *et al.* 2009; Lindström *et al.* 2013) than more resident individuals from older, established populations. The characteristics identified in highly dispersive *R. marina* are postulated to have arisen as an outcome of spatial assortative mating ('spatial sorting') interacting with natural selection to lead to spatial selection (Shine *et al.* 2011; Perkins *et al.* 2013).

The variance in dispersal ability among individuals provides an opportunity for selective processes to shape dispersal-related traits, particularly if the traits comprise an underlying dispersal syndrome. Spatial assortative mating relies on dispersal ability variation, such that individuals with traits that facilitate dispersal will accumulate at range edges and persist through proximity-limited mating between highly dispersive individuals (Travis & Dytham 2002; Phillips *et al.* 2008; Phillips *et al.* 2010b; Shine *et al.* 2011). Alternatively, individuals with increased dispersal ability may arise by natural selection for dispersal, but only if this change confers a fitness advantage (Shine *et al.* 2011). Both spatial sorting and natural selection may increase the rate of range expansion or dispersal acceleration, and consequently, the associated phenotypic, genetic, or behavioural traits may be augmented at range edges or invasion fronts. Evidence for the evolution of dispersal ability and dispersal-associated traits at range edges has been demonstrated across diverse taxa (Chuang & Peterson 2016). Broadly, traits that may characterise dispersive individuals are a larger body size (Hill *et al.* 1999; Gutowsky & Fox 2012; Kelehear *et al.* 2012; Brown *et al.* 2013; Laparie *et al.* 2013), larger locomotor appendages relative to body size (Phillips *et al.* 2006; Forsman *et al.* 2010; Therry *et al.* 2014b), a better body condition (Carol *et al.* 2009; Lopez *et al.* 2012; Rebrina *et al.* 2015), boldness, aggressiveness, or low sociality (Fraser *et al.* 2001; Duckworth 2006; Myles-Gonzalez *et al.* 2015) compared with residents. Individuals that disperse have also been found to grow or mature faster (Bøhn *et al.* 2004; Carol *et al.* 2009; Phillips 2009), and exhibit a generally higher metabolic rate (MR) at rest (Myles-Gonzalez *et al.* 2015) and during locomotion (Haag *et al.* 2005; Niitepõld *et al.* 2009).

Despite the many studies that have investigated traits associated with dispersal between core and range edge populations, and dispersal syndromes within a population, manipulative experiments that investigate trait evolution under selection for dispersal are relatively uncommon (Yano & Takafuji 2002; Friedenberg 2003; Łomnicki 2006; Bitume *et al.* 2011; Fronhofer & Altermatt 2015; Van Petegem *et al.* 2015). These types of studies are imperative to bridge the divide between studies within and among populations, by allowing evolutionary processes to be observed in real time, and under controlled conditions (Kawecki *et al.* 2012). Studies that have conducted experimental evolution by selecting for dispersal have investigated the responses of dispersal rate (e.g. Van Petegem *et al.* 2015) or life-history traits (e.g. Bitume *et al.* 2011), but typically not morphology (but see Łomnicki 2006), physiology, or behaviour. These functional, phenotypic traits are hypothesised to be crucial components of dispersal syndromes, and undoubtedly contribute to movement ability. Yet, experimental evolution has been underutilised as a tool to study the evolutionary dynamics of dispersal syndromes.

Laboratory microcosm experiments are appropriate systems to manipulate components of dispersal while controlling others, thereby focussing on specific aspects of dispersal (Bowler & Benton 2009; Bonte *et al.* 2014) and allowing the longitudinal study of functional traits under artificial selection. *Tribolium castaneum* (the red flour beetle) is an ideal model species with which to study the evolutionary dynamics of traits under selection for dispersal, as their dispersal in laboratory microcosms has been studied for more than 50 years (Naylor 1961; Prus 1963) and artificial selection for dispersal success has resulted in divergence in dispersal rate and life-history traits (Ogden 1970a; Ritte & Lavie 1977).

In the present study I address two questions: 1) can artificial selection for and against dispersal success by spatial assortative mating produce different dispersal phenotypes?; and 2) how do covarying traits evolve in response to selection based on dispersal? I aimed to determine, by spatial assortative mating, the evolutionary dynamics of traits that are predicted to constitute a dispersal syndrome, using *T. castaneum* over seven generations of artificial selection for and against dispersal success. The literature concerning dispersal syndromes describes general characteristics associated with disperser and resident traits (Ronce & Clobert 2012; Stevens *et al.* 2014; Chuang & Peterson 2016), particularly body size, locomotion apparatus size, and MR. In Chapter 3, I found significant positive associations among movement traits within a maze and relative leg length, but not body size or MR. Although the dispersal apparatus used here to select on dispersal relies on different mobility mechanisms than the maze tested in Chapter 3, it is conceivable that dispersal syndrome traits are tightly linked with general movement. Therefore, I predicted that selection at dispersal extremes would induce divergent trait evolution, such that dispersers would have longer legs relative to their body size and would be more active, moving farther and faster than residents.

Material and Methods

Animals and experimental conditions

The animals used were drawn from a laboratory population of *T. castaneum* (QTC4; Postharvest Grain Protection Team; Department of Agriculture, Fisheries and Forestry; Brisbane, QLD, Australia). Stocks were maintained on 210 g of flour medium (95% wholemeal stoneground wheat flour, 5% torula yeast) in 1 L cylindrical containers. This flour medium was also used in the 70 mL dispersal apparatus containers. All animals were housed in a controlled temperature room at 29.5 ± 1 °C and 40–60% relative humidity.

Founding animals

The founding 400 beetles (200 male and 200 female) were collected and sexed as pupae (following Halstead 1963) from a single stock that has not been experimentally evolved previously, and then divided into eight single-sex starting groups of 50. Pupae that were newly pupated (soft and translucent) or close to eclosion (partially melanised and responsive) were not used, to regulate age consistency within and among experimental cohorts. Groups of 50 individuals represented replicate lines that had a corresponding line pair of the opposite sex (Figure 5.1). Each group was housed in a 70 mL container with 15 g of flour for six days after collection as pupae. Lines were maintained as single-sex groups throughout dispersal assessment to prevent breeding, which has been demonstrated to reduce dispersal rate (Ogden 1970b) and the efficacy of artificial selection.

Dispersal apparatus and population dispersal rate

The dispersal apparatus consisted of three containers (*A*, *B*, *C*) connected in sequence by tubing inserted through the lids, containing cotton twine allowing one-way movement by climbing. Containers were 70 mL polypropylene (57 × 44 mm, Sarstedt Australia Pty. Ltd., Mawson Lakes, SA, Australia) and tubing was flexible silicone 185 mm in length (4 mm internal diameter), arched over a 165 mm distance between insertions that contained a single looped strand of cotton twine. Containers *A* and *C* held 15 g of flour, and *B* had a layer of paper to facilitate grip, but no flour. In container *A*, twine rested on the flour surface allowing beetles to climb up and into the tubing; the twine terminated 15–25 mm above the paper in *B* so that beetles could not return to *A*, and similarly, from *B* to *C*. Dispersal of *T. castaneum* through this apparatus design was found to be consistent and repeatable for both males and females (Chapter 4). Containers housing each group of 50 adults were connected to their respective apparatus (as container *A*) when beetles were 2–5 days post-eclosion. The position of each apparatus within the room was randomised at each generation. Once container *A* was connected to each apparatus, beetles were allowed to disperse for up to four days. Counts of beetles within the dispersal apparatuses were conducted at around 0900hrs and 1700hrs daily (up to 8 counts over the dispersal period). This consisted of counting individuals in the tubing between *A*–*B* and *B*–*C*, and in containers *B* and *C*. Container *C* was detached, tipped into a glass container and beetles were brought to the flour surface using a fine paintbrush, counted, and then were funnelled back into container *C* for reattachment. The number of beetles in container *A* was estimated as the starting number of individuals (50) minus the sum of beetles in all other containers and tubing. Dispersal rate was calculated as the mean number of individuals that successfully dispersed through the apparatus into container *C* each day of the measurement (n in *C*/day). This was therefore an estimate of population dispersal rate, rather than individual, which was used to compare among treatment lines. Mortality in the apparatus across all lines and throughout the experiment was < 1%.

Selection criteria and breeding design

In the first generation, the eight starting lines were run through the dispersal apparatus and used to establish 24 replicate lines in three categories of selection: (i) control; (ii) residents; and (iii) dispersers. Selection of individuals in the first generation was different to all subsequent generations (Figure 5.1). Dispersal within the first generation was monitored and ceased when approximately 15 individuals remained in container *A* and had reached *C*, respectively. The purpose of this was to ensure that 15 individuals could be classified as residents and dispersers, with the remaining 15 or more individuals in the apparatus (a subsample of the original population) randomly sampled from to establish control lines (Figure 5.1). Five of these individuals were then paired with five individuals from their matching replicate line, where breeding could occur in another 70 mL container containing 5 g of flour over five days. After breeding, the 10 individuals were placed on 3 g of fresh flour and allowed an additional five days to oviposit, after which time the adults were removed to allow the offspring to develop over a period of 23 days before collection as pupae. From this pool of pupae, 50 of each sex were chosen at random to establish the next generation of each line, and this was repeated until the seventh generation of offspring (Figure 5.1).

The remaining 10 offspring from each single-sex selected line were used for phenotyping. In generations 2–7, beetles were allowed to disperse until the following criteria were met for each of the selected lines. 1) Residents: dispersal was stopped when 15–25 individuals had not yet emigrated from container *A*. After six counts, if the number of beetles remaining in container *A* exceeded 25, these were randomly sampled from as residents. 2) Dispersers: dispersal was stopped when 15–25 individuals successfully emigrated to container *C*. After six counts, if the number of beetles that had emigrated to container *C* was less than 15, these were randomly sampled from as dispersers. 3) Controls: dispersal was stopped after 72 h (five counts). Individuals were then removed from apparatus containers, mixed, and randomly sampled from as controls.

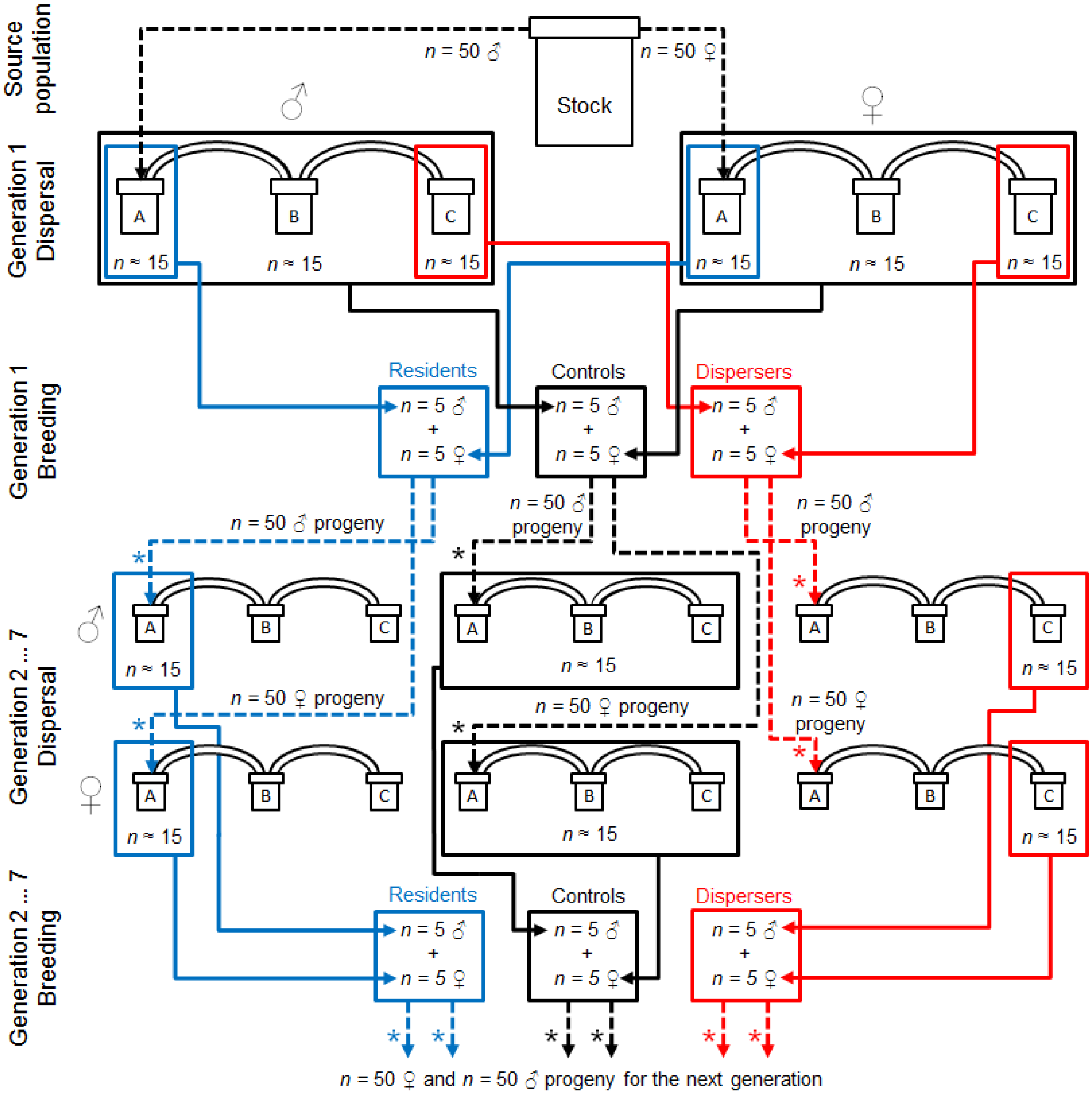


Figure 5.1 Experimental design of a single replicate (four replicates total were used) of artificial selection on dispersal. Beetles from the source population were separated into males (♂) and females (♀), then *n* = 50 were introduced into single-sex dispersal apparatuses. In generation 1, beetles dispersed until residents (red), dispersers (blue), and controls (black) had *n* ≈15 individuals that fitted each criterion (see text), then *n* = 5 males and *n* = 5 females from paired replicate lines were taken from the *n* ≈15 (solid arrows) and allowed to breed. The remaining *n* =10 individuals from each single-sex line were used for phenotyping. The progeny from the generation 1 breeders were collected and separated by sex, then *n* = 50 were used to establish the next dispersal populations (dashed arrows) as generation 2. Dispersal was allowed until each selected lines criterion was met (see text for details), then *n* = 5 of each sex from paired lines were taken from the *n* ≈15 to breed. The * symbols mark where the selection cycle repeats for generations 2–7.

Metabolic rate and activity

Rate of CO_2 production (µL CO_2/h) was measured as a proxy for metabolic rate (Lighton 2008) over 1 h and under resting conditions (29.5 ± 1 °C, dark, and fasted for 24 h). However, the beetles were highly active and resting metabolic rate (RMR) could not be established across all individuals, therefore routine metabolic rate (routine MR), which allows for some spontaneous activity, was used (Mathot & Dingemanse 2015). Individual beetles were placed in 2 mL glass chambers in-line with a flow-through respirometry system. Air drawn into the system first passed through two chemical scrubber columns (soda lime to remove CO_2 and Drierite to remove water vapour), then dry, CO_2-free air was regulated by four mass flow controllers (Aarborg Instruments and Controls, Inc.) at 25 mL/min before entering the four metabolic chambers that housed the animals. Excurrent air was passed through two LI-7000 CO_2/H_2O analysers (Li-COR Inc.) to measure CO_2 concentration with a resolution of 0.1 ppm, which was recorded at a frequency of 1 Hz using Li-Cor software. Metabolic chambers were interfaced with a LAM10H locomotion activity monitor (Trikinetics, Inc.) that used nine infrared emitter-detector beams to detect animal motion by registering each beam interruption as a value of 1. The sum of beam counts over the measurement period of 1 h was then used as a measure of spontaneous activity (counts/h) synchronous with the recording of routine MR, which was included as a covariate for all models.

Morphometrics

Individuals were weighed on a precision microbalance to attain fresh mass to 0.01 mg (XS3DU microbalance; Mettler-Toledo). Morphological measurements were taken by capturing dorsal and ventral images using a microscope-mounted camera (PL-B686; PixeLINK). Length and width of the elytron and the pronotum, and length of the hind femur were extracted using ImageJ version 1.46r software (National Institutes of Health).

Movement characteristics

Movement behaviour of individuals was characterised by assessing movement in a complex maze environment, described in detail in Chapter 2. The acrylic maze (390 × 390 × 4 mm) had 12 square passageways radiating from the central point, such that the minimum distance between each passageway was approximately equal and progression through the maze was linear. A layer of paper allowed beetles to grip the maze surface, and the transparent maze lid with the maze walls attached was placed and clamped down on the surface. Beetles were introduced into the maze through a small hole drilled in the centre of the maze lid. Each maze trial was recorded for 4 min using a webcam (1280 × 720 pixels) at 10 fps, then the video was frame-cropped from the first clear movement away from the maze centre, to 3 min (1800 frames) after. MATLAB software running a digitising tracking script (DLTdv5; Hedrick 2008) with an extended Kalman filter was used to track individual movements in the maze. Movement characteristics extracted from each track were total path length, linear distance travelled (displacement from start to end point), minimum and maximum sustained speed (5th and 95th percentiles of speed), and behavioural intermittence (the number of frames in which the animal was not detected moving).

Statistical analyses

Data were analysed using the R software environment for graphical and statistical computing version 3.2.3 (R Development Core Team 2015) using the package *lme4* (Bates *et al.* 2014). I used linear mixed effects regression (LMER) to compare the trajectories of phenotypic traits across seven generations of selection for and against dispersal. The phenotypic traits were assessed for multicollinearity, and then highly correlated variables were included in Principal Components Analyses (PCA) to simplify collinear traits. The suite of movement traits (path length, linear distance travelled, minimum speed, maximum speed and behavioural intermittence) were reduced to two traits (Table 5.1): the first two Principal Components explained 81.3% of the variance among the traits (PC1: 60.7% and PC2: 20.6%). PC1 of movement was loaded equally by path length, minimum speed, maximum speed, and behavioural intermittence, and is hereafter referred to as ‘movement ability’, and PC2 of movement was loaded almost entirely by linear distance travelled, and is hereafter referred to as ‘movement displacement’. Similarly, the suite of morphometric traits (elytron length and width, pronotum length and width, and $\sqrt[3]{\text{mass}}$) were collapsed into PC1 which explained 80.0% of the variance among the traits (Table 5.1) and is hereafter referred to as ‘body size’. Routine MR was $\log_{10}$ transformed and spontaneous activity was centred and scaled according to the *Z*-distribution for analyses. LMER models were fitted as the response trait of interest against selection, generation, sex and their interactions, while including the other phenotypic traits as covariate predictors, and respirometry channel and replicate line as random effects. Models were assessed for Gaussian residuals and error heteroscedasticity, and then ANCOVAs with type III sums of squares were calculated for each model. Due to the size of the LMER models, the full ANCOVA tables are included in the Appendix (Tables A5.1–A5.6). For data presentation, routine MR was presented as residuals that adjust for the effects of body size, spontaneous activity and age, and leg length was presented as residuals adjusting for the effect of body size. Data are presented separately for males and females, and the means ± SE of six response variables of interest under the three selection types were plotted across generations.

Table 5.1 Principal Components Analysis and relative loading of morphological and movement traits onto Principal Components that describe 'body size', 'movement ability' (PC1), and 'movement displacement' (PC2).

Loadings	Body size		Movement	
Body size traits	PC1	Movement traits	PC1	PC2
Elytron length	0.422	Path length	0.551	-0.010
Elytron width	0.473	Linear distance travelled	0.065	-0.950
Pronotum length	0.395	Minimum speed	0.455	0.249
Pronotum width	0.472	Maximum speed	0.469	-0.176
$\sqrt[3]{\text{Body mass}}$	0.468	Behavioural intermittence	-0.514	-0.069
Eigenvalue	4.00		3.034	1.030
Proportion variance	80.0%		60.7%	20.6%

Results

A note about control lines

Control lines were included to determine whether any trait changes in the resident and disperser lines were different to those that might occur by random processes. The control lines were allowed to disperse through the apparatus for 72 h, and then were mixed and randomly sampled. However, across most replicate lines, the number of control individuals that reached container *C* exceeded the number that remained in *A* before they were mixed and sampled randomly. Therefore, although the trait trajectories in the control lines were generally more similar to the dispersers than to the residents, the control lines still represent the effect of randomly selecting individuals that do or do not disperse. That variance was apparent across all traits (Figure 5.2a–l).

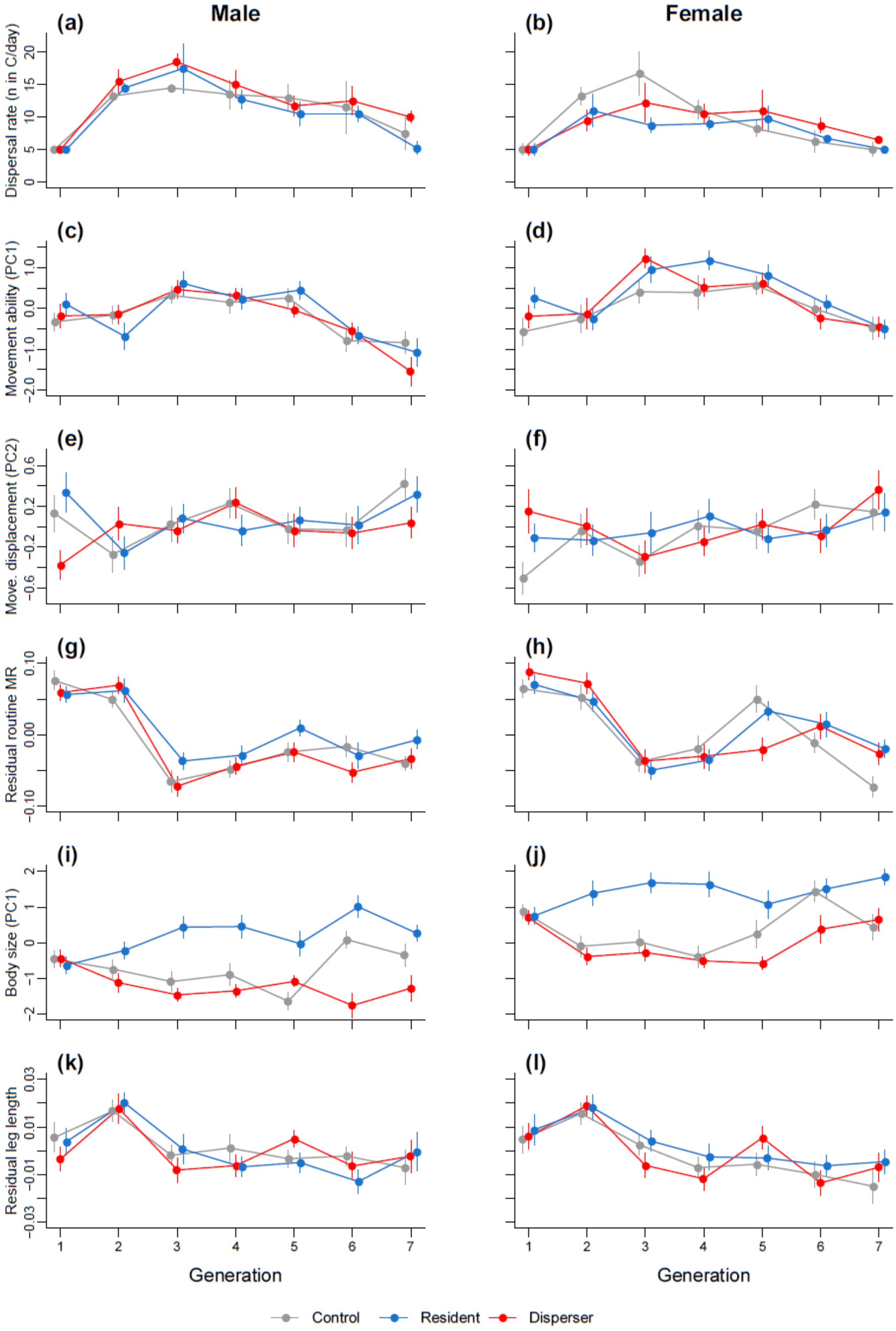


Figure 5.2 Trajectories of phenotypic traits over seven generations of artificial selection on dispersal. Males and females are presented side-by-side for visual clarity: (a, b) dispersal rate, (c, d) movement ability in the maze (PC1), (e, f) movement displacement in the maze (PC2), (g, h) residual routine MR, (i, j) PC1 score of body size, and (k, l) residual leg length. Each point is the mean of *n* ≈ 40 measurements of replicate lines ± SE.

Dispersal rate through the apparatus

The mean dispersal rate through the apparatus changed significantly throughout the experiment (full ANCOVA: Table A5.1). Dispersal rate significantly changed across generations ($F_{6,109} = 13.41$, $P < 0.001$), and this was especially apparent between generations 1 and 2 (Figure 5.2a, b). There was a marginally non-significant difference between males and females (sex; $F_{1,30} = 3.75$, $P = 0.062$), although males typically dispersed at a faster rate (Figure 5.2a, b). Despite imposing artificial selection on dispersal success, there was no difference in dispersal rate among selected lines across generations (selection × generation; $F_{12,105} = 0.21$, $P = 0.998$), however dispersers consistently dispersed faster than residents in generations 3–7 (Figure 5.2a, b). Residents took a significantly longer time for least 15 individuals to arrive in container *C* (ANOVA: $F_{1,107} = 17.52$, $P < 0.001$; Table 5.2) and between males and females (ANOVA: $F_{1,107} = 7.02$, $P < 0.01$; Table 5.2). No covariates were significantly associated with dispersal rate (Table A5.1).

Table 5.2 Summary of approximate mean time taken for ≥ 15 individuals to reach container C (h ± SE).

Generation	Time (h ± SE)			
	Residents (male)	Dispersers (male)	Residents (female)	Dispersers (female)
1	80 ± 0.0	72 ± 5.7	80 ± 0.0	78 ± 2.0
2	21 ± 0.0	24 ± 0.0	44 ± 4.0	38 ± 8.2
3	32 ± 9.8	24 ± 0.0	50 ± 8.3	24 ± 0.0
4	34 ± 5.0	26 ± 2.0	54 ± 6.0	32 ± 5.7
5	40 ± 7.3	26 ± 2.0	54 ± 11.5	40 ± 13.5
6	54 ± 10.0	30 ± 6.0	64 ± 4.6	40 ± 9.2
7	78 ± 2.0	38 ± 6.0	72 ± 5.7	58 ± 5.0
Grand mean	50.0 ± 4.3	34.3 ± 3.4	59.7 ± 3.2	44.3 ± 4.1

Movement through a complex environment

The ability of individuals to move through a complex maze environment (PC1) was not clearly altered by selection for and against dispersal success (full ANCOVA: Table A5.2). Movement ability was marginally non-significantly different among selected lines across generations (selection × generation; $F_{12,1585} = 1.63$, $P = 0.078$), but across generations movement ability varied significantly (generation; $F_{6,1586} = 26.40$, $P < 0.001$; Figure 5.2c, d). There was no clear difference among selected lines; dispersers and residents did not have consistently different movement ability from each other or the control (Figure 5.2c, d). Females, which were larger on average than males, had significantly greater movement ability (sex; $F_{1,21} = 7.90$, $P = 0.010$; Figure 5.2c, d), and movement ability was significantly positively correlated with body size ($F_{1,1559} = 13.01$, $P < 0.001$; Figure A5.1a), and with spontaneous activity ($F_{1,1581} = 11.61$, $P < 0.001$; Figure A5.1b). Movement displacement through the maze (PC2) was not clearly significantly affected by selection on dispersal (full ANCOVA: Table A5.3). Movement displacement did not differ among selected lines (selection × generation; $F_{12,1587} = 0.80$, $P = 0.654$), however it fluctuated significantly across generations (generation; $F_{6,1589} = 2.78$, $P = 0.011$; Figure 5.2e, f). Movement displacement was significantly negatively correlated with age ($F_{1,1601} = 8.49$, $P = 0.004$; Figure A5.1c) and with spontaneous activity ($F_{1,1574} = 4.52$, $P = 0.034$; Figure A5.1d).

Routine MR

Routine MR responded to artificial selection for and against dispersal success, and was associated with three covariates (full ANCOVA: Table A5.4). Routine MR significantly decreased in all selected lines over generations of selection (generation; $F_{6,1583} = 67.89$, $P < 0.001$), particularly between generations 2 and 3 where there was a considerable decrease in routine MR (mean decrease of 22% across all selected groups). Females had a higher routine MR than males (sex; $F_{1,19} = 4.70$, $P = 0.043$). Dispersers had a marginally lower routine MR than residents across generations (selection × generation; $F_{12,1583} = 1.77$, $P = 0.049$) which was more apparent in males (Figure 5.2g, h). Routine MR was highly correlated with three covariates (Figure A5.2a–c): positively with body size ($F_{1,1593} = 177.2$, $P < 0.001$), activity ($F_{1,1602} = 23.18$, $P < 0.001$), and negatively with age ($F_{1,1590} = 71.34$, $P < 0.001$), hence was presented as residuals corrected for the influence of these covariates (Figure 5.2g, h).

Body size

Body size responded rapidly to selection on dispersal, and diverged far more than any other trait measured (full ANCOVA: Table A5.5). Divergence among the selected lines occurred across generations (selection × generation; $F_{12,1583} = 3.14$, $P < 0.001$), such that dispersers and residents were distinctly different after only a single generation (Figure 5.2i, j). The divergence among selected lines continued to increase to about generation 3–4 and this difference was maintained thereafter (Figure 5.2i, j). By generation 4, dispersers had decreased in size (males by 10.4%, females by 12.6%), and residents had increased in size (males by 10.3%, females by 7.7%) such that there was a large relative size difference between dispersers and residents (20.7% in males, 22.6% in females). Females were larger than males (sex; $F_{1,18} = 27.48$, $P < 0.001$; Figure 5.2i, j) and body size was also significantly associated with five covariates (Table A5.5): positively with movement ability ($F_{1,1583} = 11.94$, $P < 0.001$; Figure A5.1a), routine MR ($F_{1,1577} = 173.58$, $P < 0.001$; Figure A5.2a), and leg length ($F_{1,1601} = 567.85$, $P < 0.001$; Figure A5.3a), age ($F_{1,1591} = 16.04$, $P < 0.001$; Figure A5.3b), and negatively with spontaneous activity ($F_{1,1597} = 5.21$, $P = 0.023$; Figure A5.3c).

Leg length

Leg length did not change under artificial selection for and against dispersal (full ANCOVA: Table A5.6), such that residents and dispersers did not have disproportionate leg lengths relative to their body size over generations (selection × generation; $F_{12,1601} = 1.16$, $P = 0.31$; Figure 5.2k, l). However, leg length varied significantly over the course of the experiment (generation; $F_{6,1601} = 14.67$, $P < 0.001$; Figure 5.2k, l). Leg length was strongly positively associated with body size ($F_{1,1593} = 590.46$, $P < 0.001$; Figure A5.3a), but no other covariates (Table A5.6).

Discussion

Much of the variation in dispersal can typically be attributed to variation in the life-history, morphological, physiological, and behavioural traits that underlie dispersal ability and constitute a dispersal syndrome (Ronce & Clobert 2012; Stevens *et al.* 2014). Spatial assortative mating has been postulated as a mechanism that leads to significant shifts in dispersal ability and to the phenotypic traits that affect dispersal at range edges (Phillips *et al.* 2006; Shine *et al.* 2011; Chuang & Peterson 2016). In the present study, I show that spatial assortative mating via artificial selection for dispersing and non-dispersing individuals can affect rapid change in some phenotypic traits. Our results demonstrate that selection for dispersal by climbing decreased both body size and activity-, age-, and size-independent routine MR, but did not significantly affect relative locomotor apparatus size, movement, activity, or even dispersal rate.

Despite the imposition of directional artificial selection for and against dispersal success, this trait did not respond strongly. While previous studies have successfully selected on dispersal success in *T. castaneum* and *T. confusum*, such responses to artificial selection vary greatly (e.g. five generations (Ogden 1970a), one generation (Ritte & Lavie 1977), and fluctuations over seven generations (Łomnicki 2006)). Highly variable rates of spread have also been demonstrated in replicate populations over 13 generations (Melbourne & Hastings 2009), and our results support the stochasticity of this system.

There are two main explanations that provide support for our findings. First, small individuals are biomechanically and energetically better suited to climbing dispersal than large individuals. Second, that spatial assortment has imposed selection on developmental rate or some aspect of energy allocation during development rather than on dispersal success *per se*. Despite dispersal rate being relatively unaffected by selection, the dispersers rapidly became smaller than residents, which contrasts with several previous insect studies that found dispersers to be larger than residents (Lawrence 1987; Anholt 1990; Łomnicki 2006; Laparie *et al.* 2013). Large body size has generally been thought to promote dispersal and invasion success, but when smaller individuals (assumed to be competitively inferior) are in a high competition environment, they may exhibit a high propensity to disperse (Bowler & Benton 2005; McCauley & Rowe 2010).

It has been argued that as larger individuals can acquire and store more energy, and if an energy reserve threshold is required for dispersal, larger individuals can disperse sooner or more frequently than smaller individuals (Bowler & Benton 2005; Benard & McCauley 2008; Laparie *et al.* 2013). The mechanisms behind this theory are that body size scales isometrically with energy stores and musculature (Lease & Wolf 2011) but allometrically with metabolic rate (White 2011), such that larger individuals have a lower resting and moving energy cost relative to their size and energy stores (Reinhold 1999). However, the absolute cost of transport is considerably lower for smaller animals (Halsey 2016), and this difference is augmented when moving up an incline. The work required to move a unit of body mass against gravity is the same irrespective of body size (Lipp *et al.* 2005), therefore climbing is ultimately less costly for individuals with less mass to move. Relatively smaller individuals will therefore have a lower cost of transport than larger ones while climbing the inclines in the dispersal apparatus. When climbing steep inclines, animals adjust their locomotor mechanics to reduce stride length and assume a more crouched posture (Birn-Jeffery & Higham 2014). Smaller individuals are able to hold their centre of mass closer to the climbing surface, and have an intrinsically shorter stride length than large individuals due to scaling with limb length (Birn-Jeffery & Higham 2014). Smaller individuals may therefore be biomechanically more effective climbers. This size-stride length relationship supports the present finding that there was no selection for disproportionately sized legs, as this may be unnecessary for small individuals to be efficient climbers. Hence, artificial selection for dispersal success favoured those individuals that were relatively small, and therefore more energetically and biomechanically efficient at climbing through the apparatus.

After two generations, dispersers also had a significantly lower routine MR than residents, particularly in males. Although few studies on dispersal have investigated MR, these have demonstrated that dispersers generally have a higher MR at rest (Myles-Gonzalez *et al.* 2015) and during flight (Haag *et al.* 2005; Niitepõld *et al.* 2009), but not always (Tracy *et al.* 2011). Glanville fritillary butterflies (*M. cinxia*) that had a high flight ability also had a high flight MR; the fitness benefits of high flight ability (predator escape, foraging, egg laying, and dispersal all require flight) are proposed to be greater than the energetic costs incurred to undertake the activities (Niitepõld *et al.* 2009). Under the allocation model (that the energy available to an animal is partitioned among resting costs and other activities; Careau *et al.* 2008), expending less of the total energy budget on routine MR is expected to allow more energy to be available for dispersal. As selection continues over generations, those individuals that disperse have a slightly lower routine MR; therefore, I cautiously infer that selection favoured individuals with efficient metabolism, allowing energy to be expended on dispersing, although the effect is small.

While the mechanism for dispersal in the present study was climbing string, movement (walking) was assessed on a horizontal plane in a maze. The two are likely mechanistically distinct; small animals are efficient at climbing but not necessarily at walking (Pontzer 2016). Although I previously found that *T. castaneum* with longer legs relative to their body size are able to travel farther and faster in a complex environment (Chapter 3), the same does not apply for climbing, where limbs that are proportionate to body size may be favoured. The finding in the present study, that relative leg length did not differ among selected lines, supports this notion.

The second plausible explanation is that spatial assortment has imposed selection on developmental rate or some aspect of energy allocation during development rather than on dispersal success, which is based on the rapid divergence in body size. In dispersers, the reduction in body size may be an indication that those individuals that dispersed fastest (those that were selected) had a faster developmental rate. Body size prior to pupation is indicative of adult size in holometabolous insects; hence those individuals that develop faster are also typically smaller (Davidowitz & Nijhout 2004). While the experimental design minimised variance in the stage of pupal development at time of collection, the emergence time was not directly constrained. Those individuals that emerged and matured earlier would likely be the ones to disperse fastest, as physiological and movement behavior responses also increase with increasing age and maturity status (Chapter 2). By the same logic, those individuals that that allocated more time and energy into growth would have emerged and matured later, and would therefore not disperse quickly (the residents). Although this explanation supports the patterns of directional selection on body size, there is little empirical data available to directly support this hypothesis. It would therefore be useful for future work to measure traits in the larval and pupal stages of development, as this may be imperative to understanding spatial mechanisms driving dispersal. The spatial assortment of phenotypes is likely to be dependent on the rate of biological processes, which should be further explored.

At the population level, maintenance of dispersal irrespective of selection may indicate prevalence of a bet-hedging strategy. This may occur as a survival mechanism such that if a local extinction event were to occur, some offspring have already dispersed among different sites and thus their genes are maintained in the metapopulation (Benton & Bowler 2012b; Kubisch *et al.* 2014). Similarly for the disperser lines, producing offspring that ubiquitously disperse may be overly risky, as there are significant potential fitness costs of dispersing (Bonte *et al.* 2012). A bet-hedging strategy that minimises the potential of fitness failure by producing offspring with a spectrum of dispersal propensities allows individuals to maximise their fitness and gene flow across multiple sites (Auld & de Casas 2013).

The present study does not provide direct support for several previously observed trait changes (e.g. increase in relative locomotor apparatus size) via spatial assortative mating in range-edge populations. However, I demonstrate that selection on dispersal success can result in shifts in body size and metabolic rate within few generations. The presence of a distinctive dispersal syndrome in *T. castaneum* is not well supported; despite artificial selection over seven generations, the movement ability, displacement, and to some extent, the dispersal rate could not be significantly distinguished among selected lines. The evolution of body size under selection on dispersal by climbing in the *Tribolium* dispersal apparatus may be explained mechanistically or with respect to growth and development, but empirical data to directly support either hypothesis is unavailable. Nevertheless, the artificial selection experiment presented here provides direct experimental evidence that morphological and physiological traits can rapidly evolve under spatial assortment, but the action of selection may not be on dispersal directly. Further investigations into the evolution of traits through spatial assortment mechanisms would benefit from exploring the allocation of energy and rates of growth and development during larval and pupal stages.

Chapter 6

No evidence for trade-offs between dispersal and reproduction after artificial selection on dispersal ability

Abstract

Reproduction and dispersal are two of the most fundamental behaviours that affect gene flow. These behaviours share significant costs; both are energetically expensive, risky, and directly affect life-history and fitness. Often, dispersal and reproduction trade off because of these shared costs, particularly regarding energy. There is also evidence that some dispersing animals can increase their fecundity by exploiting habitats that they disperse to, or by maintaining traits that counteract the trade-off, which facilitates colonisation. Here, I employed a cross-breeding experiment on a powerful model system of artificially selected lines of dispersing (disperser) and non-dispersing (resident) red flour beetles (*Tribolium castaneum*) to examine realised fecundity and sex ratio. I tested two alternative hypotheses: 1) that dispersal trades off with fecundity, such that dispersers were less fecund than residents, and 2) that dispersers were more fecund than residents, to facilitate their colonisation success. After seven generations of artificial selection, both residents and dispersers had reduced fecundity consistent with inbreeding depression, but importantly there was no difference in fecundity between dispersers and residents, which does not support either hypothesis. I propose that the lack of trade-off between dispersal and reproduction could be due to the relatively low energy cost of dispersal in this system, and that oviposition rate could be the main limitation for fecundity in residents and dispersers. The sex ratio among lines did not differ. I conclude that there was no evidence that trade-offs occur between dispersal and reproduction after artificial selection for dispersal success, and advocate that further studies on dispersal-reproduction trade-offs that investigate lifetime reproductive traits are warranted.

Introduction

Dispersal, defined as any temporal and spatial movement of an individual that has potential to contribute to gene flow (Ronce 2007), varies considerably among individuals and populations. Much of the inter-individual variation in dispersal ability can be attributed to variance in suites of covarying morphological, physiological, behavioural, and life-history traits that constitute a dispersal syndrome (Ronce & Clobert 2012; Chapters 3 and 5).

Variability in dispersal capacity through space and time leads to spatial sorting; individuals with dispersal-facilitating traits are distributed towards the edges of their geographic or home range and are proximity-limited to mating only with other highly dispersive individuals (Shine *et al.* 2011). Spatial sorting processes can lead to rapid phenotypic shifts between resident and disperser individuals especially in dispersal-related traits, including leg length (Phillips *et al.* 2006), body size (Laparie *et al.* 2013; Chapter 5), and locomotor performance (Llewelyn *et al.* 2010). In addition, life-history and fitness-related traits including growth rate, fecundity, and survival are also predicted to differ between residents and dispersers, and therefore potentially trade off with dispersal ability (Bélichon *et al.* 1996; Pärn *et al.* 2009; Phillips *et al.* 2010b). Individuals are expected to maximise their dispersal and reproductive output to distribute offspring among different habitats, thereby facilitating colonisation success and maximising the probability of their genes persisting in the metapopulation (Auld & de Casas 2013). However, dispersal and reproduction share an underlying physiological basis of assimilated energy resources (Mole & Zera 1993). Given that the two traits might unequally contribute to fitness, one behaviour may be more beneficial than the other under different circumstances (Burton *et al.* 2010; Phillips *et al.* 2010b). For example, in the event of deteriorating local conditions, individuals will likely be more fit if they invest energy in dispersing to a new habitat rather than reproducing in a declining habitat (Bell & Gonzalez 2011). Furthermore, the relationships among traits that underlie both dispersal and reproduction are complex, therefore it is difficult to predict whether increased dispersal would necessarily directly compete with reproduction (Phillips *et al.* 2010b).

Dispersal and reproduction are both energetically expensive processes that may share the same underlying energy resources and control mechanisms. A trade-off between dispersal and reproduction is supported by the results of several previous studies on insects. The energy allocated to locomotor muscles and reproductive structures and oogenesis, is derived primarily from the fat body (Lorenz & Gäde 2009) and is under hormonal control (Zera & Bottsford 2001). Consequently, dispersive individuals that invest heavily in locomotion have fewer resources to allocate elsewhere. Investment of energy into growth and somatic maintenance may further limit the capacity to maximise dispersal and reproduction concurrently (Glazier 1999; Jervis *et al.* 2005). Indeed, many studies have found that there is a reproductive cost associated with having larger flight muscles or structures and being highly dispersive (Roff 1977; Mole & Zera 1993; Zera & Denno 1997; Gu *et al.* 2006; Karlsson & Johansson 2008; Nespolo *et al.* 2008; Saglam *et al.* 2008; Khuhro *et al.* 2014; David *et al.* 2015). However, despite their being a physiological basis of energy partitioning between dispersal and reproduction, some insects are highly dispersive while still having equivalent or increased lifetime egg production, number of clutches, lower age at first reproduction, and mating advantages compared to residents (Lavie & Ritte 1978; Langellotto *et al.* 2000; Min *et al.* 2004; Hanski *et al.* 2006; Saastamoinen 2007). Highly dispersive individuals that are also highly fecund, may compensate for the potential energy deficit incurred by exploiting resources that are available in the habitat that they disperse to (Hanski *et al.* 2006; Burton *et al.* 2010). The quantity of resources an individual can acquire and allocate to life-history traits varies, and thus different reproductive strategies and dispersal traits may be selected to maximise the reproductive value (the expected ultimate genetic contribution) of their offspring (van Noordwijk & de Jong 1986; Rousset 2012).

With dispersal under both spatial sorting and natural selection, I expect that various reproductive strategies may arise in residents and dispersers (Bélichon *et al.* 1996). Two general ideas that have empirical support are that: 1) dispersal trades off with reproduction directly; and 2) dispersal does not trade off with reproduction (coloniser strategy). Dispersal trade-offs occur when dispersers have a low reproductive value because of either the energetic cost of dispersal itself, or the delay of reproduction due to the time cost of dispersal (Ziegler 1976; Hughes *et al.* 2003; Gibbs & Van Dyck 2010; Rousset 2012). In residents, the absence of dispersal costs should increase resources that are available for reproductive investment; therefore, residents have a higher reproductive value (Riddle & Dawson 1983; Gu *et al.* 2006; Pärn *et al.* 2009). A coloniser strategy occurs when dispersers develop rapidly, reproduce early, and have high fecundity relative to residents while also being dispersive (Lavie & Ritte 1978; Saastamoinen 2007; Bonte & de la Peña 2009; Burton *et al.* 2010; Bonte & Saastamoinen 2012). This strategy may be particularly effective when dispersers also have a low probability of surviving the dispersal process (Bélichon *et al.* 1996; Bonte *et al.* 2012). In addition to varied reproductive strategies, strong selection on traits can change population demographics, including offspring sex ratio. Equal sex ratios are theorised to be maintained by natural selection because each sex contributes equally to the gene pool (Fisher 1930), and this theory holds under experimental evolution (Carvalho *et al.* 1998). However, dispersal inherently changes the local density and resource competition within a population, where can alter the relative fitness of either sex, thereby the sex ratio may shift to compensate for the lower relative fitness of the less fit sex (Bulmer & Taylor 1980; Bulmer 1986; Taylor & Crespi 1994).

The present study aimed to test these ideas by specifically addressing the following questions: 1) does selection for dispersal lead to trade-offs with realised fecundity? 2) if such a trade-off exists, how does crossing the selected lines affect realised fecundity? 3) does selection for dispersal result in different demographic strategies (i.e. sex ratio)? I examined the fecundity and sex ratio of artificially selected and then cross-bred lines of resident and disperser red flour beetles (*Tribolium castaneum*). Previously, using a seven generation artificial selection experiment I have demonstrated that selecting for dispersers and residents resulted in significant divergence of body size such that residents became larger and dispersers became smaller, and that dispersers had lower metabolic rates, but that other phenotypic traits did not respond strongly to selection (Chapter 5). Given this finding, and considering that fecundity scales positively with body size in insects (Honěk 1993), I expected that residents would have greater fecundity than dispersers, supporting the dispersal trade-off hypothesis.

Material and Methods

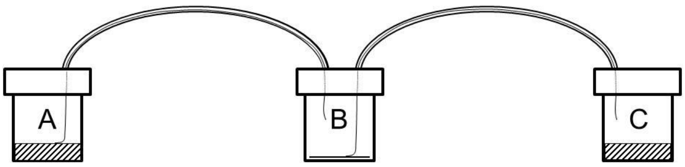
Animals and experimental conditions

Tribolium castaneum that were originally sourced from a laboratory population established in 1965 (QTC4; Postharvest Grain Protection Team; Department of Agriculture, Fisheries and Forestry; Brisbane, QLD, Australia) were used to establish a stock population for artificially selected lines. The 400 founding beetles for these selected lines were collected from a stock population, which was maintained on 210 g of flour medium (95% wholemeal stoneground wheat flour: 5% torula yeast) in 1 L containers. Breeding and ovipositing containers were 70 mL with 5 g of medium. All animals were maintained at 29.5 ± 1 °C.

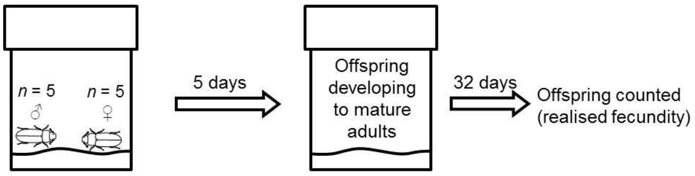
Artificial selection on dispersal

An earlier dispersal apparatus experiment used artificial selection to select for and against dispersal success (Chapter 5). Briefly, dispersal apparatuses consisted of three 70 mL containers (*A*, *B*, *C*) connected by tubing through the lids (Figure 6.1a). The tubing contained string that rested on the surface of the previous container, which beetles could climb up to move one-way through the apparatus from *A*–*B* and *B*–*C*. Fifty beetles were introduced into *A* in single-sex replicate lines, such that there were four replicate lines for each sex and each selection regime (total of 24 lines). Artificial selection was imposed by selecting the individuals that did not disperse through the apparatus (residents; R), those that successfully dispersed (dispersers; D), and by randomly sampling after 72 h in the apparatus (controls; C). After the dispersal trial, 10 individuals (five males and five females) from matching replicate lines were placed in a 70 mL breeding container with 5 g of flour, in which group mating could occur over five days (Figure 6.1b). Following the group mating period, the 10 individuals were moved to a 70 mL ovipositing container with 3 g of flour so that the females could oviposit for five days (Figure 6.1c). After 23 days of development, the offspring from the selected line pairs were sorted by sex as pupae and used to establish the male (*n* = 50) and female (*n* = 50) lines of the pair. This selection regime was maintained for seven generations.

(a) Dispersal apparatus



(b) Breeding container



(c) Ovipositing container

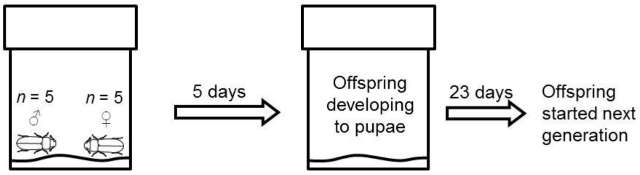


Figure 6.1 (a) Dispersal apparatus used to select for and against dispersal success. For details on the selection experiment, see text. (b) Five males and five females of each selected line pair were added to a breeding container for group mating over five days. Adults were then removed from the breeding container and the offspring produced during breeding were kept, allowed to mature for 32 days, and then counted to estimate the realised fecundity of that line pair. (c) The 10 adults that were removed from the breeding container were placed in the ovipositing container and allowed a further five days to oviposit. The resulting offspring were used as founders for the selection trials across subsequent generations.

Cross-breeding among selected lines

After completing the artificial selection experiment, the offspring of the seventh generation were used to found a large cross-breeding experiment (Figure A6.1). To determine whether reproductive output was altered by selection acting on dispersal, the selected lines were crossed. Control crosses were of the same line pair breeding used throughout the artificial selection experiment (e.g. 1C male × 1C female). Line crosses were crosses among selected lines within replicates (e.g. 1C male × 1R female). Outcrosses were crosses within the selected line type but between replicates (e.g. 1C male × 3C female). Due to the large number of possible replicate line pairs, a complete cross experiment was not logistically feasible, however, the partial cross-breeding design allowed 48 line crosses to be made, each with 10 replicate pairs of parents.

Artificial selection realised fecundity

At every generation of the artificial selection experiment, an estimate of realised fecundity for each line pair was taken by counting the number of offspring that successfully emerged as adults and reached maturity (complete sclerotisation) from the breeding containers. The offspring from the breeding containers were counted rather than those from the ovipositing containers so that the founding beetles for the following generation were not handled prior to assessing dispersal. Realised fecundity was counted 32 days following the removal of adults from the breeding container (Figure 6.1b).

Cross-breeding realised fecundity

From each line replicate pair, 10 male and 10 female pupae were randomly sampled, then randomly allocated into 10 breeding pairs. These pairs were placed in 70 mL containers with 3 g of flour medium. Emergence was checked five days later and pairs were allowed to copulate and oviposit for 10 days. Thereafter, parents were removed and the offspring were allowed to develop for 36 days until all had matured, and then the number of mature adults in each container was counted. The mean number of mature adult offspring produced across the 10 pairs was used to estimate the realised fecundity of the line crosses.

Sex ratio estimates

Following the realised fecundity estimates of the cross-breeding, the sex ratio of each line cross was determined. From each of the 48 crosses in the cross-breeding experiment, a subsample of 100 individuals per cross was taken to determine the sex ratio. This subsample was determined by first subsetting the containers of offspring to those that had 40–90 healthy offspring, and then randomly sampling three of these. The three subset containers were mixed into one container and animals were euthanized by exposure to -18 °C for 24 h. After thawing, 100 mature adults from each container were sexed under a stereomicroscope (Z45E; Cambridge Instruments, London, UK) by examining the underside of the femur on the first pair of legs for the presence of setiferous sex patches in males and their absence in females (Faustini *et al.* 1981).

Statistical analyses

All data were analysed using the R software environment for graphical and statistical computing version 3.2.3 (R Development Core Team 2015). The estimates of realised fecundity and sex ratio were normally distributed, therefore analysed using Linear Mixed Effects Regression (LMER) models using the R package *lme4* (Bates *et al.* 2014). The model for the artificial selection realised fecundity had number of offspring (response) and selection type and generation number (predictors) as fixed effects, including line replicate as a random effect. Similarly, the LMER model for cross-breeding realised fecundity had number of offspring (response) and cross type (predictor) as fixed effects, including cross-breeding pair number nested within cross replicate as random effects. Cohen's d was calculated for pairwise comparisons among crossed lines, where $0.2 < d <0.5$, $0.5 < d < 0.8$ and $d > 0.8$ were considered to be small, medium, and large effect size, respectively (Cohen 1988). The LMER for sex ratio included the ratio of male to female offspring (response) and cross type (predictor) as fixed effects, including cross replicate as a random effect.

Results

Artificial selection on dispersal: realised fecundity

Realised fecundity declined throughout the selection experiment ($F_{1,69} = 180.7$, $P < 0.001$; Figure 6.2). Between the first and seventh generations, realised fecundity decreased by 63.4% in controls, 36.3% in dispersers, and 49.2% in residents. Realised fecundity was not significantly different among selected lines over generations ($F_{2,69}$ = 1.39, P = 0.256; Figure 6.2) and the number of offspring were not different among selected lines overall ($F_{2,53}$ = 0.68, P = 0.512). That is, all selected lines declined in their reproductive fitness at similar rate and to a similar extent across replicates.

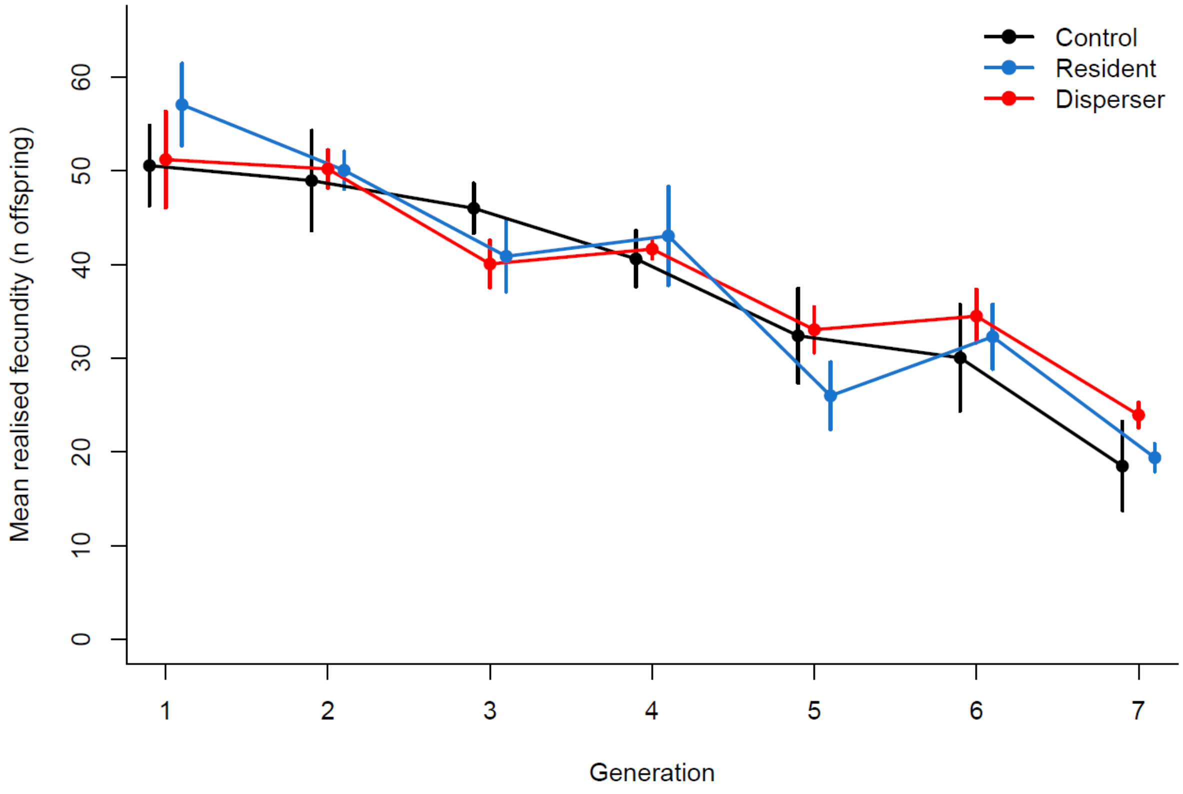


Figure 6.2 Realised fecundity (number of viable offspring produced) of selected lines for three selection regimes (controls, dispersers, and residents) over seven generations of artificial selection. Data shown are means of eight replicate lines ± SE.

Cross-breeding: realised fecundity

The number of offspring produced in each of the cross-bred lines were significantly different overall ($F_{11,431}$ = 2.01, P = 0.026; Figure 6.3). However, comparisons among any cross-bred lines within the LMER model were marginally significant (0.05 > P < 0.1; Table A6.1). Therefore, I made pairwise comparisons to determine effect sizes between control crosses, among selected line crosses, and within selected line type crosses using Cohen’s *d* (Table 6.1). Medium effect sizes were found between the resident control cross (R:R–) and: the resident-control line cross (–R:C; *d* = 0.649), control-resident line cross (–C:R;

d = 0.643), disperser-resident line cross (–D:R; *d* = 0.7), and resident outcross (–R:R$_O$; *d* = 0.732). The reciprocal resident-disperser and disperser-resident line crosses also had a medium effect size (R:D–D:R; *d* = 0.536). Comparing reciprocal crosses (e.g. R:D–D:R), I found that among all crosses, the maternal line was a stronger determinant of the number of offspring ($F_{11,398}$ = 2.2, P = 0.014) than the paternal line ($F_{11,398}$ = 1.17, P = 0.303).

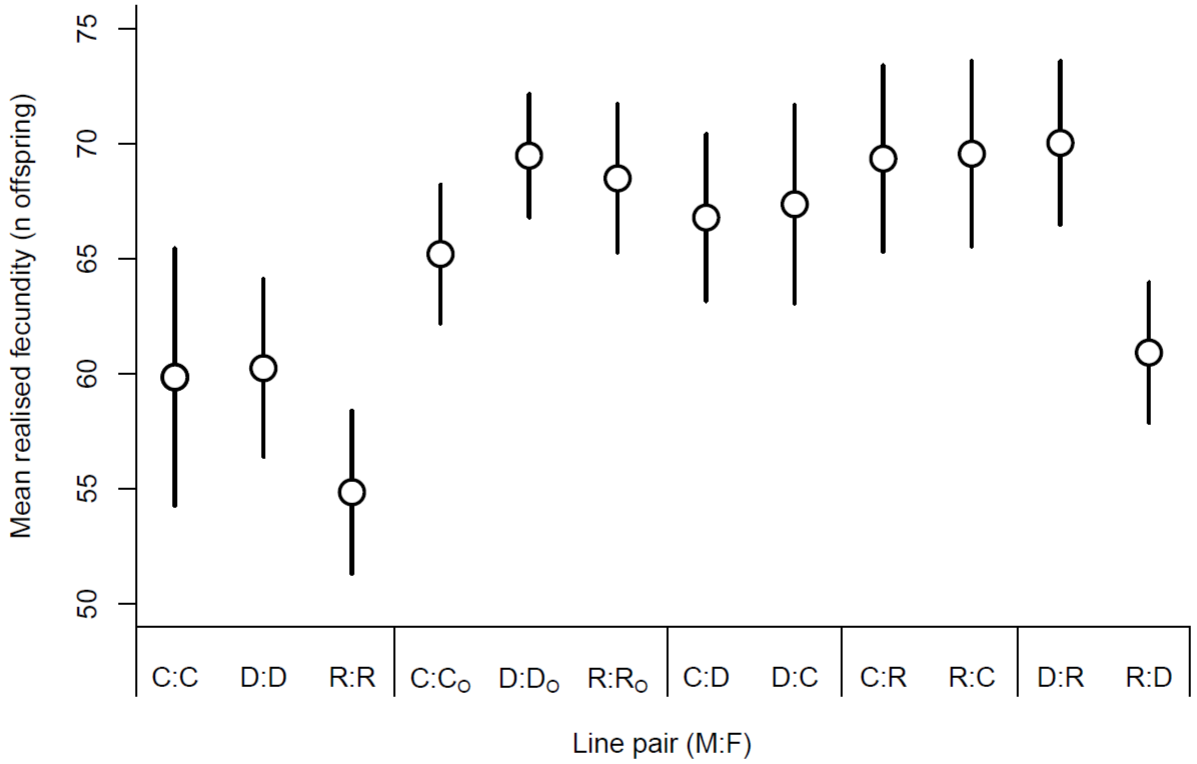


Figure 6.3 Realised fecundity (number of viable offspring produced) of various crossed line pairs among different artificially selected line crosses. C = control, D = disperser, R = resident, and $_O$ = outgroup of the same selection regime. Data shown are means of 10 replicate parent pairs ± SE.

Table 6.1 Cohen's *d* (effect size) for pairwise comparisons of cross-bred selected lines. Significant differences were considered small where $0.2 < d < 0.5$, medium where $0.5 < d < 0.8$, and large when $d > 0.8$. Medium and large effect sizes are in bold.

Within selected line replicate pairs (control cross)		Within selected line types between replicates (outcross)		Among selected line types (line cross)	
Line pair (M:F)	Cohen's *d*	Line pair (M:F)	Cohen's *d*	Line pair (M:F)	Cohen's *d*
C:C–D:D	0.014	C:C$_{\mathrm{O}}$–D:D$_{\mathrm{O}}$	0.251	C:R–R:C	0.134
C:C–R:R	0.183	C:C$_{\mathrm{O}}$–R:R$_{\mathrm{O}}$	0.178	C:D–D:C	0.165
R:R–D:D	0.250	R:R$_{\mathrm{O}}$–D:D$_{\mathrm{O}}$	0.056	R:D–D:R	**0.536**
C:C–C:D	0.251	C:C$_{\mathrm{O}}$–C:C	0.149	C:R–D:R	0.061
C:C–C:R	0.332	R:R$_{\mathrm{O}}$–R:R	**0.732**	C:D–R:D	0.216
C:C–D:C	0.256	D:D$_{\mathrm{O}}$–D:D	0.435	R:C–D:C	0.112
C:C–R:C	0.340			R:D–R:C	0.246
D:D–D:C	0.294			D:C–D:R	0.091
D:D–D:R	0.440			C:R–C:D	0.192
D:D–C:D	0.296				
D:D–R:D	0.033				
R:R–R:C	**0.649**				
R:R–R:D	0.306				
R:R–C:R	**0.643**				
R:R–D:R	**0.700**				

Cross-breeding: sex ratio

The sex ratio of offspring was not significantly different among the cross-bred lines overall ($F_{11,36} = 0.51$, $P = 0.884$; Figure 6.4), or in any pairwise comparison (Table A6.2). R:R had a slightly higher male to female ratio in both the control cross and outcross groups than D:D or C:C, but significant differences were not detected (Figure 6.4). The mean sex ratio of all lines was biased towards males (1.11 ± 0.12; $t_{11} = 3.89$, $P < 0.001$).

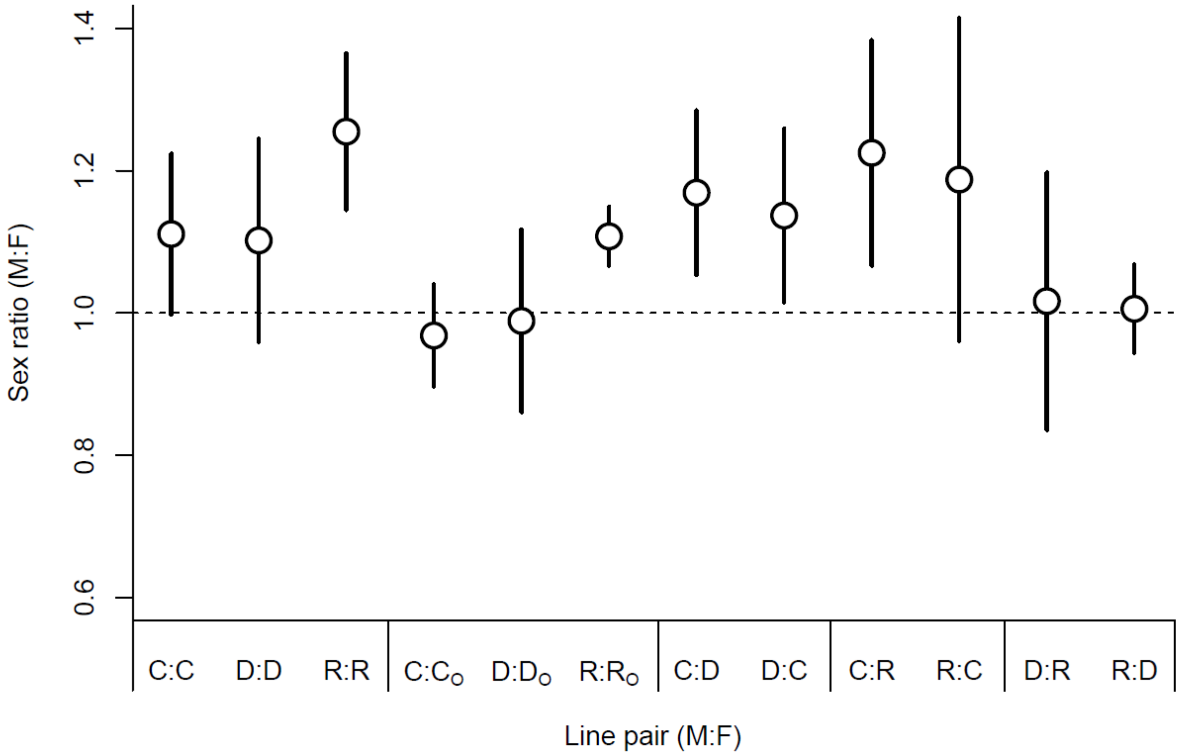


Figure 6.4 Sex ratio (M:F) of various crossed line pairs among different artificially selected line crosses. C = control, D = disperser, R = resident, and $_O$ = outgroup of the same selection regime. Dashed line represents an equal ratio of males to females (1:1). Data shown are means of four replicates ± SE and ratios were taken from a random sample of 100 individuals.

Discussion

Dispersal and reproduction share similar resources and biological constraints, however the nature of trade-offs between the two behaviours is complex. In some species, there is a clear trade-off where dispersing individuals are less reproductively fit, but in other species, dispersers are at least as fit as residents are. Here, I demonstrate that red flour beetles, *Tribolium castaneum*, that have been selected for dispersing do not have reduced fecundity compared to those selected for residency, therefore supporting the hypothesis that dispersal and reproduction do not always trade-off due to shared energy resources.

Over the seven generations of selection, for and against dispersal, the fecundity of dispersers did not differ from that of residents. However, there was a significant reduction of fecundity across all lines. This finding, coupled with the lower fecundity of control crosses compared to any other pairs in the cross-breeding experiment, is convincing evidence of an inbreeding depression (Keller & Waller 2002). Selection experiments that have relatively small population sizes inevitably restrict gene flow and potentially bottleneck populations, particularly when the selection regime further reduces the effective population size (Santiago & Caballero 1998; Kawecki *et al.* 2012). There is a strong correlation between the predicted decline in genetic variance and the population rate of increase in *T. castaneum* (McCauley & Wade 1981), and realised fecundity decreases as the level of inbreeding increases (Sharp & Agrawal 2016). Inbreeding affects life-history traits much more than morphological traits (DeRose & Roff 1999), therefore the finding that body size diverges with bidirectional selection on dispersal from our previous experiment (Chapter 5) is unlikely to be compromised by inbreeding. During dispersal and colonisation events, or in naturally transient populations, individuals that leave populations consequently reduce the size of their original population and are likely to move to habitats that, at least initially, have a small population size (Roff & Derose 2001). Therefore, some level of intermediate inbreeding may be common during dispersal events and may be associated with the phenotypic traits that describe variance in movement ability and dispersal propensity (Ronce & Clobert 2012; Auld & de Casas 2013).

Crossing selected lines can counteract the effect of inbreeding on realised fecundity (Kawecki *et al.* 2012). In our study, although the mean realised fecundity among crossed lines only differed marginally, the control crosses all had lower realised fecundity compared to the line crosses and outcrosses. I suggest that this is evidence that crossing the selected lines restored lost genetic heterozygosity and reduced the effects of inbreeding depression. The realised fecundity of the crossed lines (with one exception) did not differ, which demonstrates that none of selected lines had a reproductive advantage during early life due to their dispersal behaviour. This does not support the hypothesis that dispersal may trade-off with reproduction due to the energy cost of dispersal, as energy invested into dispersal cannot simultaneously contribute to reproduction or reproduction is delayed due to dispersal timing. The one exception was the cross between resident males and disperser females, which had similar realised fecundity to the control crosses. A recent artificial selection experiment on aerial dispersal propensity of spider mites (*Tetranychus urticae*) using cross-breeding residents and dispersers found that offspring phenotype was driven by maternal influence (Van Petegem *et al.* 2015). Maternal effects are any maternal influences on offspring phenotype that cannot be attributed to either solely offspring genotype or environment (Mousseau & Fox 1998). Given our finding that the maternal line has a stronger influence on fecundity than the paternal line, and that maternal effects can significantly affect offspring fitness, one might predict that female disperser lines may be less fit. However, under this hypothesis, I would also expect the progeny of the cross between control males and disperser females to be less fecund, but they were not. Therefore, I can only suggest that the combination of alleles between resident males and dispersers females may be less adaptive than combinations among other lines, but equivalent to that of the inbred selected lines.

I also found that the sex ratios between crossed lines did not differ, regardless of selection for or against dispersal, or crosses among lines. It is likely that natural selection acts to maintain the sex ratio at approximately 1:1, otherwise a fitness advantage would be conferred to the rarer sex by increased mating opportunity, and also by increased mate competition among the common sex, which is ultimately unsustainable (Fisher 1930; Carvalho *et al.* 1998). Artificial selection on dispersal does not appear to influence sex ratio. This finding suggests that overall, there may be a slight male-bias in *T. castaneum* sex ratio (1.11 ± 0.12), but the precision of this estimate is not high enough to rule out sampling error (see Ewen *et al.* 2004). Parents can adjust the sex ratio of their offspring in response to environmental conditions to maximise their own fitness (West & Sheldon 2002). While this finding is not dramatic, it may indicate that having more male offspring under the confined, low-density environments in which breeding occurred is advantageous, and is worth exploring further.

Overall, these results do not directly support the coloniser hypothesis either, which proposes that dispersers have a reproductive advantage over residents to facilitate colonisation. Two studies have previously investigated bidirectional selection on dispersal in *T. castaneum*: one study found that dispersers had a reproductive advantage over residents, supporting the hypothesis (Lavie & Ritte 1978). However, Zirkle *et al.* (1988) found that the reproductive output of dispersers and residents did not differ, which does not directly support the coloniser hypothesis, but still supports the lack of trade-off. Here I found that dispersers do not compromise reproduction, but also that residents do not have a reproductive advantage over dispersers, as in Zirkle *et al.* (1988). *Tribolium castaneum* is a coloniser species and has evolved to maximise both dispersal and reproduction, but the timing of dispersal and reproduction may vary among individuals (Ziegler 1976). Hence, reproductive differences between dispersers and residents may be less apparent over a relatively short window of time. Most studies that report differences in reproduction between dispersers and residents have investigated species that use flight to disperse (e.g. Min *et al.* 2004; Hanski *et al.* 2006), but insects that walk or climb to disperse may not incur as large an energy cost during locomotion. I have previously found that disperser beetles were smaller and more energy-efficient climbers, thus making it likely that their cost of dispersing would be lower still (Chapter 5).

Throughout this artificial selection experiment, dispersers became significantly smaller and residents became significantly larger (Chapter 5). Body size frequently scales with fecundity in insects (Honěk 1993) and for holometabolous insects to become larger, larvae must increase their consumption and growth rate, either investing resources into somatic growth or reproductive structures (Jervis *et al.* 2005). From these findings, I hypothesised that the larger residents would have a reproductive advantage over dispersers, but our results do not support this hypothesis. Instead, I found that residents, which were larger on average than dispersers, were equally as fecund as dispersers over the 10 day laying period. However, larger size may still confer greater lifetime reproductive success (LRS), through increased lifespan, extended reproductive period, or greater egg production (Karlsson & Wickman 1990; McCabe & Partridge 1997; Taylor *et al.* 1998), which would not be apparent if oviposition rate were similar between dispersers and residents. This is a hypothesis worth testing in future, but from the present study, it is clear that dispersers do not incur a significant reproductive cost during the important early phase of their adult life.

In summary, our findings did not support a trade-off between dispersal and reproduction in *T. castaneum* having different dispersal tendencies. I propose that this may be because dispersal by climbing is not energetically expensive for the small, energy-efficient dispersers, and therefore equivalent resources remain available for allocation to reproduction. I do not have substantial evidence to state that there is a coloniser syndrome in this species, but dispersers have equivalent reproductive ability to residents. It is surprising that the larger size of residents does not translate into a difference in reproductive success or strategy. As a possible explanation, I propose that a limit to oviposition rate could obscure differences between disperser and resident reproduction, and that residents may have greater LRS than dispersers. Exploring these hypotheses in the future would be valuable. Considering additional life-history traits such as oviposition rate, mating frequency, LRS, and lifespan when researching dispersal-reproduction trade-offs, would improve our understanding of what drives these relationships.

Chapter 7

General discussion

Overview

Variability in dispersal has significant implications for the ecology and evolution of organisms, but a thorough understanding of the complex causes and consequences of this variation is still being developed. Trait-based approaches are useful for studying the intraspecific variation in dispersal ability. In particular, investigating suites of traits, which contribute to dispersal syndromes has received much attention recently (Stevens *et al.* 2014). Many different life-history, morphological, physiological, and behavioural traits are strongly linked to dispersal, and can covary, interact, and trade-off with other traits. These traits are shaped by and contribute to the continuous evolution of dispersal. Consequently, an evolutionary perspective on dispersal-related traits contributes to a more complete understanding of the proximate and ultimate causes of dispersal (Ronce & Clobert 2012). Thus, the overall aim of the research presented in this thesis was to gain a better understanding of the relationships and evolution of traits that may constitute the dispersal syndrome, using a series of laboratory experiments using a model insect species, *Tribolium castaneum*. I investigated the dynamics of body size, metabolic rate, activity, and movement behaviour traits with life-history, the associations among these traits, the evolution of traits under selection for and against dispersal, and trade-offs between dispersal and reproduction.

Trait dynamics and associations

The age and sex of an organism can be a strong determinant of its dispersal (Bowler & Benton 2009). In Chapter 2, I provided fine-scale time-series data of the dynamics of several fundamental phenotypic traits that are thought to constitute the dispersal syndrome. First, I found that the onset of sexual maturity dramatically affected several traits that describe movement capacity, as well as spontaneous activity and routine MR in both males and females. Prior to the onset of sexual maturity, immature and unsclerotised individuals were mostly inactive, which is a protective mechanism from damage by conspecifics and the environment while the cuticle is still hardening. Immature individuals also had a low routine MR, which resulted from the low energy requirements prior to maturity and energy savings associated with inactivity. Second, after reaching sexual maturity, the mean values of these traits stabilised and remained relatively consistent throughout early adult life. These findings provide strong support for the hypothesis that dispersal, which is an energetically expensive activity reliant on movement capacity and strongly tied to reproduction, is likely to occur only after reaching sexual maturity. Importantly, the relative stability of the mean and variance of traits after the onset of maturity meant that an age range could be determined for studying trait associations and evolution in subsequent experiments (Chapters 3–5).

Although the correlations between many phenotypic traits are often thought to be robust, especially within a closed long-established laboratory population, I found the converse: trait relationships were not overly robust and showed substantial variation (Chapter 3). This result is an important finding that suggests that trait relationships do not necessarily conform to expectations derived from earlier studies that may be based on small sample sizes. Therefore, the relationships among traits should not be assumed to be stable through time and among populations. Rather, the substantial variance around trait means and trait correlations should be considered biologically meaningful, and classic assumptions or models may need to be revisited to include intra-specific variation (Bolnick *et al.* 2011).

Fundamental size relationships, i.e. the positive correlation between body size and leg length, show less variation than the relationships between morphology or routine MR and movement behaviours. Under different contexts, MR can have different biological meanings ranging from energy cost, capacity, performance, or fitness (Burton *et al.* 2011). Several-fold differences in maintenance metabolism have been observed in many species, even after accounting for body size, temperature, phylogeny, and environmental factors (Burton *et al.* 2011). Routine MR (which incorporates activity) is likely to be more variable than basal, resting, or standard MR, and therefore may be highly context-dependent. The lack of strong associations among phenotypic traits reported in Chapter 3 does not indicate that they are necessarily unrelated, as they may only be related under certain contexts, or have stronger underlying genetic correlations that may respond to selection (Wone *et al.* 2009). A logical progression from this research would be to determine the genetic underpinnings of phenotypic traits that are relevant to dispersal syndromes.

Dispersal syndromes are frequently discussed in conjunction with large body size and large locomotor apparatus relative to body size (Phillips *et al.* 2006; Forsman *et al.* 2010; Laparie *et al.* 2013). Assuming that the measures of movement ability and displacement could be reasonable proximate measures for dispersal (Hawkes 2009; Ducatez *et al.* 2012), then these measures should correlate well with body size and relative locomotor apparatus size. Only relative locomotor apparatus size was positively associated with movement ability and this mechanistic relationship still showed considerable intraspecific variation (Chapter 3). Variation is necessary for selective processes to affect trait change, and from this relationship, I hypothesised that relative leg length would be exaggerated when under directional selection for dispersal success. However, the experiment in Chapter 5 revealed that body size and routine MR were altered by selection on dispersal, but that relative leg length was not.

Before discussing these trait responses to artificial selection, it is important to first consider the relationship between the dispersal apparatus that was used for measuring dispersal and the maze environment that was used for measuring movement behaviour. The dispersal apparatus used in this body of work (Chapters 4 and 5) was based on the designs described in several previous studies that studied dispersal in *Tribolium* species (e.g. Prus 1963; Ogden 1970a; Ritte & Lavie 1977; Łomnicki 2006). The mechanism by which individual beetles could move through the apparatus was by climbing a string; initially vertically upwards, then horizontally, and then shortly downwards within a narrow, unidirectional tube. Climbing in this context is equivalent to short dispersal movements that occur within their natural and cosmopolitan habitats, especially in grain storage facilities (Semeao *et al.* 2013). Movement through the maze was on a level surface where the effects of geotaxis, slopes, and confinement are not present. Walking and climbing have not been distinguished in any previous study on *Tribolium* dispersal; most refer to movement through a dispersal apparatus as ‘walking’. However, the slope of tubing in the dispersal apparatus affected dispersal rate (Chapter 4) and movement in the maze was not different between selected dispersers and residents (Chapter 5).

Both walking and climbing in *T. castaneum* use the same locomotor appendages that share underlying musculature, however movement gait, energetic cost, and triggering conditions, are likely to differ somewhat. If selection were imposed on movement by walking rather than climbing, it is likely that the responses of other dispersal-related traits may have been different also, given the various conditions under which different dispersal mechanisms are used (Figure 1.5). The selection pressures that are imposed by routine movements are different to those driving dispersal. For example, population-level processes such as competition, density, and sex ratios are likely to drive dispersal, whereas individual-level processes such foraging and mate-searching are likely to drive routine movements (Van Dyck & Baguette 2005; Benton & Bowler 2012b). This fundamental difference in selection pressures is supported in the lack of change in movement in the maze despite selection on dispersal through the apparatus (Chapter 5). Another limitation to consider when interpreting the artificial selection results is the logistical constraint of standardising local conditions for the experiment. The specific population density, food availability, temperature, patch size, and absence of predators resulted in a specific phenotypic response, however altering these conditions would likely yield different responses. Understanding the relative dependence of the phenotype to the suite of individual- and population-level processes that influence dispersal decisions is an immense challenge in biology, but is ultimately necessary to understand dispersal ecology and evolution (Bowler & Benton 2005; Benton & Bowler 2012b; Clobert *et al.* 2012). Nevertheless, some of the measured phenotypic traits responded to artificial selection on climbing dispersal (locomotion that *T. castaneum* naturally use to disperse), and understanding the mechanistic basis for these phenotypic responses to spatial assortment provides insight into this evolutionary theory.

Spatial sorting and the evolution of dispersal-related traits

Spatial sorting proposes that the evolution of traits can be driven by proximity-limited mating between individuals that have similar dispersal-related traits (Shine *et al.* 2011). Evidence for this evolutionary process has been observed at species range edges and particularly in invasive species (Chuang & Peterson 2016). Natural selection is the conventional mechanism for evolutionary change, whereby individuals that have traits better suited to their local environment will have a fitness advantage (Darwin 1859). Both of these evolutionary mechanisms are prevalent in shaping traits that are associated with accelerating range expansion in invasive species, including birds (Berthouly-Salazar *et al.* 2012), toads (Shine *et al.* 2011; Lindström *et al.* 2013), fishes (Rehage & Sih 2004; Myles-Gonzalez *et al.* 2015), salamanders (Lowe & McPeek 2012; Davenport & Lowe 2016), mites (Van Petegem *et al.* 2015; 2016a), and insects (Piiroinen *et al.* 2011; Laparie *et al.* 2013). Understanding trait evolution is a pre-condition for elucidating factors that contribute to shifts in the distributions of invasive species; such shifts have potentially dire consequences for the ecology of native species and the conservation of natural environments (Colautti & Lau 2015).

As anthropogenic disturbance of natural environments, climate change, and global homogenisation of biota continue pervasively into the future, biological invasions are likely to become more frequent, and even more important and challenging to manage effectively (Dukes & Mooney 1999; Kokko & López-Sepulcre 2006; Ricciardi 2007; Chown *et al.* 2015; Hulme 2016). The research presented in this thesis provides a basis for understanding trait evolution that may occur during biological invasions, by investigating traits associated with dispersal syndromes and the evolution of traits through artificial spatial sorting. This knowledge could be used to inform evolutionary and invasion biology hypotheses, incorporated in spatial distribution and evolution modelling, and further tested in laboratory and field experiments. In this thesis, I have demonstrated empirically that proximity-limited mating among individuals that are categorised as dispersers or residents can result in rapid phenotypic change (Chapter 5). Additionally, I have found no evidence to suggest that dispersers and residents differ in terms of fitness (Chapter 6). A difference in fitness would be expected under natural selection; therefore the spatial sorting hypothesis is supported.

Previous studies on spatial sorting have used modelling (e.g. Travis & Dytham 2002), field observations (e.g. Laparie *et al.* 2013), or common garden experiments (e.g. Brown *et al.* 2014), but only one other study has conducted artificial selection in a spatial sorting context (Van Petegem *et al.* 2015). The present body of work is the first example of an experimental evolution approach to understanding the response of multiple phenotypic traits to spatial sorting processes. This work is also among few studies that have investigated the evolution of multiple traits simultaneously, and provides significant insight for future studies on dispersal syndromes, and trait evolution. The findings of this thesis do not support a number of observations from previous studies that investigated phenotypic differences between dispersers and residents. Clearly, relative locomotor apparatus size can have significant effects on movement ability (Chapter 3), but selection for dispersal success did not affect this trait (Chapter 5). Similarly, body size did not affect movement characteristics (Chapter 3), but was the trait most responsive to selection (Chapter 5).

Relative leg length was likely unaffected by selection on dispersal because of the mode of dispersal. That is, having relative longer legs does not facilitate climbing, but does facilitate walking. However, smaller body size is beneficial for climbing, which is probably due to the lower cost of transport while moving up a slope; therefore selection by climbing in the dispersal apparatus rapidly reduced the mean body size of dispersers. Selection against dispersal resulted in an increase in body size, which is potentially a consequence of redirected energy partitioning towards increase investment in larval growth.

In Chapter 5, I hypothesised that the significant increase in body size of residents could indicate that the energy acquired as larvae was invested into biosynthesis for reproduction; therefore residents would have a reproductive advantage over dispersers. However, in Chapter 6 I showed that there was no difference between dispersers and residents in terms of reproductive success. One reason for this could be that the short laying period (10 days) may not be representative of lifetime reproductive success (LRS), if oviposition rate did not differ between dispersers and residents. Another plausible reason could be that size increase reflects competitiveness. That is, larger individuals are usually more competitive (Bowler & Benton 2005), and residents are in a more intense competitive environment that has higher population density and declining resource quality as a result of higher density. Previous studies have found that smaller, less competitive individuals are the ones to disperse earlier (Lawrence 1987; Hanski *et al.* 1991; Léna *et al.* 1998), which also fits the patterns that I recorded in Chapter 5. Finally, the variation in dispersal rate and lack of difference in reproduction between dispersers and residents could indicate a bet-hedging strategy to produce both dispersing and non-dispersing offspring. This would be beneficial if, for example, a local extinction event occurs in a habitat with resident parents, because a proportion of their offspring would have already dispersed, and thus their genes would be maintained in the metapopulation (Kubisch *et al.* 2014). Further disentangling the relative mechanisms that determine differences between residents and dispersers is one of many possible future directions suggested by the results and implications of my work.

Future directions

In this body of work, residents and dispersers were distinguished by their ability to disperse by climbing. Although walking and climbing have not been distinguished in any previous study on *Tribolium* dispersal, it is clear from my research that the mechanistic differences between these locomotor modes affects their evolutionary responses to selection for dispersal. If individual dispersal through the apparatus had been able to be quantified, rather than measuring routine movement in the maze, it may have been possible to gain a greater understanding of individual motivation to disperse. The relative costs of different modes of locomotion and dispersal will differ dependent on conditions acting at the level of the individual rather than the population (Bonte *et al.* 2009; Bonte *et al.* 2012). Another dispersal mechanism that *T. castaneum* uses is flight, which is far more energetically expensive, but could be more effective than walking or climbing for dispersal. Artificial selection experiments that select for walking or flight dispersal could be valuable to compare with the phenotypic response of selection for climbing dispersal. Recently, large-scale experimental patch-based landscapes have been implemented to study flight dispersal in butterflies and other species, to provide a measure of dispersal more directly comparable with that of natural systems (Legrand *et al.* 2012; Bestion *et al.* 2015; Legrand *et al.* 2015). This type of experimental landscape could be useful to study the natural movement of species that use multiple modes of locomotion to disperse.

Selection on different modes of locomotion may also yield different trade-offs, therefore the relationship between flight and reproduction would be worth revisiting. It may also be valuable to substantiate the finding that there is no trade-off between dispersal and reproduction. Three approaches could be taken to achieve this aim: 1) determine if realised fecundity as measured in Chapter 6 is a useful proximate measure for LRS, and if not 2) measuring the LRS of dispersers, residents, and crossed lines, and 3) measuring lifespans and oviposition rates over the lifespan. *Tribolium castaneum* are long-lived insects, and such an experiment would take up to one year to be conducted.

Another interesting research avenue that has been raised by this body of work is how dispersers and residents differ during ontogeny, prior to the adult life stage. Body size strongly diverges with selection for residents and dispersers, and as *T. castaneum* are holometabolous insects (complete metamorphosis through four distinct life stages), body size is determined during the larval (growth) phase. Therefore, ontogenetic changes to growth and developmental rates, resource acquisition, and metabolic processing must occur in larval residents and dispersers. A critical experiment to home in on the mechanisms of body size divergence would be to quantify the food intake, growth rate, timing and size at different larval instars, and measure the MR of larvae across ontogeny. If differences in these traits are apparent between resident and disperser larvae (which seems highly probable), then it would be insightful to examine the expression of genes involved in metabolic processes and their regulation. Recent studies have found that the genetics, epigenetics, and metabolomics can differ between resident and disperser toads (Rollins *et al.* 2015) and spider mites (Van Petegem *et al.* 2016b).

The genome of *T. castaneum* has been sequenced (*Tribolium* Genome Sequencing Consortium 2008), therefore determining whether target genes are differentially expressed in residents and dispersers, and discerning their function, would be a worthwhile and attainable objective. This thesis has provided a foundational understanding of dynamics, correlations, and evolution of phenotypic traits related to dispersal, therefore a logical progression would be to investigate the genetic determinants of these phenotypic traits. A large full-sibling and half-sibling breeding design with phenotypic measurements of both parents and offspring could be used to estimate the additive genetic, phenotypic, and residual variances, and therefore the narrow-sense heritability of traits using quantitative genetic analyses. Following this experiment, top-down approaches such as quantitative trait locus (QTL) mapping, gene expression patterns, microarrays, and genomic associations can be used to work from the phenotype to the genotype to further our functional understanding of the observed phenotypes (Wheat 2012).

Candidate genes involved with metabolism and locomotor performance have been found in other dispersal studies. For examples, Glanville fritillary butterflies (*M. cinxia*) that show allelic variation in expression of the phosphoglucose isomerase (*pgi*) gene have different flight MR and flight performance (Haag *et al.* 2005; Niitepõld *et al.* 2009). Another candidate gene, *foraging* (*for*), which is associated with distance travelled while feeding and the probability of leaving a food patch has been identified in *Drosophila melanogaster* (Sokolowski 2001). Individuals with the dominant *for* allele express more of a cyclic guanosine monophosphate activated protein kinase enzyme (PKG) and exhibit different gene expression patterns in metabolic pathways compared to those with the recessive *for* allele (Kent *et al.* 2009). Subsequent studies have found numerous species that express different levels of PKG vary in locomotion behaviour and performance, suggesting it is conserved across diverse taxa (Wheat 2012). A recent genetic study on invasive cane toads has found differential expression of genes involved in metabolism and immune function between individuals from range edge and range core populations (Rollins *et al.* 2015). Those at the range edge display substantial upregulation genes involved with metabolism and activity (Rollins *et al.* 2015), which contrasts with findings from metabolomics of invasive spider mites (*Tetranychus urticae*) that have undergone a range expansion (Van Petegem *et al.* 2016b). Individuals from the expanding range edge potentially downregulate metabolic pathways associated with protein synthesis and also show differential use of amino acids to those individuals from the range core (Van Petegem *et al.* 2016b). These recent findings demonstrate the complexity of evolutionary processes at range edges that remains to be understood, and highlights the importance of integrating advances in metabolomics and epigenetic technologies. Given that many genes associated with locomotion, behaviour, and metabolism are likely to be conserved, investigating candidate genes that are differentially expressed in resident and disperser *T. castaneum* could prove to be valuable, and enhance the impact of this species as a genomic animal model.

Conclusions

Dispersal is a vital, yet immensely complex, biological process. Dispersal varies substantially among individuals, populations, and species across various contexts, therefore understanding the fundamental underlying phenotypic traits that contribute to dispersal is important. The aim of the research presented in this thesis was to gain a better understanding of the evolution of phenotypic traits that are related to dispersal, using a laboratory model species and artificial selection for different dispersal strategies. This research contributes to an extensive literature on dispersal that has broad implications, providing an insightful link among dispersal syndromes, spatial sorting, experimental evolution, and invasion biology. The major findings of this work are that the associations among morphological, physiological, and movement behaviour traits are more variable than suggested by many previous reports, and that these traits can evolve under artificial selection by spatial sorting. Relative leg length is associated with greater movement ability, but this trait does not evolve under selection for dispersal by climbing. Rather, body size and metabolic rate are important traits for dispersal by climbing, which highlights the importance of investigating the relevant traits associated with different modes of dispersal. The significant, maintained variance in dispersal rate of selected offspring also highlights the value to the insect of maintaining intra-specific variation in offspring dispersal strategy, both to optimise fitness in ephemeral habitats and to ameliorate the effects of environmental change. Overall, the findings presented in this thesis demonstrate that many phenotypic traits are important for movement behaviour and dispersal, particularly body size, and metabolic and locomotor efficiency, which provides empirical support for spatial sorting contributing to the evolution of phenotypes. These findings, taken collectively, have suggested a number of potential interesting and important research directions that could lead to a more comprehensive understanding of dispersal syndromes and variation in phenotypic traits.

References

Aguillon, S.M. & Duckworth, R.A. (2015) Kin aggression and resource availability influence phenotype-dependent dispersal in a passerine bird. *Behavioral Ecology and Sociobiology,* **69,** 625-633.

Ahmad, F., Walter, G.H. & Raghu, S. (2012) Comparative performance of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) across populations, resource types and structural forms of those resources. *Journal of Stored Products Research,* **48,** 73-80.

Alford, R.A., Brown, G.P., Schwarzkopf, L., Phillips, B.L. & Shine, R. (2009) Comparisons through time and space suggest rapid evolution of dispersal behaviour in an invasive species. *Wildlife Research,* **36,** 23-28.

Anholt, B.R. (1990) Size-biased dispersal prior to breeding in a damselfly. *Oecologia,* **83,** 385-387.

Anholt, B.R. & Werner, E.E. (1995) Interaction between food availability and predation mortality mediated by adaptive behavior. *Ecology,* **76,** 2230-2234.

Arnaud, L. & Haubruge, E. (1999) Mating behaviour and male mate choice in *Tribolium castaneum* (Coleoptera, Tenebrionidae). *Behaviour,* **136,** 67-77.

Auld, J.R. & de Casas, R.R. (2013) The correlated evolution of dispersal and mating-system traits. *Evolutionary Biology,* **40,** 185-193.

Baguette, M., Clobert, J. & Schtickzelle, N. (2011) Metapopulation dynamics of the bog fritillary butterfly: experimental changes in habitat quality induced negative density-dependent dispersal. *Ecography,* **34,** 170-176.

Bartoń, K.A. (2012) Package ‘MuMIn’. Multi-model interference. http://cran.r-project.org/web/package=MuMIn, R package verision 1.15.6.

Bartoń, K.A., Phillips, B.L., Morales, J.M. & Travis, J.M.J. (2009) The evolution of an 'intelligent' dispersal strategy: biased, correlated random walks in patchy landscapes. *Oikos,* **118,** 309-319.

Bartumeus, F. (2009) Behavioral intermittence, Lévy patterns, and randomness in animal movement. *Oikos,* **118,** 488-494.

Bates, D., Maechler, M., Bolker, B.M. & Walker, S. (2014) lme4: linear mixed-effects models using Eigen and S4. http://cran.r-project.org/package=lme4, R package version 1.1-7.

Bazazi, S., Bartumeus, F., Hale, J.J. & Couzin, I.D. (2012) Intermittent motion in desert locusts: behavioural complexity in simple environments. *PLoS Computational Biology,* **8,** e1002498.

Bélichon, S., Clobert, J. & Massot, M. (1996) Are there differences in fitness components between philopatric and dispersing individuals? *Acta Ecologica,* **17,** 503-517.

Bell, G. & Gonzalez, A. (2011) Adaptation and evolutionary rescue in metapopulations experiencing environmental deterioration. *Science,* **332,** 1327-1330.

Bell, W.J. (1990) Searching behavior patterns in insects. *Annual Review of Entomology,* **35,** 447-467.

Ben-Shlomo, R., Motro, U. & Ritte, U. (1991) The influence of the ability to disperse on generation length and population size in the flour beetle, *Tribolium castaneum*. *Ecological Entomology,* **16,** 279-282.

Benard, M.F. & McCauley, S.J. (2008) Integrating across life-history stages: consequences of natal habitat effects on dispersal. *The American Naturalist,* **171,** 553-567.

Benton, T.G. & Bowler, D.E. (2012a) Dispersal in invertebrates: influences on individual decisions. *Dispersal ecology and evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 41-49. Oxford University Press, Oxford.

Benton, T.G. & Bowler, D.E. (2012b) Linking dispersal to spatial dynamics. *Dispersal ecology and evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 251-265. Oxford University Press, Oxford.

Berthouly-Salazar, C., van Rensburg, B.J., Le Roux, J.J., van Vuuren, B.J. & Hui, C. (2012) Spatial sorting drives morphological variation in the invasive bird, *Acridotheris tristis*. *PLoS One,* **7,** e38145.

Bestion, E., Clobert, J. & Cote, J. (2015) Dispersal response to climate change: scaling down to intraspecific variation. *Ecology Letters,* **18,** 1226-1233.

Birn-Jeffery, A.V. & Higham, T.E. (2014) The scaling of uphill and downhill locomotion in legged animals. *Integrative and Comparative Biology,* **54,** 1159-1172.

Biro, P.A. & Stamps, J.A. (2008) Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution,* **23,** 361-368.

Biro, P.A. & Stamps, J.A. (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution,* **25,** 653-659.

Bishop, C.D., Erezyilmaz, D.F., Flatt, T., Georgiou, C.D., Hadfield, M.G., Heyland, A., Hodin, J., Jacobs, M.W., Maslakova, S.A., Pires, A., Reitzel, A.M., Santagata, S., Tanaka, K. & Youson, J.H. (2006) What is metamorphosis? *Integrative and Comparative Biology,* **46,** 655-661.

Bitume, E.V., Bonte, D., Magalhães, S., San Martin, G., Van Dongen, S., Bach, F., Anderson, J.M., Olivieri, I. & Nieberding, C.M. (2011) Heritability and artificial selection on ambulatory dispersal distance in *Tetranychus urticae*: effects of density and maternal effects. *PLoS One,* **6,** e26927.

Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *The Quarterly Review of Biology,* **75,** 385-407.

Bocedi, G. & Travis, J.M.J. (2016) Models of dispersal evolution highlight several important issues in evolutionary and ecological modeling. *The American Naturalist,* **187,** 143-150.

Bøhn, T., Terje Sandlund, O., Amundsen, P.-A. & Primicerio, R. (2004) Rapidly changing life history during invasion. *Oikos,* **106,** 138-150.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, **26,** 183-192.

Bonte, D. (2009) Inbreeding depresses short and long distance dispersal in three congeneric spiders. *Journal of Evolutionary Biology,* **22,** 1429-1434.

Bonte, D., Clercq, N.D., Zwertvaegher, I. & Lens, L.U.C. (2009) Repeatability of dispersal behaviour in a common dwarf spider: evidence for different mechanisms behind short- and long-distance dispersal. *Ecological Entomology,* **34,** 271-276.

Bonte, D. & de la Peña, E. (2009) Evolution of body condition-dependent dispersal in metapopulations. *Journal of Evolutionary Biology,* **22,** 1242-1251.

Bonte, D., De Roissart, A., Wybouw, N. & Van Leeuwen, T. (2014) Fitness maximization by dispersal: evidence from an invasion experiment. *Ecology,* **95,** 3104-3111.

Bonte, D. & Saastamoinen, M. (2012) Dispersal syndromes in butterflies and spiders. *Dispersal ecology and evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 161-170. Oxford University Press, Oxford.

Bonte, D., Van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Saastamoinen, M., Schtickzelle, N., Stevens, V.M., Vandewoestijne, S., Baguette, M., Barton, K., Benton, T.G., Chaput-Bardy, A., Clobert, J., Dytham, C., Hovestadt, T., Meier, C.M., Palmer, S.C.F., Turlure, C. & Travis, J.M.J. (2012) Costs of dispersal. *Biological Reviews,* **87,** 290-312.

Bowler, D.E. & Benton, T.G. (2005) Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews,* **80,** 205-225.

Bowler, D.E. & Benton, T.G. (2009) Variation in dispersal mortality and dispersal propensity among individuals: the effects of age, sex and resource availability. *Journal of Animal Ecology,* **78,** 1234-1241.

Brown, G.P., Kelehear, C. & Shine, R. (2013) The early toad gets the worm: cane toads at an invasion front benefit from higher prey availability. *Journal of Animal Ecology,* **82,** 854-862.

Brown, G.P., Phillips, B.L. & Shine, R. (2014) The straight and narrow path: the evolution of straight-line dispersal at a cane toad invasion front. *Proceedings of the Royal Society of London B: Biological Sciences,* **281,** 20141385.

Brown, S.J., Shippy, T.D., Miller, S., Bolognesi, R., Beeman, R.W., Lorenzen, M.D., Bucher, G., Wimmer, E.A. & Klingler, M. (2009) The red flour beetle, *Tribolium castaneum* (Coleoptera): a model for studies of development and pest biology. *Cold Spring Harbor Protocols,* **4,** pdb.emo126.

Bulmer, M.G. (1986) Sex ratios in geographically structured populations. *Trends in Ecology & Evolution,* **1,** 35-38.

Bulmer, M.G. & Taylor, P.D. (1980) Dispersal and the sex ratio. *Nature,* **284,** 448-449.

Burgess, S.C., Baskett, M.L., Grosberg, R.K., Morgan, S.G. & Strathmann, R.R. (2016) When is dispersal for dispersal? Unifying marine and terrestrial perspectives. *Biological Reviews,* **91,** 867-882.

Burton, O.J., Phillips, B.L. & Travis, J.M.J. (2010) Trade-offs and the evolution of life-histories during range expansion. *Ecology Letters,* **13,** 1210-1220.

Burton, T., Killen, S.S., Armstrong, J.D. & Metcalfe, N.B. (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences,* **278,** 3465-3473.

Button, K.S., Ioannidis, J.P.A., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S.J. & Munafo, M.R. (2013) Power failure: why small sample size undermines the reliability of neuroscience. *Nature Reviews Neuroscience,* **14,** 365-376.

Campbell, J.F. (2012) Attraction of walking *Tribolium castaneum* adults to traps. *Journal of Stored Products Research,* **51,** 11-22.

Campbell, J.F. & Arbogast, R.T. (2004) Stored-product insects in a flour mill: population dynamics and response to fumigation treatments. *Entomologia Experimentalis et Applicata,* **112,** 217-225.

Campbell, J.F. & Hagstrum, D.W. (2002) Patch exploitation by *Tribolium castaneum*: movement patterns, distribution, and oviposition. *Journal of Stored Products Research*, **38,** 55-68.

Campbell, J.F. & Runnion, C. (2003) Patch exploitation by female red flour beetles, *Tribolium castaneum*. *Journal of Insect Science,* **3,** 20.

Canestrelli, D., Bisconti, R. & Carere, C. (2016) Bolder takes all? The behavioral dimension of biogeography. *Trends in Ecology & Evolution,* **31,** 35-43.

Careau, V., Thomas, D., Humphries, M.M. & Réale, D. (2008) Energy metabolism and animal personality. *Oikos,* **117,** 641-653.

Carol, J., Benejam, L., Benito, J. & García-Berthou, E. (2009) Growth and diet of European catfish (*Silurus glanis*) in early and late invasion stages. *Fundamental and Applied Limnology,* **174,** 317-328.

Carvalho, A.B., Sampaio, M.C., Varandas, F.R. & Klaczko, L.B. (1998) An experimental demonstration of Fisher's Principle: evolution of sexual proportion by natural selection. *Genetics,* **148,** 719-731.

Choi, I., Shim, J.H. & Ricklefs, R.E. (2003) Morphometric relationships of take-off speed in anuran amphibians. *Journal of Experimental Zoology A,* **299,** 99-102.

Chown, S.L. & Gaston, K.J. (1999) Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews,* **74,** 87-120.

Chown, S.L., Hodgins, K.A., Griffin, P.C., Oakeshott, J.G., Byrne, M. & Hoffmann, A.A. (2015) Biological invasions, climate change and genomics. *Evolutionary Applications,* **8,** 23-46.

Chuang, A. & Peterson, C.R. (2016) Expanding population edges: theories, traits, and trade-offs. *Global Change Biology,* **22,** 494-512.

Clarke, A. & Fraser, K.P.P. (2004) Why does metabolism scale with temperature? *Functional Ecology,* **18,** 243-251.

Cline, L.D. & Highland, H.A. (1976) Clinging and climbing ability of adults of several stored-product beetles on flexible packaging materials. *Journal of Economic Entomology,* **69,** 709-710.

Clobert, J., Baguette, M., Benton, T.G. & Bullock, J.M. (2012) *Dispersal ecology and evolution*. Oxford University Press, Oxford.

Clobert, J., Le Galliard, J.-F., Cote, J., Meylan, S. & Massot, M. (2009) Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters,* **12,** 197-209.

Cohen, J. (1988) *Statistical power analysis for the behavioural sciences*. Lawrence Erlbaum Associates, USA.

Colautti, R.I. & Lau, J.A. (2015) Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. *Molecular Ecology,* **24,** 1999-2017.

Cote, J. & Clobert, J. (2010) Risky dispersal: avoiding kin competition despite uncertainty. *Ecology,* **91,** 1485-1493.

Cote, J., Clobert, J., Brodin, T., Fogarty, S. & Sih, A. (2010a) Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **365,** 4065-4076.

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T. & Sih, A. (2010b) Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society B: Biological Sciences,* **277,** 1571-1579.

Crnokrak, P. & Roff, D.A. (2002) Trade-offs to flight capability in *Gryllus firmus*: the influence of whole-organism respiration rate on fitness. *Journal of Evolutionary Biology,* **15,** 388-398.

Cuddington, K. & Yodzis, P. (2002) Predator-prey dynamics and movement in fractal environments. *The American Naturalist,* **160,** 119-134.

Cutts, C.J., Metcalfe, N.B. & Taylor, A.C. (1998) Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. *Journal of Fish Biology,* **52,** 1026-1037.

Daglish, G.J. (2006) Survival and reproduction of *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) following periods of starvation. *Journal of Stored Products Research,* **42,** 328-338.

Daglish, G.J., Nayak, M.K., Pavic, H. & Smith, L.W. (2015) Prevalence and potential fitness cost of weak phosphine resistance in *Tribolium castaneum* (Herbst) in eastern Australia. *Journal of Stored Products Research,* **61,** 54-58.

Darwin, C. (1859) *The origin of species by means of natural selection*. John Murray, London.

Davenport, J.M. & Lowe, W.H. (2016) Does dispersal influence the strength of intraspecific competition in a stream salamander? *Journal of Zoology,* **298,** 46-53.

David, G., Giffard, B., van Halder, I., Piou, D. & Jactel, H. (2015) Energy allocation during the maturation of adults in a long-lived insect: implications for dispersal and reproduction. *Bulletin of Entomological Research,* **105,** 629-636.

Davidowitz, G. & Nijhout, H.F. (2004) The physiological basis of reaction norms: the interaction among growth rate, the duration of growth and body size. *Integrative and Comparative Biology,* **44,** 443-449.

De Block, M. & Stoks, R. (2005) Fitness effects from egg to reproduction: bridging the life history transition. *Ecology,* **86,** 185-197.

Delgado, M.M. & Penteriani, V. (2008) Behavioral states help translate dispersal movements into spatial distribution patterns of floaters. *The American Naturalist,* **172,** 475-485.

Delgado, M.M., Penteriani, V., Revilla, E. & Nams, V.O. (2010) The effect of phenotypic traits and external cues on natal dispersal movements. *Journal of Animal Ecology,* **79,** 620-632.

DeRose, M.A. & Roff, D.A. (1999) A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution,* **53,** 1288-1292.

Díez, R. & López-Fanjul, C. (1979) Selection for flying ability in *Tribolium castaneum*. *Behavior Genetics,* **9,** 115-122.

Dingemanse, N.J., Both, C., van Noordwijk, A.J., Rutten, A.L. & Drent, P.J. (2003) Natal dispersal and personalities in great tits (*Parus major*). *Proceedings of the Royal Society of London B: Biological Sciences,* **270,** 741-747.

Drury, D.W., Whitesell, M.E. & Wade, M.J. (2016) The effects of temperature, relative humidity, light, and resource quality on flight initiation in the red flour beetle, *Tribolium castaneum*. *Entomologia Experimentalis et Applicata,* **158,** 269-274.

Ducatez, S., Legrand, D., Chaput-Bardy, A., Stevens, V.M., Fréville, H. & Baguette, M. (2012) Inter-individual variation in movement: is there a mobility syndrome in the large white butterfly *Pieris brassicae*? *Ecological Entomology,* **37,** 377-385.

Duckworth, R.A. (2006) Aggressive behaviour affects selection on morphology by influencing settlement patterns in a passerine bird. *Proceedings of the Royal Society of London B: Biological Sciences,* **273,** 1789-1795.

Dukes, J.S. & Mooney, H.A. (1999) Does global change increase the success of biological invaders? *Trends in Ecology & Evolution,* **14,** 135-139.

Duputié, A. & Massol, F. (2013) An empiricist's guide to theoretical predictions on the evolution of dispersal. *Interface Focus,* **3,** 20130028.

Ewen, J.G., Cassey, P. & Møller, A.P. (2004) Facultative primary sex ratio variation: a lack of evidence in birds? *Proceedings of the Royal Society of London B: Biological Sciences,* **271,** 1277-1282.

Ewing, A.W. (1963) Attempts to select for spontaneous activity in *Drosophila melanogaster*. *Animal Behaviour,* **11,** 369-378.

Faustini, D.L., Burkholder, W.E. & Laub, R.J. (1981) Sexually dimorphic setiferous sex patch in the male red flour beetle,*Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae): site of aggregation pheromone production. *Journal of Chemical Ecology,* **7,** 465-480.

Fisher, R.A. (1930) *The genetical theory of natural selection*. Claredon Press, Oxford.

Forsman, A., Merilä, J. & Ebenhard, T. (2010) Phenotypic evolution of dispersal-enhancing traits in insular voles. *Proceedings of the Royal Society of London B: Biological Sciences,* **278,** 225-232.

Fraser, D.F., Gilliam, J.F., Daley, M.J., Le, A.N. & Skalski, G.T. (2001) Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. *The American Naturalist,* **158,** 124-135.

Friedenberg, N.A. (2003) Experimental evolution of dispersal in spatiotemporally variable microcosms. *Ecology Letters,* **6,** 953-959.

Fronhofer, E.A. & Altermatt, F. (2015) Eco-evolutionary feedbacks during experimental range expansions. *Nature Communications,* **6,** 6844.

Full, R.J. & Tullis, A. (1990) Energetics of ascent: insects on inclines. *Journal of Experimental Biology,* **149,** 307-317.

Gandon, S. (1999) Kin competition, the cost of inbreeding and the evolution of dispersal. *Journal of Theoretical Biology,* **200,** 345-364.

Ghent, A.W. (1963) Studies of behavior of the *Tribolium* flour beetles. I. contrasting responses of *T. castaneum* and *T. confusum* to fresh and conditioned flours. *Ecology,* **44,** 269-283.

Gibbs, M. & Van Dyck, H. (2010) Butterfly flight activity affects reproductive performance and longevity relative to landscape structure. *Oecologia,* **163,** 341-350.

Glazier, D.S. (1999) Trade-offs between reproductive and somatic (storage) investments in animals: a comparative test of the Van Noordwijk and De Jong model. *Evolutionary Ecology,* **13,** 539-555.

Glazier, D.S. (2005) Beyond the ‘3/4-power law’: variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews,* **80,** 611-662.

Goodnight, C.J. (1989) Population differentiation and the correlation among traits at the population level. *The American Naturalist,* **133,** 888-900.

Graham, W.M. & Waterhouse, F.L. (1964) The distribution and orientation of *Tribolium* on inclines, and the concept of controls in gradient experiments. *Animal Behaviour,* **12,** 368-373.

Gu, H., Hughes, J. & Dorn, S. (2006) Trade-off between mobility and fitness in *Cydia pomonella* L. (Lepidoptera: Tortricidae). *Ecological Entomology,* **31,** 68-74.

Gutowsky, L.F.G. & Fox, M.G. (2012) Intra-population variability of life-history traits and growth during range expansion of the invasive round goby, *Neogobius melanostomus*. *Fisheries Management and Ecology,* **19,** 78-88.

Haag, C.R., Saastamoinen, M., Marden, J.H. & Hanski, I. (2005) A candidate locus for variation in dispersal rate in a butterfly metapopulation. *Proceedings of the Royal Society B: Biological Sciences,* **272,** 2449-2456.

Hagstrum, D.W. & Smittle, B.J. (1980) Age- and sex-specific tunneling rates of adult *Tribolium castaneum*. *Annals of the Entomological Society of America,* **73,** 11-13.

Halsey, L.G. (2016) Terrestrial movement energetics: current knowledge and its application to the optimising animal. *Journal of Experimental Biology,* **219,** 1424-1431.

Halstead, D.G.H. (1963) External sex differences in stored-product Coleoptera. *Bulletin of Entomological Research,* **54,** 119-134.

Hanski, I., Peltonen, A. & Kaski, L. (1991) Natal dispersal and social dominance in the common shrew *Sorex araneus*. *Oikos,* **62,** 48-58.

Hanski, I., Saastamoinen, M. & Ovaskainen, O. (2006) Dispersal-related life-history trade-offs in a butterfly metapopulation. *Journal of Animal Ecology,* **75,** 91-100.

Happ, G. (1970) Maturation of the response of male *Tenebrio molitor* to the female sex pheromone. *Annals of the Entomological Society of America,* **63,** 1782.

Haughland, D.L. & Larsen, K.W. (2004) Exploration correlates with settlement: red squirrel dispersal in contrasting habitats. *Journal of Animal Ecology,* **73,** 1024-1034.

Hawkes, C. (2009) Linking movement behaviour, dispersal and population processes: is individual variation a key? *Journal of Animal Ecology,* **78,** 894-906.

Hedrick, T.L. (2008) Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration & Biomimetics,* **3,** 034001.

Heidinger, I.M.M., Hein, S. & Bonte, D. (2010) Patch connectivity and sand dynamics affect dispersal-related morphology of the blue-winged grasshopper *Oedipoda caerulescens* in coastal grey dunes. *Insect Conservation and Diversity,* **3,** 205-212.

Hernandez Nopsa, J.F., Daglish, G.J., Hagstrum, D.W., Leslie, J.F., Phillips, T.W., Scoglio, C., Thomas-Sharma, S., Walter, G.H. & Garrett, K.A. (2015) Ecological networks in stored grain: key postharvest nodes for emerging pests, pathogens, and mycotoxins. *BioScience,* **65,** 985-1002.

Hill, J.K., Thomas, C.D. & Blakeley, D.S. (1999) Evolution of flight morphology in a butterfly that has recently expanded its geographic range. *Oecologia,* **121,** 165-170.

Hillman, S.S., Drewes, R.C., Hedrick, M.S. & Hancock, T.V. (2014) Physiological vagility: correlations with dispersal and population genetic structure of amphibians. *Physiological and Biochemical Zoology,* **87,** 105-112.

Honĕk, A. (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos,* **66,** 483-492.

Hudson, C.M., Phillips, B.L., Brown, G.P. & Shine, R. (2015) Virgins in the vanguard: low reproductive frequency in invasion-front cane toads. *Biological Journal of the Linnean Society,* **116,** 743-747.

Hughes, C.L., Dytham, C. & Hill, J.K. (2007) Modelling and analysing evolution of dispersal in populations at expanding range boundaries. *Ecological Entomology,* **32,** 437-445.

Hughes, C.L., Hill, J.K. & Dytham, C. (2003) Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. *Proceedings of the Royal Society of London B: Biological Sciences,* **270,** S147-S150.

Hui, C., Roura-Pascual, N., Brotons, L., Robinson, R.A. & Evans, K.L. (2012) Flexible dispersal strategies in native and non-native ranges: environmental quality and the ‘good–stay, bad–disperse’ rule. *Ecography,* **35,** 1024-1032.

Hulbert, A.J., Clancy, D.J., Mair, W., Braeckman, B.P., Gems, D. & Partridge, L. (2004) Metabolic rate is not reduced by dietary-restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in *Drosophila melanogaster*. *Experimental Gerontology,* **39,** 1137-1143.

Hulme, P.E. (2016) Climate change and biological invasions: evidence, expectations, and response options. *Biological Reviews***,** DOI: 10.1111/brv.12282.

Jagadeesan, R., Collins, P.J., Daglish, G.J., Ebert, P.R. & Schlipalius, D.I. (2012) Phosphine resistance in the rust red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae): inheritance, gene interactions and fitness costs. *PLoS One,* **7,** e31582.

Jennions, M.D. & Petrie, M. (1997) Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews,* **72,** 283-327.

Jervis, M.A., Boggs, C.L. & Ferns, P.N. (2005) Egg maturation strategy and its associated trade-offs: a synthesis focusing on Lepidoptera. *Ecological Entomology,* **30,** 359-375.

Karlsson, B. & Johansson, A. (2008) Seasonal polyphenism and developmental trade-offs between flight ability and egg laying in a pierid butterfly. *Proceedings of the Royal Society of London B: Biological Sciences,* **275,** 2131-2136.

Karlsson, B. & Wickman, P.O. (1990) Increase in reproductive effort as explained by body size and resource allocation in the speckled wood butterfly, *Pararge aegeria* (L.). *Functional Ecology,* **4,** 609-617.

Kawecki, T.J., Lenski, R.E., Ebert, D., Hollis, B., Olivieri, I. & Whitlock, M.C. (2012) Experimental evolution. *Trends in Ecology & Evolution,* **27,** 547-560.

Kelehear, C., Brown, G.P. & Shine, R. (2012) Rapid evolution of parasite life history traits on an expanding range-edge. *Ecology Letters,* **15,** 329-337.

Keller, L.F. & Waller, D.M. (2002) Inbreeding effects in wild populations. *Trends in Ecology & Evolution,* **17,** 230-241.

Kent, C.F., Daskalchuk, T., Cook, L., Sokolowski, M.B. & Greenspan, R.J. (2009) The *Drosophila foraging* gene mediates adult plasticity and gene-environment interactions in behaviour, metabolites, and gene expression in response to food deprivation. *PLoS Genetics,* **5,** e1000609.

Khuhro, N.H., Biondi, A., Desneux, N., Zhang, L., Zhang, Y. & Chen, H. (2014) Trade-off between flight activity and life-history components in *Chrysoperla sinica*. *BioControl,* **59,** 219-227.

Killen, S.S., Marras, S., Metcalfe, N.B., McKenzie, D.J. & Domenici, P. (2013) Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution,* **28,** 651-658.

Killen, S.S., Marras, S., Ryan, M.R., Domenici, P. & McKenzie, D.J. (2012) A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass. *Functional Ecology,* **26,** 134-143.

Kim, S.-Y., Torres, R. & Drummond, H. (2009) Simultaneous positive and negative density-dependent dispersal in a colonial bird species. *Ecology,* **90,** 230-239.

Kleiber, M. (1947) Body size and metabolic rate. *Physiological Reviews,* **27,** 511-541.

Kleiber, M. (1961) *The fire of life. An introduction to animal energetics*. Wiley, New York.

Kokko, H. & López-Sepulcre, A. (2006) From individual dispersal to species ranges: perspectives for a changing world. *Science,* **313,** 789-791.

Konarzewski, M. & Książek, A. (2013) Determinants of intra-specific variation in basal metabolic rate. *Journal of Comparative Physiology B,* **183,** 27-41.

Korona, R. (1991) Genetic basis of behavioral strategies. Dispersal of female flour beetles, *Tribolium confusum*, in a laboratory system. *Oikos,* **62,** 265-270.

Kramer, D.L. & McLaughlin, R.L. (2001) The behavioral ecology of intermittent locomotion. *American Zoologist,* **41,** 137-153.

Kubisch, A., Holt, R.D., Poethke, H.-J. & Fronhofer, E.A. (2014) Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. *Oikos,* **123,** 5-22.

Kuussaari, M., Nieminen, M. & Hanski, I. (1996) An experimental study of migration in the Glanville fritillary butterfly *Melitaea cinxia*. *Journal of Animal Ecology,* **65,** 791-801.

Kuussaari, M., Saccheri, I., Camara, M. & Hanski, I. (1998) Allee effect and population dynamics in the Glanville fritillary butterfly. *Oikos,* **82,** 384-392.

Lakovic, M., Poethke, H.-J. & Hovestadt, T. (2015) Dispersal timing: emigration of insects living in patchy environments. *PLoS One,* **10,** e0128672.

Lambin, X., Aars, J. & Piertney, S.B. (2001) Dispersal, intraspecific competition, kin competition, and kin facilitation: a review of the empirical evidence. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 110-122. Oxford University Press, New York.

Langellotto, G.A., Denno, R.F. & Ott, J.R. (2000) A trade-off between flight capability and reproduction in males of a wing-dimorphic insect. *Ecology,* **81,** 865-875.

Laparie, M., Renault, D., Lebouvier, M. & Delattre, T. (2013) Is dispersal promoted at the invasion front? Morphological analysis of a ground beetle invading the Kerguelen Islands, *Merizodus soledadinus* (Coleoptera, Carabidae). *Biological Invasions,* **15,** 1641-1648.

Larranaga, N., Baguette, M., Calvez, O., Trochet, A., Ducatez, S. & Legrand, D. (2013) Intra- and inter-individual variation in flight direction in a migratory butterfly co-vary with individual mobility. *Journal of Experimental Biology,* **216,** 3156-3163.

Lavie, B. & Ritte, U. (1978) The relation between dispersal behaviour and reproductive fitness in the flour beetle *Tribolium castaneum*. *Canadian Journal of Genetics and Cytology,* **20,** 589-595.

Lavie, B. & Ritte, U. (1980) Correlated effects of the response to conditioned medium in the flour beetle, *Tribolium castaneum*. *Researches on Population Ecology,* **21,** 228-232.

Lawrence, W.S. (1987) Dispersal: an alternative mating tactic conditional on sex ratio and body size. *Behavioral Ecology and Sociobiology,* **21,** 367-373.

Le Galliard, J.-F., Paquet, M., Cisel, M. & Montes-Poloni, L. (2013) Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. *Functional Ecology,* **27,** 136-144.

Lease, H.M. & Wolf, B.O. (2011) Lipid content of terrestrial arthropods in relation to body size, phylogeny, ontogeny and sex. *Physiological Entomology,* **36,** 29-38.

Lebeau, J., Wesselingh, R.A. & Van Dyck, H. (2016) Nectar resource limitation affects butterfly flight performance and metabolism differently in intensive and extensive agricultural landscapes. *Proceedings of the Royal Society of London B: Biological Sciences,* **283,** 20160455.

Legrand, D., Guillaume, O., Baguette, M., Cote, J., Trochet, A., Calvez, O., Zajitschek, S., Zajitschek, F., Lecomte, J., Benard, Q., Le Galliard, J.-F. & Clobert, J. (2012) The Metatron: an experimental system to study dispersal and metaecosystems for terrestrial organisms. *Nature Methods,* **9,** 828-833.

Legrand, D., Trochet, A., Moulherat, S., Calvez, O., Stevens, V.M., Ducatez, S., Clobert, J. & Baguette, M. (2015) Ranking the ecological causes of dispersal in a butterfly. *Ecography,* **38,** 822-831.

Léna, J.-P., Clobert, J., de Fraipont, M., Lecomte, J. & Guyot, G. (1998) The relative influence of density and kinship on dispersal in the common lizard. *Behavioral Ecology,* **9,** 500-507.

Lennox, R., Choi, K., Harrison, P.M., Paterson, J.E., Peat, T.B., Ward, T.D. & Cooke, S.J. (2015) Improving science-based invasive species management with physiological knowledge, concepts, and tools. *Biological Invasions,* **17,** 2213-2227.

Leslie, P.H. & Park, T. (1949) The intrinsic rate of natural increase of *Tribolium castaneum* Herbst. *Ecology,* **30,** 469-477.

Leturque, H. & Rousset, F. (2002) Dispersal, kin competition, and the ideal free distribution in a spatially heterogeneous population. *Theoretical Population Biology,* **62,** 169-180.

Lighton, J.R.B. (2008) *Measuring metabolic rates: a manual for scientists*. Oxford University Press, Oxford.

Lindström, T., Brown, G.P., Sisson, S.A., Phillips, B.L. & Shine, R. (2013) Rapid shifts in dispersal behavior on an expanding range edge. *Proceedings of the National Academy of Sciences,* **110,** 13452-13456.

Lipp, A., Wolf, H. & Lehmann, F.-O. (2005) Walking on inclines: energetics of locomotion in the ant Camponotus. *Journal of Experimental Biology,* **208,** 707-719.

Llewelyn, J., Phillips, B.L., Alford, R.A., Schwarzkopf, L. & Shine, R. (2010) Locomotor performance in an invasive species: cane toads from the invasion front have greater endurance, but not speed, compared to conspecifics from a long-colonised area. *Oecologia,* **162,** 343-348.

Łomnicki, A. (2006) Population regulation by dispersal under selection pressure for and against dispersal: an experimental test with beetles, *Tribolium confusum*. *Evolutionary Ecology Research,* **8,** 63-73.

Lopez, D.P., Jungman, A.A. & Rehage, J.S. (2012) Nonnative African jewelfish are more fit but not bolder at the invasion front: a trait comparison across an Everglades range expansion. *Biological Invasions,* **14,** 2159-2174.

Lorenz, M.W. & Gäde, G. (2009) Hormonal regulation of energy metabolism in insects as a driving force for performance. *Integrative and Comparative Biology,* **49,** 380-392.

Lowe, W.H. & McPeek, M.A. (2012) Can natural selection maintain long-distance dispersal? Insight from a stream salamander system. *Evolutionary Ecology,* **26,** 11-24.

Mahroof, R., Subramanyam, B., Throne, J.E. & Menon, A. (2003) Time-mortality relationships for *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed to elevated temperatures. *Journal of Economic Entomology,* **96,** 1345-1351.

Mancinelli, G. (2010) Intraspecific, size-dependent variation in the movement behaviour of a brackish-water isopod: a resource-free laboratory experiment. *Marine and Freshwater Behaviour and Physiology,* **43,** 321-337.

Martin, J.-R. (2003) Locomotor activity: a complex behavioural trait to unravel. *Behavioural Processes,* **64,** 145-160.

Mathot, K.J. & Dingemanse, N.J. (2015) Energetics and behavior: unrequited needs and new directions. *Trends in Ecology & Evolution,* **30,** 199-206.

Matthysen, E. (2005) Density-dependent dispersal in birds and mammals. *Ecography,* **28,** 403-416.

Matthysen, E. (2012) Multicausality of dispersal: a review. *Dispersal ecology and evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 3-18. Oxford University Press, Oxford.

McCabe, J. & Partridge, L. (1997) An interaction between environmental temperature and genetic variation for body size for the fitness of adult female *Drosophila melanogaster*. *Evolution,* **51,** 1164-1174.

McCauley, D.E. & Wade, M.J. (1981) The populational effects of inbreeding in *Tribolium*. *Heredity,* **46,** 59-67.

McCauley, S.J. & Rowe, L. (2010) *Notonecta* exhibit threat-sensitive, predator-induced dispersal. *Biology Letters,* **6,** 449-452.

McDevitt, A.D., Oliver, M.K., Piertney, S.B., Szafrańska, P.A., Konarzewski, M. & Zub, K. (2013) Individual variation in dispersal associated with phenotype influences fine-scale genetic structure in weasels. *Conservation Genetics,* **14,** 499-509.

Melbourne, B.A. & Hastings, A. (2009) Highly variable spread rates in replicated biological invasions: fundamental limits to predictability. *Science,* **325,** 1536-1539.

Merritt, L., Matthews, P.G. & White, C.R. (2013) Performance correlates of resting metabolic rate in garden skinks *Lampropholis delicata*. *Journal of Comparative Physiology B,* **183,** 663-673.

Metcalfe, N.B., Van Leeuwen, T.E. & Killen, S.S. (2016) Does individual variation in metabolic phenotype predict fish behaviour and performance? *Journal of Fish Biology,* **88,** 298-321.

Min, K.J., Jones, N., Borst, D.W. & Rankin, M.A. (2004) Increased juvenile hormone levels after long-duration flight in the grasshopper, *Melanoplus sanguinipes*. *Journal of Insect Physiology,* **50,** 531-537.

Mole, S. & Zera, A.J. (1993) Differential allocation of resources underlies the dispersal-reproduction trade-off in the wing-dimorphic cricket, *Gryllus rubens*. *Oecologia,* **93,** 121-127.

Mousseau, T.A. & Fox, C.W. (1998) The adaptive significance of maternal effects. *Trends in Ecology & Evolution,* **13,** 403-407.

Myles-Gonzalez, E., Burness, G., Yavno, S., Rooke, A. & Fox, M.G. (2015) To boldly go where no goby has gone before: boldness, dispersal tendency, and metabolism at the invasion front. *Behavioral Ecology,* **26,** 1083-1090.

Naylor, A.F. (1961) Dispersal in the red flour beetle *Tribolium castaneum* (Tenebrionidae). *Ecology,* **42,** 231-237.

Nespolo, R.F., Roff, D.A. & Fairbairn, D.J. (2008) Energetic trade-off between maintenance costs and flight capacity in the sand cricket (*Gryllus firmus*). *Functional Ecology,* **22,** 624-631.

Niitepõld, K., Smith, A.D., Osborne, J.L., Don, R.R., Carreck, N.L., Martin, A.P., Marden, J.H., Ovaskainen, O. & Hanski, I. (2009) Flight metabolic rate and *Pgi* genotype influence butterfly dispersal rate in the field. *Ecology,* **90,** 2223-2232.

Norris, M.J. (1954) Sexual maturation in the desert locust (*Schistocerca gregaria* Forskål) with special reference to the effects of grouping. *Anti-Locust Bulletin,* **18,** 1-44.

Ogden, J.C. (1969) Effect of components of conditioned medium on behavior in *Tribolium confusum*. *Physiological Zoology,* **42,** 266-274.

Ogden, J.C. (1970a) Artificial selection for dispersal in flour beetles (Tenebrionidae: *Tribolium*). *Ecology,* **51,** 130-133.

Ogden, J.C. (1970b) Aspects of dispersal in *Tribolium* flour beetles. *Physiological Zoology,* **43,** 124-131.

Park, T. (1937) The inheritance of the mutation “pearl” in the flour beetle, *Tribolium castaneum* Herbst. *The American Naturalist,* **71,** 143-157.

Park, T. & Frank, M.B. (1948) The fecundity and development of the flour beetles, *Tribolium confusum* and *Tribolium castaneum,* at three constant temperatures. *Ecology,* **29,** 368-374.

Park, T., Mertz, D.B. & Petrusewicz, K. (1961) Genetic strains of *Tribolium*: their primary characteristics. *Physiological Zoology,* **34,** 62-80.

Pärn, H., Jensen, H., Ringsby, T.H. & Sæther, B.-E. (2009) Sex-specific fitness correlates of dispersal in a house sparrow metapopulation. *Journal of Animal Ecology,* **78,** 1216-1225.

Perez-Mendoza, J., Campbell, J.F. & Throne, J.E. (2011) Effects of rearing density, age, sex, and food deprivation on flight initiation of the red flour beetle (Coleoptera: Tenebrionidae). *Journal of Economic Entomology,* **104,** 443-451.

Perkins, T.A., Phillips, B.L., Baskett, M.L. & Hastings, A. (2013) Evolution of dispersal and life history interact to drive accelerating spread of an invasive species. *Ecology Letters,* **16,** 1079-1087.

Phillips, B.L. (2009) The evolution of growth rates on an expanding range edge. *Biology Letters,* **5,** 802-804.

Phillips, B.L., Brown, G.P., Greenlees, M., Webb, J.K. & Shine, R. (2007) Rapid expansion of the cane toad (*Bufo marinus*) invasion front in tropical Australia. *Austral Ecology,* **32,** 169-176.

Phillips, B.L., Brown, G.P. & Shine, R. (2010a) Evolutionarily accelerated invasions: the rate of dispersal evolves upwards during the range advance of cane toads. *Journal of Evolutionary Biology,* **23,** 2595-2601.

Phillips, B.L., Brown, G.P. & Shine, R. (2010b) Life-history evolution in range-shifting populations. *Ecology,* **91,** 1617-1627.

Phillips, B.L., Brown, G.P., Travis, J.M.J. & Shine, R. (2008) Reid's paradox revisited: the evolution of dispersal kernels during range expansion. *The American Naturalist,* **172,** S34-S48.

Phillips, B.L., Brown, G.P., Webb, J.K. & Shine, R. (2006) Invasion and the evolution of speed in toads. *Nature,* **439,** 803.

Piiroinen, S., Ketola, T., Lyytinen, A. & Lindström, L. (2011) Energy use, diapause behaviour and northern range expansion potential in the invasive Colorado potato beetle. *Functional Ecology,* **25,** 527-536.

Pitnick, S., Markow, T.A. & Spicer, G.S. (1995) Delayed male maturity is a cost of producing large sperm in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America,* **92,** 10614-10618.

Pontzer, H. (2016) A unified theory for the energy cost of legged locomotion. *Biology Letters,* **12,** 20150935.

Potenza, L. & Mancinelli, G. (2010) Body mass-related shift in movement behaviour in the isopod *Lekanesphaera hookeri* (Isopoda, Flabellifera): a laboratory study. *Italian Journal of Zoology,* **77,** 354-361.

Prus, T. (1963) Search for methods to investigate mobility in *Tribolium*. *Ecology,* **44,** 801-803.

Prus, T. (1966) Emigrational ability and surface numbers of adult beetles in 12 strains of *Tribolium confusum* Duval and *T. castaneum* Herbst. *Ekologia Polska Seria A,* **14,** 547-588.

R Development Core Team (2015) R: a language and environment for statistical computing.

Rebrina, F., Skejo, J., Lucić, A. & Hundina, S. (2015) Trait variability of the signal crayfish (*Pacifastacus leniusculus*) in a recently invaded region reflects potential benefits and trade-offs during dispersal. *Aquatic Invasions,* **10,** 41-50.

Rehage, J.S. & Sih, A. (2004) Dispersal behavior, boldness, and the link to invasiveness: a comparison of four *Gambusia* species. *Biological Invasions,* **6,** 379-391.

Reinhold, K. (1999) Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology,* **13,** 217-224.

Ricciardi, A. (2007) Are modern biological invasions an unprecedented form of global change? *Conservation Biology,* **21,** 329-336.

Riddle, R.A. & Dawson, P.S. (1983) Genetic control of emigration behavior in *Tribolium castaneum* and *T. confusum*. *Behavior Genetics,* **13,** 421-434.

Ridley, A.W., Hereward, J.P., Daglish, G.J., Raghu, S., Collins, P.J. & Walter, G.H. (2011) The spatiotemporal dynamics of *Tribolium castaneum* (Herbst): adult flight and gene flow. *Molecular Ecology,* **20,** 1635-1646.

Ritte, U. & Lavie, B. (1977) The genetic basis of dispersal behavior in the flour beetle *Tribolium castaneum*. *Canadian Journal of Genetics and Cytology,* **19,** 717-722.

Roderick, G.K. & Caldwell, R.L. (1992) An entomological perspective on animal dispersal. *Animal dispersal* (eds N.C. Stenseth & W.Z. Lidicker), pp. 274-290. Springer Publishing, Netherlands.

Roff, D.A. (1977) Dispersal in Dipterans: its costs and consequences. *Journal of Animal Ecology,* **46,** 443-456.

Roff, D.A. (1984) The cost of being able to fly: a study of wing polymorphism in two species of crickets. *Oecologia,* **63,** 30-37.

Roff, D.A. (1986) The evolution of wing dimorphism in insects. *Evolution,* **40,** 1009-1020.

Roff, D.A. & Derose, M.A. (2001) The evolution of trade-offs: effects of inbreeding on fecundity relationships in the cricket *Gryllus firmus*. *Evolution,* **55,** 111-121.

Roff, D.A. & Fairbairn, D.J. (1991) Wing dimorphisms and the evolution of migratory polymorphisms among the Insecta. *American Zoologist,* **31,** 243-251.

Rollins, L.A., Richardson, M.F. & Shine, R. (2015) A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). *Molecular Ecology,* **24,** 2264-2276.

Ronce, O. (2007) How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology, Evolution, and Systematics,* **38,** 231-253.

Ronce, O. & Clobert, J. (2012) Dispersal syndromes. *Dispersal ecology and evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 119-138. Oxford University Press, Oxford, UK.

Rousset, F. (2012) Demographic consequences of the selective forces controlling density-dependent dispersal. *Dispersal ecology and evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 266-279. Oxford University Press, Oxford.

Royauté, R., Greenlee, K., Baldwin, M. & Dochtermann, N.A. (2015) Behaviour, metabolism and size: phenotypic modularity or integration in *Acheta domesticus*? *Animal Behaviour,* **110,** 163-169.

Saastamoinen, M. (2007) Mobility and lifetime fecundity in new versus old populations of the Glanville fritillary butterfly. *Oecologia,* **153,** 569-578.

Saglam, I.K., Roff, D.A. & Fairbairn, D.J. (2008) Male sand crickets trade-off flight capability for reproductive potential. *Journal of Evolutionary Biology,* **21,** 997-1004.

Santiago, E. & Caballero, A. (1998) Effective size and polymorphism of linked neutral loci in populations under directional selection. *Genetics,* **149,** 2105-2117.

Schimpf, N.G., Matthews, P.G.D. & White, C.R. (2012) Standard metabolic rate is associated with gestation duration, but not clutch size, in speckled cockroaches *Nauphoeta cinerea*. *Biology Open,* **1,** 1185-1191.

Schlipalius, D.I., Valmas, N., Tuck, A.G., Jagadeesan, R., Ma, L., Kaur, R., Goldinger, A., Anderson, C., Kuang, J., Zuryn, S., Mau, Y.S., Cheng, Q., Collins, P.J., Nayak, M.K., Schirra, H.J., Hilliard, M.A. & Ebert, P.R. (2012) A core metabolic enzyme mediates resistance to phosphine gas. *Science,* **338,** 807-810.

Sekar, S. (2012) A meta-analysis of the traits affecting dispersal ability in butterflies: can wingspan be used as a proxy? *Journal of Animal Ecology,* **81,** 174-184.

Semeao, A.A., Campbell, J.F., Whitworth, R.J. & Sloderbeck, P.E. (2013) Movement of *Tribolium castaneum* within a flour mill. *Journal of Stored Products Research,* **54,** 17-22.

Sharp, N.P. & Agrawal, A.F. (2016) The decline in fitness with inbreeding: evidence for negative dominance-by-dominance epistasis in *Drosophila melanogaster*. *Journal of Evolutionary Biology,* **29,** 857-864.

Shaw, A.K. & Kokko, H. (2014) Mate finding, Allee effects and selection for sex-biased dispersal. *Journal of Animal Ecology,* **83,** 1256-1267.

Shine, R., Brown, G.P. & Phillips, B.L. (2011) An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences,* **108,** 5708-5711.

Sinervo, B., Calsbeek, R., Comendant, T., Both, C., Adamopoulou, C. & Clobert, J. (2006) Genetic and maternal determinants of effective dispersal: the effect of sire genotype and size at birth in side-blotched lizards. *The American Naturalist,* **168,** 88-99.

Smith, E.H. & Whitman, R.C. (1992) *Field guide to structural pests*. National Pest Control Association, Dunn Loring, Virginia.

Socha, R. & Zemek, R. (2003) Wing morph-related differences in the walking pattern and dispersal in a flightless bug, *Pyrrhocoris apterus* (Heteroptera). *Oikos,* **100,** 35-42.

Sohal, R.S. (1982) Oxygen consumption and life span in the adult male housefly, *Musca domestica*. *Age,* **5,** 21-24.

Sokoloff, A. (1974) *The biology of Tribolium*. Clarendon Press, Oxford.

Sokoloff, A. (1977) *The biology of Tribolium with special emphasis on genetic aspects*. Clarendon Press, Oxford.

Sokolowski, M.B. (2001) *Drosophila*: genetics meets behaviour. *Nature Reviews Genetics,* **2,** 879-890.

Soliman, M.H. (1987) Ageing and parental age effects in *Tribolium*. *Archives of Gerontology and Geriatrics,* **6,** 43-60.

Speakman, J.R., Król, E. & Johnson, M.S. (2004) The functional significance of individual variation in basal metabolic rate. *Physiological and Biochemical Zoology,* **77,** 900-915.

Speakman, J.R. & Selman, C. (2003) Physical activity and resting metabolic rate. *The Proceedings of the Nutrition Society,* **62,** 621-634.

Starrfelt, J. & Kokko, H. (2012) The theory of dispersal under multiple influences. *Dispersal ecology and evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 19-28. Oxford University Press, Oxford.

Stevens, V.M., Trochet, A., Blanchet, S., Moulherat, S., Clobert, J. & Baguette, M. (2013) Dispersal syndromes and the use of life-histories to predict dispersal. *Evolutionary Applications,* **6,** 630-642.

Stevens, V.M., Trochet, A., Van Dyck, H., Clobert, J. & Baguette, M. (2012) How is dispersal integrated in life histories: a quantitative analysis using butterflies. *Ecology Letters,* **15,** 74-86.

Stevens, V.M., Whitmee, S., Le Galliard, J.-F., Clobert, J., Böhning-Gaese, K., Bonte, D., Brändle, M., Dehling, D.M., Hof, C., Trochet, A. & Baguette, M. (2014) A comparative analysis of dispersal syndromes in terrestrial and semi-terrestrial animals. *Ecology Letters,* **17,** 1039-1052.

Surtees, G. (1963) Laboratory studies on dispersion behaviour of adult beetles in grain. III. - *Tribolium castaneum* (Hbst.) (Coleoptera, Tenebrionidae) and *Cryptolestes ferrugineus* (Steph.) (Coleoptera, Cucujidae). *Bulletin of Entomological Research,* **54,** 297-306.

Taylor, P.D. & Crespi, B.J. (1994) Evolutionarily stable strategy sex ratios when correlates of relatedness can be assessed. *The American Naturalist,* **143,** 297-316.

Taylor, W.B., Anderson, R.C. & Peckarsky, L.B. (1998) Effects of size at metamorphosis on stonefly fecundity, longevity, and reproductive success. *Oecologia,* **114,** 494-502.

Teal, P.E.A. & Gomez-Simuta, Y. (2002) Juvenile hormone: action in regulation of sexual maturity in Caribbean fruit flies and potential use in improving efficacy of sterile insect control technique for Tephritid fruit flies. *IOBC-WPRS Bulletin,* **25,** 167-180.

Terblanche, J.S., Klok, C.J. & Chown, S.L. (2004) Metabolic rate variation in *Glossina pallidipes* (Diptera: Glossinidae): gender, ageing and repeatability. *Journal of Insect Physiology,* **50,** 419-428.

Therneau, T.M. (2014) A package for survival analysis in S. *R package 2.37-7***,** http://CRAN.R-project.org/package=survival.

Therry, L., Nilsson-Örtman, V., Bonte, D. & Stoks, R. (2014a) Rapid evolution of larval life history, adult immune function and flight muscles in a poleward-moving damselfly. *Journal of Evolutionary Biology,* **27,** 141-152.

Therry, L., Zawal, A., Bonte, D. & Stoks, R. (2014b) What factors shape female phenotypes of a poleward-moving damselfly at the edge of its range? *Biological Journal of the Linnean Society,* **112,** 556-568.

Thompson, J.J.W., Armitage, S.A.O. & Siva-Jothy, M.T. (2002) Cuticular colour change after imaginal eclosion is time-constrained: blacker beetles darken faster. *Physiological Entomology,* **27,** 136-141.

Tracy, C.R., Christian, K.A., Baldwin, J. & Phillips, B.L. (2011) Cane toads lack physiological enhancements for dispersal at the invasive front in Northern Australia. *Biology Open,* **1,** 37-42.

Travis, J.M.J. & Dytham, C. (2002) Dispersal evolution during invasions. *Evolutionary Ecology Research,* **4,** 1119-1129.

Tribolium Genome Sequencing Consortium (2008) The genome of the model beetle and pest *Tribolium castaneum*. *Nature,* **452,** 949-955.

Tucker, V.A. (1975) The energetic cost of moving about: walking and running are extremely inefficient forms of locomotion. Much greater efficiency is achieved by birds, fish - and bicyclists. *American Scientist,* **63,** 413-419.

Vaanholt, L.M., De Jong, B., Garland, T., Daan, S. & Visser, G.H. (2007) Behavioural and physiological responses to increased foraging effort in male mice. *Journal of Experimental Biology,* **210,** 2013-2024.

Van Dyck, H. & Baguette, M. (2005) Dispersal behaviour in fragmented landscapes: routine or special movements? *Basic and Applied Ecology,* **6,** 535-545.

van Noordwijk, A.J. & de Jong, G. (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. *The American Naturalist,* **128,** 137-142.

Van Petegem, K.H.P., Boeye, J., Stoks, R. & Bonte, D. (2016a) Spatial selection and local adaptation jointly shape life-history evolution during range expansion. *American Naturalist*.

Van Petegem, K.H.P., Pétillon, J., Renault, D., Wybouw, N., Van Leeuwen, T., Stoks, R. & Bonte, D. (2015) Empirically simulated spatial sorting points at fast epigenetic changes in dispersal behaviour. *Evolutionary Ecology,* **29,** 299-310.

Van Petegem, K.H.P., Renault, D., Stoks, R. & Bonte, D. (2016b) Metabolic adaptations in a range-expanding arthropod. *Ecology and Evolution***,** DOI: 10.1002/ece1003.2350.

Werner, E.E. & Anholt, B.R. (1993) Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *The American Naturalist,* **142,** 242-272.

West, S.A. & Sheldon, B.C. (2002) Constraints in the evolution of sex ratio adjustment. *Science,* **295,** 1685-1688.

Wheat, C.W. (2012) Dispersal genetics: emerging insights from fruitflies, butterflies, and beyond. *Dispersal ecology and evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 95-107. Oxford University Press, Oxford.

White, C.R. (2011) Allometric estimation of metabolic rates in animals. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology,* **158,** 346-357.

White, C.R. & Kearney, M.R. (2013) Determinants of inter-specific variation in basal metabolic rate. *Journal of Comparative Physiology B,* **183,** 1-26.

White, C.R. & Kearney, M.R. (2014) Metabolic scaling in animals: methods, empirical results, and theoretical explanations. *Comprehensive Physiology,* **4,** 231-256.

Whitmee, S. & Orme, C.D.L. (2013) Predicting dispersal distance in mammals: a trait-based approach. *Journal of Animal Ecology,* **82,** 211-221.

Wilbur, H.M. (1980) Complex life cycles. *Annual Review of Ecology and Systematics,* **11,** 67-93.

Willis, E.R. & Roth, L.M. (1950) Humidity reactions of *Tribolium castaneum* (Herbst). *Journal of Experimental Zoology,* **115,** 561-587.

Wilson, R.P., Griffiths, I.W., Legg, P.A., Friswell, M.I., Bidder, O.R., Halsey, L.G., Lambertucci, S.A. & Shepard, E.L.C. (2013) Turn costs change the value of animal search paths. *Ecology Letters,* **16,** 1145-1150.

With, K.A. (1994) Ontogenetic shifts in how grasshoppers interact with landscape structure: an analysis of movement patterns. *Functional Ecology,* **8,** 477-485.

Wone, B., Sears, M.W., Labocha, M.K., Donovan, E.R. & Hayes, J.P. (2009) Genetic variances and covariances of aerobic metabolic rates in laboratory mice. *Proceedings of the Royal Society of London B: Biological Sciences,* **276,** 3695-3704.

Yano, S. & Takafuji, A. (2002) Variation in the life history pattern of *Tetranychus urticae* (Acari: Tetranychidae) after selection for dispersal. *Experimental & Applied Acarology,* **27,** 1-10.

Yoder, J.M., Marschall, E.A. & Swanson, D.A. (2004) The cost of dispersal: predation as a function of movement and site familiarity in ruffed grouse. *Behavioral Ecology,* **15,** 469-476.

Zera, A.J. & Bottsford, J. (2001) The endocrine-genetic basis of life-history variation: the relationship between the ecdysteroid titer and morph-specific reproduction in the wing-polymorphic cricket *Gryllus firmus*. *Evolution,* **55,** 538-549.

Zera, A.J. & Denno, R.F. (1997) Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology,* **42,** 207-230.

Ziegler, J.R. (1976) Evolution of the migration response: emigration by *Tribolium* and the influence of age. *Evolution,* **30,** 579-592.

Ziegler, J.R. (1977) Dispersal and reproduction in *Tribolium*: the influence of food level. *Journal of Insect Physiology,* **23,** 955-960.

Ziegler, R. (1985) Metabolic energy expenditure and its hormoral regulation. *Environmental physiology and biochemistry of insects* (ed. K.H. Hoffmann), pp. 95-118. Springer Berlin Heidelberg.

Zirkle, D.F., Dawson, P.S. & Lavie, B. (1988) An experimental analysis of the genetic relationships among life-history traits and emigration behavior in *Tribolium castaneum*. *Oikos,* **53,** 391-397.

Zollikofer, C. (1994) Stepping patterns in ants - influence of body morphology. *Journal of Experimental Biology,* **192,** 107-118.

Zyromska-Rudzka, H. (1966a) Abundance and emigrations of *Tribolium* in a laboratory model. *Ekologia Polska Seria A,* **14,** 491-518.

Zyromska-Rudzka, H. (1966b) An appreciation of the laboratory set for investigating the emigratory activity of *Tribolium*. *Ekologia Polska Seria A,* **14,** 125.

Appendix

Table A3.1 Full output of model-averaged LMER where movement ability was the response variable for a suite of potentially associated traits.

Response: Movement ability	Estimate	95% CI	*Z*	*P*	Importance
(Intercept)	-6.848	-9.496 – -4.201	5.070	<0.001	
Age	-0.022	-0.028 – -0.016	6.798	<0.001	1.00
Leg length	10.790	7.245 – 14.334	5.966	<0.001	1.00
Body size	-0.578	-1.395 – 0.239	1.387	0.166	0.48
Spontaneous activity	-0.159	-0.359 – 0.042	1.553	0.120	0.23
Sex	-0.115	-0.515 – 0.285	0.562	0.574	0.16
Routine MR	-0.106	-0.281 – 0.069	1.184	0.236	0.12

Table A3.2 Full output of model-averaged LMER where movement displacement was the response variable for a suite of potentially associated traits.

Response: Movement displacement	Estimate	95% CI	*Z*	*P*	Importance
(Intercept)	1.875	0.142 – 3.608	2.121	0.034	
Leg length	-2.598	-4.699 – -0.496	2.423	0.015	0.95
Routine MR	-0.097	-0.195 – 0.001	1.949	0.051	0.23
Body size	-0.115	-0.618 – 0.387	0.451	0.652	0.20
Sex	-0.075	-0.317 – 0.166	0.613	0.540	0.11
Spontaneous activity	-0.051	-0.171 – 0.069	0.839	0.402	0.07

Table A5.1 ANCOVA results from LMER of dispersal rate through the apparatus as a response to selection for and against dispersal with various covariates.

Fixed effects	SS	MS	d.f.	*F*	*P*
Selection	2.25	1.12	2, 21	0.10	0.910
Sex	44.27	44.27	1, 30	3.75	0.062
Generation	949.63	158.27	6, 109	13.41	<0.001
Movement ability	11.33	11.33	1, 119	0.96	0.329
Movement displacement	0.18	0.18	1, 119	0.02	0.901
Body size	23.60	23.60	1, 120	2.00	0.160
Spontaneous activity	2.62	2.62	1, 114	0.22	0.638
Routine MR	10.94	10.94	1, 119	0.93	0.338
Leg length	7.83	7.83	1, 119	0.66	0.417
Selection × sex	12.31	6.16	2, 17	0.52	0.603
Selection × generation	29.35	2.45	12, 105	0.21	0.998
Sex × generation	73.26	12.21	6, 104	1.03	0.407
Selection × sex × generation	171.28	14.27	12, 104	1.21	0.287

Table A5.2 ANCOVA results from LMER of movement ability in the maze (PC1) as a response to selection for and against dispersal with various covariates.

Fixed effects	SS	MS	d.f.	*F*	*P*
Selection	2.63	1.31	2, 20	0.51	0.608
Sex	20.34	20.34	1, 21	7.90	0.010
Generation	408.04	68.01	6, 1586	26.40	<0.001
Body size	33.51	33.51	1, 1559	13.01	<0.001
Routine MR	6.72	6.72	1, 1579	2.61	0.107
Spontaneous activity	29.89	29.89	1, 1581	11.61	<0.001
Leg length	1.92	1.92	1, 1601	0.74	0.389
Age	0.51	0.51	1, 1599	0.20	0.657
Selection × sex	3.38	1.69	2, 18	0.66	0.531
Selection × generation	50.29	4.19	12, 1585	1.63	0.078
Sex × generation	22.26	3.71	6, 1585	1.44	0.195
Selection × sex × generation	16.23	1.35	12, 1584	0.53	0.900

Table A5.3 ANCOVA results from LMER of movement displacement in the maze (PC2) as a response to selection for and against dispersal with various covariates.

Fixed effects	SS	MS	d.f	*F*	*P*
Selection	0.021	0.010	2, 19	0.01	0.990
Sex	2.707	2.707	1, 21	2.68	0.116
Generation	16.839	2.806	6, 1589	2.78	0.011
Body size	0.500	0.500	1, 1545	0.50	0.482
Routine MR	0.810	0.810	1, 1583	0.80	0.370
Spontaneous activity	4.556	4.556	1, 1574	4.52	0.034
Leg length	1.439	1.439	1, 1604	1.43	0.233
Age	8.560	8.560	1, 1601	8.49	0.004
Selection × sex	1.993	0.996	2, 18	0.99	0.392
Selection × generation	9.649	0.804	12, 1587	0.80	0.654
Sex × generation	5.916	0.986	6, 1587	0.98	0.439
Selection × sex × generation	20.944	1.745	12, 1587	1.73	0.055

Table A5.4 ANCOVA results from LMER of routine MR as a response to selection for and against dispersal with various covariates.

Fixed effects	SS	MS	d.f.	*F*	*P*
Selection	0.023	0.011	2, 18	1.58	0.232
Sex	0.034	0.034	1, 19	4.70	0.043
Generation	2.909	0.485	6, 1583	67.89	<0.001
Body size	1.265	1.265	1, 1593	177.20	<0.001
Spontaneous activity	0.166	0.166	1, 1602	23.18	<0.001
Movement ability	0.016	0.016	1, 1597	2.30	0.129
Movement displacement	0.004	0.004	1, 1594	0.59	0.441
Leg length	0.003	0.003	1, 1593	0.36	0.550
Age	0.509	0.509	1, 1590	71.34	<0.001
Selection × sex	0.007	0.004	2, 18	0.51	0.612
Selection × generation	0.151	0.013	12, 1583	1.77	0.049
Sex × generation	0.101	0.017	6, 1583	2.35	0.029
Selection × sex × generation	0.112	0.009	12, 1582	1.31	0.207

Table A5.5 ANCOVA results from LMER of body size as a response to selection for and against dispersal with various covariates.

Fixed effects	SS	MS	d.f.	*F*	*P*
Selection	49.45	24.73	2, 18	13.14	<0.001
Sex	51.69	51.69	1, 18	27.48	<0.001
Generation	397.75	49.62	6, 1584	26.38	<0.001
Movement ability	22.47	22.47	1, 1583	11.94	<0.001
Movement displacement	1.53	1.53	1, 1593	0.82	0.367
Routine MR	326.51	326.51	1, 1577	173.58	<0.001
Leg length	1068.16	1068.16	1, 1601	567.85	<0.001
Spontaneous activity	9.80	9.80	1, 1597	5.21	0.023
Age	30.18	30.18	1, 1591	16.04	<0.001
Selection × sex	0.09	0.04	2, 18	0.02	0.977
Selection × generation	70.80	5.90	12, 1583	3.14	<0.001
Sex × generation	10.16	1.69	6, 1583	0.90	0.494
Selection × sex × generation	37.43	3.12	12, 1583	1.66	0.070

Table A5.6 ANCOVA results from LMER of leg length as a response to selection for and against dispersal with various covariates.

Fixed effects	SS	MS	d.f.	*F*	*P*
Selection	0.0005	0.0002	2, 1601	0.21	0.810
Sex	0.0004	0.0004	1, 1600	0.33	0.563
Generation	0.0940	0.0157	6, 1601	14.67	<0.001
Movement ability	0.0008	0.0008	1, 1532	0.73	0.395
Movement displacement	0.0013	0.0013	1, 1601	1.17	0.279
Body size	0.6306	0.6306	1, 1593	590.46	<0.001
Routine MR	0.0001	0.0001	1, 1438	0.07	0.789
Spontaneous activity	0.0013	0.0013	1, 1559	1.19	0.276
Age	0.0001	0.0001	1, 1603	0.09	0.759
Selection × sex	0.0016	0.0008	2, 1601	0.73	0.481
Selection × generation	0.0148	0.0012	12, 1601	1.16	0.310
Sex × generation	0.0048	0.0008	6, 1602	0.75	0.611
Selection × sex × generation	0.0035	0.0003	12, 1601	0.28	0.993

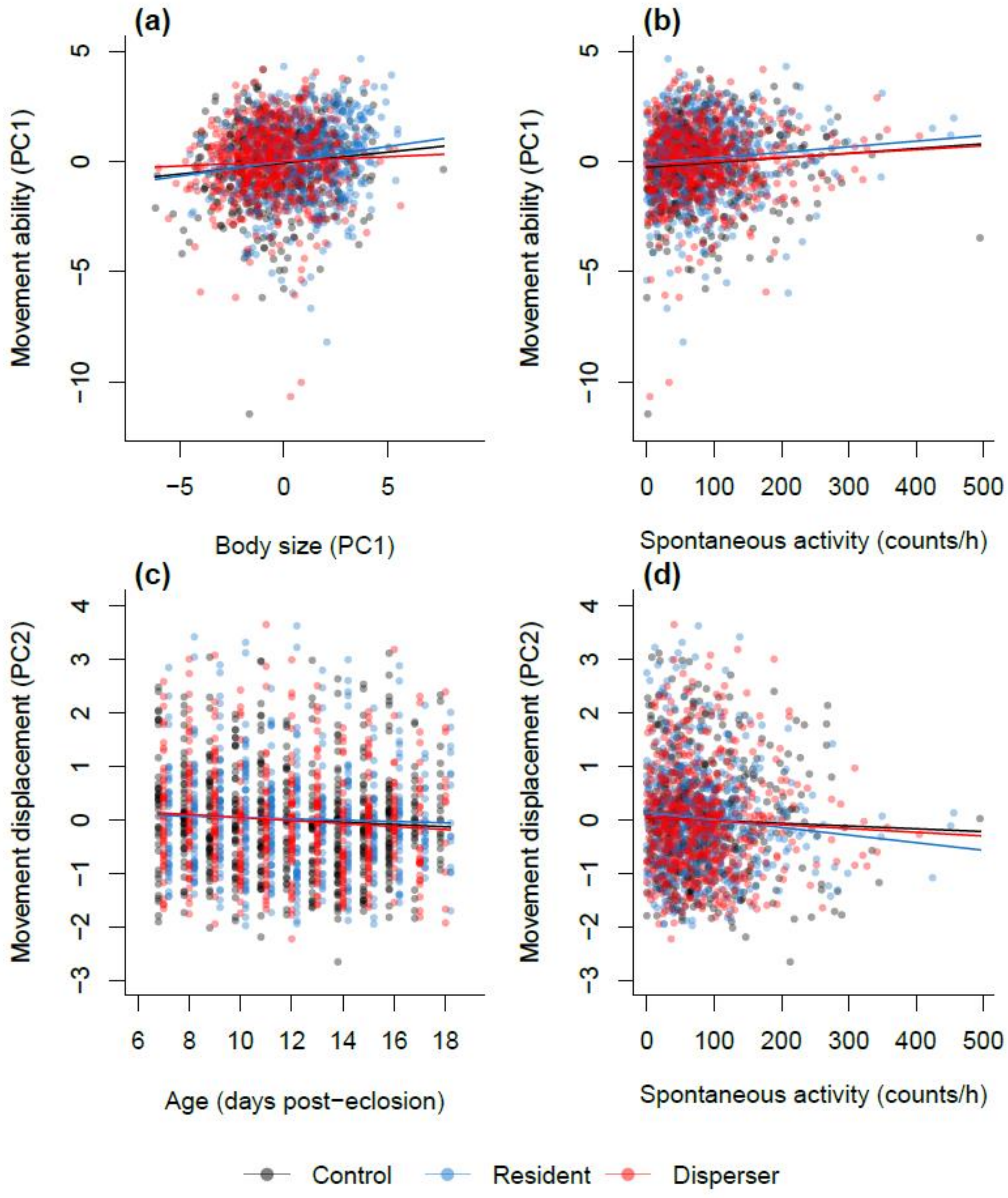


Figure A5.1 Associations among significant covariates with movement characteristics: (a) positive relationship between body size and movement ability, (b) positive relationship between spontaneous activity and movement ability, (c) negative relationship between age and movement displacement, and (d) negative relationship between spontaneous activity and movement displacement. All data are shown, both sexes and all seven generations of selection are combined, and selected lines are grouped by colour (*n* = 1651).

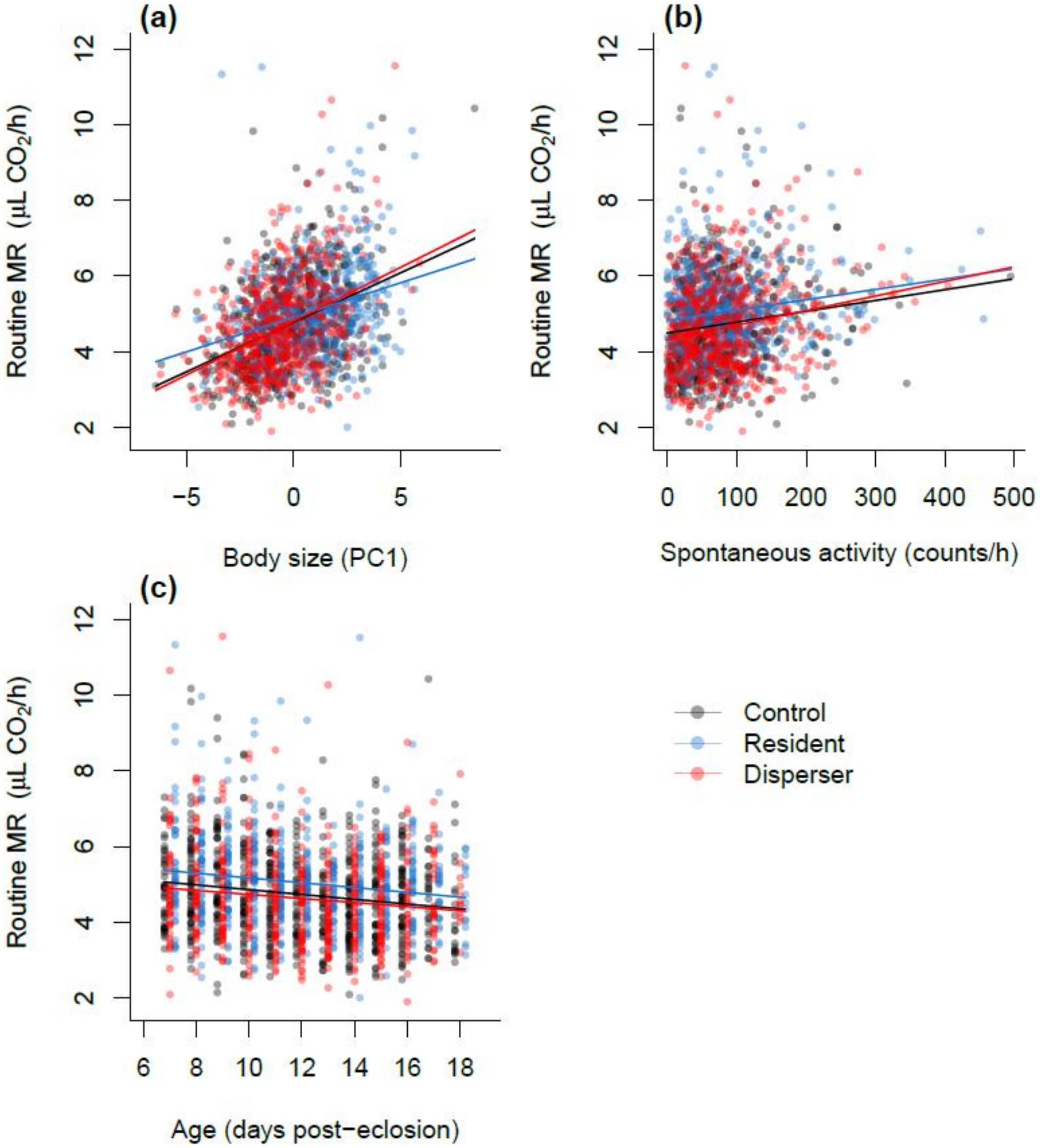


Figure A5.2 Associations among significant covariates with routine MR: (a) positive relationship between body size and routine MR, (b) positive relationship between spontaneous activity and routine MR, and (c) negative relationship between age and routine MR. All data are shown, both sexes and all seven generations of selection are combined, and selected lines are grouped by colour (*n* = 1651).

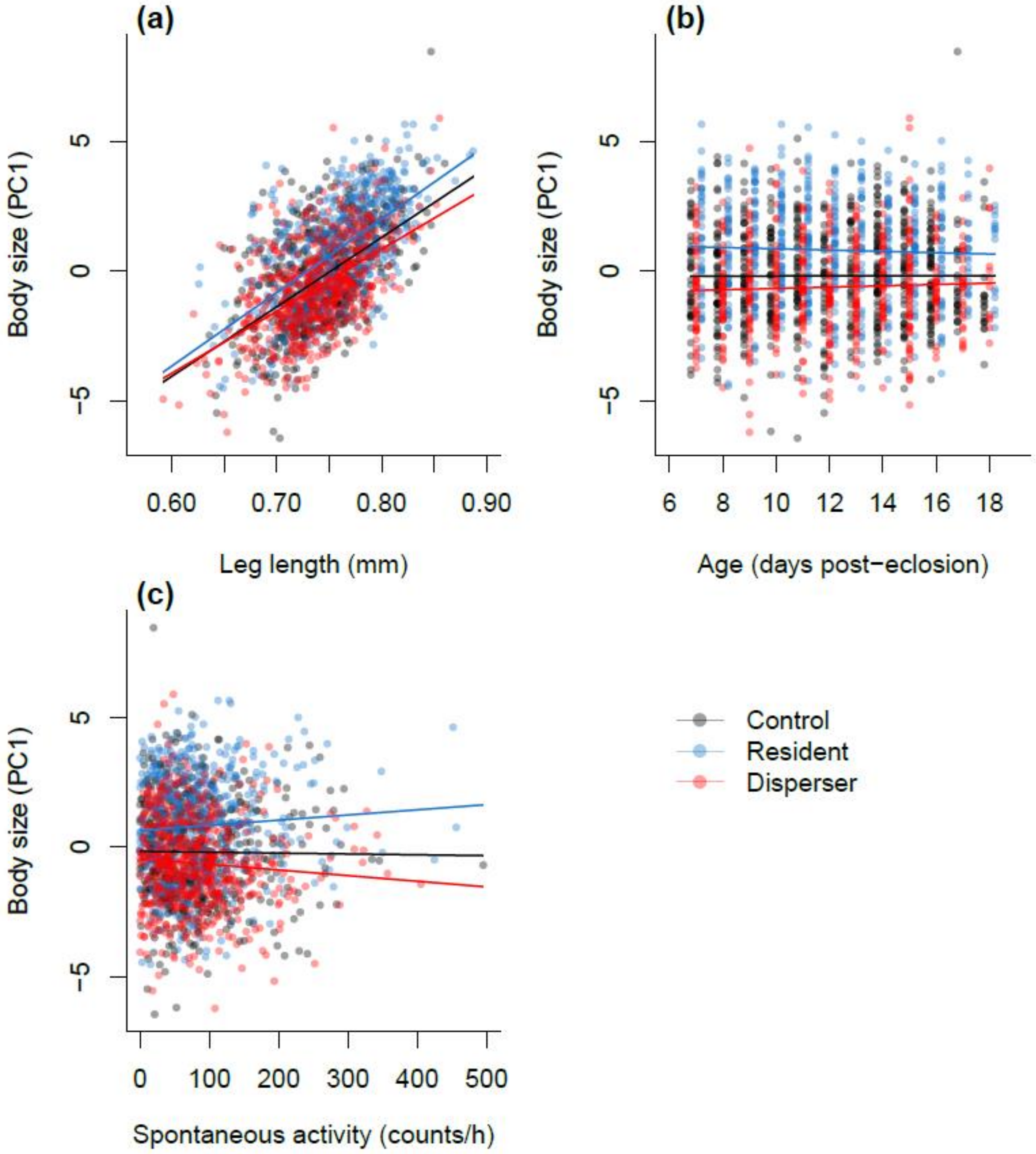
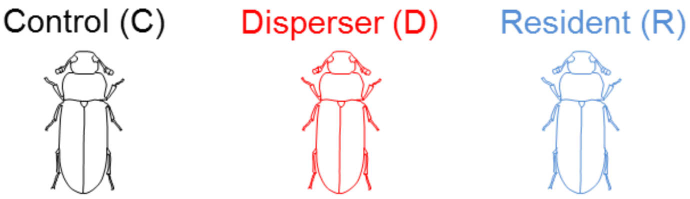


Figure A5.3 Associations among significant covariates with body size: (a) positive relationship between leg length and body size, (b) overall positive relationship between age and body size, and (c) overall negative relationship between spontaneous activity and body size. All data are shown, both sexes and all seven generations of selection are combined, and selected lines are grouped by colour (*n* = 1651).



Control crosses	Line crosses		Outcrosses
C1♂ × C1♀	C1♂ × D1♀	R1♂ × C1♀	C1♂ × C3♀
R1♂ × R1♀	C2♂ × D2♀	R2♂ × C2♀	D1♂ × D3♀
D1♂ × D1♀	C3♂ × D3♀	R3♂ × C3♀	R1♂ × R3♀
C2♂ × C2♀	C4♂ × D4♀	R4♂ × C4♀	C2♂ × C4♀
R2♂ × R2♀	D1♂ × C1♀	D1♂ × R1♀	D2♂ × D4♀
D2♂ × D2♀	D2♂ × C2♀	D2♂ × R2♀	R2♂ × R4♀
C3♂ × C3♀	D3♂ × C3♀	D3♂ × R3♀	C3♂ × C2♀
R3♂ × R3♀	D4♂ × C4♀	D4♂ × R4♀	D3♂ × D2♀
D3♂ × D3♀	C1♂ × R1♀	R1♂ × D1♀	R3♂ × R2♀
C4♂ × C4♀	C2♂ × R2♀	R2♂ × D2♀	C4♂ × C1♀
R4♂ × R4♀	C3♂ × R3♀	R3♂ × D3♀	D4♂ × D1♀
D4♂ × D4♀	C4♂ × R4♀	R4♂ × D4♀	R4♂ × R1♀

Figure A6.1 Experimental design for the cross-breeding experiment. A total of 48 crosses between lines were made, each with 10 parental pairs. D = dispersers (red), R = residents (blue), and C = controls (black).

Table A6.1 LMER model output comparing the realised fecundity of cross-bred selected lines. D = dispersers, R = residents, C = controls, and $_{\text{O}}$ = outcross, where the cross is represented as male line:female line.

Fixed effects	Estimate ± SE	d.f.	*t*	*P*
C:C (intercept)	61.15 ± 4.89	12	12.51	<0.001
C:C–D:D	0.51 ± 5.10	431	0.10	0.920
C:C–R:R	-6.85 ± 5.06	431	-1.35	0.177
C:C–C:C$_{\text{O}}$	3.86 ± 5.06	431	0.76	0.446
C:C–D:D$_{\text{O}}$	8.71 ± 5.00	431	1.74	0.082
C:C–R:R$_{\text{O}}$	7.54 ± 5.06	431	1.49	0.137
C:C–C:D	5.31 ± 5.00	431	1.06	0.288
C:C–C:R	8.65 ± 5.00	431	1.73	0.084
C:C–D:C	6.71 ± 4.97	431	1.35	0.177
C:C–D:R	9.02 ± 4.96	431	1.82	0.070
C:C–R:C	7.97 ± 5.03	431	1.59	0.114
C:C–R:D	-1.04 ± 5.00	431	-0.21	0.835

Table A6.2 LMER model output comparing the sex ratio of the cross-bred selected lines offspring. D = dispersers, R = residents, C = controls, and O = outcross, where the cross is represented as male line:female line.

Fixed effects	Estimate ± SE	d.f.	*t*	*P*
(Intercept)	1.111 ± 0.132	36	8.39	<0.001
C:C–C:C$_{\text{O}}$	-0.143 ± 0.187	36	-0.76	0.452
C:C–C:D	0.058 ± 0.187	36	0.31	0.759
C:C–C:R	0.114 ± 0.187	36	0.61	0.547
C:C–D:C	0.026 ± 0.187	36	0.14	0.890
C:C–D:D	-0.009 ± 0.187	36	-0.05	0.962
C:C–D:D$_{\text{O}}$	-0.122 ± 0.187	36	-0.65	0.519
C:C–D:R	-0.094 ± 0.187	36	-0.50	0.618
C:C–R:C	0.077 ± 0.187	36	0.41	0.685
C:C–R:D	0.144 ± 0.187	36	-0.56	0.579
C:C–R:R	-0.003 ± 0.187	36	0.77	0.448
C:C–R:R$_{\text{O}}$	-1.04 ± 0.187	36	-0.02	0.986