Beetling about: using experimental evolution to understand dispersal behaviour in the pest/model insect *Tribolium castaneum*

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Thesis submitted for the degree of Doctor of Philosophy

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January 2025

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Thesis abstract

Dispersal impacts gene flow and thus a web of eco-evolutionary processes that govern the fate of biological entities, from individuals to meta-communities. Consequently, it is crucial to understand how dispersal evolves. This thesis investigates dispersal in the crop pest and model insect, *Tribolium castaneum*, the red flour beetle. Despite its contribution to many important advances across over 100 years of experimental biology, no single source had collated this information. Therefore, in chapter two I review the extensive use of *Tribolium* beetles as a model in evolution and ecology. In chapter three I use divergent artificial selection to develop beetle lines showing repeatable dispersal phenotypes, demonstrating that the trait has a strong genetic basis, and supporting existing theory that this genetic basis may be relatively simple. Chapter four investigates the mechanistic basis of differences in dispersal. Results showed that dispersive phenotypes differ in their walking distance, straightness of travel and use of the substrate surface in ways that plausibly enhance dispersal. Chapter five investigates associations between dispersal and life-history traits, showing that dispersal is significantly associated with male mating behaviour, female reproductive investment, development time and female lifespan. These traits potentially characterise a dispersal syndrome in *T. castaneum* that shapes and constrains how the trait evolves in natural populations. In chapter six I return to the question of genetic architecture, employing genome resequencing of the selection lines to identify genomic variants associated with the dispersal phenotype. I find evidence supporting association with many candidate loci, amongst these are many known metabolic and neurophysiology genes, suggesting this may be the functional basis of dispersal variation. Finally, I synthesise the findings from each chapter and discuss what my results suggest about the evolution of dispersal in *Tribolium*, the wider relevance to our understanding of complex trait evolution and some possibilities for future research.

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Acknowledgements

That it is only my name on the cover of this document belies the many and various contributions of others, which have made the thing possible.

Most tangibly, three primary supervisors, some of whom chose me as a student and some who bore it admirably. Firstly Lewis, who inspired me to do a PhD and, even after leaving academia has continued to offer valuable advice – just in the pub now, rather than his office. Matt took up the mantle and, though we sadly didn't work closely together for very long, I continue to benefit from the fruits of his incisive scientific vision. Lastly Dave, who inherited me along with a project outside his wheelhouse, and has done more than anybody else to get us both to our current forms.

Other supervisors and colleagues have helped enormously. Simon. Mark. Will - without whom the genomic aspects of this project would still be firmly on the ground, and whose infectious enthusiasm for science I greatly admire. Thanks to Jon Slate, Harrison Ostridge and Matthieu Gautier for technical advice. I count myself lucky to have worked alongside past and present members of the Tribolium Lab; George, Ram, Ben, Ginny and Becky. The wider members of the O&E theme at UEA, with whom it has been my immense pleasure to cohabit on Floor 01. Special thanks to Jenny, Phil, Lucy, Emily, Johnny, Nat and Nathan.

Alongside my research, I've been fortunate to land other opportunities. Thanks to Diane and Ashley for the PIPS internship at JIC. The chance to work with *Heredity* I owe to the trust of Sara and James. The Peru field course was a highlight and I thank Martin and Claire for their excellent company, in sweat-soaked jungle and piranha-infested water alike. Among my most rewarding times at UEA have been those teaching, supervising, and demonstrating for undergraduate modules and project students. So I'd like to thank them too. Especially Fred.

Finally, I thank my family and friends. My mum, dad, sister and auntie Wendy, without whose tireless support and belief I could not have made it this far. My wife, Miriam, whose love, understanding and validation in times of trial have been invaluable. My friends - among whom I count many of those already named above - for the encouragement, the welcome distractions and for making this chunk of my life what it has been. Thank you.

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CHAPTER ONE

Introduction to dispersal

The perils of dispersal: *Tribolium* beetle ensnared by *Pritha garfieldi* (reproduced under a CC sharealike licence, photographer: Pars Plateau)

1.1 On dispersal

1.1.1 Dispersal

Dispersal in animals is most commonly defined as movement that influences gene flow; it is therefore a key process in the ecology and evolution of many species (Ronce 2007). While movement from one place to another may initially seem simple, its interaction with a complex web of other eco-evolutionary processes makes its causes, trade-offs and consequences difficult to untangle. In addition, dispersal bears upon many of the key problems in contemporary biology: the spread of invasive species; agricultural pests and food security; population persistence in fragmented landscapes; and range shifts in response to climate change. Illuminating the eco-evolutionary causes and consequences of dispersal therefore has implications not only for evolutionary biology, but could help to address major challenges facing modern society.

Despite its ubiquity, all dispersal is clearly not the same and many subcategories have been identified. For example, these include natal dispersal, movement from the place of birth to the site of reproduction; and breeding dispersal, movement between consecutive breeding sites (Clobert et al. 2009). Dispersal also varies largely in extent, so the terms local and long-distance dispersal have been used to distinguish these different modes, with a population’s ‘dispersal kernel’ describing its distribution of dispersal distances (Nathan et al. 2012). Similarly, any given dispersal event can be subdivided into distinct stages. Emigration (or departure) involves the leaving of the original location; transfer (or transit) involves the movement to a new location; and immigration (or settlement) describes the process of arrival and establishment in some destination (Clobert et al. 2009).

1.1.2 The individual

An individual may disperse for many reasons, e.g. to search for mates; to locate better resources, or resources for which there is less intra- and/or inter-specific competition. Kin interactions also inform movement, as dispersers seek to escape resource competition or matings with individuals to whom they are closely related (Gandon 1999). Frequently this process leads to the evolution of sex-biased dispersal strategies, though either sex may be the more dispersive (Lawson Handley and Perrin 2007).

In any dispersal event, the benefits to dispersal are balanced by potentially significant costs. Such movements are energetically expensive, might expose the individual to elevated predation risk and opportunity costs, and the mates or resources being sought might never be located (Bonte et al. 2012). The cost-benefit ratio will vary widely with the specific scenario, but depends on factors such as the relative quality of the initial and final habitat patches, quality of the intervening habitat matrix, and the phenotype of the dispersing individual (Bonte et al. 2012).

While it is apparent that some species are morphologically or physiologically better suited to dispersal, the same is less clear for conspecific individuals with a population. In the extreme, such individual variation can be seen by the presence of dispersal morphs in some insects, such as the pea aphid (*Acyrthosiphon pisum*) which plastically produces winged individuals in response to environmental cues (Brisson 2010) However, phenotypic plasticity need not be involved, and continuous variation in genetically determined traits can impart differential dispersal capacity between individuals. Such traits can be relatively simple, such as leg length in anurans allowing increased movement ability (Bredeweg et al. 2019); or more complex, like the compound-eye structure in the Fritillary butterfly enhancing its navigational capability (Turlure et al. 2016). However, for a dispersal to occur, a propensity as well as an ability to undertake a movement may be required. Dispersals exist on a continuum of conditionality; some dispersal will always be undertaken and some will depend on information and cues collected and acted upon by the organism (reviewed in Bowler and Benton 2005). For example, signals of increasing conspecific density induce the production of ballooning silk, and thus dispersal, in spiders (De Meester and Bonte 2010). Even within identical environments, how environmental information is interpreted will depend in a complex way on the individual’s ability to perceive different cues; the weightings they assign to each, based on their particular phenotype; and willingness to expose themselves to risk (Bowler and Benton 2005).

Prominent in the literature is discussion of dispersal syndromes, covariation between dispersal and other traits creating a dispersal phenotype. Dispersal-correlated traits are found widely across taxa and fall broadly into several categories; behaviours including sociality, risk-aversion and boldness; or life-history traits, such as development time and fecundity (Ronce and Clobert). While the body of evidence showing that various traits correlate with dispersal is large, there is no standard set of traits, or systematic direction of relationships with those traits, even within the same species in different environments (Clobert et al. 2009). There are several ways in which trait correlations can arise, the first being condition dependence, or phenotype matching, where an individual's phenotype influences dispersal decision-making (Ronce and Clobert). Secondly, the environment can be the mediator, underlying the manifestation of both dispersal and other traits - which then appear linked (Ronce and Clobert). The third cause is genetic, where other traits become correlated with dispersal either through linkage disequilibrium or by pleiotropic effects of major-effect loci influencing dispersal (Saastamoinen et al. 2018). For example, shared neurological, endocrine or metabolic underpinnings may cause shifts in multiple higher-level phenotypes (Sih et al. 2004). While the specific traits involved can vary widely with organisational scale, or the different stages of dispersal, each suite of traits constituting a dispersal syndrome has ultimately been shaped by divergent selection to minimise the cost:benefit ratio of opposing life-history strategies.

1.1.3 Beyond the individual

The consequences of undertaking dispersal can be great; for the individual, dispersal can mean considerable differences in fitness, but it also has effects at higher levels of biological organisation. Individual dispersal governs the area a given species occupies, and defines the limits of invasions, range expansions and contractions. Dispersal also mediates metapopulation structure, determining migration rates and gene flow which, in turn, influences evolutionary trajectories (Waters et al. 2020; Suárez et al. 2022). Consequently dispersal is also a key determinant of metapopulation persistence in fragmented, disturbed or unstable environments, where higher dispersal can allow recolonisation to balance local extinction (Eriksson et al. 2014).

Random dispersal alone is limited in its power to mould populations, however when combined with habitat selection these forces enable the maintenance of genetic variation over a far wider range of scenarios than would otherwise be possible (Barton 1992). Accordingly, the way that dispersal, especially as part of a behavioural syndrome, structures populations, can lead to non-random patterns of gene flow, with interesting knock-on effects (Edelaar and Bolnick 2012). Considering dispersal alone, in areas where highly dispersive individuals are likely to predominate, such as the frontline of an invasion or range expansion, dispersers are increasingly likely to breed with other dispersers in a process known as 'spatial sorting' (Shine et al. 2011). The result can be an acceleration of the expansion (Kot et al. 1996; Lindström et al. 2013; Weiss-Lehman et al. 2017). This was famously demonstrated in the ongoing Cane Toad (*Bufo marinus*) invasion of Australia, where, due to the evolution of increased dispersal ability and propensity, the rate of invasion has increased five-fold in less than 100 years (Phillips et al. 2006; Hudson et al. 2020; Shine et al. 2021). Further, if dispersal is linked to other traits, spatial sorting may lead to divergence in a suite of phenotypes between the range-edge and range-core populations (Clark et al. 2022). This effect is clearly important for the evolutionary ecology of populations, but when such dispersal-associated traits are functional it can have extremely large-scale implications, potentially affecting nutrient flow and trophic interactions across the meta-ecosystem (Raffard et al. 2022).

1.1.4 At the genetic level

A genetic basis has been demonstrated for many dispersal and dispersal-associated traits across a range of taxa (Saastamoinen et al. 2018), though this work has been focused on relatively few species. It is helpful to partition these traits into those associated with movement ability and those associated with movement propensity (Clobert et al. 2012). Most highly studied among movement ability traits are insect polyphenisms, a special case of plasticity where a single genotype can produce multiple discrete phenotypes. Famously, groups such as aphids, crickets and planthoppers produce winged or wingless morphs in response to environmental cues, such as density and habitat quality (Brisson 2010). These morphs differ not only in wingedness, but in a suite of correlated traits including habitat selection and flight-muscle mass, resulting in large differences in dispersal capacity and colonisation success (Zera and Denno 1997). Studies across taxa have shown high heritability values for traits such as wing length and flight muscle mass, with wing polymorphism best described as being a polygenically inherited threshold trait (Clobert et al. 2012). Variation for dispersal morphs is maintained within these populations via tradeoffs with reproductive traits such as mating success and fecundity (Mole and Zera 1994; Guerra 2011).

Realised dispersal, or dispersal propensity, has been studied more widely across taxa, including vertebrates, and particularly using wild populations; for example, with respect to migration (Clobert et al. 2012). Although migration is a special case of dispersal, constituting movement that can be reversed and/or repeated, migration has been well studied in butterflies, fish and especially birds. This literature has shown a substantial role for genetics in determining the propensity, direction and timing of migration (reviewed in Pulido 2007; Liedvogel et al. 2011). However, insects have been strongly favoured for experimental laboratory studies. Wing-monomorphic species have shown high heritability for flight incidence, duration and distance (Clobert et al. 2012). These flight characteristics also show strong genetic correlations with a range of traits commonly observed to be associated with dispersal, including fecundity, body size and lipid physiology (Ronce and Clobert; Kent and Rankin 2001).

Discovering the basis of genetic correlations between traits requires understanding of the genetic architecture underlying dispersal traits. As for other traits, the genetic basis of dispersal can be investigated by measuring the extent to which genetic loci or expression patterns co-vary with the dispersal phenotype (e.g. Caillaud et al. 2002; Fountain et al. 2016). In the genomic era, large datasets of single-nucleotide polymorphisms (SNPs) have removed the reliance on pre-identified candidate loci, allowing genome-wide association studies (Sætre and Ravinet 2019). Findings indicate that the basis of most quantitative traits is polygenic, determined by relatively small contributions from many genes (Santure et al. 2015; Kardos et al. 2016). That the same is true for dispersal is supported by work utilising the Drosophila Genetic Reference Panel that found 192 genes associated with variation in locomotion (Jordan et al. 2012). However, major-effect loci underpinning quantitative traits have been found (reviewed in Slate 2005), including some involved in dispersal. One prominent example is the *for* gene (and its homologues), a regulator encoding a signalling molecule, first identified as controlling larval foraging behaviour in *Drosophila* (Sokolowski 1980). This locus was later found to be important in locomotion and resource use in adult flies (Pereira and Sokolowski 1993). It has since been implicated in regulating movement and other behavioural traits across taxa, including in *C. elegans*, mice and humans (Anreiter and Sokolowski 2019). Another well-studied example is Pgi, best known from Glanville fritillary butterflies (*Melitaea cinxia*). This gene encodes a metabolic enzyme, and allelic variation at this locus underlies phenotypic variation in flight metabolism and dispersal propensity in wild populations (Niitepõld et al. 2009; Niitepõld and Saastamoinen 2017).

Among the vertebrates in which dispersal has been studied are macaques (*Macaca mulatta*), where dispersal tendency in young males is associated with aggression and also variation at the VNTR locus encoding a serotonin transporter (Trefilov et al. 2000). That same gene's ortholog has been implicated in dispersal and aggressive behaviour in mice (*Mus musculus domesticus*; Krackow and König 2008). Aggression is also seen to be related to dispersal in the Western bluebird (*Sialia mexicana*; Duckworth and Badyaev 2007), where it is an important factor in range expansion and interspecific competition (Duckworth 2008). In the great tit (*Parus major*), dispersal propensity and exploratory behaviour have been linked to variation at the *drd4* locus (Fidler et al. 2007), though tests of other populations and in another species, the blackcap (*Sylvia atricapilla*), have failed to find an association (Korsten et al. 2010).

1.1.5 In *Tribolium*

Tribolium beetles have a long history as laboratory models for studying ecological and evolutionary questions (see introduction to *Tribolium* as a model in chapter two), including in relation to movement and dispersal. As important agricultural pests of stored grain, and a model system representing the most species-rich order (Stork et al. 2015), understanding how and why these organisms move could provide significant agro-economic, food security and biodiversity conservation benefits.

Some of the earliest work on *Tribolium* movement was by Naylor, who saw that individuals distributed themselves according to density, aggregating at low density but dispersing uniformly at high density, in both *T. confusum* and *T. castaneum* (Naylor 1959, 1961). Females spent more time burrowing in the fodder than males, and both altered their behaviour when in mixed-sex groups (Naylor 1959, 1961). Similarly, Ghent (1963) showed that *T. castaneum* prefers fresh fodder over that conditioned by other individuals (via the secretion of chemicals), though this depended on the individual's previous environment, and was the reverse for *T. confusum*. In a series of experiments, Surtees investigated the 3-dimensional movement patterns of several grain-eating beetles within a mass of grain. He observed that *Tribolium* distributed themselves principally in response to small-scale variations in temperature and humidity and were found on the fodder surface more frequently than any of the other four species studied (summarised in Surtees 1965). Going beyond a single habitat patch, Prus (1963) pioneered the use of an arena constructed of two connected containers to assay the propensity of beetles to move from one to the other, via a connecting tube accessible from the fodder surface. Prus himself used this to show that males dispersed more than females when in single-sex groups (Prus 1963, 1966), while other later authors used variants of his setup to show that dispersal tendency is altered by population density, food availability and the age of the individuals (Ogden 1970b; Ziegler 1976, 1977).

Many of the above studies used both *Tribolium castaneum* and *T. confusum*, and found that patterns of dispersal differ between the species and between strains within species (Ghent 1963). This was an indication that *Tribolium* dispersal traits have a genetic basis, which has been independently demonstrated in several experiments through responses to artificial selection (Schurr and Bolduan 1967; Ogden 1970a; Ritte and Lavie 1977; Korona 1991; Ruckman and Blackmon 2020). The rapid response to selection observed across these studies has led some authors to postulate that the trait is controlled by very few loci (Ogden 1970a; Ritte and Lavie 1977). This was the conclusion reached by Ritte & Lavie (1977) who, after also observing differential patterns of dispersal between male and female offspring of reciprocal crosses of *T. castaneum*, suggested that dispersal was controlled by a single, sex-linked locus.

It is not only the number of loci involved in the dispersal behaviour of *Tribolium* beetles which has received attention, but also how genetic variance is partitioned. Ruckman and Blackmon (2020) used line-cross analysis to find evidence that epistatic interactions across several loci dominate additive effects in the adaptation of their experimental populations. This view fits with more recent theoretical work suggesting that rapid adaptation can also occur in more complex architectures, including in highly polygenic traits (Jain and Stephan 2017a, 2017b), counter to the prevailing wisdom that selective sweeps on large-effect loci are the predominant mode of rapid responses to selection (Messer and Petrov 2013).

However, the importance of epistasis in the findings of Ruckman & Blackmon was dependent on their observation of greater dispersal tendency among the offspring of crosses than in their high dispersal lines, a pattern not seen in earlier work which included crosses between dispersal selection lines (Ritte and Lavie 1977).

Several studies working with *Tribolium* lines created through artificial selection on dispersal have found traits that covary with movement. Dispersal propensity shows a positive relationship with rate of development to adulthood (Lavie and Ritte 1978; Lavie 1981; Zirkle et al. 1988), and these traits seem to trade-off against longevity (Lavie 1981). In *Tribolium*, dispersers also engage in less cannibalism than non-dispersers (Zirkle et al. 1988). One test showed that fecundity was greater in high dispersal lines (Lavie and Ritte 1978), suggesting that dispersers might have greater competitive ability, however this was somewhat contradicted by a separate study that showed populations of dispersers grew more slowly (Zirkle et al. 1988).

Though most work has focussed on emigration (the first phase of dispersal), and short-distance dispersal by walking, *Tribolium* are capable of longer-distance dispersal by flight, although flight initiation is common only at high temperatures, above those typically seen in grain stores (Drury et al. 2016). As for many other insect species, a genetic basis of flight ability has been shown (Díez and López-Fanjul 1979). However that study could not replicate the result in populations that had been longer in the laboratory, suggesting that variation for this trait might be lost relatively quickly in the lab (Díez and López-Fanjul 1979). Another experiment showed a positive correlation between walking and flight, as dispersal selection lines showed increased flight ability (Zirkle et al. 1988). Interestingly, flight traits covary with pesticide resistance. Due to its prolific impact as a pest of stored food products (Phillips and Throne 2010; El-Aziz 2011) much effort is spent on control of *T. castaneum*, largely via phosphine fumigation in storage facilities (Nayak et al. 2020). However, the evolution of resistance jeopardises the future utility of phosphine to manage pests (Nayak et al. 2020). Resistance is associated with genes that suppress metabolism (Schlipalius et al. 2012, 2018), and resistant beetles display reduced walking speed and flight initiation (Malekpour et al. 2016, 2018), suggesting that metabolism may constrain the ability to disperse. Further work pertaining to dispersal ability has shown that artificially selecting on individual walking distance generates lines differing in that trait, but also in duration of death-feigning response and reproductive traits (Matsumura et al. 2019, 2022). Follow-up analyses identified 311 genes differentially expressed between long and short walking lines (Matsumura et al. 2024). Differences in brain dopamine levels indicate a role for neurophysiology in this behaviour (Nakayama et al. 2012). While walking distance may contribute to dispersal ability, these studies lack a dispersal propensity component. Thus it remains unclear to what extent the walking motivation of individuals kept alone and without a source of food overlaps with the more ecologically realistic scenario of dispersal from a population inhabiting a food source.

Tribolium experiments have also contributed to understanding how spatial processes interact with dispersal during range expansion (as mentioned above). For example, beetles were used to show that rapid evolution occurring during range expansion results in greater dispersal ability at the range front (Weiss-Lehman et al. 2017). These results were supported by a contemporary study in the seed beetle, *Callosobruchus maculatus* (Ochocki and Miller 2017). We also appreciate the role of stochastic processes during expansion thanks to *Tribolium* experiments (Weiss-Lehman et al. 2017, 2019), and the repeatability of evolutionary change across the different phases of colonisation (Tittes et al. 2024).

1.1.6 Prospects

Dispersal traits constitute a node in a complex network, influencing and influenced by many other traits, and by the organism's biotic and abiotic environment. These wide-reaching effects highlight the importance of a holistic understanding of dispersal. As such, we require well replicated studies in systems with the potential to provide this comprehensive view (Zera, A. J., & Brisson, J. A. 2012). *Tribolium castaneum* is just such a species. As an important agricultural pest and an established model system much is known about its population biology and evolutionary ecology. Yet, while it has significantly advanced our understanding of the evolutionary processes accompanying dispersal, the research effort spent on characterising the trait itself has settled little definitively. Some findings are contradictory, and much of the work was conducted before the availability of modern computational and molecular genetic techniques. The emergence of the species as a genomic model has provided the means to bridge gaps in our knowledge of *Tribolium* dispersal, linking population ecology to broader spatial patterns mediated through dispersal. Doing so has the potential to add to our understanding of complex, quantitative trait evolution bring applied benefits to pest management.

1.2 This thesis

1.2.1 Thesis objectives and outline

The overarching aim of my research was to advance our understanding of dispersal in *T. castaneum* - using evolutionary experiments, simple genetic simulations, behavioural assays and molecular genomics to characterise the dispersal phenotype and its underlying mechanisms.

Below I state how this broad objective is broken down across the following five chapters, stating the specific aims of each, and the approach taken to addressing them.

Chapter two aims to collate and summarise what is known about *Tribolium* population biology, ecology and evolution from across its long use as a model system. I review the extensive use of *Tribolium* beetles as a mode, highlighting the utility of the species as a laboratory organism and teaching resource; and synthesising its contributions, particularly in the fields of: population dynamics; reproduction and sexual selection; population and quantitative genetics; and behaviour, physiology and life history.

Chapter three aims to develop a set of *Tribolium* lines differing in their dispersal behaviour with which to conduct further investigations, and estimate the genetic architecture of the dispersal trait based on the response to selection. I use divergent artificial selection to generate a robust set of beetle lines showing repeatable dispersal phenotypes, demonstrating that the trait has a strong genetic basis. Alongside, I use a simple genetic model to simulate the same selection experiment under a range of parameters: number of controlling loci, dominance, heritability. By comparing the empirical response to selection to that seen in simulated scenarios, I estimate the genetic architecture of the dispersal trait.

Chapter four aims to test for associations between possible dispersal-enhancing traits and dispersal phenotype, to investigate the proximate mechanistic basis of dispersal. I use video recordings and a machine-learning model to test for differences in activity and movement patterns between dispersal phenotypes. I develop an assay to investigate differential use of the fodder surface, and use a single-generation selection experiment to test its heritability. I then test for association between morphology and dispersal, to see whether body size or leg length could be driving differences in dispersal.

Chapter five aims to identify associations between key life history traits and the dispersal phenotype, to explore a possible dispersal syndrome. I test for differences in male mating behaviour, female reproductive investment, development time and longevity between high- and low-dispersal lines. I use the considerable literature describing *Tribolium* ecology to discuss a possible dispersal syndrome in this species and the potential adaptive significance of trait covariances.

Chapter six aims to characterise the genetic architecture of the dispersal trait, and identify genomic regions and specific genes and gene functions associated with the dispersal phenotype. I use genome resequencing from dispersal lines to identify regions of the genome associated with dispersal. I employ two complementary methods, genotype-phenotype association and a genetic divergence approach, to locate candidate regions. Genes in these regions are functionally enriched to investigate a molecular basis of the dispersal phenotype.

Finally, I bring together the results of each chapter to discuss what they suggest about the ecology and evolution of dispersal in *Tribolium*. I then outline how these findings relate to the wider fields of evolutionary biology, conservation science and applied pest management, and suggest possible future work to build upon this research.

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CHAPTER TWO

Tribolium beetles as a model system in evolution and ecology

A version of this chapter (appendix 1) is published as:

Pointer MD, Gage MJG & Spurgin LG (2021) *Tribolium* beetles as a model system in evolution and ecology. *Heredity* 126, 869–883. https://doi.org/10.1038/s41437-021-00420-1

Tribolium castaneum (Photograph: Udo Schmidt)

2.1 Abstract

Flour beetles of the genus *Tribolium* have been utilised as informative study systems for over a century and contributed to major advances across many fields. This review serves to highlight the significant historical contribution that *Tribolium* study systems have made to the fields of ecology and evolution, and to promote their use as contemporary research models. We review the broad range of studies employing *Tribolium* to make significant advances in ecology and evolution. We show that research using *Tribolium* beetles has contributed a substantial amount to evolutionary and ecological understanding, especially in the fields of population dynamics, reproduction and sexual selection, population and quantitative genetics, and behaviour, physiology and life history. We propose a number of future research opportunities using *Tribolium*, with particular focus on how their amenability to forward and reverse genetic manipulation may provide a valuable complement to other insect models.

2.2 Introduction

Research models are fundamental to scientific investigation, providing simplified systems to test and explain components within more complex ideas and hypotheses (Hartmann and Frigg 2005). Model systems can be viewed along a continuum, where simplicity is traded against complex reality, with purely theoretical models at one end, and field-based systems at the other (Winther et al. 2015). Laboratory organisms occupy an intermediate position on this continuum, offering informative opportunities to directly test ecological or evolutionary theory in complex living systems, while retaining high levels of experimental control and allowing for experimental replication. As a result, laboratory models are an essential and widely used tool in biodiversity research, and here we discuss the many applications and strengths that *Tribolium* beetles possess for research in ecology and evolution.

In choosing experimental systems, scientists face a choice between focusing effort on one of rather few 'true' model organisms (Sommer 2009), versus adding diversity to the research base, expanding the useful extrapolations that can be made (Bolker 2012). Important distinctions exist between what some consider ‘true’ model organisms, compared to those used more broadly in experimental studies to which the term is often more loosely applied (Ankeny and Leonelli 2011). Under the more stringent ‘animal model’ view, the list of model organisms is traditionally limited to very few, including *Zea mays* (maize), *Arabidopsis thaliana* (thale cress), the bacterium *Escherischa coli*, *Saccharomyces cerevisiae* (yeast), the roundworm *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster*, *Xenopus laevis* (African clawed frog), *Mus musculus* (house mouse) and *Danio rerio* (Zebrafish) (Müller and Grossniklaus 2010). Attributes that distinguish these true model organisms include established research infrastructure, high experimental tractability and the ability to represent broad ranges of both taxa and questions under study (representational scope and representational target respectively, Ankeny and Leonelli 2011). Despite these nine classic models having such attributes, it is clear that we need to consider a wider range of models for progressing research, especially where we aim to tackle questions concerning the evolutionary ecology of biodiversity in the natural environment (figure 2.1).

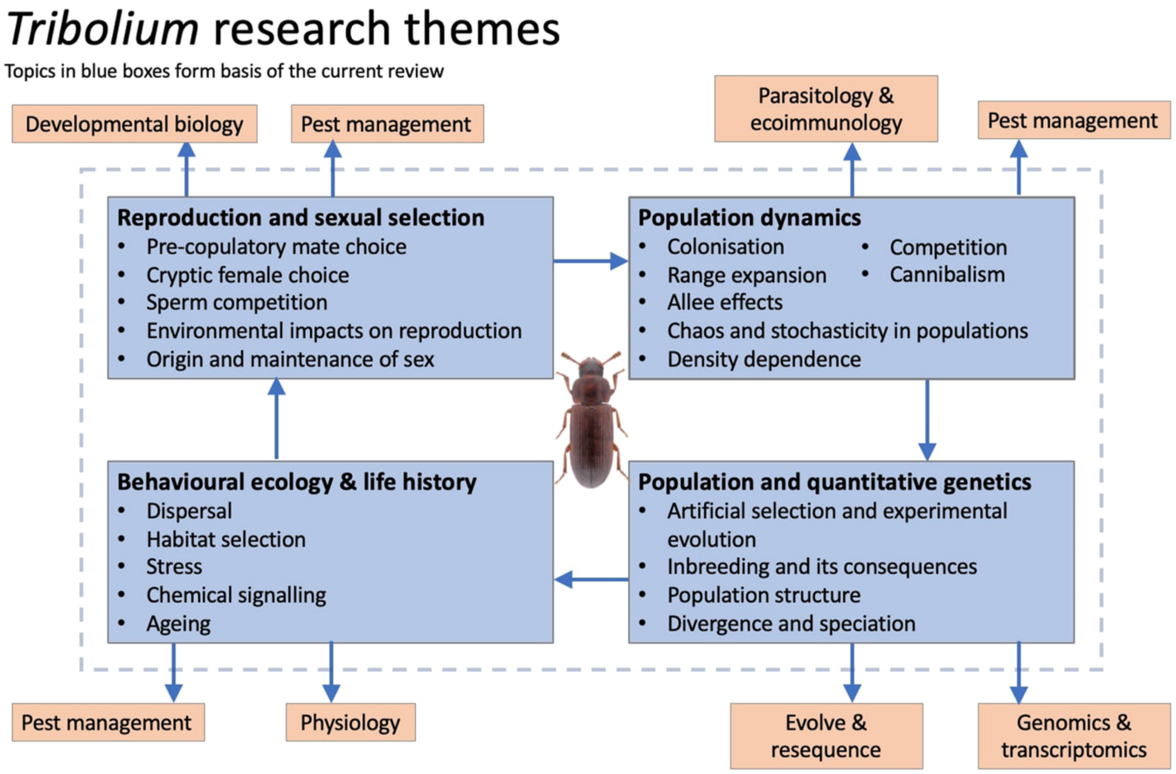

Figure 2.1. Themes from *Tribolium* research. Panels show interrelationships between research fields within evolution and ecology that have utilised *Tribolium* beetles as a research model. Those on blue backgrounds form the basis of this review

Tribolium is a genus of small tenebrionid beetles, two of which, *T. castaneum and T. confusum*, are significant global pests of stored food products (El-Aziz 2011), and widely used as experimental models (box 2.1; figure 2.2). Historically, *Tribolium* were free-living but are now mainly found infesting stored food products such as flour (Dawson 1977). The original habitat of *Tribolium* was likely beneath the bark of trees or in rotting wood, where they were secondary colonisers, characterised by rapid population growth and ready dispersal (Dawson 1977). It is not known when these pest species made the switch to a commensal lifestyle with humans, but there is evidence of *T. confusum* from Ancient Egyptian flour urns dating back ~5000 years, or ~70,000 beetle generations of selection (Andres 1931).

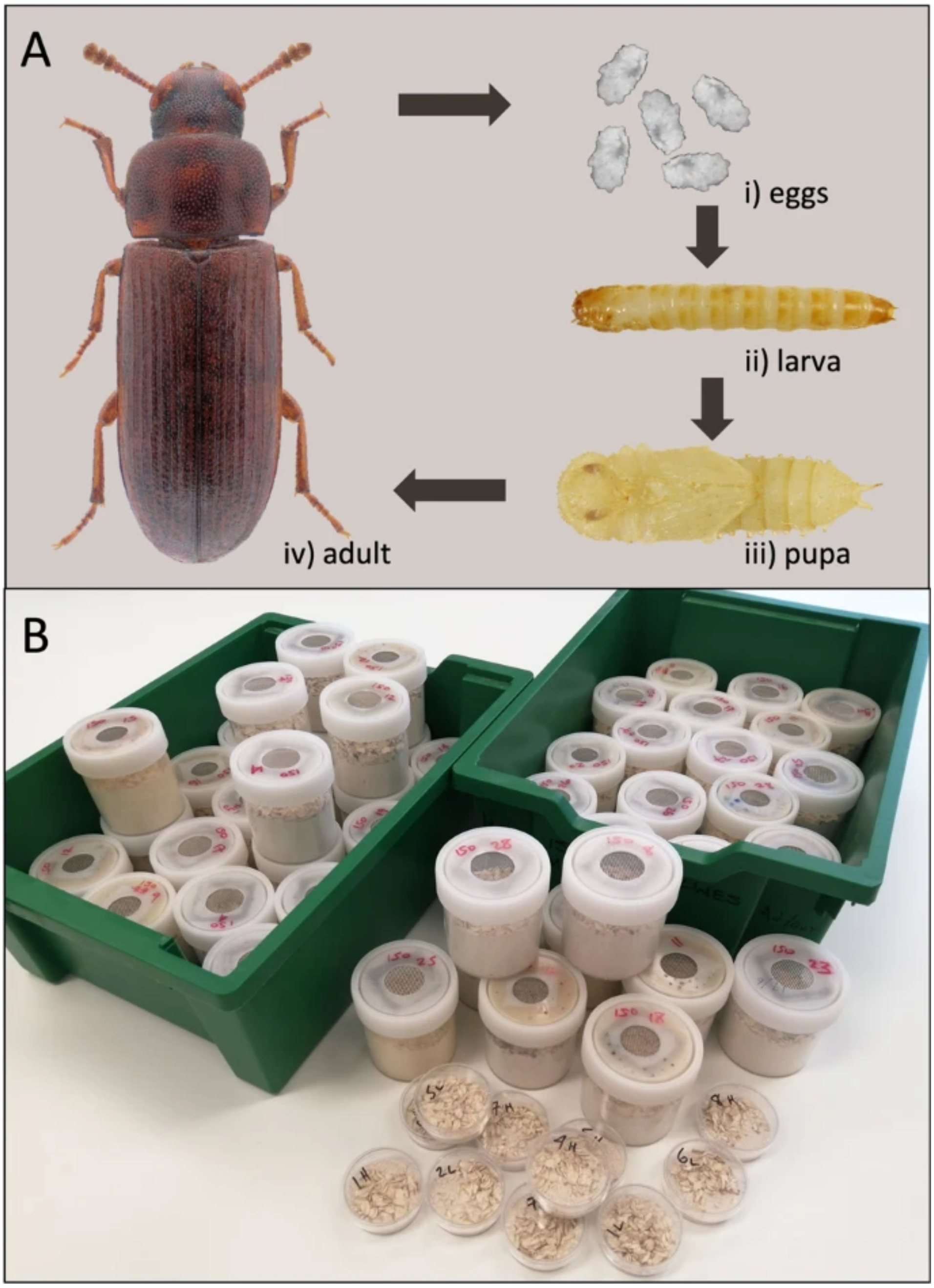

Figure 2.2. Practical *Tribolium* research. A) *Tribolium castaneum* life stages (compiled from photographs by Udo Schmidt (adult), John Obermeyer (egg), and from Khan et al. 2016). B) Experimental *Tribolium* populations housed in 250 ml screw-top containers (top) and 50 mm Petri dishes, capable of supporting ~500 and ~100 individuals respectively without significant density-dependent effects.

The purpose of this review is to highlight both the significant historical contribution from *Tribolium* flour beetles to ecological and evolutionary research, and elaborate their prominence as a contemporary study organism. *Tribolium* beetles have aided evolutionary ecology research for over a century (Chapman 1918), notably in population biology and interspecific competition (Park 1962), but also across a range of ecological and genetic disciplines. As members of the most species-rich order (Stork et al. 2015), occupying a relatively basal position among the Holometabola (metamorphosing insects), and being less highly derived than *Drosophila* (the foremost insect model), *Tribolium* beetles have broad representational scope (Brown et al. 2003). In addition, *Tribolium* is highly amenable to both forward and reverse genetic manipulation (Brown et al. 2009), a key attribute of any modern model organism (Barr 2003). *T. castaneum* was the first Coleopteran, and first agricultural pest, to have its genome sequenced (*Tribolium* Genome Sequencing Consortium et al. 2008), and annotations continue to be updated (Herndon et al. 2020). With the increasing availability of genomic data, biology is likely to become more comparative across models (Hedges 2002). *Tribolium* occupies a unique position to act as a mediary in the identification of insect orthologs of human genes, breaking a previous reliance on candidate genes from *Drosophila* and expanding the scope of insect genetics beyond highly conserved regions (Brown et al. 2003). While the *Tribolium* research infrastructure is certainly less well established than those of *Drosophila* and *C. elegans*, it is considerable and growing, with stock centres providing wild-type and many mutant strains to researchers (Brown et al. 2009), and increased availability of genomic and reverse genetic resources (Kim et al. 2010; Dönitz et al. 2015). Finally, this model is also a pest that significantly impacts global food supplies, and understanding gained in the laboratory can be applied to increase global food security (El-Aziz 2011).

Reviews of the *Tribolium* system exist, but most cover fields such as biomedicine, physiology and particularly developmental biology, where *Tribolium* is an important model due to its more representative mode of short germ band development than alternative insect models (Denell 2008; Adamski et al. 2019). Other fields where *Tribolium* are used, including parasitology, ecoimmunology, the evolutionary ecology of infectious disease and applied pest management, have relevance to the areas discussed, though we lacked the space to include them here (e.g., Kerstes and Martin 2014; Perkin and Oppert 2019; Eggert et al. 2015). Broad reviews of *Tribolium* as a model in ecology and evolution also exist but are several decades old (e.g., King and Dawson 1972; Mertz 1972; Sokoloff 1977) and much new research in this area has since emerged. We therefore aim to demonstrate that the *Tribolium* literature testifies to the model's applicability across ecological and evolutionary fields and levels of biological organisation. We break the review down into conceptual areas, focusing on: (1) population dynamics, (2) reproduction and sexual selection, (3) population and quantitative genetics, and (4) behavioural ecology, ecophysiology and life-history evolution. We also discuss implications of *Tribolium* research findings for the broader ecological and evolutionary community, and suggest some ways in which this system can continue to enhance ecological and evolutionary research. We hope that this review will be valuable both to those within the *Tribolium* community, by providing context to the often highly specialised focus of individual research groups, and to a broader readership, by synthesising the significant contributions made to ecological and evolutionary science by this often-overlooked model species.

Box 2.1. *Tribolium* ecology and experimental tractability

Many aspects of *Tribolium* ecology make them well suited as experimental organisms:

The *Tribolium* environment is also the food medium: a dry mix of fine particulates that is easily prepared and readily stored. Homogeneity within this medium eliminates micro-climatic and nutritional variation within and between replicate populations, allowing tight environmental control of both temperature and humidity, the latter being important in reducing the invasion of fungal pathogens.

Owing to *Tribolium's* long human-commensal history (~70,000 generations), the laboratory medium also has the advantage of very closely approximating its semi-wild habitat in food-storage facilities, allowing a lab environment that is less abstracted than that in other insect models.

Egg-to-adult development is completed in ~30 days (at 30 °C), with roughly 3, 20 and 4 days spent as egg, larva and pupa, respectively. Husbandry is therefore less tightly time constrained compared with faster-developing species, allowing more flexibility to generate high replicate numbers.

Adults are small (~4 mm) but dark in colour and easily distinguished from the medium. All stages, including eggs can be separated from the medium by simple sieving for counting or other experimental manipulations.

Individuals are physically and behaviourally robust to handling, without the need for anaesthetisation, allowing methods such as paint-marking of individuals for later identification.

The sexes can be distinguished in adults by the presence of a gland on the forelimbs of males, which is absent in females. Pre-reproductive individuals are most easily sexed as pupae, by their differential genital morphology, allowing single-sex cohorts or virgins to be isolated for breeding and mating studies.

Though able to fly, individuals display very limited motivation to do so in the laboratory, making containment simple relative to insects that fly. Under specific conditions, however, flight behaviour can be induced in *Tribolium*.

Tribolium mate and breed readily and promiscuously in the lab as pairs or groups, enabling manipulation of mating pattern, high levels of success in specific breeding crosses, and good opportunities for the recording of reproductive behaviour.

2.3 Population dynamics

The value of the *Tribolium* system in answering questions in population biology was recognised early in the twentieth century (Chapman 1918; Park 1934), and the system is well known for its contribution to this field. Here we break this research down into that covering (1) colonisation dynamics, (2) drivers of population size and regulation, and (3) competition. We focus on the empirical literature, but it is worth noting that *Tribolium* experiments have contributed to a large body of important mathematical modelling work aiming to predict population dynamics (Mertz 1972; reviewed in Costantino et al. 2005).

2.3.1 Colonisation and spread

Colonisation events are necessarily rare and therefore difficult to observe in nature, despite their importance. Lab models give us the opportunity to study and replicate events that determine the genetic and phenotypic make-up of contemporary populations. *Tribolium* life history is characterised by repeated episodes of dispersal and colonisation (Dawson 1977), making it an ideal system in which to investigate these processes in the laboratory. For instance, while it is well understood that the size and frequency of colonisation events are positively correlated with colonisation success (Lockwood et al. 2005), the role of genetic and demographic processes in underpinning this relationship is less clear. *Tribolium* experiments have shown that both the frequency and size of introductions affect the likelihood of establishment, with some evidence that the former may be more important (Koontz et al. 2018). Further, by manipulating levels of genetic diversity within colonising populations, an elegant set of *Tribolium* experiments have shown that demographic and genetic processes have independent roles in determining colonisation success, by affecting initial establishment success and subsequent population growth respectively (Szűcs et al. 2014; Szűcs and Melbourne et al. 2017). Similar experiments have shown that the colonising individuals' relative fitness in the new environment is as important as the number of colonisers (Vahsen et al. 2018), and that this affects population dynamics for many generations post-colonisation (Van Allen and Rudolf 2013).

Analysing the factors that underpin initial rates of population growth has also been a major aim of *Tribolium* research. It was in analysing *Tribolium* data that W.C. Allee formulated the theory that has become known as the 'Allee effect', an optimum density for initial population growth (Allee 1931; Park 1932), whereby at small population sizes mean individual fitness is reduced (Stephens et al. 1999). The rate of population growth has also been quantified across a range of environmental factors, including food quality and composition (e.g., Wong and Lee 2011), as well as life history parameters such as development time, with populations of fast-developing individuals able to grow more quickly than those in which individuals develop more slowly (Soliman 1972).

Tribolium is also well suited to the study of range expansion under a laboratory setting, using replicated populations within arrays of connected habitat patches. With such methods it has been shown that neutral stochastic processes are highly important in range expansion (Weiss-Lehman et al. 2019), with endogenous variation leading to highly unpredictable rates of spread (Melbourne and Hastings 2009). By experimentally constraining evolution, this work has shown that adaptation also plays a key role during expansion and the early stages of colonisation (e.g. Szűcs et al. 2017), though subsequent gene flow may be required into small populations to mitigate drift load which may hinder adaptation (Stewart et al. 2017). Addressing this same issue from a conservation perspective, experimental tests of both demographic and genetic rescue have shown that these interventions can reduce extinction risk and have additive effects (Hufbauer et al. 2015).

2.3.2 Population size and regulation

A large body of experimental research has used *Tribolium* populations to determine the drivers of equilibrium population size and dynamics. Early *Tribolium* work showed that equilibrium population size is mediated by habitat volume (Chapman 1928). *Tribolium* researchers have since utilised the system to investigate questions regarding effective population size, including its response to population properties such as initial density (Wade 1980a) and its relationship to census population size (Pray et al. 1996).

Studies that have paired modelling approaches with *Tribolium* population biology experiments have added greatly to our understanding of population dynamic phenomena, directly linking theory to the behaviour of real populations (reviewed in Cushing et al. 2002; Costantino et al. 2005). Demographic parameters, predicted by models to lead to chaotic dynamics, were applied experimentally to *Tribolium* populations, exposing chaotic dynamics that could be reliably disrupted by minor intervention (Costantino et al. 1995, 1997; Desharnais et al. 2001). Comparing *Tribolium* population dynamic data to model predictions has further revealed influences of stochastic processes in the behaviour of populations, as well as identifying lattice effects (dynamics arising due to the discrete nature of individual organisms, e.g., Henson et al. 2001; King et al. 2004) and accounting for the ecological synchrony between separate populations (Desharnais et al. 2018).

Tribolium populations self-regulate via complex interactions of density-dependent effects on reproductive, developmental, dispersive and cannibalistic behaviours (King and Dawson 1972). Chemical secretions (see 'life-history' section, below) accumulate in the medium in proportion to time and density in a process known as 'conditioning', and these secretions are used by the beetles as indicators of demographic parameters, such as population density (El-Desouky et al. 2018). Much of the work on this area was done in the early-mid nineteenth century and is well covered by King and Dawson (1972). Oviposition is suppressed by conditioning of the medium and increased by egg cannibalism (Sonleitner and Gutherie 1991). Recent work has shown that females evaluate current and future competitive conditions when making oviposition decisions (Halliday et al. 2019). Larval development is slowed by increasing larval and adult density (Park et al. 1939; Janus 1989). Higher density during development also has indirect negative effects on future reproductive success through reduced body weight (Assie et al. 2008). Dispersal increases with density (Ziegler 1978) and there is evidence that the genetic bases of dispersal and the reduction of oviposition in response to conditioning are linked (Lavie and Ritte 1980, see also 'Life history' below). Density-dependent processes in *Tribolium* are complex and act differentially on the sexes (Ellen et al. 2016), interact with food quality (patterns of density-dependent habitat selection depend on the foodstuffs used, Halliday et al. 2019) and weaken with deviation from thermal optimum (Halliday and Blouin-Demers 2018).

Cannibalism is consistently shown to be core to many aspects of the *Tribolium* system, including population regulation. The degree of cannibalism increases with density until a point at which the predatory individuals become satiated (Park et al. 1965). Adult cannibalism of pupae is most effective at controlling adult numbers (Young 1970), while more nutritional benefit is gained from egg-eating (Alabi et al. 2008). Females are more voracious than males, and there is no reduction in cannibalism with increasingly conditioned medium (Flinn and Campbell 2012). The extra nutrition gained from cannibalism is able to compensate for the negative effects of high density (Mertz and Robertson 1970), and to facilitate colonisation of marginal nutritional habitat (Via 1999). Populations differ in their cannibalism rates, and these differences appear stable over many tens of generations; this may be because differences in cannibalism confer no selective advantage, or perhaps because populations can occupy different peaks on the selective landscape (Stevens 1989). Kin-selection may provide a mechanism for changes in cannibalism, as reduced rates of cannibalism between certain life stages have been observed within groups of highly related individuals (Wade 1980b).

Cannibalistic behaviour creates the periodic cycles in age-structure that characterise *Tribolium* populations (Costantino and Desharnais 1991) and, by manipulating population conditions, cycles can be altered or interrupted. When cannibalism is negated, by housing life stages separately, cycles disappear (Benoit 1998) and if populations are unconfined, allowing emigration, generations become non-overlapping (Ziegler 1972). These cycling phenomena are another area in which *Tribolium* has fostered close ties between theoretical and population biologists, by providing a convenient system in which to test theories of population dynamics, such as how environmental fluctuation affects population cycling (Reuman et al. 2008).

2.3.3 Competition

Much of the reputation of the *Tribolium* system is founded on the two-species competition experiments of Thomas Park and his collaborators and their indeterminate outcomes, which helped draw attention to the role of stochastic processes in ecology (Park et al. 1964). Briefly, using experimental populations containing both *T. confusum* and *T. castaneum* it has been shown that (i) one species is almost always driven to extinction; (ii) the 'winning species' cannot be predicted by the relative performance of each species cultured alone in the focal environment; (iii) outcomes can be indeterminate under certain conditions, whereby the 'winner' is not the same in all replicates; (iv) there was no evidence for an elevated 'win rate' of populations which had won in previous experiments, suggesting that competitive ability cannot be selected on (Park and Lloyd 1955). These results spawned much work trying to account for indeterminate outcomes, including mathematical modelling (e.g., Leslie 1962). Following this early work, competition experiments in *Tribolium* have shown that demography may be more important than genetics in determining competitive outcomes in this system (Mertz et al. 1976), although inbreeding depression can cause a loss of competitive ability at low founding size (Craig and Mertz 1994). Further, a wide range of external variables have been shown to affect competition dynamics, including reduced competitive ability resulting from parasite infection and/or low natal habitat quality, and deviation from the thermal optimum (e.g., Yan et al. 1998; Van Allen and Rudolf 2015).

2.4 Reproduction and sexual selection

One of the most fundamental life processes is reproduction, yet much is still unknown about the origin and maintenance of sex, and the evolutionary forces that maintain the diversity of reproductive phenotypes observed in nature (Williams 1975). To address these, and related questions has been a rich area of *Tribolium* research. Indeed, *T. castaneum* was used in one of the first ever sperm competition and fertilisation precedence experiments (Schlager 1960). Previous broad reviews have identified ways by which females may influence paternity during and following mating, including: inhibiting sperm transfer; altering re-mating behaviour; controlling timing of spermatophore ejection (Pai and Bernasconi 2008; Fedina and Lewis 2008). These reviews also cover the male traits shown to affect paternity share in the many studies of sperm precedence conducted on *Tribolium*. We will therefore focus on subsequent advances in these areas.

Experimental studies have provided varied insights into the evolution and ecology of mating behaviour. Recent work on pre-copulatory behaviour has shown that females exhibit a preference for non-stressed males (McNemee and Marshall 2018) and that homosexual behaviour, which is quite common in *T. castaneum*, is dependent on the social environment and likely occurs due to inaccurate mate choice (Martin et al. 2015; Sales et al. 2018). Post-copulatory reproductive processes are also an important area of research, and there is much potential to track the dynamics of sperm behaviour and male–male interactions within the female tract in vivo. Advances in fluorescent tagging of sperm have made it possible to visualise sperm fate following natural matings and their movement through the tract to the fertilisation set (Droge-Young et al. 2016), and molecular methods have facilitated the study of seminal fluid proteins (South et al. 2011).

Many aspects of reproduction appear to be environment dependent, with factors such as nutrition, temperature, conditioning of medium and parasite presence shown to alter mating dynamics and reproductive fitness, both individually and in combination (Grazer et al. 2014; Khan et al. 2018; Sales et al. 2018; Vasudeva et al. 2019). Further, reproductive fitness has been shown to trade off with intrinsic factors such as walking ability (Matsumura et al. 2019) but can be enhanced by others, such as pesticide resistance (Arnaud et al. 2005). Phenotypic plasticity and genotype-by environment interactions also play an important role in determining levels of sexual conflict and adaptability (Lewis et al. 2012; Holman and Jacomb 2017).

Tribolium experiments have been used to address fundamental questions about the 'sex paradox', sexual selection and the origin of mating behaviours (Dunbrack et al. 1995). Key to these studies has been the experimental manipulation of sex ratios and mating patterns over one or multiple generations, thereby applying different sexual selection pressures, and then measuring the consequences. Using such approaches, it has been shown that levels of promiscuity increase following genetic bottlenecks, and therefore that promiscuity may provide a mechanism for avoiding genetic incompatibility (Michalczyk et al. 2011). Using long-term sexual selection lines and experimental inbreeding, *Tribolium* experiments demonstrated that sexual selection may buffer populations against extinction through purging of mutation load (Lumley et al. 2015). However, subsequent studies using alternative approaches to examine the relationship between sexual selection and mutation load have found no purging effects (e.g. Prokop et al. 2019). A population history (up to ten years) of experimentally applied, strong sexual selection has also been shown to improve the competitive ability of males and their sperm, and drive sperm morphology evolution (Michalczyk et al. 2011; Demont et al. 2014; Godwin et al. 2017), increase conspecific population invasion success (Godwin et al. 2018), enable population resilience to the extinction vortex (Godwin et al. 2020), and increase the rate of pesticide resistance evolution (Jacomb et al. 2016).

2.5 Population and quantitative genetics

Laboratory insect models have been instrumental in developing our understanding of population genetic theory, from the tracking of inversions in populations (Wright and Dobzhansky 1946) to the discovery of genetic markers (Lewontin and Hubby 1966). Though relatively understudied in comparison to *Drosophila*, many of the same features of *Tribolium* that made it originally attractive to those studying population dynamics, also make it suitable as a lens through which to view evolution in experimental populations from a genetic perspective. Since Park's competition experiments first drew the attention of geneticists towards the *Tribolium* system, genetic investigations have formed an important branch of its study (King and Dawson 1972). Genetic work on *Tribolium* spans levels of organisation, covering everything from the relationship of genotype to phenotype, through to interspecific reproductive isolation.

2.5.1 Artificial selection and experimental evolution

Early studies of adaptive evolution in *Tribolium* were based on observing phenotypic changes over generations, revealing that changes (in traits such as development time or pupal weight) can arise due to variation in productivity and cannibalism (Sokal and Sonleitner 1968; King and Dawson 1972). Similar, approaches have been used to show that the spread of a selfish genetic element through a population is proportional to the strength of its effect (Wade and Beeman 1994), and that pesticide-resistant genotypes can have increased fitness, even in the absence of pesticides (Haubruge and Arnaud 2001).

The majority of experimental studies of adaptive evolution have directly manipulated the strength of selection. Selection can be artificially applied, with those individuals who will seed the next generation being chosen by the researcher based on a given trait. Alternatively, experimental evolution can be used, where natural selection is allowed to act within populations subjected to a treatment. These techniques enable, amongst other things, the study of the relationship of genotype to phenotype. Provided that the strength of selection is known, trait heritability can be inferred from the response to selection. Alternatively, crosses between individuals of different phenotypes can reveal the genetic basis of traits. In these ways, it has been shown that traits such as pupal and adult weight are highly heritable in *Tribolium* (Wade et al. 1996). Some have moderate heritability, such as larval weight, development time and walking distance (Yamada 1974; Matsumura and

Miyatake 2015). While some, like fecundity and death-feigning duration, respond only weakly to artificial selection (Orozco and Bell 1974; Miyatake et al. 2004).

Response to selection has been shown not only in terms of the focal trait, but also in associated traits. For example, weight metrics show correlated responses to selection across life stages (Yamada 1974) and populations selected for short death-feigning duration also have short legs and lower walking motivation (Matsumura and Miyatake 2019). Lines selected for low dispersal propensity have greater longevity, poorer flight ability, are better competitors, develop more quickly and have longer generation times than dispersers (e.g., Zirkle et al. 1988). Some of these associations between traits can limit the response to selection (Irwin and Carter 2014), and attempts to break the correlations of pairs of traits through artificial selection have failed (Bell and Burris 1973; Tigreros and Lewis 2011).

Wright's shifting balance theory (Wright 1932) suggests that population structure may also influence response to selection, although there is limited evidence that this process plays an important role in nature (Coyne et al. 2000). *Tribolium* studies have applied artificial selection within sub-divide-and-merge population structures to test predictions of shifting balance theory (see Wade and Goodnight 1998). However, large panmictic populations have been found to respond better to selection for pupal weight than sub-divided, periodically mixing ones (Katz and Enfield 1977) and no difference in response was found using a similar design to select for offspring number (Schamber and Muir 2001). Further, it has been suggested that these experiments do not capture the complexity of the shifting balance process, and that doing so experimentally may not be possible (Coyne et al. 2000).

In addition to studying adaptation, *Tribolium* experiments have been used to understand the causes and consequences of stochastic processes such as mutation and drift in evolution (e.g., Rich et al. 1979). Inbreeding depression has been studied in detail in *Tribolium*, and inbreeding has been shown to have negative effects on a range of traits, including productivity and viability (López-Fanjul and Jódar 1977). Susceptibility to inbreeding depression can vary between populations and affect suites of traits within populations (Pray and Goodnight 1995; Pray 1997). Inbreeding has negative consequences for population growth and response to selection (McCauley and Wade 1981; Wade et al. 1996). However, the fitness consequences of inbreeding may only be realised in certain environments (subject to genotype-by-environment interactions arising under inbreeding), and may differ between sexes (Pray et al. 1994). The above studies generated inbreeding through enforced sib–sib matings, however more ‘realistic’ approaches have also been utilised. For example, by reducing population size but allowing free matings, bottleneck scenarios can be simulated, these have shown that subsequent stress following a bottleneck can reveal inbreeding severity, even after population size has recovered (Franklin and Siewerdt 2011).

2.5.2 Divergence and speciation

Genetic differentiation has been quantified empirically between wild *Tribolium* populations (Drury et al. 2009; McCulloch et al. 2019) and between lab populations of varying geographic origin (Yamauchi et al. 2018). While results conflict between studies, it is clear that at least some genetic structure exists within *Tribolium* species. Although there has been limited research into the drivers of population divergence, there is a substantial body of experimental research examining its consequences. Female *T. castaneum* have been shown to increase egg-laying rates when mated to inter-population males, suggesting potential inbreeding avoidance (Attia and Tregenza 2004). On the other hand, when the sperm of males from different populations compete for fertilisations, ‘home’ males can be seen to have an advantage, indicative of partial reproductive isolation (Pai and Yan 2002). Other inter-population crosses can result in perpetually immature larvae (Drury et al. 2011), and show genetic incompatibilities in *T. castaneum* that provide support for the Haldane’s rule (Demuth and Wade 2007). Partial reproductive isolation through mate choice has also been observed between populations of *T. confusum* (Wade et al. 1995).

Moving beyond populations, species across the *Tribolium* genus display a range of divergence times, making them useful for studies of reproductive isolation at the species level. Between *T. castaneum* and *T. confusum*, reproductive isolation is incomplete (Shen et al. 2016), while between *T. castaneum* and *T. freemani*, hybrids are sterile (Wade and Johnson 1994). Interspecific crosses have therefore been used to study questions related to speciation, showing for instance that significant post-copulatory prezygotic isolation can arise through asymmetric sperm precedence under interspecific male competition (Robinson et al. 1994). Crosses between *T. castaneum* and *T. freemani* have also shown that the degree to which skewed sex ratios and male deformity manifest in the F1 generation varies with the geographic origin of the *T. castaneum* strain used, even at relatively local scales (Wade et al. 1997).

The wide range of geographic and genetic distances between the species of *Tribolium*, means that the group constitutes an excellent system for comparative genomic studies seeking to understand the drivers and consequences of speciation (Brown et al. 2009), this will likely be a rich area of future study. Another area for expansion in *Tribolium* research is population genomics: the attributes that have seen the system used in population studies in the past, along with its emerging ability to generate high quality genomic data, are suited to combine in the study of genomic responses to selection. This could take the form of 'evolve and resequence' approaches that have so far struggled to make it beyond *Drosophila (*Schlötterer et al. 2015).

2.6 Behavioural ecology and life history

Research on *Tribolium* began with studies of populations, but soon branched out to include the individual-level life-history traits that underlie the dynamics of populations, often in order to correctly parameterise mathematical models. Today *Tribolium* is a model for studying life-history parameters of invertebrates in its own right. Ongoing life-history studies in *Tribolium* can mostly be divided into those concerned with dispersal, movement and habitat selection; responses to environmental stress and diet; and studies of chemical biology and ageing.

2.6.1 Dispersal, movement and habitat selection

The study of why individuals disperse, what biological and environmental factors drive this, and the population-level consequences of this process are important areas in evolutionary ecology (Bowler and Benton 2005). Laboratory insect models offer a useful opportunity to study dispersal, as individuals can be tracked, the process of dispersal can be measured in a controlled and repeatable way, phenotypes associated with dispersal can be artificially selected, and the consequences of dispersal measured. *Tribolium* offers a particularly useful model in this respect, as it has two modes of dispersal (walking and flying), and is characterised by a life history that likely requires dispersal between patchy habitats (Dawson 1977). As a result, *Tribolium* has been widely used to study a broad range of questions about the evolutionary drivers and ecological consequences of dispersal.

Like many areas of *Tribolium* research, work on dispersal began in the mid twentieth century, but has seen a modern renaissance. *Tribolium* flight is rare, and early work focused on movement by walking, defining dispersal as the tendency of adults to leave a patch of habitat within an experimental apparatus composing two connected habitat chambers (Prus 1963; reviewed in King and Dawson 1972). Variations on this set-up over many years have shown dispersal to be dependent on a complex of factors, principally an interaction between density (Zromska-Rudzka 1966) and age (see Ziegler 1976; Gurdasani et al. 2018), altered by population-age and relatedness structure (Ziegler 1978; Jasieński et al. 1988), food availability (Ziegler 1977), as well as the natal environment of a focal individual or a threshold proportion of its neighbours (Van Allen and Bhavsar 2014; Endriss et al. 2019). Adult dispersal tendency appears to have a strong genetic basis (Ritte and Agur 1977), but this tendency is not conserved within individuals between pre- and post-metamorphosis (Arnold et al. 2016). Tendency to disperse is more highly correlated with leg length than metabolic rate, indicating that dispersal phenotypes depend more on morphological than physiological traits in adults (Arnold et al. 2017). Dispersal rate and sensitivity of dispersal responses to age and environment are greater in *T. castaneum* than *T. confusum*, consistent with the former being a primary coloniser, following a more r-type strategy than that adopted by the latter (Ziegler 1976).

The last decade has seen significant attention paid to the study of flight behaviour of *T. castaneum*, with the influence of biotic and abiotic factors on flight responses being investigated in laboratory, wild and wild-caught populations. Relative humidity does not appear important, while wind speed and direction, temperature, light, resource provision, and quality, age and mating status have all been seen to alter flight initiation and/or duration (e.g., Drury et al. 2016; Gurdasani et al. 2019). Flight patterns appear to be crepuscular and show seasonality (Daglish et al. 2017; Rafter et al. 2019), though this pattern varies with latitude (Rajan et al. 2018). Consensus on the effect of sex on flight remains elusive, as the results of these studies do not agree on whether flight behaviour differs between males and females.

Movement in relation to habitat selection by different life stages has also been studied in *Tribolium*, which can occur either by walking on the surface of the medium, or tunnelling through it (Hagstrum and Smittle 1980). Larvae seeking pupation sites tend to move deeper into the medium, seeking warmth and low population density, even when this means using poor quality habitat (King and Dawson 1972; Mayes and Englert 1984; Janus 1989). Adults move in response to biotic factors, avoiding high density areas and highly conditioned media, and alter their behaviour according to their reproductive status (Naylor 1965; Wexler et al. 2017). Adult movement behaviour can also vary in response to abiotic factors, including temperature, humidity and habitat structure (Campbell and Hagstrum 2002; e.g., Halliday and Blouin-Demers 2017). Studies investigating the distribution of adults within a fodder mass have shown that fine-scale spatial and temporal structure exists, and that individual variation in patch exploitation can serve to maximise individual fitness (Surtees 1963; Campbell and Runnion 2003). This variation in movement behaviour in response to resource availability may differ when flying as opposed to walking, and between wild and laboratory populations (Ahmad et al. 2013; Ahmad et al. 2013).

2.6.2 Other life-history features

Tribolium represents a good model for studying the effects of stress due to the ease of replicability, and as a model that has relatively few ethical issues surrounding its use. Stress, in the form of starvation, heat or cold shock, or combinations thereof (Shostak et al. 2015), has been experimentally applied to show negative effects on reproductive output and behaviour, movement patterns, and immune response (Sbilordo and Gräzer 2011; Eggert et al. 2015; Wexler and Scharf 2017). Alternatively, effects of tolerance to stress can be the response variable, and this has been used to show that the ability to tolerate stress is affected by a range of factors including parental age (Halle et al. 2015), thermal acclimation regime (Izadi et al. 2019) and rearing conditions (Scharf et al. 2015). Later-life effects of natal/juvenile stress have also been shown in *Tribolium*, with the natal environment affecting adult dispersal (Van Allen and Bhavsar 2014), competitive dynamics (Van Allen and Rudolf 2015), productivity and rates of cannibalism (Boyer 1976).

Tribolium excrete a range of chemicals into their environment, some of which are aggregation pheromones (mainly 4,8 dimethyldecanal, Suzuki 1980), whereas others are a defence against predators and microorganisms whilst playing an important role in population dynamics as indicators of population density (Arnaud et al. 2000). The chemicals used as pheromones differ across the genus (Arnaud et al. 2002), and are produced predominantly by males, eliciting the strongest responses from females (Stevenson et al. 2017). Diet alters the chemical composition of secretions, but may not alter their efficacy (Ming and Lewis 2010). Defensive compounds such as benzoquinones have antimicrobial properties (Prendeville and Stevens 2002) and show genetically controlled differential production across individuals (Yezerski et al. 2004). Due to the shared benefit of their action through density regulation, and the individual costs associated with their production, this can represent a social dilemma (Gokhale et al. 2017). The distinction between these two classes of secretions is not clear, with benzoquinones also shown to possess pheromone-like attractive properties (Verheggen et al. 2007). Understanding *Tribolium* chemobiology represents a fascinating future challenge, given the potential of secretions to influence individual, population and interspecies processes.

Considering its role as a pest, there has been much interest in the ability of *Tribolium* to utilise different food resources and the effect that these have on its life history. Early authors list a wide range of products on which *Tribolium* has been found, including flours of many grains, peas, beans, nuts, chocolate and several spices (e.g., Chittenden 1896). Grain preference was among the first tests performed by Chapman, the man credited with pioneering the use of *Tribolium* for experimental study, who found a preference for wheat flour, and an inability to feed on whole grains (Chapman 1918; Park 1934). Very many studies of responses to diet and diet quality in *Tribolium* have since been published, which show that natural or artificial diets result in large effects on a broad range of fitness parameters (e.g., Sinha 1966; Sokoloff et al. 1966; Wong and Lee 2011).

Finally, there is a limited body of research on ageing in *Tribolium. T. confusum* was the organism in which exposure to radiation was first shown to increase longevity (Davey 1917); this work was subsequently expanded on and replicated widely among insects (Ducoff 1986; Calabrese 2013). However, recent decades have seen *Tribolium* superseded as an invertebrate model of ageing by relatively short-lived alternatives in *Drosophila*, *C. elegans* and yeast (Kennedy 2008). Nonetheless, age-related changes have been shown in individual-level morphological, physiological, biochemical, behavioural and pathological traits (Soliman 1987). Ageing research in *Tribolium* has generated important insights into the link between development and ageing and their genetic basis and the evolution of senescence (Soliman and Lints 1975; Mertz 1975). Some more recent work has highlighted parental-age effects on development and stress tolerance (Halle et al. 2015) and a decline in metrics of movement with increasing age (Wexler et al. 2016). As a relatively long-lived arthropod model (adults can live for up to 4 years, Good 1936) *Tribolium* may have utility for understanding ageing in slower-senescing animals such as vertebrates.

2.7 Future directions and conclusions

We have highlighted some of the broad range of fields in which *Tribolium* has been successfully used as a research model. However, we believe that there is still much untapped potential from this organism for addressing several research areas, particularly the expansion of historically productive *Tribolium* fields in combination with genomic data. Studies of divergence can continue to exploit the genus' diversity and mating ecology, in combination with genomic techniques, to probe deeper into the process of species formation. Population dynamic and artificial selection responses can be investigated at the level of the entire genome, expanding the complexity of our understanding. Pest management can employ genomic information to increase the efficacy and specificity of its techniques, minimising the collateral damage while maximising benefits.

Importantly, *Tribolium* research can also incorporate work on climate change, which represents an enormous threat to biodiversity, with insects likely to be severely affected due to their short life cycles and temperature sensitivity (Bale et al. 2002). There is great scope for *Tribolium* as a model in which to study the effects of climate change on insects, for example by studying their physiological, ecological and evolutionary responses to experimental evolution under increased temperature. Many of the characteristics lending research utility to *Tribolium* (Box 1) also make it highly suitable as a research-led teaching resource (Hoste 1968), a role in which we feel it has been underutilised in the past.

Tribolium beetles possess many attributes that have made them a desirable study organism through a long history and breadth of application. They have contributed to many important past discoveries, and continue to be employed in addressing fundamental questions across fields in evolution and ecology. The utility of *Tribolium* spans levels of organisation, and a great responsiveness to genetic manipulation (Brown et al. 2009) promises to extend their relevance far into the genomic age. The ever-growing research infrastructure, and ability to integrate knowledge from across fields, makes *Tribolium* a valuable model system to complement the established invertebrate models of *Drosophila* and *C. elegans*.

2.8 Contributions

The study was conceived by Lewis G Spurgin, in discussion with Michael D Pointer. MDP researched and wrote the manuscript, with help from both LGS and Matthew JG Gage in structuring and review.

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CHAPTER THREE

Genetic architecture of dispersal behaviour in the post-harvest pest and model organism *Tribolium castaneum*

A version of this chapter (appendix 2) is published as:

Pointer MD, Spurgin LG, Gage MJG *et al.* (2023). Genetic architecture of dispersal behaviour in the post-harvest pest and model organism *Tribolium castaneum*. *Heredity* **131**, 253–262 https://doi.org/10.1038/s41437-023-00641-6

Marked males of *T. castaneum* in a Petri dish

3.1 Abstract

Dispersal behaviour is an important aspect of the life-history of animals. However, the genetic architecture of dispersal-related traits is often obscure or unknown, even in well studied species. *Tribolium castaneum* is a globally significant post-harvest pest and established model organism, yet studies of its dispersal have shown ambiguous results and the genetic basis of this behaviour remains unresolved. We combine experimental evolution and agent-based modelling to investigate the number of loci underlying dispersal in *T. castaneum*, and whether the trait is sex-linked. Our findings demonstrate rapid evolution of dispersal behaviour under selection. We find no evidence of sex-biases in the dispersal behaviour of the offspring of crosses, supporting an autosomal genetic basis of the trait. Moreover, simulated data approximates experimental data under simulated scenarios where the dispersal trait is controlled by one or few loci, but not many loci. Levels of dispersal in experimentally inbred lines, compared with simulations, indicate that a single locus model is not well supported. Taken together, these lines of evidence support an oligogenic architecture underlying dispersal in *Tribolium castaneum*. These results have implications for applied pest management and for our understanding of the evolution of dispersal in the coleoptera, the world's most species-rich order.

3.2 Introduction

Dispersal is important in the ecology and evolution of many species (Ronce 2007) and plays a key role in species' ability to cope with habitat fragmentation and anthropogenic climate change (Travis et al. 2013). Conversely, dispersal also drives inadvertent introductions and invasions of non-native species (Renault et al. 2018). Knowledge of the movement of pests, and its genetic basis, will also allow us to better preserve biodiversity, forecast outbreaks, and to design management and control strategies (Jeger 1999). The consequences of dispersal can be considerable for the organism; as a result of moving to different abiotic, biotic, and/or reproductive environments, individuals may experience extensive differences in fitness, but there are also effects at higher levels of organisation (i.e. at the population or species level compared to at the individual level; Clobert et al. 2012). As such, individual dispersal decisions combine to shape the distribution of individuals and populations and thus govern the area a given species occupies, defining the limits of range expansions and shifts (Kokko and López-Sepulcre 2006). Dispersal is also a key determinant of metapopulation persistence in fragmented, disturbed, or unstable environments, where it can allow recolonisation to balance local extinction (Eriksson et al. 2014). Dispersal also mediates metapopulation structure, determining migration rates and gene flow, which in turn influence evolutionary trajectories of dispersal and on-dispersal traits (Suárez et al. 2022). In both cases, the genetic architecture of the focal trait is an important factor, for example the number of controlling loci can affect the rate of dispersal evolution (Weiss-Lehman and Shaw 2022) and epistatic variance in any trait can be converted to additive variance by drift during founding events, or vice versa as a result of later additional gene flow into the population (Wade and Goodnight 1998; Hill 2017).

A genetic basis to dispersal has been shown in a wide variety of animal species, however, the identity, number, and mode of action of the genes underlying dispersal vary widely (Saastamoinen et al. 2018). Large-effect loci underlying dispersal have been identified in various species (e.g. Trefilov et al. 2000; Fidler et al. 2007; Krackow and König 2008; Edelsparre et al. 2014). Contrastingly, a powerful study of dispersal utilised the *Drosophila* Genetic Reference Panel and found 192 genes associated with variation in locomotion (Jordan et al. 2012). This finding agrees with the view that polygenic architectures generally underlie complex quantitative traits (e.g. Husby et al. 2015; Santure et al. 2015), such as dispersal. However, the genetic basis of dispersal remains obscure in most species.

Tribolium castaneum is a significant pest of stored food products (El-Aziz 2011), responsible for a portion of the ~10% of total grain lost to insects during storage (Boxall 2001), and therefore their control is of biological and economic importance. This species is also an important laboratory model across a range of disciplines, from evolutionary ecology to development (Denell 2008; chapter 2, Pointer et al. 2021). Additionally, as Coleopterans, they are members of the most species-rich order and, being an early diverging lineage in the phylogeny of metamorphosing insects, *T. castaneum* is considered highly representative of other insect species (Brown et al. 2003; Stork et al. 2015). Understanding the genetic causes and consequences of dispersal in *Tribolium* could have significant pest control benefits, as well as adding to our knowledge of how populations evolve during range expansion (Weiss-Lehman and Shaw 2022) and how insects may respond to habitat suitability shifts under climate change.

Several studies on *Tribolium* have demonstrated a genetic basis to dispersal through responses to artificial selection (Schurr and Bolduan 1967; Ogden 1970a, b; Ritte and Lavie 1977; Korona 1991; Ruckman and Blackmon 2020); though many of these measured dispersal only in single-sex groups. The rapid response to selection observed across these experiments, has led some authors to postulate that the trait is controlled by very few loci (Ogden 1970a; Ritte and Lavie 1977). For example, evidence of differential dispersal in the sexes between reciprocal crosses, have led to suggestion that dispersal is controlled by a single, sex-linked locus (Ritte and Lavie 1977). In contrast, line cross analysis on a similar set of crosses has provided evidence that epistatic interactions across loci dominate additive effects in dispersal adaptation (Ruckman and Blackmon 2020). Despite considerable effort spent investigating dispersal in *Tribolium* little is known definitively; findings are contradictory (Ritte and Lavie 1977; Ruckman and Blackmon 2020), and many studies are only modestly replicated (Ritte and Lavie 1977), and/or were conducted before the availability of modern computational and molecular genetic techniques.

Here, we combine quantitative genetic, population genetic, and computational approaches—comparing results from replicated experiments and agent-based simulations—to understand the genetic basis of *Tribolium* dispersal. Dispersal is known to consist of three distinct phases, emigration, transit, and immigration (Ronce 2007). While we acknowledge that the focus of this study and previous studies is technically emigration, local movement tendency has been linked to longer distance dispersal by flight (Zirkle et al. 1988) and we use the term dispersal for consistency with the existing *Tribolium* literature. Further, our experimental setup requires individuals to traverse an empty resource patch before they are deemed to have dispersed, making the movement trait studied here more emigration-like than previous work in the system (e.g. Ritte and Lavie 1977). Specifically, by comparing the results of artificial selection for dispersal to agent-based simulations modelling artificial selection under a range of genetic architectures we investigate the genetic basis of the trait. Next, by comparing the dispersal behaviour of experimental crosses to crosses simulated under either sex-linked or non-sex-linked architectures, we explicitly test the hypothesis of a single sex-linked dispersal locus proposed by Ritte and Lavie (1977). If this holds true we predict that simulations under a single-locus architecture will best fit the observations from experimental selection, and that behaviour of males and females will differ in the F1 generation of experimental and simulations under sex-linked architectures but not under non-sex-linked architectures. Lastly, we compare dispersal in experimentally inbred beetle lines and inbreeding simulations, gathering further evidence on the genetic basis of dispersal in this system.

3.3 Materials and methods

3.3.1 Beetles and husbandry

Beetles used were of the Krakow super-strain (KSS), bred to combine global *Tribolium castaneum* genetic variation (Laskowski et al. 2015). Stock populations were maintained at Ne ≈ 300 within 1.2 L plastic containers covered with lids into which 70 × 70 mm windows of fine mesh had been inserted for ventilation. Populations were kept on a ‘standard fodder’ medium of 90% organic wheat flour and 10% brewer’s yeast at 30°C, 60% relative humidity, and a 12:12 light-dark cycle (light from 8 am–8 pm) at the University of East Anglia. These are the same conditions under which the stock populations have been kept for ~12 years. The standard husbandry cycle consisted of two phases; during the oviposition phase adult (12+/−3 days post-eclosion) beetles chosen to parent the next generation were removed from their populations and placed into fresh fodder for seven days of mating and egg-laying. Following this period, adults were sieved from the fodder and discarded, beginning the 35-day development phase—during which time eggs in the fodder developed through the larval and pupal stages to become adults. By preventing any interaction between sexually mature adults and offspring reduces the risk of negative density-dependent effects, removes the opportunity for intergenerational interactions, such as egg-cannibalism, and allows accurate tracking of passing generations.

3.3.2 Experimental methods — dispersal phenotyping assay

To investigate the genetic basis of dispersal behaviour and its response to selection we established selection lines by breeding from individuals based on their behavioural phenotypes, as determined using dispersal assays. Dispersal arenas were constructed that consisted of two square, 1.2 L plastic containers with removable lids, connected by a length of rigid PVC tubing with 8 mm internal diameter (Figure 3.1A). When 200 ml of fodder was placed into container A and made level, the surface of the fodder intersected the opening of the tube. Stoppers made from baked Fimo polymer clay were used to block either end of the tube when required. On the first day of the assay the tube was blocked and 200 ml of fodder was placed into container A, made level and topped with oats to aid traction. Next, 200 adult beetles of mixed sex (12+/−3 days post eclosion) were added to container A and given a 2 h acclimation period. After this time, the tube was unblocked, beginning a 20 h dispersal period. The walls of both containers were smooth and prevented climbing, so individuals that fell from the tube to the floor of container B were unable to return to container A. Beetles in container B at the end of the period were considered to have dispersed. We utilised two types of dispersal assay, differing in the number of dispersal opportunities they afforded: a 1-opportunity assay, (described above) and a 3-opportunity assay, which provided greater resolution at which to measure behaviour. During the latter, following the first dispersal period, individuals from container B were marked with a dot of paint on the dorsal thorax, using a paint marker pen (Posca, 0.7 mm). The contents of container A were separated by sieving. The fodder was replaced in container A, flattened and topped with the same oats. The original 200 individuals from a single replicate, retrieved from container A or B, were returned to container A for a second round of dispersal. This process was repeated twice so each individual had three opportunities to disperse, and its number of dispersals recorded. To mitigate effects of unavoidable small differences in arena construction, lines were assigned randomly to arenas each generation. Processing each line was time-consuming so, in order to guard against time-of-day effects, lines were divided evenly between two temporal blocks (lines 1–8 for both treatments in block 1 and lines 9–16 in block 2). Some component of the measured behaviour may be due to learning across dispersal opportunities within generations, however, as any effect of learning would be consistent across generations we expect the overall impact to be minimal.

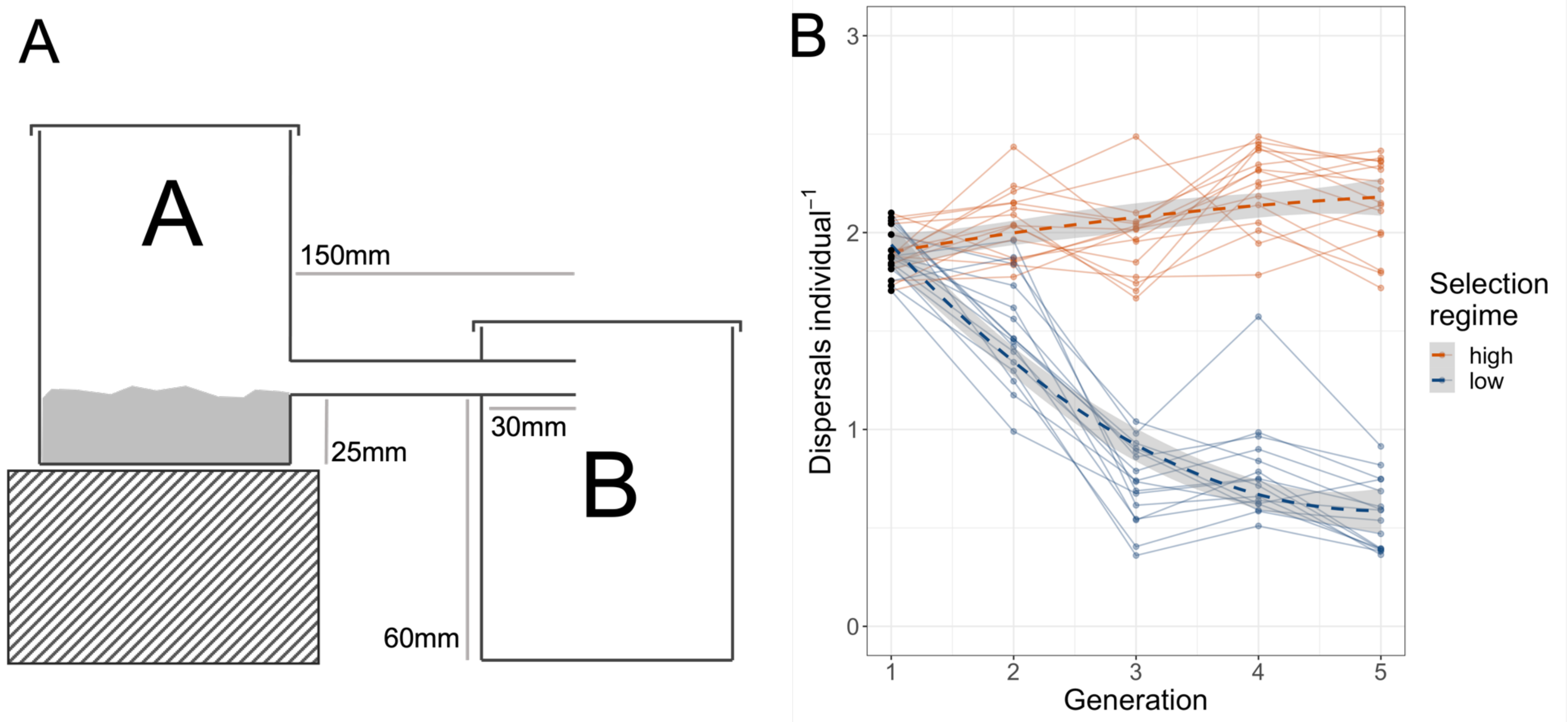

Figure 3.1. **Artificial selection on dispersal.** A Experimental arena setup used to assay the dispersal behaviour of experimental *Tribolium castaneum* populations and provide a basis on which to artificially select individuals displaying high and low dispersal propensity. B Mean dispersals per individual (of a maximum of three) for each *T. castaneum* selection line across four generations of selection on high and low dispersal (generation 1 represents behaviour in the stock population). Orange and blue represent high and low selection regimes respectively. Solid lines connect repeated measurements from the same selection line, while dashed lines show predictions generated by a GLM fitting the interaction of selection regime and generation modelled as a second order polynomial.

3.3.3 Experimental methods — breeding dispersal lines by artificial selection

Initial 3-opportunity dispersal assays were conducted and the selection criteria were three dispersals for high-dispersal lines and zero dispersals for low-dispersal lines. Assays were conducted on 16 replicate groups of stock beetles, with individuals meeting the selection criteria used to found 16 high- and 16 low-dispersal lines respectively. Where possible, 30 individuals were taken to produce the next generation in each replicate of each treatment, otherwise as many as met the selection criteria. This was only ever <30 in the initial generation; of the 32 created lines, the minimum population size was 21 (mean = 27.56, SD = 3.35). In each of generations 2–5, dispersal behaviour in high- and low-dispersal lines was retested with 3-opportunity assays. From individuals meeting the selection criteria of the relevant treatment, 30 were randomly selected to produce the next generation, all other individuals were discarded.

To maximise the effectiveness of selection, it was desirable that all offspring were parented by individuals meeting the selection criteria of the relevant regime, *i.e*. from the group of 30 parental individuals. However, during the assay, individuals selected to produce the next generation could potentially mate with others not from this group, for example, females selected to propagate the high dispersal regime could have been inseminated by non-highly-dispersing males. To mitigate this, we isolated the parental group of 30 individuals for 72 h of intragroup mating, allowing the strong last male precedence displayed by *Tribolium* (Lewis and Austad 1990) to minimise the number of offspring sired by non-group males. After this time, fodder potentially containing non-group eggs was discarded and beetles were transferred to fresh fodder for 7 days of oviposition. Following oviposition, eggs were left to develop in the fodder, adults were removed, frozen, and sexed by the presence of odiferous glands on male upper forelegs (Hinton 1942). Sexing allowed us to ensure that sex ratios were not overly skewed among reproductive groups, and to evaluate sex bias in dispersal behaviour itself. Using this procedure 16 high-dispersal and 16 low-dispersal lines were selected over 5 generations. Hereafter, referred to as 1–16H and 1–16 L respectively.

After generation 5, a 1-opportunity assay was used every other generation (to gen 15) to monitor dispersal propensity and select 30 individuals to produce the next generation. In non-assayed generations, 100 individuals were randomly selected to propagate each line. Selection lines were housed within 250 ml PVC containers with a circular base of 34 mm radius and 100 mm in height, round (20 mm radius) windows of fine metal mesh were inserted in the screw-on lids.

3.3.4 Experimental methods — phenotyping crosses within and between dispersal treatments

To investigate whether dispersal was under the control of a single, sex-linked locus we performed reciprocal crosses within and between selection lines and assayed the dispersal behaviour of offspring. High and low dispersal lines were paired according to their number (1H with 1 L, *etc*.) and four types of cross (within-line and reciprocal between-line crosses; figure 3.2) were performed on each pair. We began by obtaining virgin individuals, taken as pupae from the relevant populations on day 20 of the development phase and raised to adulthood in single-sex groups in 57 ml (61 mm diameter, 27 mm height) plastic containers with hinged lids and ventilation holes. Once mature, males were marked with a coloured dot on the dorsal thorax using a paint marker (0.7 mm; Uniposca, www.posca.com), a method shown not to alter behaviour (Sales et al. 2018). At 12+/−3 days post-eclosion, a single male and female were paired in a 5 ml vial on 0.5 ml of fodder for 48 h of mating opportunity, before males were discarded and females transferred to oviposit on fresh fodder for seven days in 57 ml containers. Each type of cross between a single pair of individuals was replicated five times for each pair of lines. After the females were removed following oviposition, egg-containing fodder from the five replicate crosses was combined so that offspring development took place in non-sibling groups. Each female oviposited on 10 ml of fodder, to maintain the per-female fodder volume from the selection lines, and maintain equal developmental population density. From these new populations, pupae were sexed and kept in single-sex groups until males could be marked post-eclosion. At 8+−3 days post-eclosion 100 males and 100 females were combined to form a cohort of 200 beetles with a 1:1 sex ratio, four days later this population entered a 1-opportunity dispersal assay.

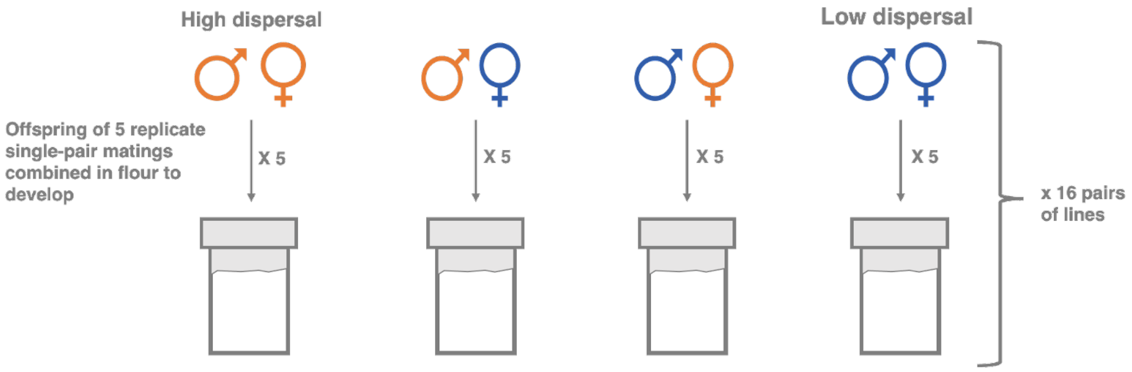

Figure 3.2. Experimental cross design. Representation of the method used to obtain offspring for dispersal assays of reciprocal crosses between pairs of high- and low-dispersal selection lines of *T.castaneum*. For each of 16 pairs of lines, each of 4 cross types was replicated with 5 single-pair matings, with egg-containing fodder combined to develop as a population.

3.3.5 Experimental methods — breeding and phenotyping inbred lines

We established highly inbred lines by repeated sib–sib mating to remove genetic variation within each line. Intense inbreeding will increase homozygosity, potentially fixing alleles at each locus. If dispersal is a single-locus trait, highly inbred lines should fix either the high or low dispersal allele, with probabilities proportional to the starting allele frequencies, and accordingly display either high or low dispersal behaviour, equivalent to that seen in artificially selected lines. By comparing the dispersal behaviour of these lines to predictions generated by simulation under different assumed genetic architectures we have another way to evaluate the genetic basis of this trait. To create the inbred lines, 110 KSS males and 110 KSS females were paired 1:1 (using the same method as for the previous experiment) and used to found 110 lines. Each generation for 10 generations, three male and three female pupae were sexed from each line at 20 days following the end of the oviposition period and left to eclose as virgins in single-sex single-line groups. Resulting adults were paired to create three replicate single-pair matings, coded A, B, and C—to guard against lines going extinct through failure to mate. Pupae used to parent the following generation were always taken from replicate A, unless it was extinct whereon B was used, etc. The fecundity of individual females is reduced by inbreeding depression (Fernández et al. 1995), so to obtain the 200 individuals required for a dispersal assay an additional generation was bred, from a group of 30 beetles per line. As this breeding immediately followed a single pair mating, these 30 individuals were siblings, meaning a total of 11 generations of inbreeding were conducted, following which 64 lines of the initial 110 were left extant. Dispersal behaviour of inbred lines was then measured using a 1-opportunity dispersal assay.

3.3.6 Simulation methods — agent-based simulation to model artificial selection on dispersal

A population genetic simulation of the dispersal selection experiment was created in R (ver.2021.09.1—R Core Team 2020). This agent-based simulation assumed combinations of several genetic (sex-linkage state, dominance value, trait heritability, starting dispersal allele frequency) and demographic parameter values, while incorporating some stochasticity in how they affected simulated populations. By running simulations across the available parameter space we were able to compare simulated data with experimental observations and make qualitative estimates of unknown parameters within our experimental populations.

We simulated starting populations of 200 individuals, equal to that of experimental assays, and assigned genotypes probabilistically using specified starting allele frequencies (in runs using a sex-linked architecture the probability of receiving a Y as the second allele was equal to the sex ratio). Individuals were each assigned binary dispersal outcomes for each dispersal opportunity of the simulated assay, according to their genotype. The following equations were used to calculate the contribution of a single locus to the probability of dispersing given specified values of dispersal trait heritability (h) and dominance (d), which were then used to draw outcomes from a Bernoulli distribution. For an aa genotype this was:

1) $$\frac{1-h}{2}$$

for an Aa genotype;

2) $$\frac{2hd-h+1}{2}$$

And for an AA genotype;

3) $$\frac{h+1}{2}$$

Figure 3.3 shows how the probability of dispersal varied for each genotype across the possible combinations of h and d. Following the format of the dispersal selection experiment, in the model 30 individuals were chosen at random from those that emigrated zero or three times to act as parents of the subsequent generation of low and high selection regime lines respectively. From this point, the model simulated and tracked low and high lines as discrete populations. Within each selection regime, each parental female was mated with between one and three males, with equal probability. The number of offspring produced by each female was chosen from a normal distribution with a mean of 100 and standard deviation of 25 (approximating typical values for *T. castaneum*, e.g. Vasudeva et al., 2021). At each simulated locus independently, each of these offspring was assigned its first allele randomly chosen from the female parent and its second allele from one of that female's mates with equal probability. These new individuals were then assigned dispersal outcomes as above, and the cycle of reproduction and dispersal assays repeated over a total of 5 generations.

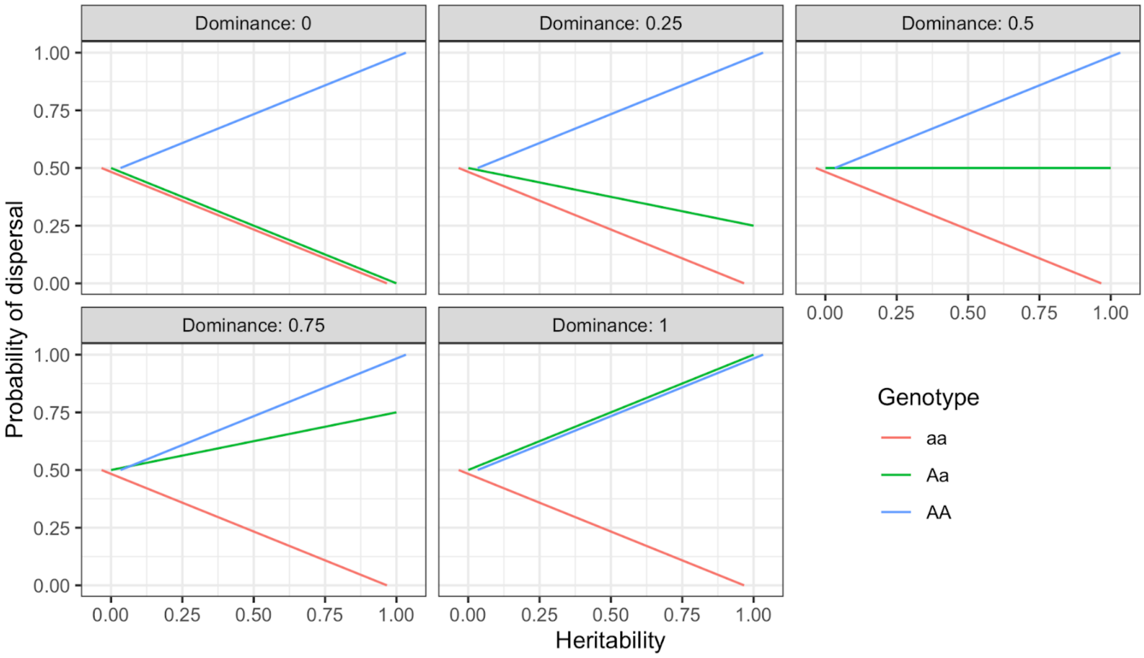

Figure 3.3. Simulated dispersal probabilities. Dispersal probabilities of simulated individuals having each possible single-locus biallelic genotype, at combinations of heritability, and dominance (panels). Where lines occupy the same plot space they have been shifted so that both are visible, i.e. blue and red lines show reflective symmetry across the line y=0.5.

To estimate the contribution of dominance, heritability, and starting allele frequency to dispersal outcomes, we used the simulation model to perform a parameter scan across a range of values, so that we might compare the response to selection of dispersal behaviour in simulated populations to that observed in experimental populations. Parameter scans comprised a model run for each of 100 different combinations of dominance, heritability, and starting dispersal allele frequency values (table 3.1). Scenarios containing allele frequencies of zero and one, or a heritability of zero, were excluded because evolution is not possible under these conditions.

Table 3.1. Simulated parameter combinations. The genetic architectures simulated, and the range and increments used for each of the parameters heritability (h), dominance (d) and starting allele frequency (A). One combination of these parameters is termed a 'scenario'. The model was run using all 100 unique scenarios for each of the 5 genetic architectures tested.

Genetic architecture		Simulated scenarios: a unique combination of h, d and A		
		Range [increment] of simulated parameters		
N loci	Sex-linked	Heritability (h)	Dominance (d)	Starting allele freq (A)
1	Yes	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]
1	No	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]
3	No	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]
5	No	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]
10	No	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]

To investigate the number of genes that might underlie dispersal, we developed versions of the simulation model that each assumed a different trait architecture, where dispersal was controlled by 1, 3, 5, and 10 additive, unlinked, biallelic loci, each inherited as described above, with no recombination. For architectures with multiple loci, an individual's dispersal probability was the mean of probabilities calculated independently for each locus. Parameter scans were carried out for each architecture, where each consisted of 50 independent repeats (each equivalent to one high and one low line from the experimental selection) and simulated populations were tracked across 4 generations of selection, to mirror the experimental procedure. For the single locus model, we studied an additional case where the trait was X-linked. *Tribolium castaneum* follows the XX/XY sex-determination system (Juan and Petitpierre 1991). Here females received two alleles, which combined to determine their phenotype, as described above, but males received only one dispersal-determining allele and a Y, which did not contribute to the dispersal phenotype.

3.3.7 Simulation methods — phenotyping crosses within and between dispersal treatments

The simulation model was extended to mirror the crosses performed with experimental beetle lines. This allowed us to generate predictions of the dispersal behaviour of offspring for comparison with results from experimental crosses. For each of the sex-linked and non-sex-linked versions of the single-locus architecture, we began by taking the parameter scan outputs from scenarios that best approximated the experimental data—for both architectures this was $h = 0.6$, $d = 0.5$, $A = 0.8$ (see results). These outputs contained simulated individuals that were the result of four generations of selection. We then simulated an additional mating, in the same way as before except, where during selection females were mated to males that matched their phenotype, here females were assigned to one of four cross treatments and mated to males with corresponding phenotypes. These cross types were: within high selection regime (Hf-Hm); high selection regime female x low selection regime male (Hf-Lm); low selection regime female × high selection regime male (Hm-Lf); within low selection regime (Lf-Lm; figure 3.2). Their simulated offspring were then put through a simulated 1-opportunity dispersal assay, where binary dispersal outcomes were assigned probabilistically according to individual genotype, dominance and heritability.

3.3.8 Simulation methods — simulating and phenotyping inbred lines

This simulation was adapted from the dispersal selection simulation, and simulated 10 generations of inbreeding through individual sibling-sibling matings and one additional generation parented by 30 individuals. Following inbreeding, a 1-opportunity dispersal assay was simulated on inbred lines, in the same way as described for the dispersal selection model. This was done separately for 250 replicates of each trait architecture (single-, 3-, 5- and 10-locus), using all sets of parameters values for each that gave highest agreement with experimental results during the parameter scan ($R^2 > 0.8$). In this way we generated predictions about the dispersal behaviour of individuals of highly inbred lines, under different assumptions regarding the genetic control of the trait.

3.3.9 Statistical methods

All data wrangling and analysis were performed in R ver.2021.09.1 (R Core Team 2020) and data-wrangling largely used the Tidyverse packages (Wickham et al. 2019). Generalised linear mixed models (GLMMs) were fitted using ‘Lme4’ (Bates et al. 2015) and Satterthwaite method p-values were obtained using ‘lmerTest’ (Kuznetsova et al. 2017; Luke 2017).

3.3.10 Statistical methods used for experimental data

To investigate the effectiveness of selection on dispersal behaviour, we employed a GLMM. The response variable was the mean number of dispersals per beetle (maximum of 3) for each selection line each generation. Selection regime and block were entered as fixed effects, alongside generation fitted as a second-order polynomial, and the interaction of generation and selection regime. Line ID was fitted as a random factor. Additionally, a GLMM was applied separately to high-selection data to test for changes in dispersal propensity over generations. This model was fitted twice, first with generation as a linear predictor, then as a second-order polynomial.

We tested for sex bias in dispersal behaviour in the initial generation using chi-squared tests on sex ratios of 3-time and 0-time emigrants summed across all replicates. We also analysed the sex of emigrants using generalised linear models (GLMs) with a binomial error distribution and a logit link function; proportion of males was the response variable and sample sizes were entered as weightings (Wilson and Hardy 2002). The model was fitted separately to data on numbers of 0- and 3-time dispersers, with the effect of generation, fitted as a linear fixed effect, on sex bias in the high selection regime and the low selection regime.

To compare the dispersal behaviour of male and female offspring from different types of crosses between dispersal lines, we employed a GLMM with number of dispersals as the response variable. Because the density of organisms is known to affect dispersal rates (e.g. Ogden 1970b), assay-level means of disperser numbers were preferred to individual-level outcomes to avoid non-independence within replicate populations. A first model testing the individual effects of cross type and sex contained these as fixed predictors, along with block, while line ID was entered as a random predictor. A second model contained the interaction of cross type and sex, along with block as fixed predictors and line ID as a random factor. Hartigan's dip test (Hartigan and Hartigan 1985) was used to test for multimodality in the distribution of dispersal propensity of inbred lines, with p-values obtained by 10,000 Monte Carlo simulations using R package 'diptest' (Maechler 2021).

To ensure the continuity of inferences between 1- and 3-opportunity dispersal assays, we used dispersal data from generation 5 and fitted the same model to both 1- and 3-opportunity data and compared the results. First a model was fitted to the full 3-opportunity dataset, then the same model was separately fitted to the same dataset filtered to just the first of three dispersal opportunities, to represent a 1-opportunity assay. Initially we applied the same GLMM to the 1- opportunity data as has been described in the main analysis, however with the lower amount of data these models had singular fit (the variance explained by the random effect was zero). We therefore simplified the effects structure by dropping the random effect of selection line and fitted a GLM to both datasets - incorporating the response variable, with selection regime and block ID as fixed effects. Data from both 1- and 3-opportunity dispersal assays were capable of showing that individuals from the high emigration selection regime were significantly more likely to emigrate than low regime beetles. The greater resolution of data over three dispersal opportunities led to a larger effect of selection regime using these data (GLM, $\beta=-1.58$, SE=0.06, $p<0.001$).

3.3.11 Statistical methods used for simulated data

To obtain information on the likely genetic architecture, starting allele frequency, dominance and heritability in experimental populations, we compared results from empirical selection to results of simulations. We did this by starting with the parameter estimates generated by the statistical model applied to the experimental data (above). We then fit those parameter estimates to the output of each simulated scenario to generate residuals. From these we manually computed R^2 values, to assess how well each simulated scenario approximated the experimental data. This analysis was performed for each genetic architecture and for autosomal and sex-linked versions of the single-locus simulation. To assess whether the sex-linked architecture led to sex-biased dispersal, we computed the difference in dispersal tendency of males and females for each scenario under both single-locus simulation and compared these visually using a heatmap of parameter space. Sex difference in dispersal was computed as the mean dispersal tendency in females across replicates subtracted from the same metric computed for males.

On each output from simulated crosses between selection lines, for sex-linked and non-sex-linked single-locus architectures we employed GLMMs as for the experimental cross data (see above). However, fitting these directly resulted in singularity as the random effect of line accounted for none of the variance in the data, we therefore removed this and fitted the same fixed effects as a GLM. We also fitted the statistical model generated using the experimental dataset to the simulated output from each architecture, using predicted values to obtain residuals and calculated an R^2 to compare the fits.

Hartigan's dip tests (as above) were used to test for multimodality of distributions of (i) mean allele frequencies within lines within each genetic architecture, and (ii) of numbers of dispersers in each line. Initially, this was done using 250 replicate simulated lines, but for architectures showing significant multimodality these 250 replicates were randomly subsampled down to 64 to match the number of experimental inbred lines and retested.

3.4 Results

3.4.1 Experimental results — dispersal in artificially selected lines

Dispersal behaviour differed between selection regimes after only one generation of selection, as evident from the non-overlapping 95% CIs of model predictions (figure 3.1B). This was a result of dispersal falling in the low selection regime lines until generation 3, then remaining low, while dispersal in the high selection lines showed a small but significant increase when generation was fitted as a linear predictor (GLMM, $\beta = 0.07$, SE = 0.01, $p < 0.001$). A significant interaction between selection regime and generation was found when the effect of generation was modelled as a quadratic (table 3.2). Individual variation in dispersal propensity was present in all generations of both treatments, but a shift towards higher and lower propensities was observed in high and low lines respectively. For a comparison of results from 1- and 3-opportunity assays see supplementary material.

Within the low dispersal regime, we observed a male bias among dispersers ($\chi^2 = 26.79$, df=1, $p < 0.001$, $n = 344$; figure 3.4), which decreased across generations ($\beta = 0.10$, SE = 0.03, $p < 0.001$). The proportion of males among dispersers in high dispersal lines was lower than expected ($\chi^2 = 5.14$, df = 1, $p = 0.02$, $n = 467$; figure 3.4).

Table 3.2. Response to selection LMM. A linear mixed model of the response to selection in experimental *T. castaneum* populations artificially selected for high and low dispersal behaviour. With mean dispersals per individual as the dependent variable, block was fitted as a fixed effect to account for potential variation in experimental conditions, generation was fitted as a second order polynomial and 'high' is the reference category for selection regime. As a random effect we modelled a random intercept of line ID (Var<0.01, SD = 0.05).

	Estimate	SE	p
(Intercept)	2.16	0.04	<0.001
Generation	2.21	0.30	<0.001
Generation^2	-0.75	0.30	0.013
Selection regime	-1.10	0.04	<0.001
Block	-0.06	0.04	0.146
Generation x Selection regime	-5.64	0.42	<0.001
Generation^2 x Selection regime	6.26	0.42	<0.001

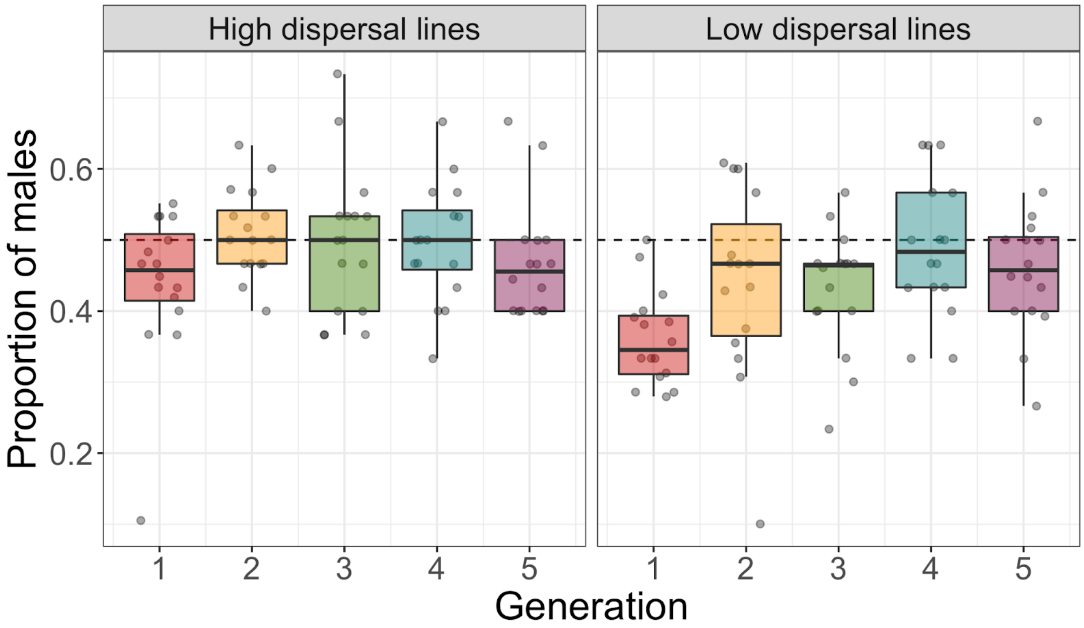

Figure 3.4. Sex ratios of dispersers. Sex ratio within groups of 30. *Tribolium castaneum* beetles selected to parent the next generation of high and low dispersal lines during an artificial selection experiment. The dashed line represents an equal sex ratio.

3.4.2 Experimental results – phenotyping crosses within and between dispersal treatments

When testing the individual fixed effects of whether cross type and sex predicted dispersal, we found a significant effect of cross type (figure 3.5; Table 3.3). Within-high regime crosses showed significantly higher dispersal than all other crosses, while dispersal from within-low regime crosses was significantly lower than all other cross types. Both reciprocal between regime crosses showed dispersal propensities intermediate to the within-regime crosses but did not different significantly from each other. No effect of sex on dispersal was found. No significant interaction was seen between cross type and sex (Table 3.3).

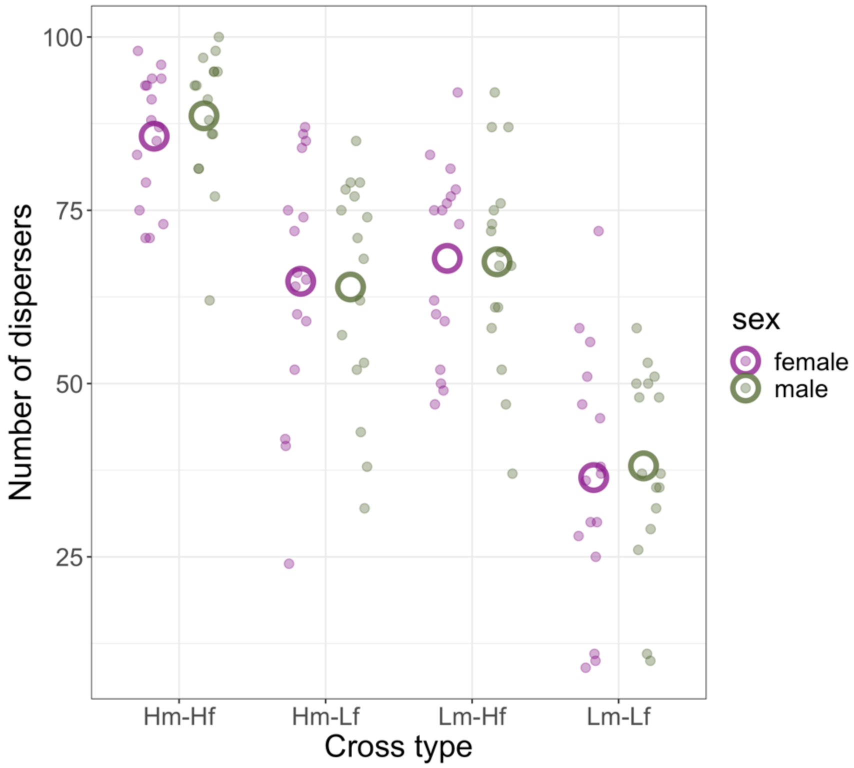

Figure 3.5. Dispersal of crosses. Number of dispersers from assayed mixed-sex populations of 200 *T. castaneum* offspring from crosses within and between dispersal selection regimes. On the X-axis, ‘H’ and ‘L’ represent the selection regime and ‘m’ and ‘f’ the sex of parents, e.g. Hm-Lf indicates that the male parent came from a high selection line and the female parent from a low selection line. Data for males and females are shown in purple and green respectively. Small filled points show experimental measures from individual crosses, while large hollow points show predictions of a GLMM fitting the effect of the interaction of sex and cross type on number of dispersers, while controlling for line ID.

Table 3.3. Dispersal of empirical crosses GLM. Mixed models testing the dispersal behaviour of offspring of reciprocal crosses within and between *T. castaneum* lines experimentally selected for high and low dispersal propensity. Above the dashed line results are from a model fitting the individual effects of predictors, below the line results are from an equivalent model fitting the interaction between sex and cross type, with 'Hm-Lf' as the reference category. In both, we modelled the random intercept of line ID (model 1: Var = 116.26, SD = 10.78; model 2: Var = 116.26, SD = 10.78).

	Estimate	***SE***	**p**
(Intercept)	63.93	3.33	<0.001
Cross type [Hm-Lf]	(reference)		
Cross type [Hm-Hf]	22.81	2.47	<0.001
Cross type [Lm-Hf]	3.47	2.47	0.162
Cross type [Lm-Lf]	-21.16	2.47	<0.001
Sex	0.83	1.74	0.636
Cross type [Hm-Lf] x sex [female]	(reference)		
Cross type [Hm-Hf] x sex	3.75	4.98	0.453
Cross type [Lm-Hf] x sex	0.31	4.98	0.95
Cross type [Lm-Lf] x sex	2.5	4.98	0.62

3.4.3 Experimental results — dispersal phenotypes of inbred lines

Inbred lines displayed a range of dispersal propensities, from 0–188/200 beetles dispersing (figure 3.6 row A), with a distribution not differing from unimodal (D = 0.04, *p* = 0.72).

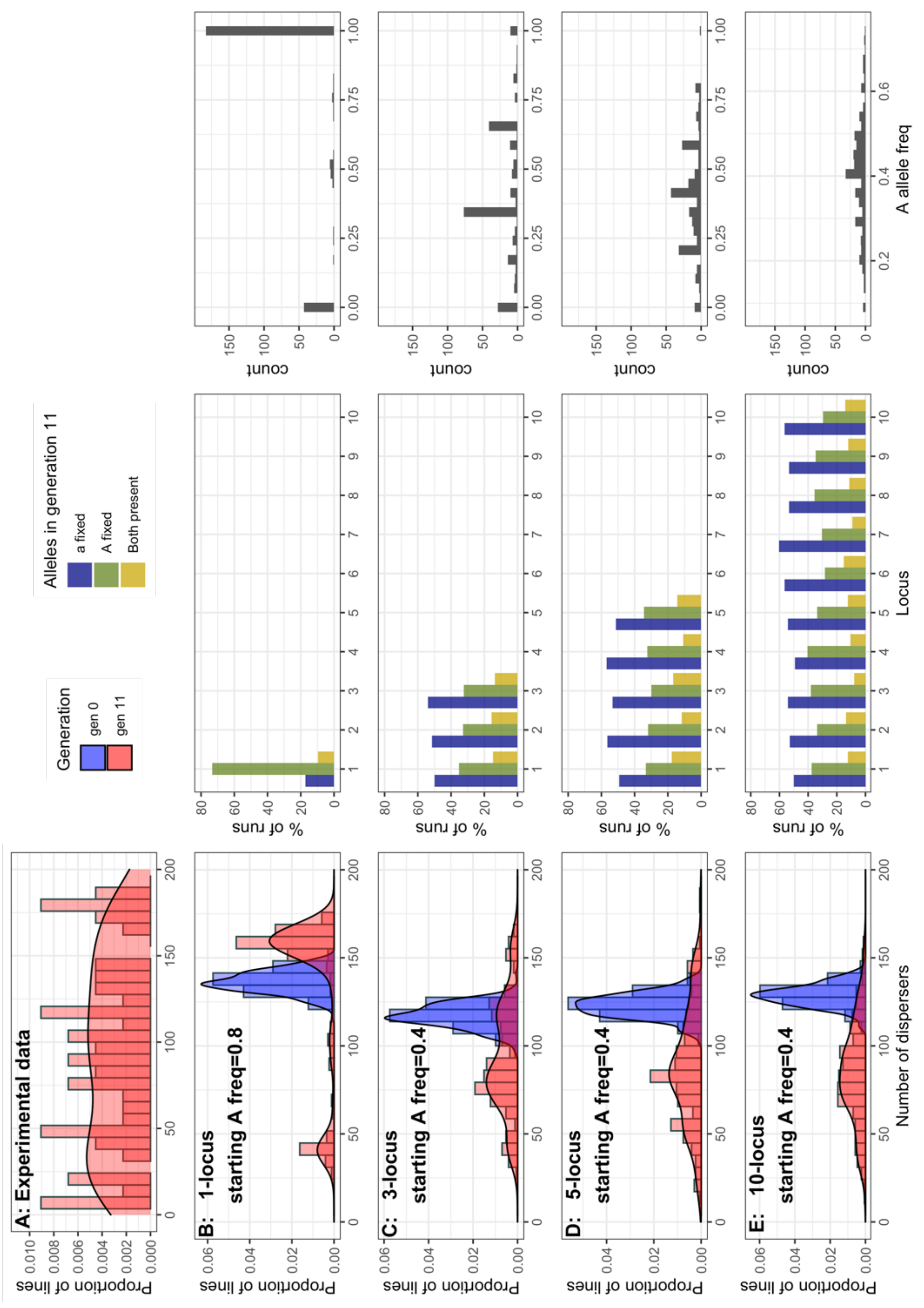

Figure 3.6. Dispersal in experimental and simulated populations. Combined data from experimental (column 1, row A) and simulated (all other panels) evolution experiments selecting on dispersal behaviour. Column 1: (A) Experimental results showing the number of dispersers from each of 64 lines of T. castaneum following 11 generations of extreme inbreeding and (B–E) data from agent-based simulations designed to model the same

inbreeding design and dispersal assay as used experimentally. Numbers of dispersers are shown prior to (blue) and following (red) 11 generations of inbreeding. Sample sizes for simulations were 250 independent populations for each genetic architecture, each modelling the trait as controlled by either 1, 3, 5 or 10 additive biallelic loci (rows B-E respectively). For each simulated architecture, starting dispersal allele frequencies were 0.8, 0.4, 0.4 and 0.4 in these architectures respectively, this and other parameters were selected as those maximising agreement with experimental results during a parameter scan (figure 3.7). For simulated data, additional columns display: 2) Allele outcomes at each locus at generation 11, i.e. whether either allele had become fixed, or both alleles remained in the population. 3) The distribution of allele frequencies averaged across loci for each individual simulated line.

3.4.4 Simulated results — comparison of simulated to experimental selection on dispersal

Considering only non-sex-linked architectures, which varied in the number of underlying loci, 20 of 400 scenarios had R^2 values of > = 0.80, representing a good fit of the experimental model to the simulated data. Of these, 15 scenarios came from single-locus simulations, 11 from 3-locus, seven from 5-locus and one from a 10-locus simulation. Architectures varied in the parameter space over which they could simulate a good fit to the experimental data (figure 3.7), with less functional parameter space observed with increasing number of loci. Individual scenarios with good fit were observed across a wide range of parameter values.

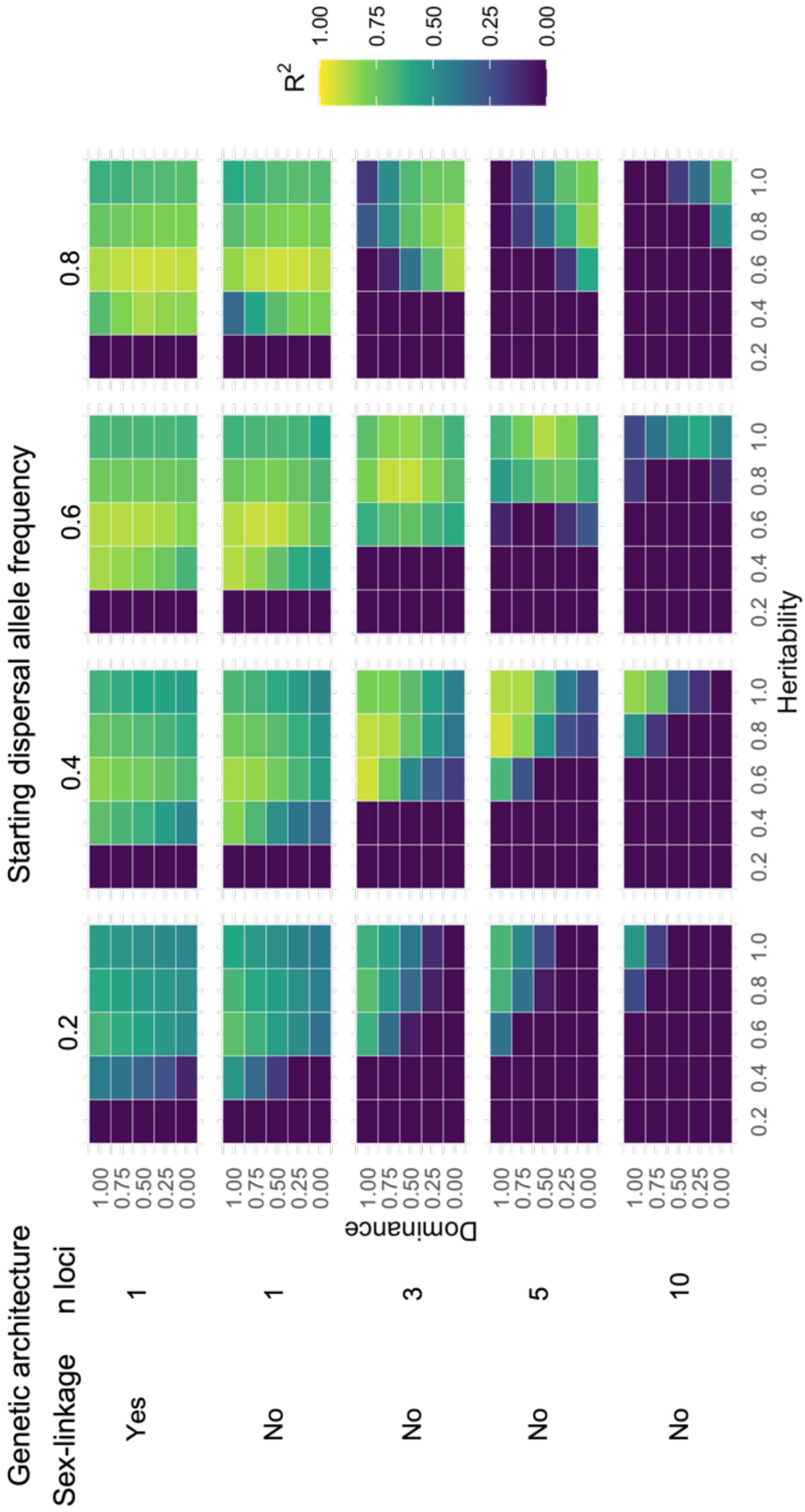
Genetic architecture
Sex-linkage
n loci
Yes
No
No
No
No
1
1
3
5
10
Dominance
1.00
0.75
0.50
0.25
0.00
Starting dispersal allele frequency
0.2
0.4
0.6
0.8
Heritability
0.2 0.4 0.6 0.8 1.0
R^2
1.00
0.75
0.50
0.25
0.00

Figure 3.7. Comparison of dispersal in simulated and empirical populations. Parameter scans using an agent-based simulation designed to model an emigration selection experiment using *T. castaneum* under different trait architectures. Colours represent R^2 values assessing the fit of a model defined on experimental data, and applied to simulated data across scenarios comprising combinations of dominance (d), trait heritability (h) and starting dispersal allele frequency (A), across a sex-linked single-locus, and unsex-linked single-, 3-, 5- and 10 locus architectures.

Heritability values (h) required for good model fits were $h \geq 0.6$ under single- and 3-locus architectures (figure 3.7). Good model fits generally required higher heritability values when dominance (d) was low, especially at higher numbers of controlling loci. Within each architecture, fits were poorest at low starting dispersal allele frequency (A = 0.2), though good fits were seen across the rest of the range of this parameter ($A = 0.4$, $A = 0.8$). Dominance (d) showed a strong negative interaction with starting dispersal allele frequency (A), with higher values of dominance required when dispersal allele frequency was low.

Considering the sex-linked single-locus model, the functional parameter space and parameter values of maximum fits were almost identical to the non-sex-linked version. Sex biases in dispersal were observed to be greater in sex-linked than non-sex-linked simulations under some parameter scenarios, though these differences were not of large magnitude. In high dispersal lines simulated with a sex-linked architecture, high dominance values ($d = 1$) resulted in greater dispersal in females than in males, with this pattern being strongest at low starting dispersal allele frequency and becoming minimal at the highest tested starting dispersal allele frequency ($A = 0.8$). In low selection lines, the inverse pattern was observed, with the lowest dominance values having male-biased dispersal, most pronounced at the maximum starting dispersal allele frequency and minimal at $A = 0.2$. In contrast, the non-sex-linked scenarios showed small stochastic variations across parameter space in both high and low selection lines.

Simulated crosses between selection lines generated divergent outcomes between sex-linked and non-sex-linked single-locus architectures (Table 3.4). Under both architectures, the offspring of within-high and within-low regime crosses showed high and low dispersal behaviour respectively, in both sexes. In contrast, behaviour of offspring of between regime crosses differed between architectures: as expected, both sexes showed intermediate behaviour under a non-sex-linked architecture, as did the female offspring under sex-linkage; but male offspring from this architecture inherited the behavioural phenotype of their mother's regime. This pattern was reflected by the model, where a significant interaction was seen between cross type and sex under the sex-linked, but not non-sex-linked architecture (Table 3.4). Applying the mixed model fitting the interaction of cross type and sex, generated using experimental data, to the output from simulating each architecture resulted in better fit to the non-sex-linked data ($R^2 = 0.63$) than to the sex-linked data ($R^2 = 0.50$).

Table 3.4. Dispersal of simulated crosses GLM. General linear models of the dispersal behaviour of offspring of reciprocal crosses within and between simulated lines selected for high and low dispersal propensity, under sex-linked and unsex-linked genetic architectures. Above the dashed line results are from a model fitting the individual effects of predictors, below the line results are from an equivalent model fitting the interaction between sex and cross type, with 'HmLf' as the reference category (model 1, sex-linked: Var = 116.26, SD = 10.78; model 2: Var = 116.26, SD = 10.78).

	Sex-linked			**Unsex-linked**		
	Est	**SE**	**p**	**Est**	**SE**	**p**
(Intercept)	34.46	1.37	<0.001	51.04	0.69	<0.001
Cross [HmLf]	(reference)					
Cross [HmHf]	45.58	1.73	<0.001	28.33	0.87	<0.001
Cross [LmHf]	30.26	1.73	<0.001	-0.66	0.87	0.447
Cross [LmLf]	-14.94	1.73	<0.001	-30.19	0.87	<0.001
Sex	0.48	1.26	0.695	-0.15	0.61	0.807
Cross [HmLf] x [female]	(reference)					
Cross [HmHf] x male	29.32	1.71	<0.001	-2.62	1.73	0.13
Cross [LmHf] x male	59.96	1.71	<0.001	1.8	1.73	0.298
Cross [LmLf] x male	30.08	1.71	<0.001	0.54	1.73	0.755

When simulating inbreeding with different numbers of loci, the patterns of alleles remaining in generation 11 were always similar across loci within each architecture. Patterns were also similar across architectures with the same starting allele frequency, with the frequency of an allele leading to its being fixed more often (figure 3.6, rows B-E, column 2).

3.4.5 Simulated results — dispersal of simulated inbred lines

Within the simulated inbred lines the distribution of dispersal at generation 0 was unimodal with mean values of 100–150 dispersers under all architectures (figure 3.6; column 1). By generation 11 the distribution displays two obvious modes when modelled with a single-locus architecture. These observations were confirmed with dip tests for multimodality, indicating that the single-locus simulation showed a significantly non-unimodal distribution of numbers of dispersers under the parameter set that gave the greatest agreement with results of experimental selection ($D = 0.06$, $p < 0.001$). This remained true under all other single-locus scenarios which resulted in high agreement (R^2 of >0.8; $D < 0.14$, $p < 0.001$). As expected, the same pattern was observed in allele frequencies; both the best performing ($D = 0.09$, $p < 0.001$) and all other high performing parameter sets ($R^2 > 0.8$) showed significantly non-unimodal distributions ($D < 0.18$, $p < 0.001$).

Under the 3-, 5- and 10-locus architectures the distribution of dispersal outcomes in generation 11 was shifted to the left but retained an apparently unimodal distribution. However, many scenarios under the 3- and 5-locus architectures showed statistical multimodality, even after adjustment for multiple comparisons (3-locus: $0.03 < D < 0.09$, $0.4 < p < 0.001$; 5-locus: $0.02 < D < 0.04$, $0.02 < p < 0.99$). All scenarios under these architectures also showed multimodality of allele frequency distributions ($D < 0.13$, $p < 0.001$). When the trait was controlled by 10 loci, neither the number of dispersers ($D = 0.02$, $p = 0.86$), nor the underlying allele frequency showed significantly multimodal distributions ($D = 0.03$, $p = 0.12$). Across all architectures, peaks in disperser numbers appear to correspond to peaks in dispersal allele frequency (figure 3.6, columns C and D).

When single-locus simulation results were subsampled down to experimental sample size ($n = 64$) the bimodal pattern remained strong, though the pattern was no longer significantly different from unimodality (Hartigan's dip test; $D = 0.06$, $p = 0.07$), but did show multimodal allele frequency distribution ($D = 0.07$, $p < 0.05$).

3.5 Discussion

Tribolium beetles are significant agricultural pests and important model organisms, whose life history is characterised by bouts of dispersal and colonisation (Dawson 1977), yet the genetic basis of dispersal behaviour in this species is unresolved. Here, we demonstrate rapid evolution of dispersal behaviour under strong selection, mostly as a response to selection for low dispersal but also as a significant increase in dispersal in high selection lines. We find no evidence of sex biases in dispersal in offspring of crosses, suggesting that the trait is not sex-linked. Simulations show that feasible parameter space over which simulated data can approximate experimental data exists under scenarios where the dispersal trait is controlled by one or few loci, but not many loci. However, levels of dispersal in experimentally inbred lines as compared with simulations, indicate that a single locus model is not well supported. Considered together, we suggest that these findings support an oligogenic architecture underlying dispersal in *Tribolium castaneum*.

Past work on *Tribolium* has provided evidence of a genetic basis to dispersal behaviour, which our results corroborate (Schurr and Bolduan 1967; Ogden 1970a, 1970b; Ritte and Lavie 1977; Korona 1991; Ruckman and Blackmon 2020). We saw a rapid response to selection on dispersal, creating extreme divergence between the behaviour of high and low dispersal selection regimes over just two generations in our *T. castaneum* lines. Such a result clearly demonstrates that variation for the trait was present in our stock *T. castaneum* population, despite more than ten years in the laboratory. This raises the question of how variation for dispersal behaviour is maintained in laboratory populations with no ability to disperse, especially since dispersal appears to trade-off with fecundity (Zirkle et al. 1988). It may be that positive correlates of dispersal observed in this species (e.g. shorter development time; Zirkle et al. 1988) are sufficient to keep dispersal phenotypes common. Interestingly, a greater reduction was observed in dispersal in low selection lines than the increase seen in high dispersal lines, indicating that dispersal tendency was already high in the original population. This is in contrast to previous studies which have shown lower initial rates and larger increases in dispersal in high selection lines (Ritte and Lavie 1977; Ruckman and Blackmon 2020). One explanation for this may be differences in methodology; our approach, with assays conducted on mixed-sex groups having had prior opportunity to mate, is more ecologically realistic than methods measuring dispersal in sibling groups (Ritte and Lavie 1977) and single-sex groups of virgins (Ruckman and Blackmon 2020), where movement may be driven by mate-searching. This observation may be explained by differences in the lines used; our KSS populations, having been bred to combine global *T. castaneum* diversity, are likely diverse relative to other strains and perhaps better capture the high level of dispersal present in wild populations existing in patches of ephemeral habitat, even though the strains used in both other studies were recently collected from the wild. Results are also consistent with differences in husbandry practices between laboratories (personal comm. Blackmon), our laboratory may have inadvertently selected for increased dispersal in stock populations prior to the study, perhaps by retrieving breeding individuals from the surface of the fodder to parent each subsequent generation. If this is the case, it suggests a relationship between dispersal and use of different strata of the fodder, which may warrant further investigation.

Our results, comparing experimental selection to selection simulated over a range of genetic parameters and architectures, broadly support an oligogenic architecture of dispersal. Models involving one, three and five, and to a lesser degree 10, additive loci were capable of agreeing with our empirical results under at least one combination of heritability, dominance and starting dispersal allele frequency. Therefore our findings suggest that if additive variance predominates in the genetic determination of dispersal, few loci are major contributors to the trait. However, we find no evidence that there needs to be only a single locus to account for the observed rapid change in behaviour under selection. Indeed, results from simulations and experiments using inbred lines provide tentative evidence that the trait involves more loci. Theoretically, under architectures with fewer interacting loci, fixation of alleles resulting from inbreeding has a larger impact on the focal trait. We observed this in simulations, where under a single locus the fixation of dispersal alleles was detectable as multimodality in the dispersal distribution. Importantly, the experimental results did not show multimodality in their dispersal distribution, suggesting that the trait may involve more than one locus. This finding is counter to the conclusions of Ritte and Lavie (1977), who postulated that *Tribolium* dispersal was controlled by a single locus. Recent theoretical work suggests that rapid adaptation can also occur with complex genetic architectures, including highly polygenic ones (Jain and Stephan 2017a, 2017b), and that the evolution of dispersal may be more rapid under the control of larger number of loci (Weiss-Lehman and Shaw 2022); counter to the prevailing wisdom that selective sweeps on large-effect loci are the predominant mode of rapid responses to selection (Messer and Petrov 2013). Such rapid evolution of dispersal can be important, for example, during range expansion; there is theoretical evidence that evolving increased dispersal at the range front can prevent the accumulation of expansion load (Peischl and Gilbert 2020). As a species that relies on ephemeral habitat (Dawson 1977), and has undergone extensive natural and human-mediated range expansion, this process may well have been important in the evolutionary history of *T. castaneum*. Oligogenic architectures of dispersal traits have been found in other insect taxa, notably in the Glanville Fritillary butterfly (*Melitaea cinxia*), where an epistatic interaction between two genes influences metapopulation dynamics through variation in dispersal (Hanski 2011; Niitepõld and Saastamoinen 2017).

We found that offspring of crosses between high and low dispersal lines demonstrated intermediate dispersal behaviour. This is consistent with some prior work (Ritte and Lavie 1977), but contrasts with one study which found that the dispersal of between treatment crosses was higher than in their high dispersal lines (Ruckman and Blackmon 2020). This elevated dispersal behaviour of cross offspring contributed heavily to their conclusion that epistatic variance outweighs additive variance in dispersal. This difference could, again, be due to their use of virgin single-sex groups, as opposed to mixed-sex groups, in dispersal assays, though further experiments would be needed to determine this. We were able to approximate through simulation both the response to selection and behaviour of the offspring of crosses observed in experiments, using only simple additive interactions between loci, without invoking epistasis. This is not to say that epistasis could not also account for the observed data, which we did not test, but that our observations are consistent with a simple additive architecture.

When we crossed experimental lines selected for high and low dispersal, offspring displayed the pattern expected under a non-sex-linked genetic architecture. Further, we included a single-locus sex-linked architecture among those which we simulated selection on dispersal. Experimental results agreed more closely with simulated crosses performed under an assumption of non-sex-linkage than with sex-linkage. These findings suggest that the dispersal trait on which we have selected is not controlled by a single sex-linked locus, as posited by Ritte and Lavie (1977).

Sex-biased dispersal is common in nature (Trochet et al. 2016), and sex-linkage of dispersal genes is likely important to its evolution (Brom et al. 2018). In our simulations, we saw sex-biased dispersal in only a small subset of scenarios of single loci sex-linked architecture. Complete dominance of either allele resulted in sex-biased dispersal, with females dispersing more when the dispersal allele was dominant and males dispersing more when the non-dispersal allele was dominant, as expected in an XX/XY system. When dominant alleles are rare, the strength of this effect is increased, explaining the interaction we observed between sex-biased dispersal and starting allele frequency in these simulations. Experimentally, while we saw no sex bias in the dispersal of high selection lines, we saw evidence of a weak female bias in low selection lines—a pattern not seen in our simulations, suggesting a cause not captured by our model. Similar results have been obtained by other studies of mixed-sex populations (Ogden 1970b; Ziegler 1977), suggesting that the male-biased dispersal seen in groups of siblings (Ritte and Lavie 1977) or single-sex virgins (Prus 1963; Ruckman and Blackmon 2020), is the result of either the mating-status, population sex ratio or relatedness. All of these are known to be important mediators of individual dispersal in other systems (Clobert et al. 2012), and in the case of relatedness, in *Tribolium* larvae (Jasieński et al. 1988). In particular mating status has recently been shown to alter dispersal decisions in the northern tamarisk beetle, *Diorhabda carinulata* (Clark et al. 2022). The contrast between our results and those using virgin or mated single-sex groups of *Tribolium* (Ritte and Lavie 1977; Ruckman and Blackmon 2020) highlights the importance of mating status and/or social environment in this system, and the ability of individuals to perceive and alter behaviour in response. Social environment is known to modify developmental life-history traits in *Tribolium* (Ellen et al. 2016), however, tests of mating status on flight have shown mixed results (Perez-Mendoza et al. 2011).

Our results support the view that dispersal in *Tribolium* is under the control of a small number of loci. A logical next step would be to investigate where these genes are and how they act to determine the phenotype. That *Tribolium* dispersal appears to be oligogenic (rather than polygenic) means that a genome-wide association approach may be able to detect loci across lines selected for differential dispersal. Known genes with large effects on dispersal in insects commonly have broad physiological, metabolic or neurological functions and are important beyond their effects on movement (Goossens et al. 2020); and it will be interesting to discover how genes are acting to control dispersal in *Tribolium*. It would also be instructive to explore whether dispersers and non-dispersers differ in measures such as leg length, activity and movement pattern. For example, a difference in dispersal but not leg length or movement pattern might suggest a physiological rather than a morphological or neurological basis. Such findings could then be thought of in relation to any candidate genes identified using genomics. *Tribolium* is a leading model system for RNA interference (Klingler and Bucher 2022), and candidate dispersal genes would be attractive targets for knockouts which, if effective, could present a valuable method of controlling this prolific pest.

Dispersal is an important aspect of the life history of many species, and its study in taxa such as pests and invaders could bring significant agroeconomic and biosecurity benefits. However, as a complex behaviour is challenging to study. We provide evidence that dispersal in the pest insect *T. castaneum* is under oligogenic control, opening up the possibility of identifying the loci involved using molecular genomics.

3.6 Data availability

All data files, simulation scripts and analysis scripts associated with this article are available in Dryad (https://doi.org/10.5061/dryad.c866t1gcb).

3.7 Contributions

The study was conceived by Lewis G Spurgin and Michael D Pointer with input from Matthew J G Gage, and the experimental procedure designed by LGS and MDP. MDP carried out all experiments, data analysis and wrote the manuscript with input from David S Richardson. The manuscript was then reviewed by all authors but MJG, before revision by MDP and DSR.

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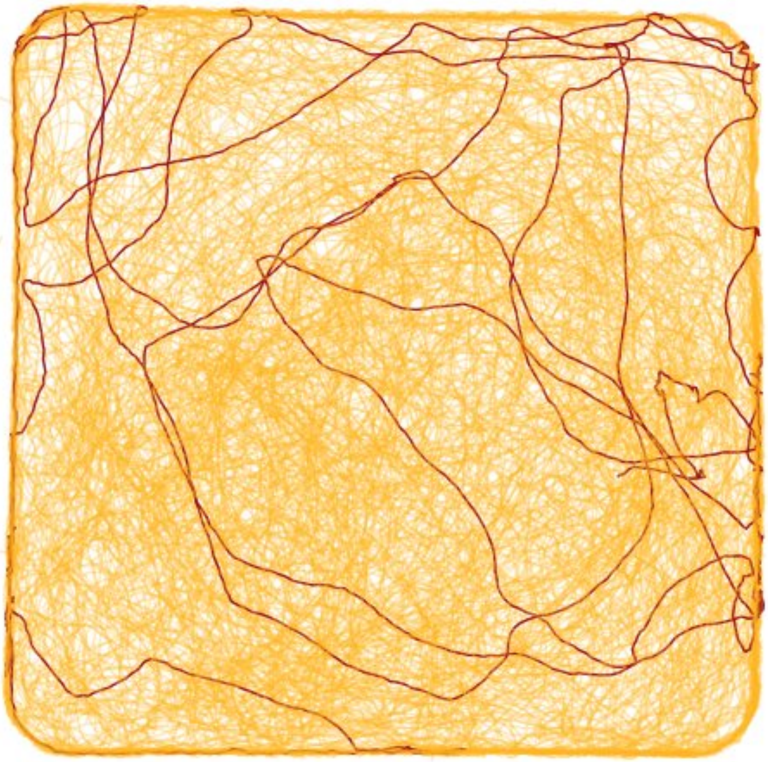
CHAPTER FOUR

Traits Underlying Experimentally Evolved Dispersal Behavior in Tribolium castaneum

A version of this chapter (appendix 3) is published as:

Pointer MD, Spurgin LG, Vasudeva R *et al.* (2024) Traits Underlying Experimentally Evolved Dispersal Behavior in *Tribolium castaneum*. *J Insect Behav* 37, 220–232. https://doi.org/10.1007/s10905-024-09862-x



The track of a single beetle overlaid on the tracks of all beetles from its population

4.1 Abstract

Dispersal is an important behaviour in many animals, with profound effects on individual fitness and the evolutionary trajectories of populations. This is especially true within taxa with particular life-history strategies, for example those that exploit ephemeral habitat. Further, dispersal is commonly seen to be part of behavioural syndromes - suites of traits that covary across behavioural contexts. The red flour beetle, *Tribolium castaneum* (Coleoptera, Tenebrionidae), is a major post-harvest crop pest responsible for large losses through the infestation of stored grain. In this system dispersal is known to have a strong genetic basis and differential artificial selection on dispersal traits produces strong phenotypic divergence. However, it is unknown which traits are able to rapidly evolve to produce these results, or which behavioural components underlie differences in dispersal. Using replicate lines of *T. castaneum* previously selected for divergent dispersal behaviour, we test for correlated activity and movement patterns, morphology and substrate surface use. We find robustly repeatable associations between the dispersal phenotype and higher activity, straighter paths, larger body size (but not relative leg length) and increased tendency to remain at the surface of fodder. Together our results suggest that dispersal is part of a syndrome of traits in *T. castaneum*, and must be treated as such when considering the evolution of dispersal in this system, and in attempting to predict and control its spread.

4.2 Introduction

Dispersal is a life-history trait with great importance in the ecology and evolution of many species, and across different levels of organisation (Clobert et al. 2012). For the individual, the fitness consequences of relocation to a new environment can be enormous (Clobert et al. 2012), while individual dispersal outcomes aggregate to determine species' ranges and metapopulation structures through effects on gene flow (Kokko and Lopez-Sepulcre 2006; Ronce 2007). Consequently dispersal is a key parameter underlying evolutionary trajectories and metapopulation persistence in fragmented and unstable habitats (Suarez et al. 2022; Eriksson et al. 2014).

Dispersal is also central to several problems in contemporary biology. Greater insight into dispersal processes will hopefully enable us to better understand and predict species' ability to cope with anthropogenic changes to climates and landscapes (Travis et al. 2013). This will also make us better prepared to combat the introduction and spread of non-native species, a major driver of biodiversity loss (Renault et al. 2018). Research into the factors underlying intraspecific variation in dispersal, and the traits associated with dispersal strategies will also allow the integration of evolutionary theory into pest management (Mazzi and Dorn 2012); improved knowledge of the movement of pests will allow us to better forecast outbreaks, design pest management strategies and improve global food security (Jeger 1999).

A genetic basis of dispersal-related traits has been shown in a range of species (Saastamoinen et al. 2018), though the mechanistic basis of effects on dispersal are more difficult to study and often remain unknown. The genetic architecture of dispersal is usually thought to be polygenic (e.g. Jordan et al. 2012); however, genes with large effects on dispersal have been identified across taxa; and can be broadly separated into those with either metabolic (Niitepõld and Saastamoinen 2017) or neurophysiological (Trefilov 2000; Fidler et al. 2007; Krackow and Konig 2008; Sokolowski 1980; Anreiter and Sokolowski 2019) effects on movement. Neurophysiological variation can underlie suites of traits within an individual, leading to consistent and correlated responses that differ among individuals, called personality, typically studied in terms of traits such as activity and boldness (Roche et al. 2016). Non-behavioural traits can also be part of syndromes, for example genetic differences in morphology can be correlated with, or directly affect, dispersal. Classically, a wing-polyphenism in the pea aphid *Acyrthosiphon pisum* is under the control of a single sex-linked locus determining the presence/absence of wings (Caillaud et al. 2002), however, in other species morphological differences associated with movement can be more quantitative. Within species, overall body size often covaries with dispersal, though the picture is complex and the direction of the relationship is dependent on the system (Bowler and Benton 2005). In many cases the key morphological trait may be the size or shape of a specific functional structure, such as a leg or pelvis (Losos 1990; Hudson et al. 2016).

The red flour beetle *Tribolium castaneum* (Coleoptera, Tenebrionidae) is a globally significant post-harvest agricultural pest and an established model organism (Boxall 2001; El-Aziz 2011; chapter 2, Pointer et al. 2021). Dispersal is an important aspect of *Tribolium* ecology (Dawson 1977) and dispersal within and between food storage facilities frustrates efforts to control their impact as pests (Semeao et al. 2013). *Tribolium castaneum* moves by both walking and flight, but flies under only certain environmental conditions (Drury et al. 2016), is only very rarely seen to fly inside and is caught outside in traps targeting walking individuals (Semeao et al. 2013). Therefore, while longer dispersals are likely by flight (Ridley et al. 2011), walking is the most common mode of dispersal. A body of previous work in this system has used artificial selection or experimental evolution to demonstrate strong genetic control of locomotive dispersal and rapidly generate large phenotypic differences in dispersal propensity between lines (E.g. Prus (1966); Ogden 1970a); Ritte and Lavie (1977); Korona (1991); Melbourne and Hastings (2009); Weiss-Lehman et al. (2017); Ochocki and Miller (2017); Ruckman and Blackmon 2020; Arnold et al. 2023; chapter 3, Pointer et al. 2023). Correlations between dispersal and other life history traits have been found (Ritte and Lavie 1978; Lavie 1981; Zirkle et al. 1988; chapter 5, Pointer et al. 2024), however, the traits providing the proximate mechanisms underlying differences in dispersal have received little investigation outside of morphology (Arnold et al. 2017, 2023). Even here, results are equivocal, with leg length seen to vary positively with movement ability (Arnold et al. 2017), negatively with dispersal (Arnold et al. 2023) and variously with walking distance (Matsumura and Miyatake 2018, 2019; Matsumura et al. 2019). Hence, it is not currently known how dispersal is evolving in these populations, or which traits are able to respond to novel rapid selection on dispersal. Further, lines subject to negative selection for dispersal in Pointer et al. (chapter 3, 2023) had the greatest phenotypic response, showing almost no dispersal propensity after five generations of selection. Identifying traits responsible for a loss of dispersal may be of particular interest in fields where the spread of organisms may be problematic, such as invasion biology or pest management.

Here we use lines of *T. castaneum* previously selected for differential dispersal (chapter 3, Pointer et al. 2023) to investigate associations between dispersal and other traits to understand the proximate mechanism leading to differences in dispersal tendency, and determine whether dispersal in this system is part of a behavioural syndrome. Specifically we test for differences in activity and movement patterns, morphology, and use of the surface of the habitat medium, that could result in the observed differences in levels of dispersal.

4.2 Methods

4.2.1 Beetles and Dispersal Propensity

The *Tribolium castaneum* beetles used in this study were from 44 experimental lines: 16 high dispersal lines and 16 low dispersal lines (referred to collectively as dispersal regimes) and 12 unselected control lines from the same original Krakow super-strain (KSS) stock (Laskowski et al. 2015), maintained under the same conditions as the selection lines for 5 generations prior to experiments. Full details of the selection experiment and husbandry procedures can be found in Pointer et al. (chapter 3, 2023) but in brief, high (1–16 H) and low (1–16 L) dispersal lines were generated by five generations of divergent artificial selection, using a dispersal assay. In this assay, each individual was given three opportunities to “disperse” from a mixed-sex group of 200 conspecifics (i.e., leave a patch of suitable habitat (120 × 120 × 200 mm container filled to 50 mm with a 9:1 mixture of organic wheat flour and brewer’s yeast, and topped with oats for traction); cross a short distance of unsuitable habitat (150 mm of plastic tubing) and not return – see Fig. S1). Individuals that dispersed three times out of the three opportunities were considered to display a dispersive phenotype, and individuals that dispersed zero times from the three opportunities were considered to display a not-dispersive phenotype. Individuals of each of these phenotypic extremes were bred to produce the subsequent generation, while intermediate phenotypes were discarded. After a single generation of selection, mean dispersal phenotypes (measured as the mean number of dispersal events per individual out of three opportunities) between the treatments were significantly different. After five generations of selection, dispersal phenotypes were strongly divergent, and the distributions of dispersal phenotypes between the two treatments were non-overlapping (chapter 3, Pointer et al. 2023). Thereafter, in order to reduce experimental effort, selection was applied only in even numbered generations up to generation 16. In generation 17 we conducted an assay - using the above procedure as above - on populations of 200 beetles randomly selected from each experimental line. Individuals were marked after each dispersal opportunity and dispersal propensity in each line was scored as the mean number of realised dispersals per individual (out of the three opportunities).

4.2.2 Activity and Movement Pattern

For an individual to disperse in an assay, it had to encounter the opening of the tube to the second container of the dispersal arena (figure 4.1). More active individuals would be more likely to encounter the opening and therefore disperse more often than less active individuals. Thus it is possible that differential levels of locomotor activity and/or movement are driving differential dispersal in our selection lines, so we developed an activity assay to test for this difference.

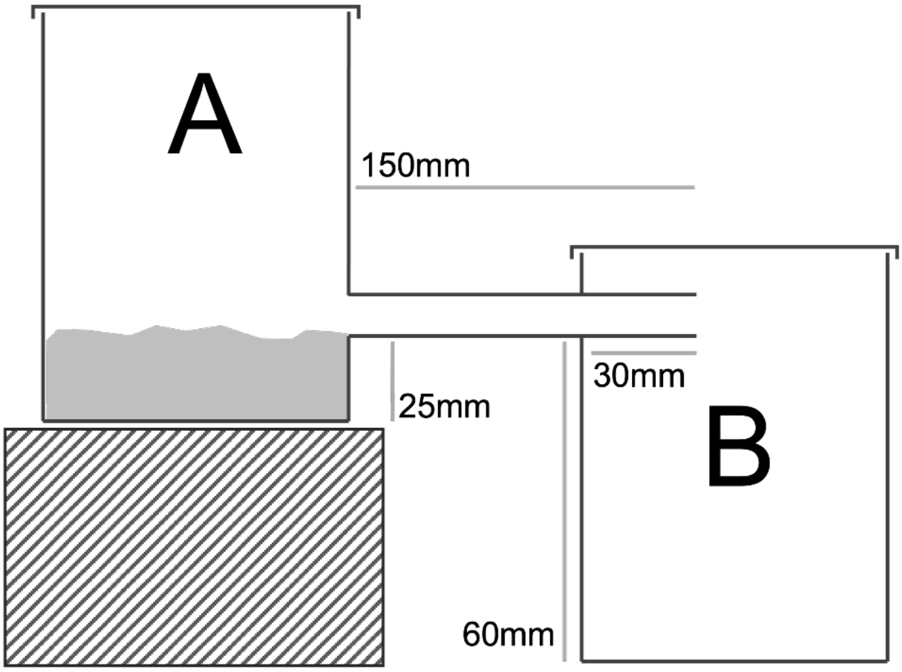


Figure 4.1. Dispersal arena. Experimental arena setup used to assay the dispersal behaviour of experimental *Tribolium castaneum* populations and provide a basis on which to artificially select individuals displaying high and low dispersal propensity.

Activity arenas were constructed by cutting around the sides of a 1.2 L plastic tub 20 mm from its bottom, to remove the base. This was then attached to a large white clay tile wrapped in laboratory tissue paper, using hot glue on the outer surface (figure 4.2 A). This created a square area within which beetles were able to grip and could move freely, but could not escape by climbing the smooth walls of the tub. A set of twelve arenas were arranged such that each was positioned directly below a video camera (models: Sony HDRCX115E; Sony HDRCX190E; Sony HDRCX405) mounted to a horizontal board 300 mm above (figure 4.2 B). Arenas were lit by LED strips attached to the underside of the board. Pupae from each of the 44 experimental lines were sexed and sorted to form test populations of 10 individuals at 1:1 sex ratio, as mating status has been shown to alter movement pattern (Wexler et al. 2017). At this stage the experiment was blinded, line identities were replaced with three figure codes to mitigate unconscious bias during data collection and analysis. Two temporal blocks were used, block 1 consisted of high dispersal lines 1–8, low dispersal lines 1–8 and control lines 1–6, block 2 consisted of high dispersal lines 9–16, low dispersal lines 9–16 and control lines 7–12. At 7 ± 1 days post-eclosion, each test population was placed into an experimental arena, given 10 min acclimation time, then recorded for 10 min at 25 frames per second. Assays were conducted either in the afternoon (1400–1600), or in the evening (1700–1900) in order to include the time of day when activity is known to peak in this species (Rafter et al. 2019). The timing of assays was randomised across all treatments. Event logging software BORIS (Friard and Gamba, 2016) was used to manually record the time of any escapes from activity arenas for each video.

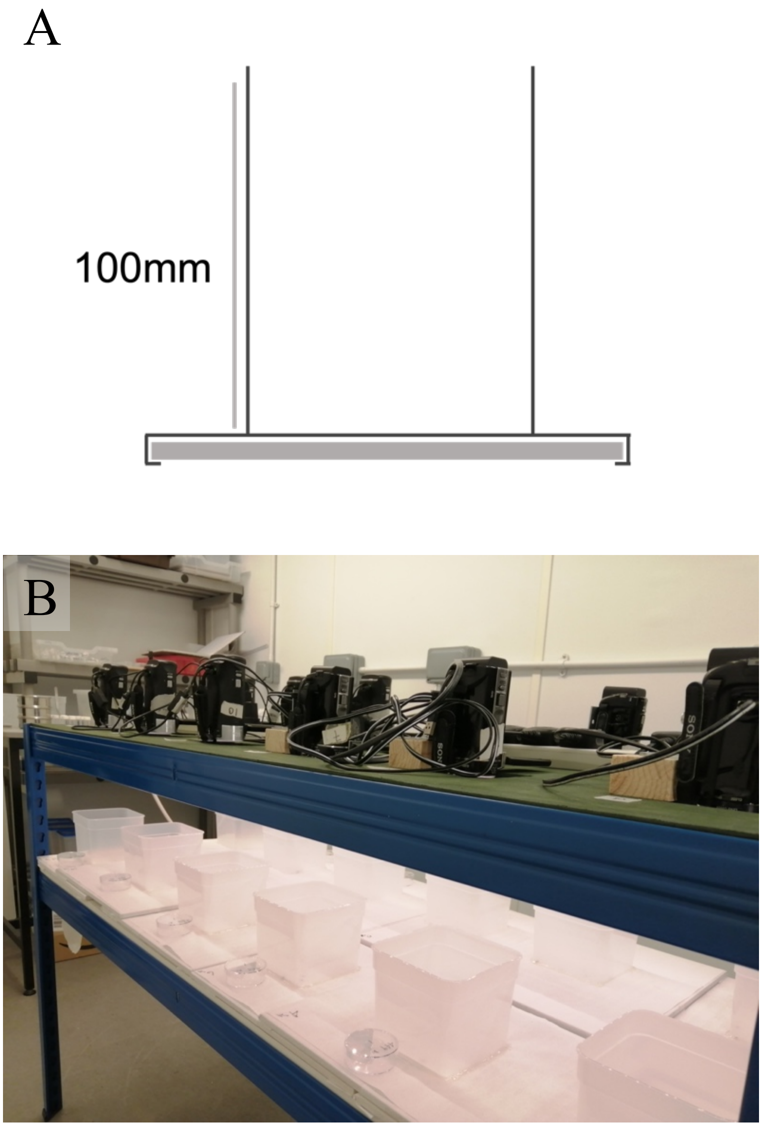


Figure 4.2. Activity assay setup. Experimental setup used to assay the movement behaviour of experimental *Tribolium castaneum* populations. A) Schematic of the arena, consisting of a plastic food container with the base removed, attached using hot glue to a ceramic tile wrapped in white laboratory tissue paper (shown in grey). B) How the arenas shown in A were arranged beneath a custom-made tabletop, to which 12 video cameras were mounted to film video clips.

4.2.3 Surface Affinity

Over the course of the selection experiment, we observed anecdotally that fewer beetles were present on the fodder surface in replicates of low dispersal selection lines than high dispersal lines. It is possible that individual decisions on whether to remain on the fodder surface are driving differences in dispersal (as only individuals on the surface encounter the dispersal tube). To test this we assayed the surface affinity of populations under the same conditions experienced during dispersal assays. Arenas were identical to pot A of the dispersal arenas used during artificial selection for dispersal (figure 4.1), but lacking the opening of the tube leading to a second pot. Test populations were placed onto the surface of the fodder in an arena. After 2 h, photographs were taken of the surface of the fodder and the number of beetles remaining on the surface of the fodder in each replicate was determined. This may have been an underestimate as some individuals were likely obscured by the oats, but gave a minimum value.

To determine if surface affinity is under genetic control we conducted selection on this trait over a single generation. The experimental setup was exactly as described above, but in addition the arena contained a horizontal slot through which a thin plastic separator could be pulled, partitioning the arena contents (figure 4.3). The slot was positioned such that, when the arena contained 250 ml of fodder and the separator was pulled across, fodder within 8 mm of the fodder surface was above the partition, and fodder more than 8 mm from the fodder surface was below the partition. Early trials suggested that an 8 mm distance provided the best separation of surface from not-surface individuals. Populations of 200 unselected stock beetles were placed into arenas, and after two hours the separator was pulled across and the beetles in each partition sieved from their fodder. Thirty individuals from each group of "surface" and "not-surface" beetles were then isolated together for three days, before being transferred to fresh fodder to oviposit. The adults were removed after 7 days and the eggs left to develop to adulthood. At 12 ± 3 days post-eclosion the offspring were assayed using the same procedure (above) as their parents to ascertain their surface affinity. The timing of assays was controlled, as above, by randomising all replicate trials across periods before and during the peak of activity.

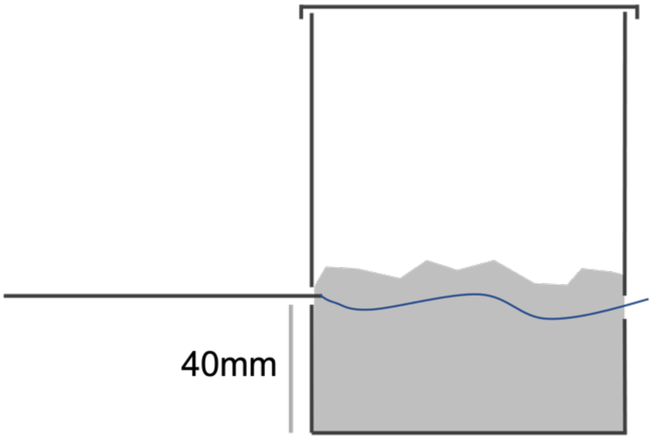


Figure 4.3. Surface-use assay setup. Experimental arena used to assay the surface affinity behaviour of experimental *Tribolium castaneum* populations and provide a basis on which to artificially select individuals displaying high and low surface affinity. By pulling a string (blue) threaded through small holes in the walls of the container, a stiff plastic separator could be pulled through a slit in the opposite wall. This completely isolated fodder and beetles within 8mm of the fodder surface from those below.

4.2.4 Morphology

Beetles from the 44 experimental lines were collected 12 ± 3 days post eclosion and frozen at -80 C until thawed for dissection. The left elytrum and rear left leg were dissected out from 780 individual beetles, 15 of each sex from each of lines 1–10 L, 1–10 H and 1–6 C. Dissections were carried out under an Olympus SZX9 microscope using fine tip watchmaker's forceps, in 30 µl insect saline solution on a clean glass slide. Body parts were imaged under 4X using a dark field phase contrast microscope and an Olympus BX41 camera through GX capture v8.5 software. A 1 mm calibration slide was used between sessions. ImageJ software (Schneider et al. 2012) was used to measure the length of the elytrum, femur, tibia and first tarsus segment, along with femur width at the widest point. *Tribolium* leg segments show some curvature; however, we were interested in their linear extent as the determinant of stride-length. For individuals where an accurate measurement could not be made from the photograph, no measurement was taken. To standardise the procedure as far as possible, all dissection and photography was carried out by RV, using the outer surfaces of the right leg, and all measurements were taken by

MDP. For a subset of 90 individuals, measurements were repeated four days after the initial measurements for analysis of repeatability.

4.2.5 Statistical Methods

All data wrangling and analyses were performed in R (ver.4.3.1; R core team 2023). Mixed models were fitted using package 'lme4' (Bates et al. 2015) with p-values added with 'lmerTest' (Kuznetsova et al. 2017). Summary statistics are presented as means ± standard error throughout.

To test for a difference in dispersal propensity we fitted a linear model (Table 1). The response variable was a population-level measure of the mean number of dispersals per individual, this was used as the density dependence of dispersal behaviour (e.g. Ogden 1970b) means that individual dispersal events are non-independent.

Movement tracking was performed on 110 10 min (15000 frame) video clips using a machine learning algorithm in the software package Loopy (http://loopbio.com/loopy/; settings are provided in table 4.1). Additionally, eight 400 frame clips were human-annotated to ground-truth the model. While individuals were tracked, due to the difficulty associated with tracking individuals moving over each other, tracks were aggregated to give a measure of activity at the population-level for each replicate. Location data from tracking software was used to derive three metrics of activity for each population.

1) As a replicate-level measure of activity, path lengths were calculated from location data as the total distance travelled, in pixels, by each individual per second.

Path lengths were calculated from location data for each detected object as:

$$\sum_{i=min}^{max} \sqrt{(x_{i-1} - x_i)^2 + (y_{i-1} - y_i)^2}$$

Where each i is a frame of the video and x and y are horizontal and vertical coordinate locations respectively, in pixels. These values were then summed across all objects from a single replicate to give a total path length for that population. Note that the tracking model struggles to follow individuals moving over each other, therefore an 'object' does not correspond to an individual beetle over the length of the recording, but a feature in the recording with locations assigned to it by the tracking model over a series of consecutive frames - as such the number of objects detected in a recording is many more than the number of individual beetles, because many objects may be different components of the same individual's path.

To account for beetles escaping during the observation period, time of escapes was noted from videos and the sum taken of length of time each beetle was present to give a measure of the number of beetle-seconds represented in each video observation. Total path lengths were divided by beetle-seconds per replicate to give the measure of mean distance traveled per individual per second.

Pearson's correlation was very high between path distances calculated from human-annotated clips and the same clips tracked with the machine learning model providing confidence in the tracking model (*n* = 8, rho = 0.99, 95%CIs = 0.98, 0.99).

Table 4.1. Model structure used in each test of a specific movement variable. Models containing only fixed factors were fitted as GLMs, those containing random factors were fitted as GLMMs. Interactions are shown with the ' *' symbol, representing an interaction term alongside each of the interacting terms added individually

Test	**Independent variable**	**Fixed factor/s**	**Random factor/s**
Dispersal	Mean dispersals per individual	Dispersal regime	
Path length	Path length	Dispersal regime	Block ID, Line ID, camera ID
Sinuosity	Sinuosity	Dispersal regime	Line ID, camera ID
Edge affinity	Edge affinity	Dispersal regime	Line ID, camera ID
Surface affinity - populations	Number on surface	Dispersal regime	Block
Genetic control of surface affinity	Proportion on surface	Selection regime	Block
Overall body size	Morphological PC1	Dispersal regime*sex	Line ID
Relative leg length	Leg length (femur + tibia)	Dispersal regime *sex, morphological PC1	Line ID

Table 4.2. Machine learning parameters. Values of parameters used when applying a trained keypoint pose predictor algorithm to track *Tribolium castaneum* beetles from pre-recorded videos in software LOOPY (LoopBio).

Parameter	**Value**
Score	0.5
Max detections per class	10
Min detection distance	8
Max tracking distance	22
Death threshold	10

2) Sinuosity of beetle paths was calculated per replicate, as a measure of movement tortuosity/straightness of travel, according to the method of (Benhamou 2004). Sinuosity (S) was calculated from location data as:

$$S \ = \ 2[\,P(\frac{1-c^2 - s^2}{(1-c)^2 \ + \ s^2} + b^2)]^{-0.5}$$

Where P = the physical distance between location readings, b = the coefficient of variation of P, c = the mean sine of all turning angles within a path, s=the mean cosine of all turning angles within a path (Benhamou, 2004). Prior to the calculation, object tracks were trimmed to the longest sequence where no location was within 1/35 of the width of the area from any edge - because encountering the edge would influence the path. Object tracks were then 'rediscretised' (given a standardised physical distance between recorded locations) of p - this accounts for spatial autocorrelation of turning angles in data with high measurement density (Benhamou 2004). Sinuosity was independently computed for each tracked object and a mean taken across all objects from a single video file.

3) Edge affinity was computed for each replicate as the proportion of recorded beetle locations that were within 10 mm of an edge of the arena, with edges of the arena defined as the maximum and minimum X and Y locations recorded across the whole replicate recording.

During data exploration, all records from a single camera (11) in block 2 were identified as extreme outliers and omitted from the analysis. The same linear mixed effects model (GLMM) structure was used to model the three movement metrics, path length, sinuosity and edge affinity (Table 1). The sinuosity model was fitted twice, once for each of two rediscretisation distances ($p = 10$ and $p = 20$), a parameter used to control for non-independence of consecutive turns (see Supplementary methods), results presented are from models using data where $P = 10$, however using $P = 20$ did not qualitatively change the results and these are presented in Supplementary results.

The surface affinity of populations and the genetic control of surface affinity were each analyzed with GLMMs (Table 1). Repeatability of morphological data was assessed by calculating Spearman's rank correlation between repeated measurement taken from the same individual. As all morphological variables were highly correlated ($r > 0.6$), we condensed the information using factor analysis, on a correlation matrix using the function *prcomp* from the R package 'stats' (R Core Team 2023). However, factor analysis cannot deal with missing data, of which there was a high proportion for femur length due to residual thorax tissue preventing accurate measurement; we therefore took forward only records for which all metrics had been quantified (n = 330). A single principal component (PC1) accounted for 74% of the total variation (figure 4.4). Each variable contributed roughly equally to PC1 (23–26%) and all were negatively correlated (table 4.2), we therefore flipped the sign of PC1 to make the interpretation more intuitive. The remaining PCs each captured ≤ 10% of the total variation and were less biologically interpretable. We therefore took forward only PC1 into further analyses, as a proxy for overall body size. We used GLMMs to test separately for overall size differences (represented by PC1) between selection regimes and sexes (Table 1) and for a difference in leg length relative to overall body size (Table 1). Where interaction terms were non-significant they were removed and models refit to test the independent effects of fixed factors.

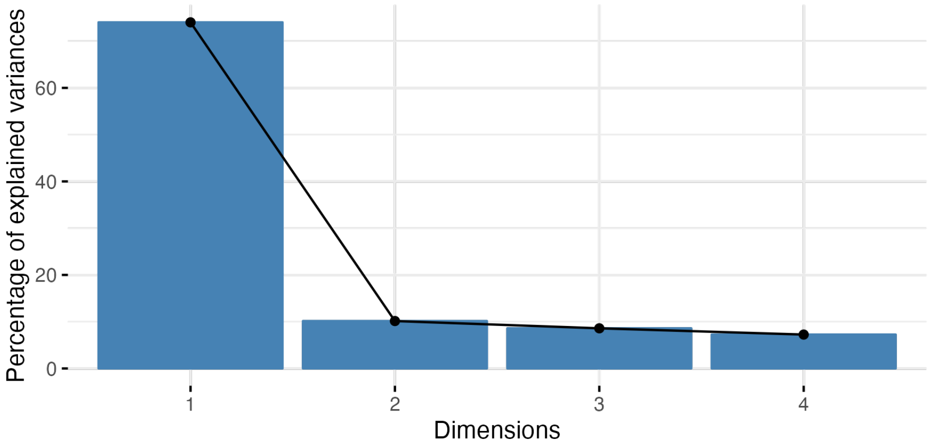


Figure 4.4 Explanatory power of morphological PCs. The variance explained by each principal component generated by factor analysis on morphological variables measured from *T. castaneum* individuals taken from replicate lines experimentally evolved for high (h), control (no selection; KSS) or low (l) levels of dispersal behaviour.

Table 4.3. Loadings of morphological PCA. The loadings of each principal component generated by factor analysis on morphological variables measured from *T. castaneum* individuals taken from replicate lines experimentally evolved for high (H), control (no selection; KSS) or low (L) levels of dispersal behaviour.

Variable	PC1	PC2	PC3	PC4
Elytra length	-0.5024428	-0.05924221	-0.8126212	0.2892894
Femur length	-0.5082749	0.20898609	0.5291783	0.6464919
Femur width	-0.4888609	-0.77011670	0.2402095	-0.3320160
Tibia width	-0.5002229	0.59977949	0.0437777	-0.6229969

4.3 Results

4.3.1 Dispersal Propensity

Beetles from low dispersal lines (0.70 ± 0.06) dispersed significantly less than those from high dispersal lines (2.44 ± 0.04; LM, β=-1.74, se = 0.07, $p < 0.001$; figure 4.5) and significantly less than unselected control lines (1.94 ± 0.06; LM, β=-1.23, se = 0.08, $p < 0.001$; figure 4.5). High dispersal lines dispersed more than control lines, with the magnitude of the difference being greater than that between low dispersal lines and controls (LM; β = 0.51, se = 0.08, $p < 0.001$; figure 4.5).

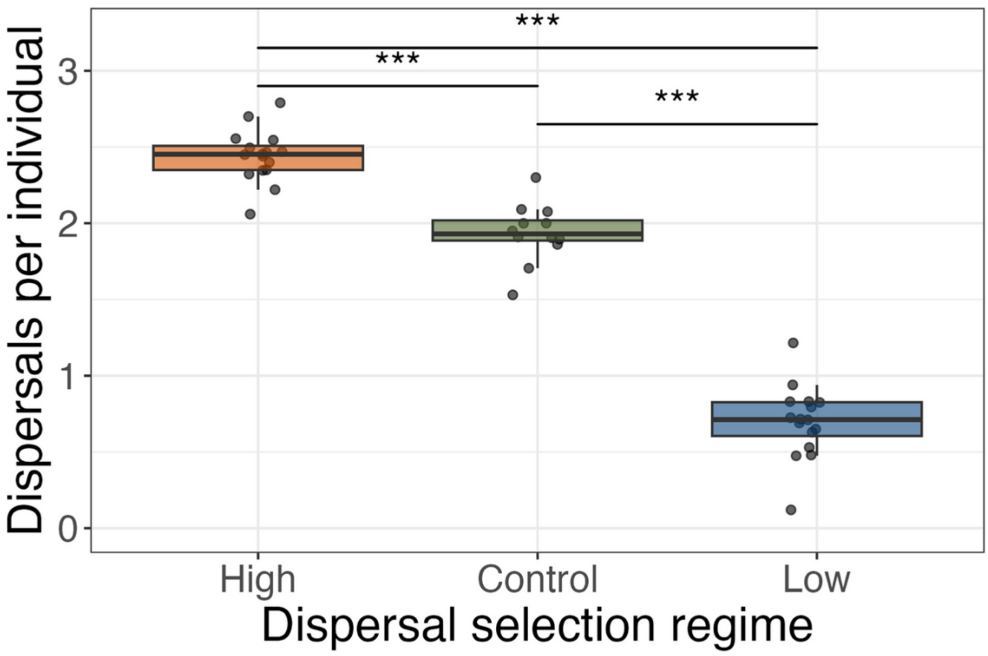


Figure 4.5. Generation 17 dispersal phenotypes. Mean number of dispersals per individual (out of a maximum of three) in populations of 200 *Tribolium castaneum* flour beetles, taken from lines artificially selected for high (*n* = 16) or low (*n* = 16) levels of dispersal behaviour or from unselected control lines (*n* = 12)

4.3.2 Activity and Movement Pattern

Average distances (pixels second^{-1}) traveled by beetles from low dispersal lines (111 ± 1.46) were shorter than those traveled by either high dispersal (129 ± 1.76; GLMM: β = -17.94, SE = 3.14, $p < 0.0001$; figure 4.6 A) or control (125 ± 1.6; β=-14.24, SE = 3.35, $p < 0.001$) regimes, which did not differ from each other (β = 3.70, SE = 3.40, $p = 0.28$).

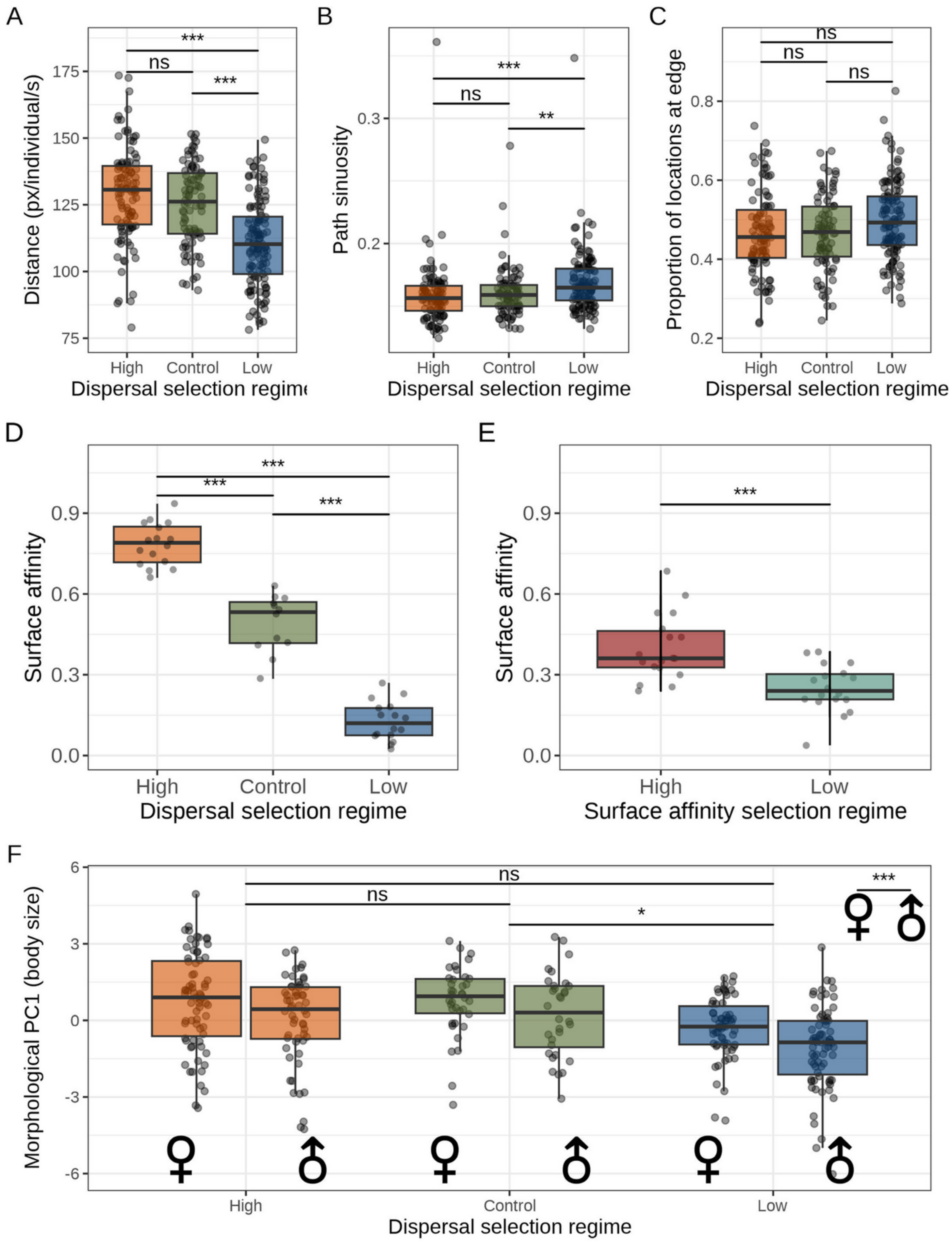


Figure 4.6. Variation in dispersal traits across selection lines. Activity and movement traits in dispersal selection lines are shown as (A) tracked path length; B Path sinuosity; C Edge use. Surface affinity (proportion of 200 beetles remaining on the surface after 2 h) in lines artificially selected for (D) dispersal propensity over nine generations and (E) surface

affinity over a single generation. F Body size represented by morphological PC1 in dispersal selection lines. Boxes display interquartile ranges and bold lines show medians

Beetles from low dispersal lines moved with more sinuous paths than did beetles from high dispersal lines ($P = 10$, GLMM: $P = 10$, β = 0.01, SE = 0.003, $p < 0.001$; figure 4.6B), and also moved more sinuously than control lines ($P = 10$, β = 0.009, SE = 0.003, $p = 0.01$). The control treatment sinuosity was intermediate between high and low lines, but did not differ from high lines ($P = 10$, β=-0.002, SE = 0.003, $p = 0.48$). Sinuosity models using the larger rediscretisation distance (P=20) produced qualitatively the same results as those using P=10, for completeness we give the full results for P=20 here. Beetles from low dispersal lines moved with more sinuous paths than did beetles from high dispersal lines (P=20, β=0.007, SE=0.002, p<0.01), and also moved more sinuously than high dispersal lines (P=20, β=0.005, SE=0.003, p=0.049). The control treatment sinuosity was intermediate between high and low lines, but did not differ from high lines (P=20, β=-0.002, SE=0.003, p=0.42).

Edge use in low dispersal lines (0.50 ± 0.10) was not significantly higher than in high dispersal lines (0.47 ± 0.11; β = 0.03, SE = 0.02, $p = 0.06$; figure 4.6 C) or controls (0.47 ± 0.09; β = 0.03, SE = 0.02, $p = 0.15$). Edge affinity of high dispersal lines did not differ significantly from that of controls (β=-0.006, SE = 0.02, $p = 0.76$).

4.3.3 Surface Affinity

The surface affinity of high dispersal lines (157.00 ± 3.48) was greater than that of low dispersal lines (57.90 ± 6.60, GLMM; β = 131.25, SE = 5.96, $p < 0.001$; figure 4.6D) and controls (99.2 ± 9.33, GLMM; β = 58.63, SE = 6.43, $p < 0.001$). Low dispersal lines had lower surface affinity than did controls (β=-72.63, SE = 6.43, $p < 0.001$).

The offspring of unselected stock parents collected from the fodder surface were found on the surface significantly more than the offspring of parents collected from below the surface of the fodder (GLMM; β = 0.15, SE = 0.03, $P < 0.001$, figure 4.6E).

4.3.4 Morphology

Measurements of elytra length, femur length, femur width and tibia length were all highly repeatable (Spearman’s rank correlation, $p < 0.001$; table 4.3). Length of the first tarsus segment had substantially lower repeatability ($N = 55$, $r = 0.73$, $p < 0.001$) and was dropped from further analyses.

Table 4.4. Repeatability of morphological measures. Repeatability of morphological measurements taken from *Tribolium castaneum* beetles artificially selected for dispersal propensity.

		Repeatability	**Spearman’s rank**
Variable	**N**	**rho**	**p**
Elytra length	90	0.99	<0.001
Femur length	51	0.98	<0.001
Femur width	82	0.91	<0.001
Tibia length	83	0.92	<0.001
First tarsus segment length	55	0.73	<0.001

Sex was a strong predictor of PC1, with males being smaller than females (-0.42 ± 0.14 versus 0.39 ± 0.12 respectively; GLMM; $\beta = 0.82$, SE = 0.16, $p < 0.001$; figure 4.6F). PC1 was significantly lower in the low selection lines (-0.70 ± 0.13) than in controls (0.45 ± 0.16; GLMM, β=-1.10, SE = 0.52, $p = 0.049$), but did not differ either between high dispersal lines (0.52 ± 0.18) and controls (β=-0.32, SE = 05.2, $p = 0.55$), or between low and high dispersal lines (GLMM; β=-0.79, SE = 0.4, $p = 0.09$). There was no significant interaction effect between selection regime and sex on PC1 (GLMM; all $p > 0.07$). When controlling for PC1 as a measure of body size, leg length did not differ significantly between any combination of selection regime and control lines (GLMM; all $p > 0.42$), nor between males and females ($p = 0.07$), nor was any interaction significant between the effects of selection regime and sex (GLMM; all $p > 0.68$).

4.4 Discussion

We found that *Tribolium castaneum* populations selected for divergent dispersal behaviour differ in levels of activity and movement pattern, use of the substrate surface and body size. The consistency of these effects across many independently evolving replicate lines (see figure 4.5) indicates that the coevolution of these traits is robustly repeatable. The tested traits are commonly seen as part of dispersal syndromes in other taxa, suggesting that dispersal in *Tribolium* should be considered in the context of a broad life-history strategy.

We observed that artificial selection on dispersal propensity generated significantly different levels of path sinuosity and activity in lines of *T. castaneum*. Previous studies in *Tribolium* have shown that movement distance is heritable, generating large differences in the trait under divergent artificial selection (Matsumura and Miyatake 2015), but have not assayed the dispersal propensity in these populations. Our finding agrees with many other studies across animals showing that dispersal is associated with exploratory activity (Krackow 2003; Cote et al. 2010b). The picture is similar for sinuosity, with exploratory individuals in many species moving on straighter paths (Brown et al. 2014; Klarevas-Irby et al. 2021), as we have shown for *T. castaneum*. Further, such activity and movement traits are commonly seen as personality traits and observed as components of behavioural syndromes, suites of traits that covary and show consistency within individuals (Wolf and Weissing 2012). In addition, we observe large variation within treatments of all activity and movement traits. High variance in movement traits, including dispersal itself, has also been seen as a feature of populations where dispersal is evolving (Melbourne and Hastings 2009; Ochocki and Miller 2017; Weiss-Lehman et al. 2017). However, we do not see similarly high variance in our measure of dispersal. Therefore, it may be that each movement trait is contributing only a small amount to the large overall difference in realised dispersal. It is also possible that the population-level approach we took to measuring activity and the AI tracking methodology introduced measurement error and inflated variances. If true, this may have prevented us from detecting differences in movement traits between high dispersal lines and controls, where the magnitude of the dispersal differences is less than between low dispersal lines and controls (reasons why dispersal may have responded more strongly to negative selection are discussed in more detail in Pointer et al. (chapter 3, 2023).

For organisms living within a three-dimensional matrix - such as dead wood and grain masses, the ancestral and contemporary habitats of *T. castaneum* (Dawson 1977) - presence at the fodder surface increases risk. Therefore, an individual's willingness to expose itself to this risk may well be indicative of boldness. Boldness - broadly defined as risk-taking (Sloan Wilson et al. 1994) - is another prominent animal personality trait, which shows considerable individual variation in a broad range of taxa, from humans to cockroaches (Sloan Wilson et al. 1994; Stanley et al. 2017). Our results show that individuals from high dispersal lines spend more time at the fodder surface than those from low dispersal lines, suggesting a higher level of boldness. Dispersal is known to correlate with boldness across animal taxa, with important implications for invasiveness (Cote et al. 2010a; Myles-Gonzalez et al. 2015) and are thought to be mediated through common physiology and/or endocrinology (Cote et al. 2010a). This study examined surface use, as a measure of boldness, in the absence of predators. As predation represents one of the main risks to individuals, it may be that the presence of predators would modify surface use, and thus change, or reveal more about variation in, individual boldness. In the cockroach *Gromphadorhina portentosa*, past predator interaction reduces boldness (but not activity; McDermott et al. 2014). In contrast, boldness and predator avoidance represent separate axes of behaviour in the ground beetle *Nebria brevicollis* (Labaude et al. 2018). Measuring surface use in the presence versus absence of a predator would resolve which of these patterns is followed in *Tribolium*, and even whether the response to predation risk varies across dispersal phenotypes. Additionally, time of day might play a role in mediating boldness and movement traits. Our measures here were averages across the on- and off-peak activity periods for *Tribolium* (Rafter et al. 2019), but future work performing separate tests across these times would reveal if dispersers show differential circadian cycles of activity than not-dispersers, however this was beyond the scope of the current project.

In addition to the effects of behaviour, animal movement patterns also often differ as a result of quantitative individual differences in morphology. For example, sprint speed in lizards is dependent upon leg length (Losos 1990). Current evidence for associations between morphology and movement in *Tribolium* is not simple to interpret. Greater leg length, but not body size, was related to increased movement ability when tested within a single generation (Arnold et al. 2017), suggesting a simple mechanistic relationship between morphology and movement ability. Recent work measuring body size and femur length following artificial selection on dispersal showed that body size and mass were inversely related to dispersal propensity (Arnold et al. 2023). In addition, both body size and leg length (controlling for body size) were correlated with walking distance in lines selected for death-feigning duration (Matsumura and Miyatake 2019). However, separate studies have shown the opposite relationship, with shorter leg length in males artificially selected for walking distance (Matsumura et al. 2019), and no relationship in females (Matsumura and Miyatake 2018). In the current study we did not test morphology directly against movement, but tested both in lines selected for differential dispersal propensity. We found that lines selected for low dispersal propensity have experienced a concomitant reduction in body size - so in that sense dispersers have longer legs - but that leg length did not differ relative to body size. Hence, our results agree with the general expectation that dispersive individuals are larger (Renault 2020), and with specific examples where invasive beetles are larger at the range front (Laparie et al. 2013; Yarwood et al. 2021). However, we find a contrast with Arnold et al. (2023), which at first appears to be very similar work to ours in terms of comparing morphology in dispersal lines. On closer examination, the dispersal assays employed in each study differ markedly, with the present study requiring walking, whereas Arnold et al. required individuals to climb to disperse. As Arnold et al. note, “smaller individuals are biomechanically and energetically better suited to dispersal by climbing”, in contrast to dispersal by walking, which may explain the differential findings. Interestingly, Arnold et al. (2023) also find that the sexes respond differently to selection on dispersal when using a design that selects separately on males and females in isolation. Thus, in their study dispersal included mate-finding behaviour. In contrast, we aimed to exclude mate-finding by selecting on dispersal in mixed-sex groups of beetles and found no difference in morphological response between males and females. In this light, our results support the suggestion of Arnold et al. that mate-finding, which drives higher dispersal in males in the absence of females (Prus 1966; Ogden 1970b), may determine sex differences in morphology. Overall, such patterns serve to highlight the complexity of studying dispersal, and support the thesis that measures of movement traits are sensitive to differences in experimental design, setup and arena settings, as found by a recent study using *Tribolium* (Scharf et al. 2023).

Body size is a trait commonly seen as part of dispersal syndromes - the suites of traits associated with dispersal - though the direction of the relationship between size and dispersal is dependent on the specifics of the system, and even on environmental conditions (Bowler and Benton 2005). Dispersal in *Tribolium* is usually thought of as a way for small individuals who may be poor competitors to avoid resource competition (Zirkle et al. 1988; Arnaud et al. 2005), so a larger body size of dispersers suggests that other factors might also be at work. Greater size of dispersers is observed in many animal species, including insects (Anholt 1990; Bowler and Benton 2005). Possibly larger size is mechanistically beneficial for efficient movement, or it may be that larger size/better condition is required to undertake movement (Bowler and Benton 2005).

Since dispersal is “any movement of individuals or propagules with potential consequences for gene flow across space” (Ronce 2007), rather than a behaviour per se, individual activity can be defined as a tendency that contributes to dispersal behaviour (Benton et al. 2012). High dispersal lines were more active and moved on straighter paths, and it is difficult to imagine that such behaviour would not tend to be more dispersive than the converse. Moving with greater activity and in straighter lines would achieve a given dispersal distance in less time, with less energy expenditure and less exposure to risk. Surface use also seems straightforwardly related to dispersal; the first stage of dispersal is emigration; in order to disperse an individual must leave its initial location (Clobert et al. 2012), a necessary first step in dispersing to a new patch might be choosing not to burrow into the substrate but to remain on the surface. We also observed that surface use responded significantly to a single generation of selection, suggesting that this trait has a simple genetic basis. There is evidence for an oligogenic genetic architecture of dispersal in *Tribolium* (chapter 3, Pointer et al. 2023), and the tight correlation between dispersal and surface use may point to either a shared mechanism or causal relationship between these traits. As commonly associated personality-related traits, activity and boldness may be underpinned by shared neurophysiological pathways, such as those related to dopamine signalling (Silva et al. 2020; Wu and Seebacher 2022). Loci with neurophysiological effects on dispersal include those relating to dopamine signalling in birds and mammals (Fidler et al. 2007; Krackow and Konig 2008; Trefilov 2000). In insects, the *foraging* gene, coding for a signalling regulator, affects dispersal in the diptera, lepidoptera and orthoptera (Sokolowski 1980; Anreiter and Sokolowski 2019). Dopamine and other biogenic amines have been previously linked to behaviour in *T. castaneum* (Miyatake et al. 2008; Nishi et al. 2010). It would be instructive to investigate molecular genomic variation between the lines used in this study and sites related to dopamine signalling seem to be reasonable a priori candidates based on our results.

Overall, our results suggest that dispersal is associated with a suite of traits in *T. castaneum*. The presence of a such a dispersal syndrome, or personality-dependent dispersal, is known from many taxa, from single-celled organisms to mammals, including many insects (Cote et al. 2010a; Clobert et al. 2012; Fronhofer et al. 2018; Renault 2020). Further, dispersal syndromes may be part of overarching pace-of-life syndromes linking personality to multiple behaviours (Réale et al. 2010). Some traits in a syndrome may directly assist dispersal, whereas others mitigate costs, although the line between these categories is often blurred (Cote et al. 2010a). The set of traits tested in this study: activity pattern; morphology; surface use, all covary with dispersal tendency in a direction that plausibly enhances the dispersal in high dispersal lines and/or mitigates the costs of dispersing. Deeper understanding of the dispersal syndrome in *T. castaneum* requires genomic study and multivariate analysis of a broad range traits, across a large set of lines. Ideally these lines would be isogenic to remove individual-level variation and enable characterisation of traits and genotypes in genetically identical, and genetically stable, populations across space and time. Dispersal interacts with many aspects of ecology and life-history, and traits of interest might include those related to development, reproduction, population dynamics and social environment, physiology, and senescence – all of which have been subjects of *Tribolium* research, but not in a framework capable of resolving their interrelatedness or genetic underpinnings. Knowledge of eco-evolutionary dynamics is key to understanding biogeography and changes in range (Wellenreuther et al. 2022), which is especially important for species with significant effects on the environment or human populations. Our findings suggest that suites of correlated traits enable species to respond to selection on dispersal; therefore, this context must be considered when investigating the limits of dispersal evolution, and in attempting to predict and control the spread of organisms such as invasive species, crops pests and disease vectors.

4.5 Data Availability

The data files and analysis scripts used in this study are openly available from Mendeley data (https://doi.org/10.17632/zcb97xf8xt.1).

4.6 Contributions

Michael D Pointer conceptualised the work with David S Richardson and Lewis G Spurgin, who also acquired the funding. MDP developed the methodology with help from LGS and Ramakrishnan Vasudeva, and carried out investigation with help from RV. MDP conducted the analyses, prepared figures and drafted the original manuscript, and was assisted in reviewing and editing by Mark McMullan, Simon Butler and DSR. Project administration was done by DSR, who also provided supervision with MM and SB.

4.7 References

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CHAPTER FIVE

Life history correlations and trade-offs resulting from selection for dispersal in Tribolium castaneum

A version of this chapter (appendix 4) is published as:

Pointer MD, Spurgin LG, McMullan M, Butler S, Richardson DS (2024) Life history correlations and trade-offs resulting from selection for dispersal in *Tribolium castaneum*. *Journal of Evolutionary Biology* 37, 748–757. https://doi.org/10.1093/jeb/voae041

Tribolium larva (Klingler, 2004)

5.1 Abstract

Dispersal is an important facet of the life history of many organisms and is, therefore, subject to selective pressure but does not evolve in isolation. Across nature, there are examples of dispersal syndromes and life history strategies in which suites of traits coevolve and covary with dispersal in combinations that serve to maximize fitness in a given ecological context. The red rust flour beetle, *Tribolium castaneum*, is a model organism and globally significant post-harvest pest that relies on dispersal to reach new patches of ephemeral habitat. Dispersal behaviour in *Tribolium* has a strong genetic basis. However, a robust understanding of the relationship between dispersal and other life-history components, which could elucidate evolutionary processes and allow pest managers to control their spread and reduce the impact of infestation, is currently lacking. Here, we use highly replicated lines of T. castaneum previously artificially selected for divergent small-scale dispersal propensity to robustly test several important life history components: reproductive strategy, development time, and longevity. As predicted, we find that a suite of important changes as a result of our selection on dispersal: high dispersal propensity is associated with a lower number of longer mating attempts by males, lower investment in early life reproduction by females, slower development of later-laid offspring, and longer female life span. These findings indicate that correlated intraspecific variation in dispersal and related traits may represent alternative life history strategies in T. castaneum. We therefore suggest that pest management efforts to mitigate the species' agro-economic impact should consider the eco-evolutionary dynamics within multiple life histories. The benefits of doing so could be felt both through improved targeting of efforts to reduce spread and also in forecasting how the selection pressures applied through pest management are likely to affect pest evolution.

5.2 Introduction

Dispersal is the movement of individuals with the potential to induce gene flow and, therefore, plays a crucial role in evolutionary ecology (Ronce, 2007). It is an important element of the life history of many organisms, with effects ranging from the individual level to the spatial dynamics of meta-populations (Matthysen, 2012; Benton & Bowler, 2012). A greater understanding of the ecology and evolution of dispersal will help in addressing several key problems in contemporary biology, including species' responses to climate change, spatial functioning of populations under habitat fragmentation, and the spread of invasive species (Travis et al., 2013; Renault et al., 2018).

Dispersal shows associations with many other traits across a broad range of taxa, and these suites of correlated phenotypes can be termed dispersal syndromes. Where there is individual variation in dispersal within species, high and low dispersal may represent different life history strategies (Clobert et al., 2012). As movement is energetically costly, it might be assumed that correlations between dispersal and other traits may be negative simply because the portion of any resource expended on dispersal is unavailable to be invested elsewhere. In reality, however, the picture is far more complex, and the presence, strength, and direction of relationships, as well as the traits involved, are highly context dependent (Bonte & Dahirel, 2017) and vary even with the individual components of dispersal (Jervis et al., 2007). The concerted evolution of suites of traits serves to mitigate the significant costs of dispersal and maximize individual fitness within the specific ecology of different systems (Bonte et al., 2012). Dispersal syndromes, and the underlying correlations and trade-offs in phenotypes associated with dispersal, have attracted much attention (Ronce & Clobert, 2012) as they provide insight into the multivariate nature of selection on life history traits and the constraints on their evolution (Bonte et al., 2012; Clobert et al., 2012). Most notably, dispersal consistently shows a relationship between fecundity and survival across taxonomic orders; however, even these correlations are far from universal, depending on a range of factors, including locomotion strategy and mode of thermoregulation (Stevens et al., 2014). The majority of research has focussed on vertebrates where individual tracking is possible (e.g., Cote & Clobert, 2012) and on wing-dimorphic insects where the dispersal phenotypes are discontinuous (Zera & Denno, 1997; Guerra, 2011). Less has been done on wing-monomorphic insects where dispersal phenotypes are more difficult to quantify. Among these wing-monomorphic insects, the majority of studies are on species where flight is the major mode of movement, and therefore, differences in energetic output between dispersal phenotypes are likely to be much more important than in systems where dispersal is by walking. Studies are required within more diverse systems to enable the recognition of more general patterns and to identify the extent to which causal factors and evolutionary consequences are dependent on the evolutionary ecology of the individual taxa (Bonte & Dahirel, 2017; Ronce & Clobert, 2012).

The red flour beetle, *Tribolium castaneum*, is a globally significant post-harvest crop pest (El-Aziz, 2011) long used as a model system in evolutionary and population biology (chapter 2, Pointer et al., 2021). The species is long-lived for a small beetle, having a short egg, larval, and pupal stages (<5, <20, and <5 days, respectively, under near optimal conditions) followed by a long adult stage of up to 4 years (Good, 1936; Pointer et al., 2021). Historically, *Tribolium* were free-living, likely beneath the bark of trees or in rotting wood, where they were secondary colonizers characterized by rapid population growth, intense intraspecific competition, and ready dispersal (Dawson, 1977). At an unknown time in its history, the species switched to being predominantly human-commensal, infesting a wide variety of stored food products (Dawson, 1977). While they are capable of long-distance movement by flight, *Tribolium* beetles fly only in high temperatures (~>25 °C; Drury et al., 2016), and therefore, dispersal movements by flight are likely limited to outdoor movements in the tropics. In contrast, dispersals in the species' introduced, temperate range, or within artificially cooled food storage facilities, are likely to be predominantly by walking. Recent work in this system has shown variation in life history traits in natural populations (Matsumura et al., 2023), also that local dispersal (specifically emigration, but we keep the term dispersal for consistency with the existing literature) in *Tribolium* has a strong genetic basis, and that the architecture of the trait may be relatively simple (chapter 3, Pointer et al., 2023). Furthermore, dispersal propensity is correlated with several functionally dispersal-promoting traits (activity, movement pattern, affinity for the surface of the medium; chapter 4, Pointer et al. 2024) related to activity and boldness, generally considered to be important dimensions of animal personality (Roche et al., 2016). As an established model system, the evolutionary ecology of *Tribolium* is relatively well understood (chapter 2, Pointer et al., 2020), yet despite the importance of dispersal to the species and to human pest management (Dawson, 1977; Jeger, 1999), little is known about a possible dispersal syndrome among life history traits. Such knowledge could help to predict the evolutionary consequences of management practices (Karlsson Green et al., 2020). The only previous studies used poorly replicated selection lines, selected under suboptimal conditions (Lavie & Ritte, 1978; Zirkle et al., 1988 using lines from Ritte & Lavie, 1977), or focused on correlations with walking motivation (distance walked by an individual in 30 min in the absence of food or conspecifics; e.g., Matsumura & Miyatake, 2019, using lines from Matsumura & Miyatake, 2015). As both the propensity to leave the fodder and directionality of movement are important aspects of walking dispersal in *Tribolium* (Korona, 1991; chapter 4, Pointer et al. 2024), walking motivation is a single component of dispersal and a potential dispersal-enhancing trait, rather than dispersal per se, whereas the dispersal selection assay we employ explicitly captures all these components of dispersal behaviour.

Here, we use highly replicated lines of *T. castaneum* previously artificially selected for high and low levels of local dispersal propensity to investigate correlations between dispersal and several life history traits. The first trait we evaluate is male mating success. In wing-dimorphic insects, dispersal is known to show a negative association with mate acquisition traits (Guerra, 2011). Evidence for a similar trade-off in *Tribolium* has come from studying walking motivation lines (e.g., Matsumura et al., 2019). We expand on this previous work by using lines selected for a trait that better reflects dispersal and using mating assays within an ecologically realistic social environment, incorporating female choice and male–male competition, which are important aspects of reproductive biology in this system (Fedina & Lewis, 2008). If dispersal trades off with an investment in competitive ability, we expect to see highly dispersive males achieving fewer matings than less dispersive males.

We also evaluate female reproduction using a measure of fecundity. Fecundity is often seen to covary with dispersal, but the direction of the relationship is dependent on the cause (Stevens et al., 2014; Campos-Candela et al., 2019). The release from competition experienced by successful dispersers often means that they are able to invest more in reproduction (Burton et al. 2010). In contrast, the oogenesis-flight syndrome known from flying insects results from energetic constraint, meaning that dispersive individuals invest less in reproduction, particularly in early life (Johnson, 1969; Harshman & Zera, 2007; Tigreros & Davidowitz, 2019; Asplen, 2020). For this reason, we also include a temporal component, comparing fecundity before and after peak dispersal. Given the importance of intraspecific competition in *Tribolium* (Dawson, 1977), we expect population dynamic processes to dominate and, therefore, expect to observe increased female fecundity in highly dispersive lines, especially following the time of peak dispersal.

Another trait often seen to covary with dispersal is development time (Guerra, 2011). Associations between dispersal and development time in insects vary widely in strength and direction (Guerra, 2011), though these studies are overwhelmingly focussed on wing-dimorphic insects (but see Goodwyn & Fujisaki, 2007 in water-striders, Heteroptera: Gerridae). Such species are adapted to very different ecological conditions than the boom-bust cycles experienced by *Tribolium* populations, with bust phases characterized by cycles of very high intraspecific competition (including prolific larval and adult cannibalism of eggs and pupae [Stevens, 1989] and scarce resources [Dawson, 1977]). Under such conditions, a reversed dispersal-development pattern might be expected, in which a high-competition environment selects for rapid development to mitigate the risk of cannibalism by larvae to later-hatching eggs, with this pressure being relaxed among the offspring of dispersers in less competitive environments. Following this logic leads us to expect that a release from competition gained through dispersal will result in dispersive phenotypes being associated with slower development.

Finally, we examine longevity. As for fecundity, if energetic constraints influence life span, we might expect to see a negative relationship between dispersal and longevity. However, we expect high dispersal lines to have longer life spans, in line with the relationship observed among terrestrial animals, where greater longevity is thought to allow more time for a suitable reproductive habitat to be located (Stevens et al., 2014). This may be particularly strong considering the release from intense competition potentially afforded by successful dispersal in this system. Overall, if a dispersal syndrome exists in this system, we expect to see significant correlations between dispersal phenotype and many of the studied traits. We discuss our results in the context of a dispersal syndrome in *T. castaneum*, the relevance to both the evolution of this and similar species, and pest management aiming to control this prolific pest.

5.3 Materials and methods

5.3.1 Beetles and dispersal propensity

The *T. castaneum* beetles used in this study were from 44 experimental lines: 16 high dispersal lines, 16 low dispersal lines previously bred from an outbred stock population and 12 unselected control lines from the same original Krakow super-strain stock (Laskowski et al., 2015), maintained under the same conditions as the selection lines for five generations prior to experiments. High and low dispersal lines were bred under divergent artificial selection over five generations, using a dispersal assay in which each individual was given three opportunities to “disperse” from a mixed-sex group of 200 conspecifics (i.e., leave a patch of suitable habitat (120 mm × 120 mm × 200 mm container filled to 50 mm with a 9:1 mixture of organic wheat flour and brewer’s yeast, and tops with oats for traction); cross a short distance of unsuitable habitat (150 mm of plastic tubing) and not return). Individuals that dispersed three times out of the three opportunities were considered to display a dispersive phenotype, and individuals that dispersed zero times from the three opportunities were considered to display a non-dispersive phenotype. Individuals of each of these extreme phenotypes were bred to produce the subsequent generation, while intermediate phenotypes were discarded. Further details of the selection experiment and of husbandry procedures can be found in Pointer et al. (chapter 3, 2023). After a single generation of selection, mean dispersal phenotypes (measured as the mean number of dispersals per individual out of three opportunities) between the treatments were significantly different. After five generations of selection, dispersal phenotypes were strongly divergent, and the distributions of dispersal phenotypes between the two treatments were non-overlapping (chapter 3, Pointer et al., 2023). The lines were split into two temporal blocks to manage workload: block one consisted of high dispersal lines 1–8, low dispersal lines 1–8, and control lines 1–6, and block two consisted of high dispersal lines 9–16, low dispersal lines 9–16 and control lines 7–12. Individuals used for all experiments were taken from the same generation as those in which dispersal was quantified in Pointer et al. (chapter 4, 2024), where dispersal was found to be still strongly divergent and non-overlapping between treatments (dispersals per individual out of a maximum of three, low dispersal lines = 0.70 ± 0.05; high dispersal lines = 2.44 ± 0.07). All experiments in this study used the 32 selected lines; in experiments where we were less constrained by limits imposed by experimental effort, we also incorporated 12 unselected control lines. Throughout all experiments, populations were kept on a fodder mix of 90% organic white bread flour and 10% brewer yeast; environmental conditions were maintained at 30 °C and 60% relative humidity—hereafter referred to as "standard conditions." This research consisted of non-invasive observations of the natural behaviour of insects, and no ethical approval was required in the United Kingdom. Nevertheless, all experiments were carried out according to good scientific and ethical practice.

5.3.2 Male mating behaviour

Mating behaviour was scored from 305 video recordings. Full details are given in Pointer et al. (chapter 4, 2024), but briefly, test populations of 10 individuals at a 1:1 sex ratio were taken from the 44 experimental lines in 2 temporal blocks. Each assay consisted of recording one test population for 10 min within a 120 mm × 120 mm arena, which was smooth-sided to prevent climbing. The number of mountings and total duration of mounting within a 10-min window was scored for each replicate using event logging software BORIS (Friard & Gamba, 2016). It was impossible to entirely prevent escapes during recording; therefore, some replicates represent fewer beetles for part of the assay. To account for this, trait measures for each replicate were adjusted by the number of "beetle-seconds" described by that replicate (trait measure divided by the sum of the length of time each individual was present in the assay arena). This results in small values but represents the most accurate way to present the data.

5.3.3 Female reproductive output

The 32 dispersal selection lines were split into 2 temporal blocks. Twenty-two virgin adults of each sex were obtained per line by sexing as pupae and raising in single-sex groups of 20. Males were marked with a dot of paint on the dorsal thorax using a method that was shown not to affect reproductive output (Sales, 2018). Males and females were paired in vials for 48 hr of mating opportunity, after which the males were discarded. At 4 ± 2 days post-eclosion, females were transferred into pots to oviposit for 8 days, then transferred to a fresh pot for an additional 8 days of oviposition (hereafter termed oviposition periods). These timings were chosen to encompass the known dispersal peak of *T. castaneum*, which occurs at ~10 days post-eclosion (Ziegler, 1976). In the second temporal block of lines, the first 8 hr of oviposition were in a third pot to obtain individuals whose laying time was more precisely known to allow measurement of development time (below); however, offspring from the first 8 hr and the rest of the 8 days were combined in evaluating reproductive output. Oviposition pots were kept under standard conditions for 35 days after the removal of the female before being frozen until adult offspring could be counted. Offspring were allowed to develop in excess of fodder to eliminate any effect of density or resource limitation. A female's reproductive output was taken as the number of adult offspring resulting from a given period of oviposition. Replicates producing no offspring were removed from the analysis as they are far more likely to represent male reproductive failure rather than a lack of female fecundity, which is the variable of interest here. This is because rates of female infertility are extremely low in this system relative to those of males (Pai, 2001; Matsumura et al., 2023).

5.3.4 Development time

During the reproductive fitness assay, females from lines in the second temporal block (176 females from each dispersal regime) oviposited in a separate pot for the first 8 hr to obtain a cohort whose laying time was more precisely known. The offspring developing in these pots were observed every 24 hr, and each pupation day was recorded. We use pupation time as a measure of development to allow direct comparison with existing work (Zirkle et al., 1988).

5.3.5 Longevity

Within the 2 temporal blocks, 10 males and 10 females from each of 44 experimental lines were sexed as pupae and raised in single-sex sex groups until 6 ± 3 days post-eclosion. At this point, each virgin individual was placed into a separate vial with 3-ml fodder. Each individual was observed every 14 days until day 462, with the time of deaths recorded until all individuals were dead.

5.3.6 Statistical methods

All data wrangling and analyses were performed in R (ver.4.3.1; R Core Team, 2021). Mixed models were fitted using package "*lme4*" (Bates et al., 2015) with *p*-values added with "*lmerTest*" (Kuznetsova et al., 2017). Model validation used the check_model function from the "*performance*" package (Lüdecke et al., 2021) and/or simulated residuals with DHARMa (Hartig, 2022). Most experiments included temporal block as a control variable. This could not be modelled as a random factor in GLMMs as it has only two levels, and random effects with few levels are known to produce imprecise estimates (Hodges, 2013). We had no reason to believe that block would have any effect on the traits under study, and the study design balanced dispersal treatments across the two blocks. Therefore, to simplify the main models in each analysis, we first checked that temporal block was not a significant predictor of the dependent variable, and did not include it in the main models.

Both total reproductive output and timing of reproduction were modelled with LMMs (table 5.1). To test the timing of reproduction, we initially modelled the difference in reproductive output between oviposition periods one and two; however, high overdispersion in these models prevented an adequate investigation. We therefore focussed on reproductive output in the first oviposition period, as early life reproduction is likely to be the most relevant for dispersal trade-offs (Zera & Denno, 1997).

Table 5.1. Life history model structures. Structure of models used to fit the effect of artificial selection for dispersal on several life history traits in replicate lines of *Tribolium castaneum*. The first column gives the biological trait of interest to a part of the analysis, and the second column gives the measured included as the dependent variable in the model, including standardization or codings.

Trait	Dependent variable	Fixed factor/s	Random factor/s
Number of matings by males	Number of matings (per individual per second)	Dispersal regime	Line ID
Duration of mating by males	Mating duration (per individual per second)	Dispersal regime	Line ID
Total reproductive fitness	Total reproductive output	Dispersal regime	Line ID
Timing of reproductive fitness	Reproductive output in first oviposition period	Dispersal regime	Line ID
Development time	Pupation time	Dispersal regime × ovipos period	
Longevity	Alive (0)/dead (1)	Dispersal regime × sex	Individual ID, time

Neither development time nor longevity data conformed to the proportional hazards assumption of Cox PH models. Seeing as the pupation rate among offspring in the development analysis was 100%, and no individuals needed to be censored from the dataset, we modelled time to pupation using a GLM with a Poisson error distribution (Table 1). Initially, we included female ID as a random factor to account for the fact that multiple individuals had the same mother; however, it was removed as it accounted for 0 variance in development time.

As Cox models were not appropriate, we modelled longevity using event history analysis (Keiding, 2014), which is qualitatively similar but without the assumption of proportional hazards. Rather than considering the time until some event (death), this approach considers the risk of an event occurring; hence, longevity is evaluated as a mortality risk. Accordingly, we modelled event (death) risk per time period using a binomial distribution; at each observation, individuals were assigned “0” if the event had not occurred and assigned “1” once the event took place. Repeated measures from each individual were included in the model via a random effect of individual ID. Line ID was initially included as a random effect but was removed as it added a large amount of complexity to the model structure without significantly improving model performance. Longevity models were also fitted for each sex independently, using the same structure but excluding sex as a fixed factor. The structure of GLMMs used to conduct these event history analyses is given in Table 1.

5.4 Results

Temporal block was not a significant predictor of mating duration (LM: β=-2.22e-3, SE=2.45e-3, p=0.365). Nor did block have a significant effect on the number of mating attempts (LM: β=-5.67e-5, SE=3.70e-5 , p=0.13). A GLM fitting the effect of block on total reproductive output were non-significant (β=-8.13, SE=5.91, p=0.170). An equivalent model fitted to just data from oviposition period one was also non-significant (β=5.03, SE=2.86, p=0.079). Considering longevity, a binomial GLMM fitting the effect of temporal block on time of death for showed no significant effect of block (β=-0.11, SE=0.09, p=0.233). Neither did a similar model fitted to only male data demonstrate a significant effect of block on time of death (β=-0.09, SE=0.15, p=0.551), and the same result was found for females (GLMM: β=-0.19, SE=0.11, p=0.090). Therefore, to simplify the main models in each analysis, we did not include block (see statistical methods)

5.4.1 Male mating behaviour

The number of mating attempts (mountings per individual per second, adjusted to account for escapes) observed in low dispersal lines (mean ± SE = 7.13e−4 ± 3.01e−5) was greater than that in either high dispersal (5.10e−4 ± 3.17e−5, GLMM: β = 2.01e−4, SE = 6.23e−5, *p* < 0.01; 5.1Ai) or control lines (4.81e−4 ± 2.74e−5, GLMM: β = 2.38e−4, SE = 6.65e−5, *p* < 0.001). High dispersal lines did not differ from controls in their number of mating attempts (β = 3.49e−5, SE = 6.76e−5, *p* = 0.61). The total duration of mating (seconds per individual, adjusted to account for escapes) was not different between high dispersal lines (mean ± SE = 2.1e−2 ± 2.3e−3) and low dispersal lines (2.5e−2 ± 1.9e−3, GLMM: −3.9e−3, SE = 3.5e−3, *p* = 0.27; figure 5.1Aii), between high dispersal lines and controls (2.0e−2 ± 2.2e−3, GLMM: β = 9.2e−4, SE = 3.8e−3, *p* = 0.8), or between low dispersal lines and controls (GLMM: β = 4.8e−3, SE = 3.7e−3, *p* = 0.20).

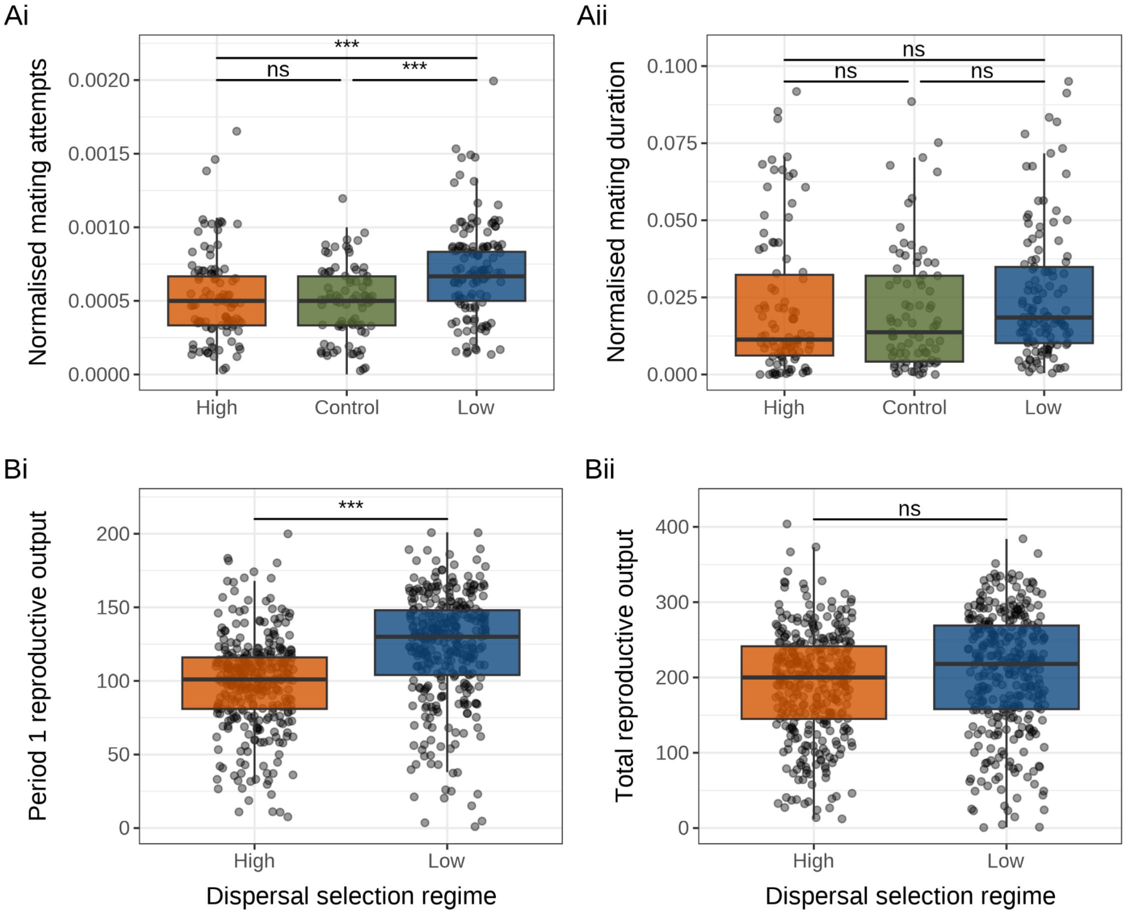

Figure 5.1. Reproductive traits in experimental dispersal lines. Reproductive traits assaying in *Tribolium castaneum* lines selected for high or low dispersal behaviour and unselected controls. A) mating traits scored from ten minute video recordings of mixed-sex populations of ten individuals, showing i) number of mating attempts by males (normalised to be expressed per beetle per second) and ii) total mating duration (per beetle per second). These data are normalised by the number of 'beetle-seconds' represented in replicate recordings, to account for uneven representation due to escapes (trait measure divided by the sum of the number of seconds each beetle was present in the assay arena). B) Reproductive output (number of adult offspring produced) of individual females, i) in total across two eight day periods of oviposition, roughly equating to before and after the peak of dispersal, and ii) reproductive output in the first eight day period of oviposition. Points are semi-transparent so that dark points indicate multiple overlapping points. *** indicates a significance level of <0.001. 'ns' indicates a non-significant difference.

5.4.2 Reproductive output

Early life reproduction was significantly different between high and low selection lines, with high dispersal lines laying significantly fewer eggs in the first oviposition period (98.60 ± 1.74) than low dispersal lines (122.58 ± 2.08; LMM, $\beta = -23.89$, SE = 4.43, $p < 0.001$; figure 5.1Bi). However, the total reproductive output across two oviposition periods did not differ between pairs from high (mean ± SE = 191.1 ± 3.9) and low dispersal lines (207.3 ± 4.4, GLMM: $\beta = 16.00$, SE = 8.73, $p = 0.077$; figure 5.1Bii).

5.4.3 Development time

The pupation time of high dispersal lines (mean ± SE = 23.92 ± 0.08) was not significantly different from that of low dispersal lines (23.45 ± 0.08; figure 5.2A; table 5.2). Pupation time was significantly higher in the second oviposition period (24.44 ± 0.09) than the first (23.05 ± 0.07; figure 5.2A; table 5.2). There was also a significant interaction between the dispersal and oviposition period, with high dispersal lines in the second oviposition period having significantly lower pupation probability than expected from the additive effects of high dispersal and period 2 alone (figure 5.2A; table 5.2).

Table 5.2. Pupation time GLM. General linear model of the pupation time (days since the egg was laid) of offspring laid on either day 4 ± 2 or 13 ± 2 of adult life span across replicate lines selected for either high or low dispersal propensity. *Note*: These time points correspond to day 1 of oviposition period 1 and day 1 of oviposition period 2, which are chosen to represent before and after the peak of dispersal in unselected *Tribolium* populations.

	β	**SE**	***p***
Intercept	23.12	0.10	<0.001
Dispersal regime (low)	−0.14	0.15	0.34
Oviposition period (2)	1.66	0.15	<0.001
Dispersal regime (low): Period (2)	−0.64	0.22	0.004

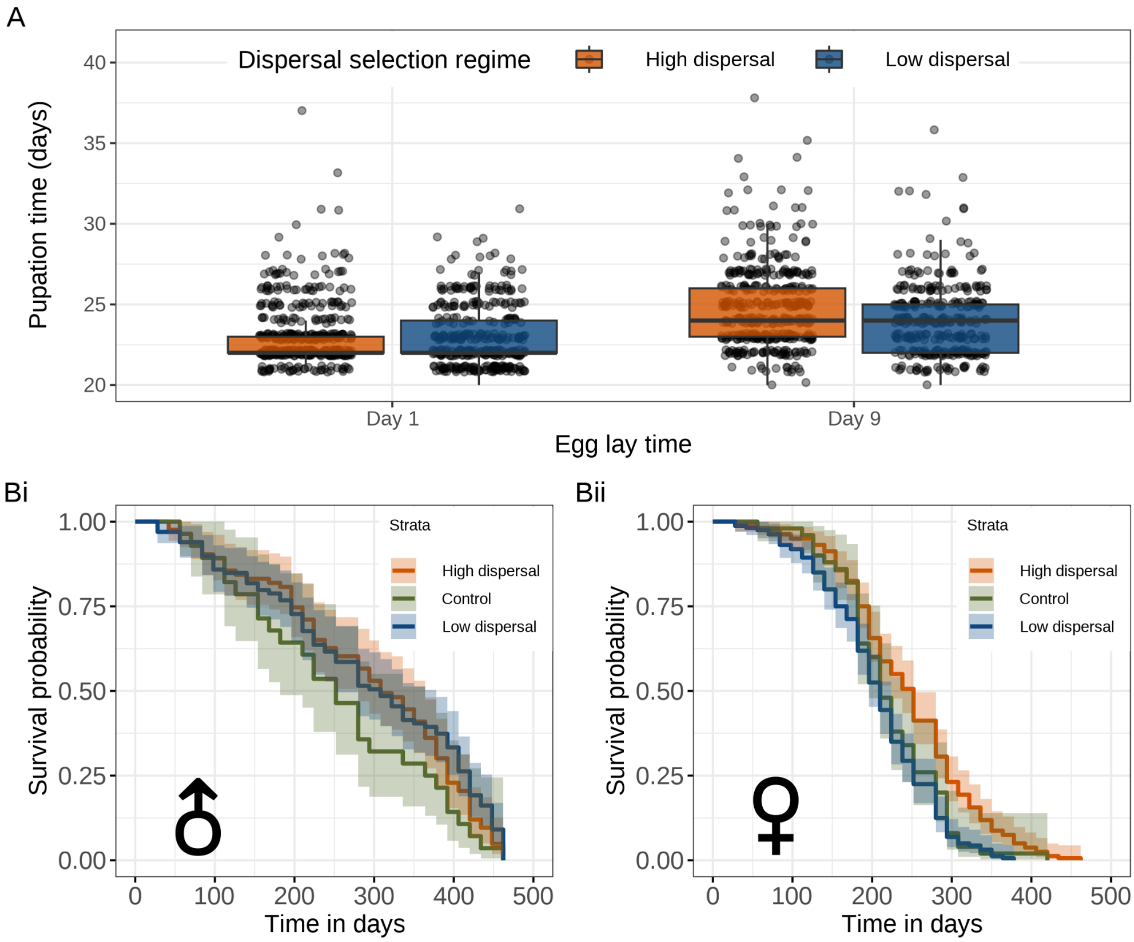

Figure 5.2. Life history traits in experimental dispersal lines. Life history traits assayed in lines of *Tribolium castaneum* selected for high or low dispersal behaviour. A) pupation time (days since egg was laid) of offspring laid on either day 4±2 or 13±2 of adult lifespan (day 1 of oviposition period 1 and day 1 of oviposition period 2 - these time points correspond to before and after the peak of dispersal in *Tribolium*), across dispersal regimes. B) survival probability across dispersal regimes for i) males and ii) females. Points are semi-transparent so that dark points indicate multiple overlapping points.

5.4.4 Longevity

High dispersal lines (median life span in days [mld] = 252) had significantly lower mortality risk than either low dispersal lines (mld = 224; table 5.3) or controls (mld = 224; table 5.3). Male mortality risk (mld = 294) was significantly lower than that of females (mld = 224; table 5.3). There was a significant interaction between the dispersal regime and sex, with males from high dispersal and control regimes having greater mortality risk than expected under the additive effects of sex and dispersal regime alone (table 5.3). Low dispersal males had lower mortality risk than predicted by a purely additive model. Post hoc tests individually for each sex showed that the difference in mortality risk between dispersal phenotypes was absent in males, where high dispersers (mld = 308) did not have significantly longer life spans than either control (mld = 252; GLMM mortality risk, $\beta = 0.30$, SE = 0.23, $p = 0.186$; figure 5.2Bi) or low dispersal lines (mld = 308; GLMM mortality risk, $\beta = -0.12$, SE = 0.16, $p = 0.433$; figure 5.2Bi). Instead, the overall difference was driven by a significant difference in females, where high dispersers (mld = 252) had longer life spans than control (mld = 210; GLMM mortality risk, $\beta = 0.41$, SE = 0.20, $p = 0.043$; figure 5.2Bii) and low dispersal lines (mld = 210; GLMM mortality risk, $\beta = 0.58$, SE = 0.14, $p < 0.001$; figure 5.2Bii).

Table 5.3. Longevity GLMM. Generalised linear mixed model, using an event history approach to model longevity as mortality risk across time using a binomial distribution in lines of *Tribolium castaneum* artificially selected for dispersal propensity. *Note*: Data above the dashed line are from a model in which “high dispersal” was the reference category, below the dashed line “low dispersal” was the reference.

	Log odds	**SE**	***z***	**Pr(>$\lvert z \rvert$)**
(Intercept)	−2.58	0.37	−6.89	<0.001
Low dispersal	0.51	0.12	4.13	<0.001
Unselected dispersal control	0.35	0.17	1.98	0.047
Male	−0.72	0.15	−4.77	<0.001
Unselected dispersal control: Male	0.03	0.29	0.12	0.908
Low dispersal: Male	−0.63	0.20	3.09	0.002
(Intercept)	−2.07	0.38	−5.46	<0.001
High dispersal	−0.50	0.13	−3.91	<0.001
Unselected dispersal control	−0.16	0.18	−0.85	0.369
High dispersal: Male	0.63	0.20	3.10	<0.01
Unselected dispersal control: Male	0.69	0.30	2.33	0.024

5.4 Discussion

Our results show that aspects of mating behaviour, reproductive timing, development time, and longevity are covaried, with the tendency to initiate local dispersal by walking in *Tribolium castaneum*. Specifically, high dispersal propensity is significantly associated with fewer mating attempts, lower investment in early-life reproduction, slower development of later-laid offspring, and longer female life span.

Reproductive traits are commonly seen to vary with dispersal, and there are many possible reasons why mating behavior and dispersal might coevolve. For example, an individual might alter its reproductive environment through dispersal, changing the level of male–male competition or sexual antagonism it experiences. Alternatively, resources may need to be directed away from reproductive effort in preparation for dispersal. Correlations in both directions exist between reproductive traits and dispersal among insects, e.g., mating frequency is higher for dispersive Glanville fritillary butterflies (*Melitaea cinxia*, Bonte and Saastamoinen 2012) but lower for more dispersive long-winged male crickets (*Velarifictorus* spp., Zeng & Zhu, 2012; Zhao et al., 2017). In *T. castaneum*, we observed fewer matings overall among the high dispersal phenotype. While total mating duration was equal across the high and low dispersal phenotypes, we observed fewer matings among the high dispersers, suggesting that each high dispersal line mating is of longer duration. There are a number of plausible explanations for this observation; similar patterns are seen in other beetle species, where the balance of mating duration and remating frequency is adjusted in response to investment in other physical or behavioural traits (Simmonds & Kotiaho, 2007; Yamane et al., 2010). More specifically, the longer mating duration could, for example, be driven by prolonged mate-guarding in these lines (Fedina & Lewis, 2008), potentially as a result of uncertainty about the mating environment in a post-dispersal habitat. Alternatively, greater investment in peri-copulatory mechanisms might compensate for poorer sperm competition of dispersers (Matsumura et al., 2019). This represents an interesting opportunity for further work to investigate questions such as whether sperm competitiveness differs with dispersal, whether the mating behaviour of males differs before and after the age of peak dispersal, and whether there is an effect of social environment on each reproductive strategy.

With regard to the reproductive output of females, in the present study, we saw no overall difference in the number of eggs laid between dispersal phenotypes, which aligns with the finding that reproductive output did not differ in *Tribolium* lines divergently selected specifically for walking distance (Matsumura & Miyatake, 2018). However, that study did not have a temporal component, whereas in the present study, we saw less investment in early reproduction in high dispersal lines. “Early” and “late” here, while both relatively early in the long lifetime of *Tribolium*, roughly coincide with before and after the age of peak dispersal, which occurs at ~10 days post-eclosion (Ziegler, 1976). Two possible, though not mutually exclusive, explanations for this observation are that (i) high investment in movement early in life reduces reproductive investment through energy constraint, and (ii) dispersers delay reproductive investment until after dispersal “in expectation” of finding a more favourable environment. Existing examples of delay in reproduction appear to be limited to flying insects and are attributed to energetic constraints (e.g., Zera & Zhao, 2006). However, in this case, the fact that total reproductive output is equal over both oviposition periods seems to lend support to the latter explanation. Similar findings have come from rhesus macaques (*Macaca mulatta*), where reproductive timing and dispersal are associated with the serotonin transporter gene genotype (Trefilov et al., 2000). This gene has been linked to aggressive behaviour and risk-taking, traits commonly seen as important elements of animal personality and behavioural syndromes (Roche et al., 2016). More research is required to uncover whether either i, ii, or both of these causes are driving reproductive timing differences between dispersal phenotypes, as well as the genetic and physiological mechanisms.

In our experiments focussed on development time, we observed no average difference in development time with dispersal phenotype over the whole laying period, in contrast to a previous study using *Tribolium*, which showed slower development of dispersers (Lavie & Ritte, 1978). However, we found that eggs laid later developed more slowly, and this effect was strongest in high dispersal lines. It is likely that this pattern is driven by the effects of cannibalism acting at different strengths across different temporal and spatial scenarios. Evidence is provided by a study in which populations selected for increased population size also showed increased cannibalism and shorter development times (Wade, 1979). A similar pattern has been seen in invasive cane toad populations (*Rhinella marina*), where tadpoles in high-density populations evolved cannibalistic feeding behaviour and, in response, reduced development time (DeVore et al., 2021). However, while these examples are suggestive, the situation in *Tribolium* is very complex, and to confirm a causative role of cannibalism, more information is needed on the relative strengths of different types of cannibalism (larvae eating eggs, larvae eating pupae, adults eating eggs, adults eating pupae) in different dispersal contexts. Interestingly, our results show some agreement with an experimental evolution study in *Tribolium*, where dispersal and other traits were allowed to coevolve as a population expanded across a landscape of connected habitat patches (Weiss-Lehman et al., 2017). Here, populations at the range front evolved high dispersal and low intrinsic growth rates. Our finding is that development time increases post-dispersal in dispersive lines, which might contribute to low growth rates in range-front populations.

We found that highly dispersive females had greater longevity than females from low dispersal lines but saw no difference among males. This result was opposite to that found in the only previous comparable study (Lavie, 1981; further discussed below). Among insects, most data on dispersal and longevity suggests that highly dispersive individuals have shorter life spans (Hanski et al., 2006; Gu et al., 2006). However, as with other traits, those studies on insects overwhelmingly focus on species where flight is the major mode of movement and differences in energetic output between dispersal phenotypes are likely to be much more important than in *Tribolium*. Indeed, the *Tribolium* dispersal phenotype has been shown previously to lack any association with a higher metabolic rate (Arnold et al., 2017), which might be expected to lead to early mortality (Lints, 1989). Our own experimental lines show differences in dispersal-enhancing traits that would not be expected to require higher metabolic output, such as straightness of movement and disinclination to burrow (chapter 4, Pointer et al. 2024). Rather, our findings fit better with the most common relationship between these traits more broadly across terrestrial animals, where longevity is higher in dispersers, potentially allowing a longer time in which to locate a suitable habitat patch (Stevens et al., 2014). This may be additionally explained by competitive pressures in *Tribolium*, where the particularly intense competition experienced by residents selects for competitive ability over the life span, and escape from this competition could allow longer life in dispersers. In other systems, highly competitive environments are shown to select for higher metabolic rates (Hack, 1997; Pettersen et al., 2020). That the effect is seen only in females is interesting. Male *T. castaneum* ordinarily live far longer than females, so the observed effect served to equalize the life span of the sexes in high dispersal lines. Possibly, female longevity is suppressed more than male longevity under high competition, for example, by the known effect of male harassment (e.g., Attia et al., 2023), and release from competition allows them to approach the greater life span of males.

Previous investigations into life history correlations with dispersal in *Tribolium* have either utilized poorly replicated selection lines generated under potentially unreliable methodologies (Ritte & Lavie, 1977; Lavie & Ritte, 1978; Lavie, 1981; Zirkle et al., 1988) or tested for associations with walking motivation as a proxy for dispersal, rather than dispersal per se (Matsumura & Miyatake, 2015, 2018). As well as serving as a robust, explicit test of a dispersal syndrome in *Tribolium*, we can compare the results of this study to previous work to assess the consistency of effects across independent studies using this system. Overall, our results conflict with those of earlier tests of life history traits using dispersal lines derived from Ritte and Lavie (1977), which found greater fecundity, faster development, and shorter life span among dispersers (Lavie & Ritte, 1978; Lavie, 1981; Zirkle et al., 1988). Some issues in the original selection experiment may contribute to these differences, as sample sizes were small (3 replicates per treatment vs. 16 in the present study), dispersal assays were conducted on groups of full siblings, and the strength of selection was highly inconsistent between generations (Ritte & Lavie, 1977). Nevertheless, it seems surprising that these methodological differences could flip the direction of relationships between traits. The present study finds greater agreement with work on lines selected for walking motivation by Matsumura and Miyatake (2015), who have subsequently shown that individuals with higher walking motivation have shorter matings and equal fecundity to those with lower walking motivation (Matsumura & Miyatake, 2018; Matsumura et al., 2019). In combination, data from these studies indicate that life history correlations in *Tribolium* are not necessarily robust to variations in methods used to generate and measure dispersal phenotypes. Consequently, the effects of all possible contributing factors must be considered when designing artificial selection regimes. We consider this study the most rigorous effort to date addressing these questions in the *Tribolium* system.

Taken together, our results suggest that correlations between life history traits and dispersal in *Tribolium* are qualitatively different from insects whose principal dispersal mode is by flight, where bioenergetic constraints are responsible for trade-offs. Instead, we suggest that the coevolution of life history and dispersal in *Tribolium* is shaped by dispersal, facilitating escape from intense intraspecific competition that characterizes the species' ecology (Dawson, 1977). It is important to note here that our investigations focus on emigration and walking movement as the mode of travel likely most relevant within temperature-controlled food storage facilities. It is possible that experimental selection on flight might uncover a set of correlations and trade-offs more akin to those seen in flying insects, but this is beyond the scope of the current study.

The observation of associations between traits inevitably raises questions about the mechanistic basis of such patterns. Genetic correlations between life history traits may be due to linkage-disequilibrium between causal loci or because of pleiotropy with key genes underlying multiple traits. Characterizing correlations in our *T. castaneum* lines opens up the potential for investigating the links between traits using molecular genomics. Current theory suggests that the genetic basis of *Tribolium* dispersal is relatively simple (Ritte & Lavie, 1977; chapter 3, Pointer et al., 2023) or at least involves only a few genes of large effect. Major effect loci implicated in dispersal and life history variation in insects are known (Saastamoinen et al., 2018), the best-studied example being the Pgi locus in the Glanville Fritillary butterfly (Klemme & Hanski, 2009; Niitepõld & Saastamoinen, 2017). However, the most comprehensive studies are in *Drosophila* and have identified hundreds of genes differentially expressed in common between lines independently artificially selected for aggression, mating behaviour and locomotor activity (Jordan et al., 2007). Resolving the genetic basis of dispersal and its association with life history variation may be highly instructive in considering how these traits evolve and how they may be managed.

Overall, our findings suggest that correlated intraspecific variation in local dispersal and related traits may represent alternative life history strategies in *T. castaneum*. We therefore suggest that pest management efforts to mitigate the species' agro-economic impact should consider the eco-evolutionary dynamics within multiple life histories. The benefits of doing so could be felt both through improved targeting of efforts to reduce spread and also in forecasting how the selection pressures applied through pest management are likely to affect pest evolution.

5.5 Data availability

Raw data files used in this study can be found on Dryad 10.5061/dryad.j6q573nn8 Representative image files can be found on Zenodo at https://doi.org/10.5281/zenodo.10852855.

5.6 Contributions

Michael D Pointer conceptualised the study with David S Richardson. MDP led the investigation and drafted the manuscript, assisted by DSR, who also administered the project. All authors reviewed the MS and commented on later versions.

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CHAPTER SIX

Genomic signatures of adaptation to artificial selection on dispersal in Tribolium castaneum; a model insect and agricultural pest

Supplementary material is provided as appendix 5

T. castaneum, false colour scanning electron micrograph (reproduced under CC BY-NC-ND 2.0 UK from Wellcome Images. Photographer: Kevin MacKenzie)

6.1 Abstract

Dispersal is key to the ecology and evolution of many organisms, and bears upon many of the largest challenges facing human society, including biosecurity, food security and biodiversity conservation. Consequently, understanding how dispersal evolves is an important aim of contemporary biology. Yet, the complexity of dispersal poses significant challenges and well-replicated studies in tractable systems are required to tease out evolutionary signatures. Here we utilise whole-genome re-sequencing of individuals from thirty-two lines of *Tribolium castaneum*, previously selected for divergent dispersal propensity, and displaying robustly repeatable behaviour, to identify the genetic architecture of adaptation to selection on dispersal. Using complementary genotype-phenotype association (BayPass) and divergence (F_{ST}) analyses, we identify regions of the genome associated with the phenotype. Results support a polygenic basis of dispersal, observing many candidates genes across the genome associated with the dispersal phenotype. The functions of candidates suggest that both metabolic and neurophysiological mechanisms contribute to dispersal evolution in this system.

6.2 Introduction

Dispersal is a complex life history trait with a critical role in the ecology and evolution of many species (Ronce 2007). Individual movements influence population size, density and range (Kokko and López-Sepulcre 2006), and thus the metapopulation dynamics that contribute to population expansion or to population persistence in fragmented landscapes (Clobert 2012; Legrand *et al.* 2017). Furthermore, via effects on patterns of gene flow, dispersal determines patterns of genetic variation within and among populations, with implications for their evolutionary trajectories (Holsinger and Weir 2009).

Across taxa, dispersal is also commonly seen as part of suites of coevolving phenotypes, referred to as behavioural syndromes (Clobert 2012). Individual variation in such syndromes may represent different life-history strategies (Sih *et al.* 2004). Patterns of association between these traits are complex and highly context-dependent, and the mechanistic basis of such associations is poorly understood (Clobert 2012). In addition to its effects on the evolution of other traits, recent work has revealed that under conditions such as range expansion, dispersal itself can rapidly evolve over short timescales (Ochocki and Miller 2017; Weiss-Lehman *et al.* 2017; Simcox *et al.* 2024). For example, dispersal evolution during the cane toad (*Bufo marinus*) invasion of Australia has accelerated the advance of the range front by fivefold in less than 100 years (Shine *et al.* 2021).

Dispersal is also an important aspect of evolutionary responses to anthropogenic climate change, habitat fragmentation, and the dynamics of biological invasions (Travis *et al.* 2013; Legrand *et al.* 2017; Renault *et al.* 2018). Thus, it is essential to understand the causes and consequences of dispersal evolution (Ronce 2007). Uncovering the genetic architecture of dispersal traits will clarify how variation underlying dispersal is maintained in populations, aid in elucidating the basis of behavioural syndromes, inform our ability to trace how molecular variation leads to phenotypic differences in movement patterns, and enable predictions around the adaptive potential of dispersal and its consequences (Saastamoinen *et al.* 2018).

The genetic architecture of a trait is key to how the trait responds to selection (Pritchard and Di Rienzo 2010; Le Corre and Kremer 2012). For example, in sexually reproducing organisms, a larger number of causative loci is expected to increase the evolutionary rate of traits, due to increased potential for recombination (Goddard *et al.* 2005; Pritchard *et al.* 2010). However, simulation studies modelling the rate of dispersal evolution under different architectures have shown conflicting results (Saastamoinen *et al.* 2018; Weiss-Lehman and Shaw 2022). The genetic basis of dispersal-related traits has been described in a range of species, revealing highly varying genetic architectures (Saastamoinen *et al.* 2018; Dochtermann *et al.* 2019). Dispersal is usually thought to be a polygenic trait (Merilä and Sheldon 1999; Pritchard and Di Rienzo 2010), an idea supported by work in insects and vertebrates (Jordan *et al.* 2012; Saatoglu *et al.* 2024). However, in some taxa there are large-effect loci influencing dispersal (Saastamoinen *et al.* 2018), which can be separated into those with metabolic (Niitepõld and Saastamoinen 2017) or neurophysiological (Sokolowski 1980; Trefilov *et al.* 2000; Fidler *et al.* 2007; Krackow and König 2008; Anreiter and Sokolowski 2019) effects on movement. Notable examples include the *For* gene (*Foraging*; and its homologues), a neuro-signalling regulator linked to movement behaviour in taxa from *C.elegans* to humans (reviewed in Anreiter and Sokolowski 2019); and *Pgi*, which underlies phenotypic variation in flight metabolism and dispersal propensity in wild populations of butterflies (reviewed in Niitepõld and Saastamoinen 2017).

Genomic sequencing of individuals across populations that differ in dispersal phenotypes can be used to identify regions of the genome associated with adaptive evolution, by scanning the genome for characteristic patterns of DNA polymorphism (Hahn 2018). Between-population F_{ST} is used to identify regions of high divergence (Hahn 2018; Sætre and Ravinet 2019). Additional metrics such as π and Tajima's D can summarise variations and provide additional evidence of selection. However, some such approaches rely on assumptions about the demographic and temporal context of selection that may be violated in experimental scenarios. When seeking the genetic basis of a particular trait, genome-wide association studies based on single marker regression can be powerful tools (Korte and Farlow 2013; Santure and Garant 2018), but require individual-level phenotypes and large sample sizes to detect all but the strongest quantitative trait loci (Hayes 2013). An alternative approach is to use methods developed to test for links between genotype and environment (Coop *et al.* 2010), but which are also applicable to population-level genotype-phenotype associations (GPA). Early implementations of these GPA models were effective at finding selected loci but suffered from high false discovery rates because they did not consider the contribution of neutral demographic processes to observed associations (De Mita *et al.* 2013). Subsequent methods that account for processes such as gene flow and drift have far lower false discovery rates while retaining the ability to detect selection, especially when population structure is non-hierarchical, or adaptation is polygenic (de Villemereuil *et al.* 2014; Lotterhos and Whitlock 2015). Modern implementations of GPA models such as BayPass (Gautier 2015) have successfully identified genomic associations with many environmental, phenotypic and experimental conditions, including climate (Stonehouse *et al.* 2024), invasiveness (Olazcuaga *et al.* 2020), urbanness (Babik *et al.* 2023) and pathogen prevalence (Sheppard *et al.* 2022, 2024).

Whole genome re-sequencing of replicated populations under experimental evolution regimes (Schlötterer *et al.* 2015), allows powerful statistical methods to be leveraged to resolve genotype-phenotype associations (Coop *et al.* 2010; Gautier 2015; Olazcuaga *et al.* 2020). The emergent genomic model *Tribolium castaneum* is highly suited to experimental evolution studies (chapter 2, Pointer *et al.* 2021; Campbell *et al.* 2022). The species is a globally significant pest, responsible for large economic losses and impacts on food security (Phillips and Throne 2010), and consequently its dispersal ecology is of great applied interest. Previous work on *T. castaneum* has indicated that dispersal may be a component of a behavioural syndrome, covarying with key life-history traits (Lavie and Ritte 1978; Zirkle *et al.* 1988; chapter 5, Pointer *et al.* 2024). Individual-level dispersal variation seems to be driven by activity levels and movement patterns (chapter 4, Pointer *et al.* 2024), suggesting that, as in other systems, the phenotype may stem from differences in neurophysiology and/or metabolism. Some genes whose expression covaries with walking motivation have been identified (Matsumura *et al.* 2024), yet no study to date has investigated the molecular genomic basis of dispersal in this system.

Here we utilise whole genome re-sequencing of individuals from replicated (n=32) lines of *T. castaneum*, previously selected for divergent dispersal propensity, and displaying robustly repeatable behaviour (chapter 3, Pointer *et al.* 2023; chapter 4, Pointer *et al.* 2024), to identify the genetic architecture of adaptation to selection on dispersal. We employ BayPass, leveraging the power of the study's population-level replication, to highlight SNPs significantly associated with dispersal selection regimes. We complement this nuanced, line-level analysis with an F_{ST} approach to identify patterns of divergence between individuals pooled within each selection regime. We then functionally enrich candidate genes to explore how genetic variation might be linked to dispersal. We find many genes associated with the dispersal phenotype, supporting a polygenic trait architecture. The functions of candidate genes suggest that both metabolic and neurophysiological mechanisms contribute to dispersal evolution in this system.

6.3 Methods

6.3.1 Beetles and husbandry

The Krakow super-strain (KSS) of *Tribolium castaneum* flour beetles was created by combining 14 laboratory strains from across the world (Laskowski et al. 2015). This strain has been maintained at a census size of 600 individuals for ~150 generations. All beetle populations throughout were kept on a fodder medium of 90% organic flour 10% brewers yeast, on a constant 12:12 light:dark cycle, with relative humidity of 60% and a regime of non-overlapping generations. At 12±3 days post eclosion, adults are sieved from the fodder and a randomly chosen subset are combined in fresh fodder to begin a seven-day mating and oviposition period, after which adults are removed. Eggs remaining in the fodder then develop over a 35-day development period. By preventing any interaction between sexually mature adults and offspring this method reduces the risk of negative density-dependent effects, removes the opportunity for intergenerational interactions, such as egg-cannibalism, and allows accurate tracking of passing generations.

6.3.2 Artificial selection for dispersal

Thirty-two experimental lines were founded from KSS stock and artificially selected for dispersal propensity as described by Pointer et al. (chapter 3, 2023). Briefly, high (n=16) and low (n=16) dispersal lines were bred under divergent artificial selection over five generations, using a dispersal assay in which each individual, housed within groups of 200, was given three opportunities to 'disperse': i.e. leave a patch of suitable habitat, cross a short distance of unsuitable habitat and not return. Individuals that dispersed three times out of the three opportunities were considered to display a dispersive phenotype. Individuals that dispersed zero times were considered to display a non-dispersive phenotype. Thirty individuals of each of these phenotypes were selected to sire the subsequent generation of the relevant treatments, while individuals of intermediate phenotype were discarded.

After a single generation of selection, the mean dispersal phenotype (mean number of dispersals per individual out of three opportunities) between the treatments was significantly different. After five generations of selection, the distribution of the dispersal phenotype between the two treatments was non-overlapping (chapter 3, Pointer *et al.* 2023). Between generations six and 16, a less stringent selection regime was applied. In odd-numbered generations a reduced assay, which allowed a single dispersal opportunity, was used to phenotype the dispersers and non-dispersers, of which 30 of the relevant type were selected to parent the subsequent generation of each line. In odd-numbered generations 100 randomly chosen individuals parented the next generation. In generation 17, dispersal was quantified and found to be still strongly divergent and non-overlapping between treatments (chapter 4, Pointer *et al.* 2024); dispersals per individual out of a maximum of three, low dispersal lines = 0.70±0.06; high dispersal lines = 2.44±0.04).

6.3.3 Sample preparation and sequencing

In generation 17, samples were collected for sequencing from dispersal selection lines, to investigate genomic differences underlying the phenotype, and from the ancestral KSS population, to examine change in response to selection. Adult females were sampled and flash-frozen in liquid nitrogen. For each individual, DNA extraction was conducted using the DNeasy blood and tissue kit (insect tissue protocol, Qiagen), with the whole individual ground in liquid nitrogen. The extract was then purified using a 1x bead cleanup with the AMPure XP SPRI protocol (Beckman Coulter). Library preparation and sequencing were performed at the Earlham Institute (Norwich, UK) using the low-input transposase-enabled (LITE) pipeline (see supplementary methods). Samples were then sequenced on two S4 flowcells over two lanes on the Illumina Novaseq 6000 platform. Sequences were obtained from 210 individuals, six from each of 32 dispersal lines and 18 from the KSS stock.

6.3.4 Variant calling and filtering

Reads were trimmed using Trimmomatic v.039 (Bolger, Lohse and Usadel, 2014) and mapped to the T.cast5.2 reference genome (10.1186/s12864-019-6394-6), using BWA-MEM v0.7.17 (Li, 2013). Mapping was followed by SAMtools v1.18 fixmate and sort to ensure BAM files were correctly formatted and sorted prior to removing duplicates (Danecek et al., 2021). PCR duplicates were then removed using Picard v2.26.2 RemoveDuplicates (Broad Institute, 2019). Finally, mappings were filtered for complete read pairs and those with a mapping quality (MAPQ) >25 using SAMtools view.

Joint genotyping was conducted using BCFtools v1.18.0 mpileup (Danecek et al., 2021). BCFtools call was then used to call all sites under the multi-allelic model (-m). BCFtools filter was used to remove variants within 3bp of other variants, with a variant quality score < 30, that were at a locus with sequencing depth less than 578 and greater than 5201 (+/- 3 times average sequencing depth), and were represented by data at that locus in less than 50% of individuals (-g 3 -G 3 -e 'DP < 578 || DP > 5201 || F_MISSING > 0.5 || QUAL < 30'). The resulting file is referred to hereafter as the allsites vcf.

At this point, from the allsites vcf, single nucleotide polymorphisms (SNPs) were extracted using BCFtools view. At this stage, they were further filtered to remove sites with minor allele count <3. This file is referred to hereafter as the SNP vcf.

To prepare the allsites vcf for further analysis, variant and invariant sites were handled separately: Invariant sites were extracted using VCFtools 0.16.0 (Danecek *et al.* 2011), and stored in a separate file. Variant sites were filtered using VCFtools so as to only contain biallelic SNPs that did not deviate significantly from Hardy-Weinberg Equilibrium (HWE p-value < 0.001). Following this filter, invariant and variant sites were concatenated using BCFtools index and concat. This file is referred to hereafter as the filtered allsites vcf.

QC of the SNP vcf revealed three individuals (10HT, 11HT3, 11HT4) to exhibit huge counts of singleton SNPs and indels compared to other samples. As this is likely a sign of sequencing error, we excluded reads from these samples from the raw sequence data and reran the above steps to regenerate both the SNP vcf and the allsites VCF.

6.3.5 Linkage disequilibrium

The SNP vcf was down-sampled to 1 SNP per 0.5kb and used to calculate pairwise linkage disequilibrium (LD) between SNPs up to a maximum distance of 5Mb, using VCFtools. As per-line sample sizes were not sufficient to reliably estimate LD, estimates were obtained from ‘populations’ consisting of all individuals from within each treatment (high dispersal, low dispersal, KSS) and within all treatments combined. Using a custom R script, we calculated mean LD within distance bins of 1kb and plotted this to visualise LD in the data (appendix 5 figure S1). In each treatment LD halved from the maximum at ~50kb, we therefore took this as a window size with which to begin to examine patterns across the genome.

6.3.6 Population structure

Principal component analysis (PCA) was performed using plink (v1.9; Purcell *et al.* 2007) to assess how genetic variation between samples was partitioned across replicate populations, and across dispersal selection treatments. The SNP vcf was pruned for linkage with bcftools (+prune -m 0.3 -w 50kb) before PCA with plink2 (Chang *et al.* 2015).

6.3.7 Identification of candidate loci

We performed a genome-wide scan for selection using *BayPass* v2.4 (Gautier 2015), implementing a Baysian framework sensitive to demography. BayPass estimates a background allele frequency (omega) matrix across populations, to account for the confounding effect of demography which can frustrate the identification of selected variants (Günther and Coop 2013; Gautier 2015). This approach allowed us to control for unquantified differences in relatedness among individuals used to found each selection line. The BayPass model uses the omega matrix to correct for neutral correlations when testing allele frequencies for population divergence or association with environmental/trait variables. Within BayPass, we utilised the contrast statistic, C_2, which contrasts SNP allele frequencies between two groups of populations specified by a binary trait (Olazcuaga *et al.* 2020). This method outperforms others in identifying SNPs under selection in scenarios analogous to our experiment (Olazcuaga *et al.* 2020). We computed C_2 across 32 dispersal lines, with the dispersal selection treatment as the binary covariable.

To avoid the impact of small, annotation-sparse, unplaced scaffolds in the reference genome, we ran BayPass on the 10 linkage-group-level scaffolds. We computed the BayPass background matrix using a curated subset of independent, high confidence, highly representative, putatively neutral SNPs which afforded the best opportunity to estimate the neutral covariance in allele frequencies across the genome (omega dataset; see supplementary methods). The omega dataset consisted of 12,232 non-exonic SNPs, evenly representing the genome. The dataset used in BayPass analysis runs contained a less stringently filtered set of 3,240,899 sites, derived from the SNP vcf (see Supplementary Methods).

We performed two independent Baypass runs with different random seed initiators and computed correlations to test the consistency of model performance with our data (Olazcuaga *et al.* 2020; Dickson *et al.* 2020). The C_2 estimates were calibrated using a pseudo-observed dataset (POD; Gautier 2015; see supplementary methods). The 0.999 quantile of C_2 values from the POD analysis was used as the outlier threshold for empirical C_2 values. C_2 candidate regions were defined as those containing >=2 outlier SNPs separated by <50kb, and C_2 candidate SNPs were outlier SNPs within candidate regions (Gautier 2015).

We computed between-treatment F_{ST} by pooling data from samples representing each of the 16 lines within each selection treatment, to form two larger treatment-level ‘populations’: high and low dispersal. We computed the weighted Wier & Cockerham F_{ST} between these populations in both 10kb and 50kb windows with *pixy* (Korunes and Samuk 2021), from the allsites VCF. Though this method lacks the resolution of data at the line level, it offers a complementary approach that aims to identify strong signal from regions differing consistently between dispersal treatments. and remove noise associated with having low sample sizes per replicate population. Directly neighbouring F_{ST} outlier windows were combined to form contiguous F_{ST} candidate regions. We considered outlier F_{ST} windows above the 0.99 and 0.999 quantile to test whether C_2 outliers were also F_{ST} outliers.

Nucleotide diversity (π) and absolute nucleotide divergence (D_{xy}) were computed in 10kb and 50kb non-overlapping windows along the genome using *pixy*, from the allsites VCF. Tajima’s D was computed in the same windows (vcftools --TajimaD). F_{ST}, π, D_{xy} and Tajima’s D in the regions around candidate SNPs were visualised using a custom R script that smoothed the curve by taking a rolling mean across five consecutive windows. Aggregate genome-wide π was computed as the mean across all 10kb windows from linkage-group level scaffolds.

6.3.8 Characterisation of candidate genes

Genes associated with selection candidates were identified by intersecting their positions with the annotation (Tribolium_castaneum.T.cas5.2.59.gff3; bedtools *intersect*). To obtain a holistic picture of selection, and contrast the findings of GPA and F_{ST} methods, we used five different sets of candidates as input: 1) F_{ST} outlier regions that overlapped with a C2 candidate region; 2) candidate SNP positions, returning genes containing a candidate SNP; 3) C_2 candidate region locations, returning all genes overlapping with C_2 candidate regions; 4) F_{ST} candidate region locations, returning all genes overlapping with F_{ST} candidate regions; 5) Genes within the highest F_{ST} windows of peaks significant at the 0.999 quantile threshold. We used the returned gene lists as input to g:Profiler (Reimand *et al.* 2007;

Kolberg *et al.* 2023) to test for enrichment of functional terms derived from gene ontology (GO). All other settings were the G:profiler defaults, the background used was all genes in the *T. castaneum* genome. The OSG3 annotation (https://ibeetle-base.uni-goettingen.de/download/species/Tcas/OGS3.gff.gz) and the iBeetle-Base database (Dönitz *et al.* 2018) were used to manually identify gene functions, and orthologous genes in *Drosophila* melanogaster were subsequently researched using Flybase (Öztürk-Çolak *et al.* 2024).

6.4 Results

6.4.1 DNA sequencing

Following adapter trimming, 199,248 - 32,028,177 reads per sample were mapped to the Tcas5.2 reference. Following removal of PCR duplicates and quality filtering 106,340 - 13,399,500 reads per sample remained, representing 0.09 - 11.83x mean coverage (appendix 5 table S1). The filtered allsites vcf contained 37,840,898 sites and the SNP vcf contained 4,418,680 SNPs.

6.4.2 Linkage disequilibrium

Linkage disequilibrium (LD) was similar in high dispersal, low dispersal and all treatments combined, peaking at Ca. R^2=0.2, and halving from the maximum at 0.6-0.7Mb (appendix 5 figure S1A;B;C). The KSS control samples had the highest LD, with a peak of 0.25 and halving at ~1.3Mb (appendix 5 figure S1D), although this estimate is less reliable due to smaller sample size (n=18 vs n>=93).

6.4.3 Population structure

An LD pruned and MAF filtered set of 223,034 SNPs was used to perform PCA. The scree plot of variance explained by each principal component (PC) showed a drop off in explanatory power after the first two PCs (appendix 5 figure S2A), which explained 6.15% and 5.95% of the total variance respectively. Samples from the same replicate line clustered closely together (appendix 5 figure S2B). PC1 was most associated with the division between dispersal treatments (appendix 5 figure S2C), with low dispersal lines having lower PC1 values than high dispersal lines, and KSS samples being intermediate between them.

6.4.4 Identification of candidate loci

Visualising the background allele frequency, or omega, matrix, generated by using BayPass on a set of 12,232 putatively neutral SNPs, showed no genome-wide pattern of relatedness across individual replicate lines (appendix 5 figure S4). BayPass analysis showed high repeatability, with the C_2 estimates associated with SNPs being highly correlated across two replicate runs with different starting seeds (Pearson's r = 0.964). Of the 3,240,899 sites in the dataset, we identified 267 SNPs as C_2 outliers, with representation of all ten linkage groups from the assembly (figure 1A). Linkage groups two, three and four contained the most outlier SNPs (44, 93 and 32 respectively). Following grouping SNPs following Galthier (2015), we recover 22 candidate regions for association with dispersal, representing 256 candidate SNPs (appendix 5 figure S5).

Calculation of F_{ST} using 10kb and 50kb windows gave similar results (appendix 5 figure S6). Therefore, we opted to take forward the 10kb output, to use the greater resolution it afforded to identify functional regions. Mean windowed F_{ST} between pooled high and low dispersal samples reached values of 0.18 (figure 1B), indicating regions of the genome displaying consistent differences in allele frequency between dispersal treatments. We identified 17 regions of elevated F_{ST}, using a 0.99 quantile threshold (figure 1C). Using a more stringent 0.999 quantile threshold gave five significant peaks, on linkage groups 2, 3, 10 and X.

A single candidate region from BayPass (chrLG2:9994244-9994716) was also located within an F_{ST} outlier window, under both thresholds. This region contained three SNPs separated by 472bp, lying within an intron of the gene TC001033 (figure 1Dii). The F_{ST} outlier region that overlapped the candidate region covered ~200kb and incorporated the highest F_{ST} values genomewide.The remaining population genetic statistics computed, π, D_{xy} and Tajima's D, showed no diagnostic patterns across candidate regions. Genome-wide π in the ancestral KSS population, averaged across 10kb windows was 0.018, se = 8.47×10^{-5}.

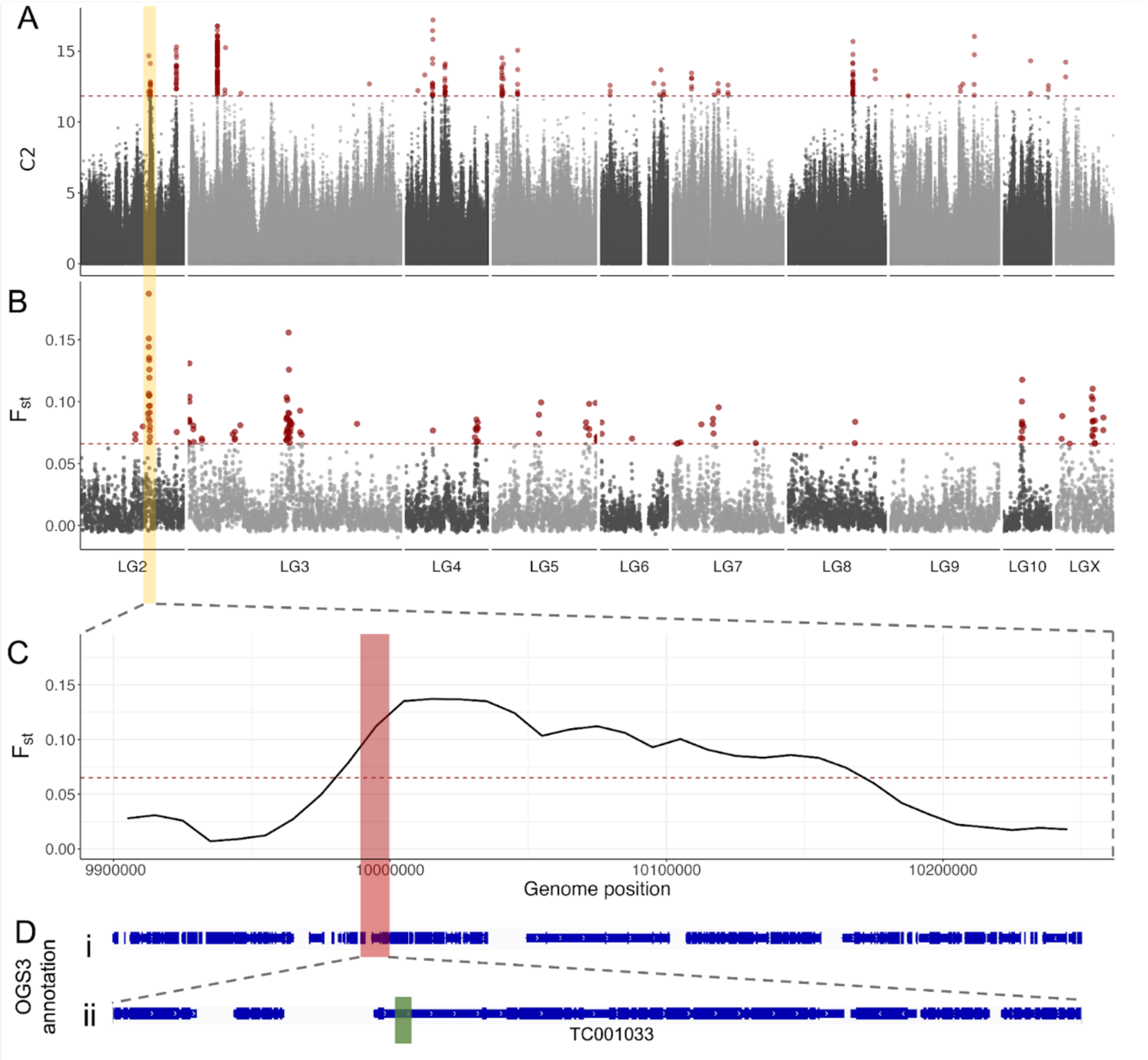

Figure 6.1. Selection scan for association with dispersal. Genomic variation associated with divergent artificial selection for dispersal propensity in 32 independently evolving lines of *Tribolium castaneum*. A) Values of the C_2 statistic generated by BayPass analysis as a measure of a SNP's association with the direction of selection. Red points are in excess of the 0.999 quantile of C_2 values from a BayPass run on a pseudo-observed dataset of putatively neutral SNPs, shown by the dotted line. B) 10kb windowed mean F_{ST} between all individuals from each dispersal selection regime considered as a single population (i.e. Combining the 16 replicate lines in each treatment). Red points are in excess of the 0.99 quantile of F_{ST} values genomewide. Yellow shading in A and B indicates the region identified as outlier in both BayPass and F_{ST} analyses. C) An expanded view of F_{ST} across the yellow shaded region. The plotted line represents a rolling mean of F_{ST} computed across five 10kb windows. Red shading indicates the local C_2 candidate region, ±5kb for visibility. D) Tcas5.3 OGS3 gene annotations, i) across the extent of the elevated F_{ST} region shown in C, and ii) around the local C_2 candidate region. Green shading indicates a 472bp intronic section of TC001033, containing the top three outlier SNPs of the C_2 peak.

6.4.5 Characterisation of candidate genes

Using a strict positional intersection between the Tcas5.2 annotation and the 256 C_2 candidate SNPs showed that 217 fell within genes, 70 in exons and 147 in introns. Returning all genes overlapping with candidate regions identified 104 unique genes (appendix 5 table S2).

GO analysis was performed independently on five sets of genes. Genes containing candidate SNPs were enrichment for functions related to DNA topological change (table 2), specifically activity of topoisomerase enzymes involved in regulating torsional stress and tangling in DNA (McKinnon 2016). Genes within C2 candidate regions were enriched for 15 enriched terms, across molecular functions, biological processes and KEGG pathways, with the most significant being related to peptidase activity, specifically serine-type endopeptidases (table 6.1). Genes overlapping F_{ST} candidate regions were enriched for UDP-glucose 6-dehydrogenase activity, an enzyme crucial to carbohydrate metabolism. Genes within the strongly divergent region on LG2 were enriched for functions related to the synthesis of amino acids, specifically L-proline via the activity of Pyrroline-5-carboxylate reductase. The top F_{ST} windows of the most highly significant peaks did not show functional enrichment, however the eight genes they contained may have been too few to analyse effectively. Strikingly, the majority of these genes (TC001035, TC001034, TC003206, TC010853, TC010535) have *Drosophila* homologs (swiss cheese, noisette, highwire, CG42673, casein kinase IIα, firelighter) known to be involved in either energy metabolism or neuronal functioning linked to locomotor behaviour.

Table 6.1. Gene ontology analysis using G:profiler. Functional enrichment among genes in candidate regions located by BayPass and F_{ST} outlier analyses between two sets of *Tribolium castaneum* populations artificially selected for divergent dispersal propensity.

Gene set	GO/KEGG category	GO/KEGG term	P_{adj}
Genes containing candidate SNPs (n=225)	GO:MF	DNA topoisomerase type I (single strand cut, ATP-independent) activity	<0.001
	GO:MF	DNA topoisomerase activity	<0.05
	GO:BP	DNA topological change	<0.05
Genes in C_2 candidate regions (n=104)	GO:MF	Serine-type endopeptidase activity	<0.001
	GO:MF	Serine-type peptidase activity	<0.001
	GO:MF	Serine hydrolase activity	<0.001
	GO:MF	Endopeptidase activity	<0.001
	GO:MF	Peptidase activity	<0.001
	GO:MF	DNA topoisomerase type I (single strand	<0.05
	GO:BP	Blood coagulation	<0.01
	GO:BP	Hemostasis	<0.01
	GO:BP	Coagulation	<0.01
	GO:BP	Reproduction	<0.01
	GO:BP	Wound healing	<0.01
	GO:BP	Regulation of body fluid levels	<0.01
	GO:BP	Multicellular organism reproduction	<0.05
	GO:BP	Response to wounding	<0.05
	KEGG	ABC transporters	<0.05
Genes in F_{ST} candidate regions (n=258)	GO:MF	UDP-glucose 6-dehydrogenase activity	<0.05
Genes in F_{ST} peak region on LG2 (n=37)	GO:MF	Pyrroline-5-carboxylate reductase activity	<0.01
	GO:BP	L-proline biosynthetic process	<0.05

	GO:BP	L-amino acid biosynthetic process	<0.05
	GO:BP	Proteinogenic amino acid biosynthetic	<0.05
	KEGG	Biosynthesis of amino acids	<0.05

6.5 Discussion

We set out here to use replicated lines of flour beetles, artificially selected for differential dispersal behaviour, to undertake the first study of the genomic basis of this trait in *Tribolium*. Using whole genome resequencing with genotype-phenotype association (GPA) and F_{ST} approaches, we identify candidate loci contributing to the evolution of dispersal in this system.

Previous work on dispersal in *T. castaneum* suggests that the trait has a relatively simple genetic basis in this species (chapter 3, Pointer *et al.* 2023), possibly a single locus of large effect (Ogden 1970; Ritte and Lavie 1977). In the present study, only one gene (TC001033) was found to be associated with dispersal, by both the GPA (BayPass) and F_{ST} analyses. However various other loci were also identified as being associated with dispersal treatment by one of the two methods. The *D. melanogser* homolog of TC001033 (multiple ankyrin repeats single KH domain; *mask*) is known to enable protein kinase binding activity, and be involved in several biological processes including eye formation via regulation of the sevenless pathway. It also plays a role in mitochondrial functioning, linked to muscular maintenance and flight performance in adult flies (Smith *et al.* 2002; Zhu *et al.* 2015; Katzemich *et al.* 2015). Given that dispersal propensity is known to covary with activity in the beetle lines used in this study, it seems highly plausible that variation at the *mask* locus could contribute to driving the phenotype via mitochondrial performance - as seen with the *pgi* locus in Lepidoptera (Niitepõld *et al.* 2009; Niitepõld and Saastamoinen 2017).

Though we find a single gene with the strongest support for association with dispersal, in tentative support of a single-gene theory of *Tribolium* dispersal evolution, we also identify many other dispersal-associated genes across the genome. It is possible that selection initially drove divergence at a single causal locus, and at its physical neighbours through linkage, with these changes leading to a subsequent re-tuning at many other loci across the genome, generating their secondary association with dispersal. Sequencing data were obtained 17 generations after the onset of selection, providing time for this process to occur. However, it seems more likely that the response to selection was polygenic and many loci are driving the phenotype. The strength of signals from GPA exceeded that of *mask* in many cases and, while it was the highest genomewide, the F_{ST} around *mask* was relatively small to account for a large difference in phenotype. With our current data it is not possible to definitively distinguish between scenarios. Furthermore, whilst a rapid response to selection (as seen in our selection lines) was suggested by Ritte and Lavie (1977) to be an indicator of a simple genetic basis, recent theory indicates that rapid phenotypic change can result from relatively small shifts across many loci, especially under a strong novel selection pressure (Pritchard and Di Rienzo 2010; Jain and Stephan 2017).

We may have further evidence to support a polygenic basis. The candidate regions we identified with GPA and F_{ST} were not associated with signatures in pi, D_{xy} or Tajima's D, population genetic parameters traditionally used as secondary indicators of selection. The lack of signal in Tajima's D is likely due to the nature of the experiment; the statistic relies on assumptions about the demographic and temporal context of the selection (Hahn 2018; Sætre and Ravinet 2019). Due to the extremely recent short-duration selection on our populations, these assumptions are unlikely to be satisfied and thus the characteristic signature won't be present (Saetre and Ravinet 2019). The strongly negative local Tajima's D expected following a selective sweep is due to the accumulation of new, low-frequency mutations after the sweep, expected even if the selection was relatively 'recent' in evolutionary time (Hahn 2018; Sætre and Ravinet 2019). Selection on our lines was very recent (17-12 generations before sequencing), and the populations of sufficiently small size, that we would not expect to observe new mutations within this timeframe. Consequently we do not consider an absence of negative Tajima's D to be evidence of a lack of selection, even under a sweep scenario. However, we expect π and D_{xy} to reflect the loss of variation around a sweep, even under extremely rapid recent selection. Consequently, a lack of signal in these parameters supports a polygenic response to selection, via small shifts in allele frequency, preserving variation surrounding selected sites and not suppressing local measures of π and D_{xy}.

Given that we accept the weight of our evidence suggesting that multiple loci are associated with dispersal, then our results agree with findings from other insect and vertebrate systems, showing a complex genetic basis of dispersal-related traits. Utilising the Drosophila Genetic Reference Panel, Jordan *et al.* (2012) identified 192 genes associated with locomotion. Similarly, dispersal in the House sparrow (*Passer domesticus*) is polygenic, with a complex basis involving both genes and environment (Saatoglu *et al.* 2024). Comparing our results to the only other genetic analysis of *Tribolium* dispersal showed no overlap in the genes we identify with those seen to differ in expression between lines selected for high- and low-walking behaviour (Matsumura *et al.* 2024). However, results from that study may not have well-represented the phenotype due to a lack of specificity in both the tissue types and the life-stage examined.

Taking into consideration all the loci we identified as associated with dispersal through either method (GPA or F_{ST}) provides a set of candidate genes. These genes showed functions (through *Drosophila* homology) plausibly related to dispersal via both metabolic and neurophysiological mechanisms, as seen in other insects (Saastamoinen *et al.* 2018). Metabolism was implicated through *mask*, and, while topoisomerase activity (to maintain cellular function via DNA maintenance) and UDP-glucose 6-dehydrogenase are involved in various cellular processes, important under metabolic/oxidative stress (Egger *et al.* 2011; Chen *et al.* 2013; Milano *et al.* 2024). The high F_{ST} region around *mask* was associated with proline synthesis. Proline is used by many insects to fuel highly energy intensive activity, such as locomotion or flight (Bursell 1981). Knockdown of a gene related to proline metabolism inhibited dispersal in the Colorado potato beetle (*Leptinotarsa decemlineata; Wan et al. 2015)*, providing further support for a role for energy metabolism in the dispersal phenotype investigated here. We also identify genes with neurological and neuroendocrine functions, such as development and activity of brain structures, cell signalling, sensory perception and response to sensory cues (*Swiss cheese*, Dutta *et al.* 2016; *Fzr*, Kaplow *et al.* 2008; *Tace*, Zang *et al.* 2022; *Ras85D*, Sepp and Auld 2003; *Tmhs*, Cosetti *et al.* 2008; *PsGEF*, Zwarts *et al.* 2015). Among these, *Fzr*, *CkIIα* (*casein kinase IIα*; Lin *et al.* 2002; Akten *et al.* 2009; Zwarts *et al.* 2017) and *Highwire* (Strauss 2002; Huang *et al.* 2012) are known to affect locomotor behaviour, memory and aggression in flies. Such findings are intriguing, as they link to key traits within dispersal and broader behavioural syndromes, thought to covary via shared neuroendocrine mechanisms (Sih *et al.* 2004). While further investigation is needed to make robust mechanistic conclusions, it is already known that the focal beetle lines differ in traits such as boldness, and movement pattern (chapter 4, Pointer *et al.* 2024).

Precisely characterising polygenic adaptation is extremely challenging (Wellenreuther and Hansson 2016), however the model system and experimental setup we employ here could be used powerfully to this end. To describe a trait as having a polygenic architecture tells us how many loci might affect it. However this does not define what the response to selection might be in terms of the contribution of individual alleles, the redundancy in their effects, or their repeatability across multiple replicate populations - parameters known as the adaptive architecture of a trait (Barghi *et al.* 2020). The best opportunity to study adaptive architecture is afforded by time-series data from highly replicated experimental evolution studies (Schlötterer *et al.* 2015; Barghi *et al.* 2020). An extension of the dispersal selection experiment we undertake here, using pooled sequencing to obtain a robust time-series of allele frequencies, would provide an excellent system to study the adaptive architecture of dispersal.

Having identified candidate loci for association with dispersal, the logical next step is to characterise their impact on the trait. *Tribolium* are amenable to genetic manipulation tools (Posnien *et al.* 2009; Gilles *et al.* 2015). It would be simple to use RNAi knock-down to silence the expression of a candidate gene and thus observe its broad effect on the phenotype. However, using CRISPR to knock-in individual variants of interest would allow more subtle insights into their functions, while retaining the gene's native context (Wang and Doudna 2023). Knock-ins to isofemale lines would isolate the variants on consistent genetic backgrounds and allow robust quantification of their effects on dispersal.

Many of the genes identified had known functions related to the development of sensory organs and/or responses to sensory cues. The importance of chemical signalling and density-dependent dispersal to *Tribolium* is well known (King and Dawson 1972; Ziegler 1978; El-Desouky *et al.* 2018). Hence, it would be interesting to investigate whether selection for high/low dispersal has altered the shape of the dispersal-density relationship. Similarly, many candidate genes were linked to flight phenotypes in other insects, and it seems a reasonable hypothesis that these loci would also affect flight performance in

Tribolium. Testing this might be a step towards integrating flight (long distance dispersal) with the far more advanced study of *Tribolium's* short-distance dispersal, necessary to gain a more complete understanding of dispersal in this system.

We recover candidate loci across the genome showing associations with dispersal. Enrichment for functions related to metabolism and neurophysiology indicate the molecular basis of how selection acts on dispersal in this system. The data and approaches we use here prime the field to develop a more complete understanding of the eco-evolution of dispersal, including through the avenues for future research that we highlight.

6.6 Contributions

This work was led by Michael D Pointer with support from David S Richardson and Will J Nash. MDP led the investigation with support from WJN. Lewis G Spurgin and Mark McMullan provided conceptual input, and the project was administered by DSR with Simon Butler.

Sequencing was performed at the Earlham Institute, funded by a Biotechnology and Biological Sciences Research Council (BBSRC) studentship to MDP (BB/M011216/1) and provided via the Core Capability Grant BB/CCG2220/1 at the Earlham Institute and its constituent work packages (BBS/E/T/000PR9818 and BBS/E/T/000PR9819), and the Core Capability Grant BB/CCG1720/1 and the National Capability at the Earlham Institute BBS/E/T/000PR9816 (NC1—Supporting EI's ISPs and the UK Community with Genomics and Single Cell Analysis), BBS/E/T/000PR9811 (NC4—Enabling and Advancing Life Scientists in data-driven research through Advanced Genomics and Computational Training), and BBS/E/T/000PR9814 (NC 3 - Development and deployment of versatile digital platforms for 'omics-based data sharing and analysis). Authors also acknowledge support from BBSRC Core Capability Grant BB/CCG1720/1 and the work delivered via the Scientific Computing group, as well as support for the physical HPC infrastructure and data centre delivered via the NBI Computing infrastructure for Science (CiS) group. Part of this work was delivered via the BBSRC funded National Bioscience Research Infrastructure (BBS/E/ER/23NB0006)

at Earlham Institute by members of the Technical Genomics and Core Bioinformatics Groups.

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CHAPTER SEVEN

General discussion

T. castaneum scales a mesh (reproduced under a CC licence, photographer: Victor Engel)

7.1 Overview

In this thesis, I have explored dispersal in *Tribolium castaneum* flour beetles. Building upon existing research, using more robust sample sizes, modern computational methods and molecular genetic techniques, I aimed to extend our understanding of the evolution of short-distance dispersal in this system. In this final chapter, I bring together the main results from each earlier chapter to discuss what my results suggest about dispersal in *Tribolium*, their wider relevance to the field and some possible directions for future research.

Chapter two aimed to collate research from across the long use of *Tribolium* as a model system. I produced a review which now serves as a resource for the Tribolium research community (published in Heredity in 2021 with 65 citations at the time of writing) and provides a base of eco-evolutionary knowledge on which subsequent investigation of dispersal in this species can be grounded. In chapter three I aimed to take a quantitative genetic look at the genetic architecture of this change in dispersal. The lines I bred showed a strong genetic basis of the trait, as evidenced by the rapid response to artificial selection. Simulation modelling and crosses between lines supported the earlier theory that the genetic architecture could be relatively simple, but refuted the idea that the trait was sex-linked. In chapter four I then aimed to investigate the mechanistic basis of this dispersal. I showed that dispersers differ from non-dispersers in their activity, patterns of movement, and use of habitat, in ways that plausibly enhance dispersal. Chapter five was then aimed at exploring a potential dispersal syndrome in *Tribolium*. I showed that dispersal covaries with male mating behaviour, female reproductive investment, development time and female lifespan. In concert, these key traits may represent alternative life-history strategies. Finally, chapter six aimed to identify regions of the genome underlying the dispersal phenotype as selected for in my experiments. I found associations between dispersal and many loci across the genome. Among these candidates were many known metabolic or neurophysiological genes, suggesting that this is likely the functional molecular basis of dispersal in this system.

Overall, this thesis represents a logical and coherent research programme aimed at elucidating the evolutionary ecology of *Tribolium* dispersal. When combined, the results provide insight into the key drivers of dispersal evolution, across levels of biological organisation, and suggest several areas where further study could build upon this work.

7.2 Synthesis

A strong theme evident in the results of this thesis is the importance of intraspecific interactions in dispersal. This is a well-known feature of *Tribolium* ecology. Indeed the first experimental use of *Tribolium* was in studying competition (Park, 1962). For this reason, I was conscious of designing assays to measure behaviour in a relatively representative competitive and reproductive environment - at least as far as this is possible in the laboratory. Hence I was able to study emigration from a mixed-sex population inhabiting a viable food source, where both mates and sustenance were available. I see this as an important factor, separating dispersal from foraging or mate-searching (Van Dyck and Baguette, 2005), and a limitation of much of the previous research on *Tribolium* dispersal. The centrality of intraspecific competition in *Tribolium* ecology is reemphasised in the correlations observed between life-history traits and dispersal (Chapter 5). The pattern of association with male mating behaviour, female oviposition and development time suggest distinct life-history strategies shaped by competition. One strategy centres on investment in being competitive: remaining in the patch, competing for matings and ovipositing to maximise the chances of the offspring. The alternative is to disperse and attempt to reach a new patch where competition for resources is relaxed.

In *Tribolium*, population density, and therefore competition intensity, is signalled via ‘conditioning’ of the environment by chemical secretions (El-Desouky et al., 2018). Dispersal varies with density (Ziegler, 1978) and, interestingly, several of the genes I identified as dispersal candidates (Chapter 6) were related to the development of sensory organs and/or responses to sensory cues. It seems reasonable to hypothesise that decreased sensory acuity or sensitivity might reduce the response to density cues and result in lower dispersal propensity. Further investigation of how selection for high/low dispersal has altered the shape of the dispersal-density relationship would provide deeper insight into how intraspecific competition mediates population dynamics.

Two chapters (three and six) address the genetic architecture of dispersal. By simulating the selection experiment in silico, under a range of parameters, chapter two provided evidence that the response to selection was consistent with an oligogenic architecture. Chapter six points to a more polygenic genetic basis of the trait, with many regions across the genome showing significant association with dispersal. Hence, while both chapters refute the existing theory of a single-gene basis (Ritte and Lavie, 1977), they point to different numbers of underlying loci. Of the two methods, the molecular approach provides far more robust evidence, directly measuring signatures of selection from the genome, compared to the indirect simulation. The discrepancy indicates that the simulation, which was necessarily a simplified representation, did not capture important aspects of how the trait evolves. Notably, the model considered only additive variance, did not allow for recombination, and constrained all simulated loci to the same values of starting allele frequency, dominance and heritability (see Chapter 3). It is possible simulating with a pre-built software, such as SLiM (Haller and Messer, 2023), designed to handle complex processes such as recombination, would have yielded results that better fit with the findings from the genomic selection scan.

As well as being a model system to study eco-evolution, *Tribolium* is a globally significant pest species, whose study can yield applied benefits (Brown et al., 2009). The fields of insecticide-resistance evolution and genetic manipulation are key areas of pest management research, and dispersal relates to them both. Resistance to insecticide has been shown to evolve in response to selection pressure applied by fumigating food storage facilities infested with *Tribolium*. High rates of dispersal could lead to the escape of resistance genes into un-treated populations. However, it is known that resistance is associated with lower metabolic rate (Schlipalius et al., 2012, 2018) and reduced flying and walking dispersal (Malekpour et al., 2016, 2018), and therefore likely to the reduced spread of dispersal alleles. My results from this thesis suggest that resistant individuals of a less dispersive phenotype, as seen among phosphine-resistant individuals, may be more likely to have greater competitive ability, facilitating the spread of resistance genes when those genes do reach new populations.

Genetic manipulation as a means of pest control is a growing area of research. Technologies such as RNA-interference and CRISPR have the potential to provide enormous benefits to food security, biosecurity and human health. For example, enabling us to develop synthetic gene drives, artificially increasing the transmission of traits, such as sterility, to eradicate targeted populations (Champer et al., 2016). Dispersal is known to be a key parameter in the outcomes of such projects (Legros et al., 2013, 2021), some of which wish to increase dispersal to maximise the spread of the gene drive mechanism, while others wish to limit dispersal to prevent ‘escape’ beyond the intended target area. Identification of gene candidates underlying dispersal provides the potential to edit loci involved in dispersal alongside the main gene drive target, effectively ‘coupling’ the desired dispersal phenotype to the trait of interest. While further work is required to know whether mask - the strongest candidate identified by my genomic analysis - has a large enough effect on dispersal to be a viable target of *Tribolium* control strategies, the possibility is intriguing.

7.3 Future directions

This thesis has provided new insight into *Tribolium* dispersal, but also leaves many open questions and potential ways to extend this research programme. I have built upon work focussed on short-distance dispersal by walking but now, with this basis established, short-distance dispersal can be integrated with *Tribolium* dispersal by flight to provide a holistic view of dispersal. In many respects, I would expect the two modes of dispersal to covary. For example, increased metabolic performance increases enabling both walking and flying (Malekpour et al., 2016, 2018). There is some evidence from other insects that the kinds of dispersal-enhancing movement patterns I identify in dispersal lines are consistent across walking and flying modes of dispersal (Socha and Zemek, 2003). However, the relationship between walking and flight dispersal is one aspect that requires formal investigation before we can fully understand dispersal in the *Tribolium* system.

For a dispersal event to be undertaken both an ability and a propensity are required in an individual. I identified candidate dispersal loci with both metabolic and neurophysiological functions, and it is tempting to see these as underlying dispersal ability and propensity respectively. However, such a hypothesis evidently requires testing. While a link between dispersal ability and metabolic performance is well established (Reinhold, 1999; Niitepõld and Saastamoinen, 2017; Saastamoinen et al., 2018), the picture is more complex for neurology. Extensive research in Drosophila indicates that distinct neural pathways modulate movement coordination and responses to sensory cues (Gowda et al., 2021). Thus it seems likely that genetic variation in neurophysiology is contributing to both dispersal ability and propensity in *Tribolium*. The respective underpinnings of ability and propensity, along with the degree of covariance between them, are interesting angles for future study

Having supplied evidence that dispersal is a polygenic trait in this system, how does it evolve? Does selection consistently act via the same set of genes and genetic variants, or is there redundancy among the many loci that contribute to dispersal? Is the genetic architecture of the adaptive response predictably modified by altering the exact nature of the selection pressure? Addressing these questions is central to understanding how complex traits evolve under natural selection. However, the study of polygenic adaptation presents many challenges, including disentangling the small individual effects of numerous loci and distinguishing between adaptive signals and background variation (Wellenreuther and Hansson, 2016). Despite these difficulties, emerging concepts such as ‘adaptive architecture’, and modern molecular techniques such as pooled-sequencing, provide opportunities to detect and characterise the genetic changes underlying adaptation (Kofler and Schlötterer, 2014; Barghi et al., 2020). When combined with experimentally tractable systems, these methods provide powerful frameworks to test fundamental hypotheses about the evolution of quantitative traits (Kofler and Schlötterer, 2014; Barghi et al., 2020). I have shown that dispersal in *Tribolium* is a polygenic trait and highly responsive to artificial selection. Further research, building on these results and using pool-seq to obtain time series of allele frequencies would provide scope to contribute to our understanding of complex quantitative trait evolution.

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APPENDIX ONE

Published version of chapter two:

Pointer MD, Gage MJG & Spurgin LG (2021) *Tribolium* beetles as a model system in evolution and ecology. *Heredity* 126, 869–883. https://doi.org/10.1038/s41437-021-00420-1

Heredity (2021) 126:869–883
https://doi.org/10.1038/s41437-021-00420-1

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REVIEW ARTICLE

Tribolium beetles as a model system in evolution and ecology

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Received: 19 October 2020 / Revised: 19 February 2021 / Accepted: 19 February 2021 / Published online: 25 March 2021
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Abstract

Flour beetles of the genus *Tribolium* have been utilised as informative study systems for over a century and contributed to major advances across many fields. This review serves to highlight the significant historical contribution that *Tribolium* study systems have made to the fields of ecology and evolution, and to promote their use as contemporary research models. We review the broad range of studies employing *Tribolium* to make significant advances in ecology and evolution. We show that research using *Tribolium* beetles has contributed a substantial amount to evolutionary and ecological understanding, especially in the fields of population dynamics, reproduction and sexual selection, population and quantitative genetics, and behaviour, physiology and life history. We propose a number of future research opportunities using *Tribolium*, with particular focus on how their amenability to forward and reverse genetic manipulation may provide a valuable complement to other insect models.

Introduction

Research models are fundamental to scientific investigation, providing simplified systems to test and explain components within more complex ideas and hypotheses (Hartmann and Frigg 2005). Model systems can be viewed along a continuum, where simplicity is traded against complex reality, with purely theoretical models at one end, and field-based systems at the other (Winther et al. 2015). Laboratory organisms occupy an intermediate position on this continuum, offering informative opportunities to directly test ecological or evolutionary theory in complex living systems, while retaining high levels of experimental control and allowing for experimental replication. As a result, laboratory models are an essential and widely used tool in biodiversity research, and here we discuss the many applications and strengths that *Tribolium* beetles possess for research in ecology and evolution.

In choosing experimental systems, scientists face a choice between focusing effort on one of rather few ‘true’ model organisms (Sommer 2009), versus adding diversity to the research base, expanding the useful extrapolations that can be made (Bolker 2012). Important distinctions exist between what some consider ‘true’ model organisms, compared to those used more broadly in experimental studies to which the term is often more loosely applied (Ankeny and Leonelli 2011). Under the more stringent ‘animal model’ view, the list of model organisms is traditionally limited to very few, including *Zea mays* (maize), *Arabidopsis thaliana* (thale cress), the bacterium *Escherischa coli*, *Saccharomyces cerevisiae* (yeast), the roundworm *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster*, *Xenopus laevis* (African clawed frog), *Mus musculus* (house mouse) and *Danio rerio* (Zebrafish) (Müller and Grossniklaus 2010). Attributes that distinguish these true model organisms include established research infrastructure, high experimental tractability and the ability to represent broad ranges of both taxa and questions under study (representational scope and representational target respectively, Ankeny and Leonelli 2011). Despite these nine classic models having such attributes, it is clear that we need to consider a wider range of models for progressing research, especially where we aim to tackle questions concerning the evolutionary ecology of biodiversity in the natural environment (Fig. 1).

Tribolium is a genus of small tenebrionid beetles, two of which, *T. castaneum and T. confusum*, are significant global

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Associate editor: Frank Hailer

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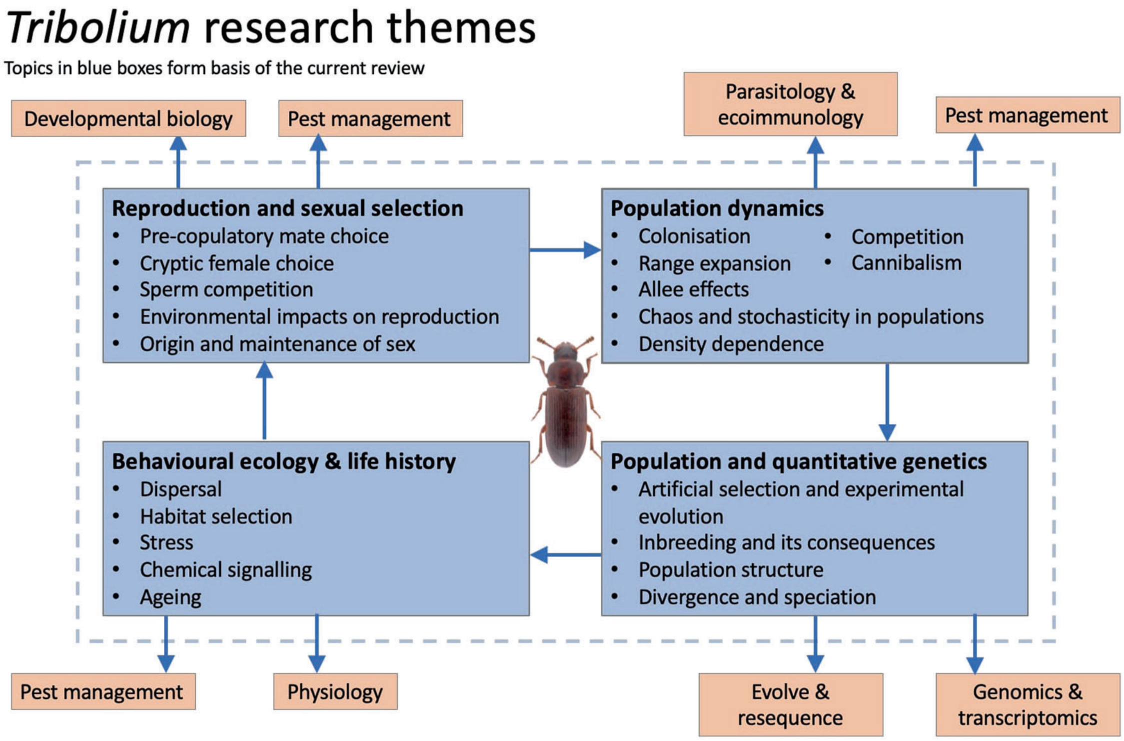

Fig. 1 ***Tribolium* research themes.** Panels show interrelationships between research fields within evolution and ecology that have utilised *Tribolium* beetles as a research model. Those on blue backgrounds form the basis of this review (colour figure online).

pests of stored food products (El-Aziz 2011), and widely used as experimental models (Box 1; Fig. 2). Historically, *Tribolium* were free-living but are now mainly found infesting stored food products such as flour (Dawson 1977). The original habitat of *Tribolium* was likely beneath the bark of trees or in rotting wood, where they were secondary colonisers, characterised by rapid population growth and ready dispersal (Dawson 1977). It is not known when these pest species made the switch to a commensal lifestyle with humans, but there is evidence of *T. confusum* from Ancient Egyptian flour urns dating back ~5000 years, or ~70,000 beetle generations of selection (Andres 1931).

The purpose of this review is to highlight both the significant historical contribution from *Tribolium* flour beetles to ecological and evolutionary research, and elaborate their prominence as a contemporary study organism. *Tribolium* beetles have aided evolutionary ecology research for over a century (Chapman 1918), notably in population biology and interspecific competition (Park 1962), but also across a range of ecological and genetic disciplines. As members of the most species-rich order (Stork et al. 2015), occupying a relatively basal position among the Holometabola (metamorphosing insects), and being less highly derived than *Drosophila* (the foremost insect model), *Tribolium* beetles have broad representational scope (Brown et al. 2003). In addition, *Tribolium* is highly amenable to both forward and reverse genetic manipulation (Brown et al. 2009), a key attribute of any modern model organism (Barr 2003). *T. castaneum* was the first Coleopteran, and first agricultural pest, to have its genome sequenced (Tribolium Genome Sequencing Consortium et al. 2008), and annotations continue to be updated (Herndon et al. 2020). With the increasing availability of genomic data, biology is likely to become more comparative across models (Hedges 2002). *Tribolium* occupies a unique position to act as a mediary in the identification of insect orthologs of human genes, breaking a previous reliance on candidate genes from *Drosophila* and expanding the scope of insect genetics beyond highly conserved regions (Brown et al. 2003). While the *Tribolium* research infrastructure is certainly less well established than those of *Drosophila* and *C. elegans*, it is considerable and growing, with stock centres providing wild-type and many mutant strains to researchers (Brown et al. 2009), and increased availability of genomic and reverse genetic resources (Kim et al. 2010; Dönitz et al. 2015). Finally, this model is also a pest that significantly impacts global food supplies, and understanding gained in the laboratory can be applied to increase global food security (El-Aziz 2011).

Reviews of the *Tribolium* system exist, but most cover fields such as biomedicine, physiology and particularly developmental biology, where *Tribolium* is an important model due to its more representative mode of short germ band development than alternative insect models

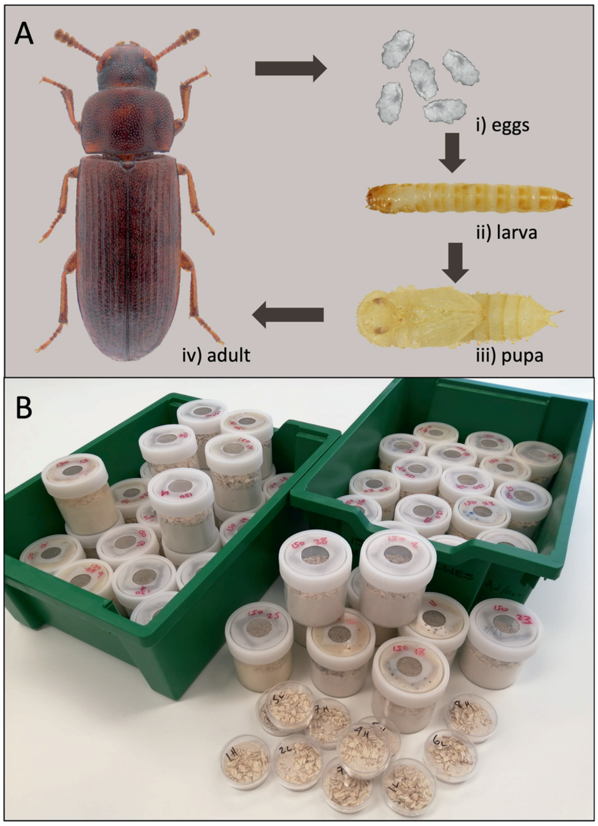

Fig. 2 ***Tribolium*** **life history and husbandry. A** *Tribolium castaneum* life stages (compiled from photographs by Udo Schmidt (adult), John Obermeyer (egg), and from Khan et al. 2016). **B** Experimental *Tribolium* populations housed in 250 ml screw-top containers (top) and 50 mm Petri dishes, capable of supporting ~500 and ~100 individuals respectively without significant density-dependent effects.

(Denell 2008; Adamski et al. 2019). Other fields where *Tribolium* are used, including parasitology, ecoimmunology, the evolutionary ecology of infectious disease and applied pest management, have relevance to the areas discussed, though we lacked the space to include them here (e.g., Kerstes and Martin 2014; Perkin and Oppert 2019; Eggert et al. 2015). Broad reviews of *Tribolium* as a model in ecology and evolution also exist but are several decades old (e.g., King and Dawson 1972; Mertz 1972; Sokoloff 1977) and much new research in this area has since emerged. We therefore aim to demonstrate that the *Tribolium* literature testifies to the model's applicability across ecological and evolutionary fields and levels of biological organisation. We break the review down into conceptual areas, focusing on: (1) population dynamics, (2) reproduction and sexual selection, (3) population and quantitative genetics, and (4) behavioural ecology, ecophysiology and life-history evolution. We also discuss implications of *Tribolium* research findings for the broader ecological and evolutionary community, and suggest some ways in which

Box 1. *Tribolium* ecology and experimental tractability

Many aspects of *Tribolium* ecology make them well suited as experimental organisms:

- The *Tribolium* environment is also the food medium: a dry mix of fine particulates that is easily prepared and readily stored. Homogeneity within this medium eliminates micro-climatic and nutritional variation within and between replicate populations, allowing tight environmental control of both temperature and humidity, the latter being important in reducing the invasion of fungal pathogens.
- Owing to *Tribolium's* long human-commensal history (~70,000 generations), the laboratory medium also has the advantage of very closely approximating its semi-wild habitat in food-storage facilities, allowing a lab environment that is less abstracted than that in other insect models.
- Egg-to-adult development is completed in ~30 days (at 30 °C), with roughly 3, 20 and 4 days spent as egg, larva and pupa, respectively. Husbandry is therefore less tightly time constrained compared with faster-developing species, allowing more flexibility to generate high replicate numbers.
- Adults are small (~4 mm) but dark in colour and easily distinguished from the medium. All stages, including eggs can be separated from the medium by simple sieving for counting or other experimental manipulations.
- Individuals are physically and behaviourally robust to handling, without the need for anaesthetisation, allowing methods such as paint-marking of individuals for later identification.
- The sexes can be distinguished in adults by the presence of a gland on the forelimbs of males, which is absent in females. Pre-reproductive individuals are most easily sexed as pupae, by their differential genital morphology, allowing single-sex cohorts or virgins to be isolated for breeding and mating studies.
- Though able to fly, individuals display very limited motivation to do so in the laboratory, making containment simple relative to insects that fly. Under specific conditions, however, flight behaviour can be induced in *Tribolium*.
- *Tribolium* mate and breed readily and promiscuously in the lab as pairs or groups, enabling manipulation of mating pattern, high levels of success in specific breeding crosses, and good opportunities for the recording of reproductive behaviour.

this system can continue to enhance ecological and evolutionary research. We hope that this review will be valuable both to those within the *Tribolium* community, by providing context to the often highly specialised focus of individual research groups, and to a broader readership, by synthesising the significant contributions made to ecological and evolutionary science by this often-overlooked model species.

Population dynamics

The value of the *Tribolium* system in answering questions in population biology was recognised early in the twentieth century (Chapman 1918; Park 1934), and the system is well known for its contribution to this field. Here we break this research down into that covering (1) colonisation dynamics, (2) drivers of population size and regulation, and (3) competition. We focus on the empirical literature, but it is worth noting that *Tribolium* experiments have contributed to a large body of important mathematical modelling work aiming to predict population dynamics (Mertz 1972; reviewed in Costantino et al. 2005).

Colonisation and spread

Colonisation events are necessarily rare and therefore difficult to observe in nature, despite their importance. Lab models give us the opportunity to study and replicate events that determine the genetic and phenotypic make-up of contemporary populations. *Tribolium* life history is characterised by repeated episodes of dispersal and colonisation (Dawson 1977), making it an ideal system in which to investigate these processes in the laboratory. For instance, while it is well understood that the size and frequency of colonisation events are positively correlated with colonisation success (Lockwood et al. 2005), the role of genetic and demographic processes in underpinning this relationship is less clear. *Tribolium* experiments have shown that both the frequency and size of introductions affect the likelihood of establishment, with some evidence that the former may be more important (Koontz et al. 2018). Further, by manipulating levels of genetic diversity within colonising populations, an elegant set of *Tribolium* experiments have shown that demographic and genetic processes have independent roles in determining colonisation success, by affecting initial establishment success and subsequent population growth respectively (Szűcs et al. 2014; Szűcs and Melbourne et al. 2017). Similar experiments have shown that the colonising individuals' relative fitness in the new environment is as important as the number of colonisers (Vahsen et al. 2018), and that this affects population dynamics for many generations post-colonisation (Van Allen and Rudolf 2013).

Analysing the factors that underpin initial rates of population growth has also been a major aim of *Tribolium* research. It was in analysing *Tribolium* data that W.C. Allee formulated the theory that has become known as the 'Allee effect', an optimum density for initial population growth (Allee 1931; Park 1932), whereby at small population sizes mean individual fitness is reduced (Stephens et al. 1999). The rate of population growth has also been quantified across a range of environmental factors, including food quality and composition (e.g., Wong and Lee 2011), as well as life history parameters such as development time, with populations of fast-developing individuals able to grow more quickly than those in which individuals develop more slowly (Soliman 1972).

Tribolium is also well suited to the study of range expansion under a laboratory setting, using replicated populations within arrays of connected habitat patches. With such methods it has been shown that neutral stochastic processes are highly important in range expansion (Weiss-Lehman et al. 2019), with endogenous variation leading to highly unpredictable rates of spread (Melbourne and Hastings 2009). By experimentally constraining evolution, this work has shown that adaptation also plays a key role during expansion and the early stages of colonisation (e.g. Szűcs et al. 2017), though subsequent gene flow may be required into small populations to mitigate drift load which may hinder adaptation (Stewart et al. 2017). Addressing this same issue from a conservation perspective, experimental tests of both demographic and genetic rescue have shown that these interventions can reduce extinction risk and have additive effects (Hufbauer et al. 2015).

Population size and regulation

A large body of experimental research has used *Tribolium* populations to determine the drivers of equilibrium population size and dynamics. Early *Tribolium* work showed that equilibrium population size is mediated by habitat volume (Chapman 1928). *Tribolium* researchers have since utilised the system to investigate questions regarding effective population size, including its response to population properties such as initial density (Wade 1980a) and its relationship to census population size (Pray et al. 1996).

Studies that have paired modelling approaches with *Tribolium* population biology experiments have added greatly to our understanding of population dynamic phenomena, directly linking theory to the behaviour of real populations (reviewed in Cushing et al. 2002; Costantino et al. 2005). Demographic parameters, predicted by models to lead to chaotic dynamics, were applied experimentally to *Tribolium* populations, exposing chaotic dynamics that could be reliably disrupted by minor intervention (Costantino et al. 1995, 1997; Desharnais et al. 2001). Comparing

Tribolium population dynamic data to model predictions has further revealed influences of stochastic processes in the behaviour of populations, as well as identifying lattice effects (dynamics arising due to the discrete nature of individual organisms, e.g., Henson et al. 2001; King et al. 2004) and accounting for the ecological synchrony between separate populations (Desharnais et al. 2018).

Tribolium populations self-regulate via complex interactions of density-dependent effects on reproductive, developmental, dispersive and cannibalistic behaviours (King and Dawson 1972). Chemical secretions (see 'life-history' section, below) accumulate in the medium in proportion to time and density in a process known as 'conditioning', and these secretions are used by the beetles as indicators of demographic parameters, such as population density (El-Desouky et al. 2018). Much of the work on this area was done in the early-mid nineteenth century and is well covered by King and Dawson (1972). Oviposition is suppressed by conditioning of the medium and increased by egg cannibalism (Sonleitner and Gutherie 1991). Recent work has shown that females evaluate current and future competitive conditions when making oviposition decisions (Halliday et al. 2019). Larval development is slowed by increasing larval and adult density (Park et al. 1939; Janus 1989). Higher density during development also has indirect negative effects on future reproductive success through reduced body weight (Assie et al. 2008). Dispersal increases with density (Ziegler 1978) and there is evidence that the genetic bases of dispersal and the reduction of oviposition in response to conditioning are linked (Lavie and Ritte 1980, see also 'Life history' below). Density-dependent processes in *Tribolium* are complex and act differentially on the sexes (Ellen et al. 2016), interact with food quality (patterns of density-dependent habitat selection depend on the foodstuffs used, Halliday et al. 2019) and weaken with deviation from thermal optimum (Halliday and Blouin-Demers 2018).

Cannibalism is consistently shown to be core to many aspects of the *Tribolium* system, including population regulation. The degree of cannibalism increases with density until a point at which the predatory individuals become satiated (Park et al. 1965). Adult cannibalism of pupae is most effective at controlling adult numbers (Young 1970), while more nutritional benefit is gained from egg-eating (Alabi et al. 2008). Females are more voracious than males, and there is no reduction in cannibalism with increasingly conditioned medium (Flinn and Campbell 2012). The extra nutrition gained from cannibalism is able to compensate for the negative effects of high density (Mertz and Robertson 1970), and to facilitate colonisation of marginal nutritional habitat (Via 1999). Populations differ in their cannibalism rates, and these differences appear stable over many tens of generations; this may be because differences in cannibalism confer no selective advantage, or perhaps because populations can occupy different peaks on the selective landscape (Stevens 1989). Kin-selection may provide a mechanism for changes in cannibalism, as reduced rates of cannibalism between certain life stages have been observed within groups of highly related individuals (Wade 1980b).

Cannibalistic behaviour creates the periodic cycles in age-structure that characterise *Tribolium* populations (Costantino and Desharnais 1991) and, by manipulating population conditions, cycles can be altered or interrupted. When cannibalism is negated, by housing life stages separately, cycles disappear (Benoit 1998) and if populations are unconfined, allowing emigration, generations become non-overlapping (Ziegler 1972). These cycling phenomena are another area in which *Tribolium* has fostered close ties between theoretical and population biologists, by providing a convenient system in which to test theories of population dynamics, such as how environmental fluctuation affects population cycling (Reuman et al. 2008).

Competition

Much of the reputation of the *Tribolium* system is founded on the two-species competition experiments of Thomas Park and his collaborators and their indeterminate outcomes, which helped draw attention to the role of stochastic processes in ecology (Park et al. 1964). Briefly, using experimental populations containing both *T. confusum* and *T. castaneum* it has been shown that (i) one species is almost always driven to extinction; (ii) the 'winning species' cannot be predicted by the relative performance of each species cultured alone in the focal environment; (iii) outcomes can be indeterminate under certain conditions, whereby the 'winner' is not the same in all replicates; (iv) there was no evidence for an elevated 'win rate' of populations which had won in previous experiments, suggesting that competitive ability cannot be selected on (Park and Lloyd 1955). These results spawned much work trying to account for indeterminate outcomes, including mathematical modelling (e.g., Leslie 1962). Following this early work, competition experiments in *Tribolium* have shown that demography may be more important than genetics in determining competitive outcomes in this system (Mertz et al. 1976), although inbreeding depression can cause a loss of competitive ability at low founding size (Craig and Mertz 1994). Further, a wide range of external variables have been shown to affect competition dynamics, including reduced competitive ability resulting from parasite infection and/or low natal habitat quality, and deviation from the thermal optimum (e.g., Yan et al. 1998; Van Allen and Rudolf 2015).

Reproduction and sexual selection

One of the most fundamental life processes is reproduction, yet much is still unknown about the origin and maintenance of sex, and the evolutionary forces that maintain the diversity of reproductive phenotypes observed in nature (Williams 1975). To address these, and related questions has been a rich area of *Tribolium* research. Indeed, *T. castaneum* was used in one of the first ever sperm competition and fertilisation precedence experiments (Schlager 1960). Previous broad reviews have identified ways by which females may influence paternity during and following mating, including: inhibiting sperm transfer; altering re-mating behaviour; controlling timing of spermatophore ejection (Pai and Bernasconi 2008; Fedina and Lewis 2008). These reviews also cover the male traits shown to affect paternity share in the many studies of sperm precedence conducted on *Tribolium*. We will therefore focus on subsequent advances in these areas.

Experimental studies have provided varied insights into the evolution and ecology of mating behaviour. Recent work on pre-copulatory behaviour has shown that females exhibit a preference for non-stressed males (McNemee and Marshall 2018) and that homosexual behaviour, which is quite common in *T. castaneum*, is dependent on the social environment and likely occurs due to inaccurate mate choice (Martin et al. 2015; Sales et al. 2018). Post-copulatory reproductive processes are also an important area of research, and there is much potential to track the dynamics of sperm behaviour and male–male interactions within the female tract in vivo. Advances in fluorescent tagging of sperm have made it possible to visualise sperm fate following natural matings and their movement through the tract to the fertilisation set (Droge-Young et al. 2016), and molecular methods have facilitated the study of seminal fluid proteins (South et al. 2011).

Many aspects of reproduction appear to be environment dependent, with factors such as nutrition, temperature, conditioning of medium and parasite presence shown to alter mating dynamics and reproductive fitness, both individually and in combination (Grazer et al. 2014; Khan et al. 2018; Sales et al. 2018; Vasudeva et al. 2019). Further, reproductive fitness has been shown to trade off with intrinsic factors such as walking ability (Matsumura et al. 2019) but can be enhanced by others, such as pesticide resistance (Arnaud et al. 2005). Phenotypic plasticity and genotype-by environment interactions also play an important role in determining levels of sexual conflict and adaptability (Lewis et al. 2012; Holman and Jacomb 2017).

Tribolium experiments have been used to address fundamental questions about the ‘sex paradox’, sexual selection and the origin of mating behaviours (Dunbrack et al. 1995). Key to these studies has been the experimental manipulation of sex ratios and mating patterns over one or multiple generations, thereby applying different sexual selection pressures, and then measuring the consequences. Using such approaches, it has been shown that levels of promiscuity increase following genetic bottlenecks, and therefore that promiscuity may provide a mechanism for avoiding genetic incompatibility (Michalczyk et al. 2011). Using long-term sexual selection lines and experimental inbreeding, *Tribolium* experiments demonstrated that sexual selection may buffer populations against extinction through purging of mutation load (Lumley et al. 2015). However, subsequent studies using alternative approaches to examine the relationship between sexual selection and mutation load have found no purging effects (e.g. Prokop et al. 2019). A population history (up to ten years) of experimentally applied, strong sexual selection has also been shown to improve the competitive ability of males and their sperm, and drive sperm morphology evolution (Michalczyk et al. 2011; Demont et al. 2014; Godwin et al. 2017), increase conspecific population invasion success (Godwin et al. 2018), enable population resilience to the extinction vortex (Godwin et al. 2020), and increase the rate of pesticide resistance evolution (Jacomb et al. 2016).

Population and quantitative genetics

Laboratory insect models have been instrumental in developing our understanding of population genetic theory, from the tracking of inversions in populations (Wright and Dobzhansky 1946) to the discovery of genetic markers (Lewontin and Hubby 1966). Though relatively understudied in comparison to *Drosophila*, many of the same features of *Tribolium* that made it originally attractive to those studying population dynamics, also make it suitable as a lens through which to view evolution in experimental populations from a genetic perspective. Since Park’s competition experiments first drew the attention of geneticists towards the *Tribolium* system, genetic investigations have formed an important branch of its study (King and Dawson 1972). Genetic work on *Tribolium* spans levels of organisation, covering everything from the relationship of genotype to phenotype, through to interspecific reproductive isolation.

Artificial selection and experimental evolution

Early studies of adaptive evolution in *Tribolium* were based on observing phenotypic changes over generations, revealing that changes (in traits such as development time or pupal weight) can arise due to variation in productivity and cannibalism (Sokal and Sonleitner 1968; King and Dawson 1972). Similar, approaches have been used to show that the spread of a selfish genetic element through a population is proportional to the strength of its effect (Wade and Beeman 1994), and that pesticide-resistant genotypes can have increased fitness, even in the absence of pesticides (Haubruge and Arnaud 2001).

The majority of experimental studies of adaptive evolution have directly manipulated the strength of selection. Selection can be artificially applied, with those individuals who will seed the next generation being chosen by the researcher based on a given trait. Alternatively, experimental evolution can be used, where natural selection is allowed to act within populations subjected to a treatment. These techniques enable, amongst other things, the study of the relationship of genotype to phenotype. Provided that the strength of selection is known, trait heritability can be inferred from the response to selection. Alternatively, crosses between individuals of different phenotypes can reveal the genetic basis of traits. In these ways, it has been shown that traits such as pupal and adult weight are highly heritable in *Tribolium* (Wade et al. 1996). Some have moderate heritability, such as larval weight, development time and walking distance (Yamada 1974; Matsumura and Miyatake 2015). While some, like fecundity and death-feigning duration, respond only weakly to artificial selection (Orozco and Bell 1974; Miyatake et al. 2004).

Response to selection has been shown not only in terms of the focal trait, but also in associated traits. For example, weight metrics show correlated responses to selection across life stages (Yamada 1974) and populations selected for short death-feigning duration also have short legs and lower walking motivation (Matsumura and Miyatake 2019). Lines selected for low dispersal propensity have greater longevity, poorer flight ability, are better competitors, develop more quickly and have longer generation times than dispersers (e.g., Zirkle et al. 1988). Some of these associations between traits can limit the response to selection (Irwin and Carter 2014), and attempts to break the correlations of pairs of traits through artificial selection have failed (Bell and Burris 1973; Tigreros and Lewis 2011).

Wright's shifting balance theory (Wright 1932) suggests that population structure may also influence response to selection, although there is limited evidence that this process plays an important role in nature (Coyne et al. 2000). *Tribolium* studies have applied artificial selection within sub-divide-and-merge population structures to test predictions of shifting balance theory (see Wade and Goodnight 1998). However, large panmictic populations have been found to respond better to selection for pupal weight than sub-divided, periodically mixing ones (Katz and Enfield 1977) and no difference in response was found using a similar design to select for offspring number (Schamber and Muir 2001). Further, it has been suggested that these experiments do not capture the complexity of the shifting balance process, and that doing so experimentally may not be possible (Coyne et al. 2000).

In addition to studying adaptation, *Tribolium* experiments have been used to understand the causes and consequences of stochastic processes such as mutation and drift in evolution (e.g., Rich et al. 1979). Inbreeding depression has been studied in detail in *Tribolium*, and inbreeding has been shown to have negative effects on a range of traits, including productivity and viability (López-Fanjul and Jódar 1977). Susceptibility to inbreeding depression can vary between populations and affect suites of traits within populations (Pray and Goodnight 1995; Pray 1997). Inbreeding has negative consequences for population growth and response to selection (McCauley and Wade 1981; Wade et al. 1996). However, the fitness consequences of inbreeding may only be realised in certain environments (subject to genotype-by-environment interactions arising under inbreeding), and may differ between sexes (Pray et al. 1994). The above studies generated inbreeding through enforced sib–sib matings, however more 'realistic' approaches have also been utilised. For example, by reducing population size but allowing free matings, bottleneck scenarios can be simulated, these have shown that subsequent stress following a bottleneck can reveal inbreeding severity, even after population size has recovered (Franklin and Siewerdt 2011).

Divergence and speciation

Genetic differentiation has been quantified empirically between wild *Tribolium* populations (Drury et al. 2009; McCulloch et al. 2019) and between lab populations of varying geographic origin (Yamauchi et al. 2018). While results conflict between studies, it is clear that at least some genetic structure exists within *Tribolium* species. Although there has been limited research into the drivers of population divergence, there is a substantial body of experimental research examining its consequences. Female *T. castaneum* have been shown to increase egg-laying rates when mated to inter-population males, suggesting potential inbreeding avoidance (Attia and Tregenza 2004). On the other hand, when the sperm of males from different populations compete for fertilisations, 'home' males can be seen to have an advantage, indicative of partial reproductive isolation (Pai and Yan 2002). Other inter-population crosses can result in perpetually immature larvae (Drury et al. 2011), and show genetic incompatibilities in *T. castaneum* that provide support for the Haldane's rule (Demuth and Wade 2007). Partial reproductive isolation through mate choice has also been observed between populations of *T. confusum* (Wade et al. 1995).

Moving beyond populations, species across the *Tribolium* genus display a range of divergence times, making them useful for studies of reproductive isolation at the species level. Between *T. castaneum* and *T. confusum*, reproductive isolation is incomplete (Shen et al. 2016), while between *T. castaneum* and *T. freemani*, hybrids are sterile (Wade and Johnson 1994). Interspecific crosses have therefore been used to study questions related to speciation, showing for instance that significant post-copulatory prezygotic isolation can arise through asymmetric sperm precedence under interspecific male competition (Robinson et al. 1994). Crosses between *T. castaneum* and *T. freemani* have also shown that the degree to which skewed sex ratios and male deformity manifest in the F1 generation varies with the geographic origin of the *T. castaneum* strain used, even at relatively local scales (Wade et al. 1997).

The wide range of geographic and genetic distances between the species of *Tribolium*, means that the group constitutes an excellent system for comparative genomic studies seeking to understand the drivers and consequences of speciation (Brown et al. 2009), this will likely be a rich area of future study. Another area for expansion in *Tribolium* research is population genomics: the attributes that have seen the system used in population studies in the past, along with its emerging ability to generate high quality genomic data, are suited to combine in the study of genomic responses to selection. This could take the form of ‘evolve and resequence’ approaches that have so far struggled to make it beyond *Drosophila* (Schlötterer et al. 2015).

Behavioural ecology and life history

Research on *Tribolium* began with studies of populations, but soon branched out to include the individual-level life-history traits that underlie the dynamics of populations, often in order to correctly parameterise mathematical models. Today *Tribolium* is a model for studying life-history parameters of invertebrates in its own right. Ongoing life-history studies in *Tribolium* can mostly be divided into those concerned with dispersal, movement and habitat selection; responses to environmental stress and diet; and studies of chemical biology and ageing.

Dispersal, movement and habitat selection

The study of why individuals disperse, what biological and environmental factors drive this, and the population-level consequences of this process are important areas in evolutionary ecology (Bowler and Benton 2005). Laboratory insect models offer a useful opportunity to study dispersal, as individuals can be tracked, the process of dispersal can be measured in a controlled and repeatable way, phenotypes associated with dispersal can be artificially selected, and the consequences of dispersal measured. *Tribolium* offers a particularly useful model in this respect, as it has two modes of dispersal (walking and flying), and is characterised by a life history that likely requires dispersal between patchy habitats (Dawson 1977). As a result, *Tribolium* has been widely used to study a broad range of questions about the evolutionary drivers and ecological consequences of dispersal.

Like many areas of *Tribolium* research, work on dispersal began in the mid twentieth century, but has seen a modern renaissance. *Tribolium* flight is rare, and early work focused on movement by walking, defining dispersal as the tendency of adults to leave a patch of habitat within an experimental apparatus composing two connected habitat chambers (Prus 1963; reviewed in King and Dawson 1972). Variations on this set-up over many years have shown dispersal to be dependent on a complex of factors, principally an interaction between density (Zromska-Rudzka 1966) and age (see Ziegler 1976; Gurdasani et al. 2018), altered by population-age and relatedness structure (Ziegler 1978; Jasieński et al. 1988), food availability (Ziegler 1977), as well as the natal environment of a focal individual or a threshold proportion of its neighbours (Van Allen and Bhavsar 2014; Endriss et al. 2019). Adult dispersal tendency appears to have a strong genetic basis (Ritte and Agur 1977), but this tendency is not conserved within individuals between pre- and post-metamorphosis (Arnold et al. 2016). Tendency to disperse is more highly correlated with leg length than metabolic rate, indicating that dispersal phenotypes depend more on morphological than physiological traits in adults (Arnold et al. 2017). Dispersal rate and sensitivity of dispersal responses to age and environment are greater in *T. castaneum* than *T. confusum*, consistent with the former being a primary coloniser, following a more r-type strategy than that adopted by the latter (Ziegler 1976).

The last decade has seen significant attention paid to the study of flight behaviour of *T. castaneum*, with the influence of biotic and abiotic factors on flight responses being investigated in laboratory, wild and wild-caught populations. Relative humidity does not appear important, while wind speed and direction, temperature, light, resource provision, and quality, age and mating status have all been seen to alter flight initiation and/or duration (e.g., Drury et al. 2016; Gurdasani et al. 2019). Flight patterns appear to be crepuscular and show seasonality (Daglish et al. 2017; Rafter et al. 2019), though this pattern varies with latitude (Rajan et al. 2018). Consensus on the effect of sex on flight remains elusive, as the results of these studies do not agree on whether flight behaviour differs between males and females.

Movement in relation to habitat selection by different life stages has also been studied in *Tribolium*, which can occur either by walking on the surface of the medium, or tunnelling through it (Hagstrum and Smittle 1980). Larvae seeking pupation sites tend to move deeper into the medium, seeking warmth and low population density, even when this means using poor quality habitat (King and Dawson 1972; Mayes and Englert 1984; Janus 1989). Adults move in response to biotic factors, avoiding high density areas and highly conditioned media, and alter their behaviour according to their reproductive status (Naylor 1965; Wexler et al. 2017). Adult movement behaviour can also vary in response to abiotic factors, including temperature, humidity and habitat structure (Campbell and Hagstrum 2002; e.g., Halliday and Blouin-Demers 2017). Studies investigating the distribution of adults within a fodder mass have shown that fine-scale spatial and temporal structure exists, and that individual variation in patch exploitation can serve to maximise individual fitness (Surtees 1963; Campbell and Runnion 2003). This variation in movement behaviour in response to resource availability may differ when flying as opposed to walking, and between wild and laboratory populations (Ahmad et al. 2013; Ahmad et al. 2013).

Other life-history features

Tribolium represents a good model for studying the effects of stress due to the ease of replicability, and as a model that has relatively few ethical issues surrounding its use. Stress, in the form of starvation, heat or cold shock, or combinations thereof (Shostak et al. 2015), has been experimentally applied to show negative effects on reproductive output and behaviour, movement patterns, and immune response (Sbilordo and Gräzer 2011; Eggert et al. 2015; Wexler and Scharf 2017). Alternatively, effects of tolerance to stress can be the response variable, and this has been used to show that the ability to tolerate stress is affected by a range of factors including parental age (Halle et al. 2015), thermal acclimation regime (Izadi et al. 2019) and rearing conditions (Scharf et al. 2015). Later-life effects of natal/juvenile stress have also been shown in *Tribolium*, with the natal environment affecting adult dispersal (Van Allen and Bhavsar 2014), competitive dynamics (Van Allen and Rudolf 2015), productivity and rates of cannibalism (Boyer 1976).

Tribolium excrete a range of chemicals into their environment, some of which are aggregation pheromones (mainly 4,8 dimethyldecanal, Suzuki 1980), whereas others are a defence against predators and microorganisms whilst playing an important role in population dynamics as indicators of population density (Arnaud et al. 2000). The chemicals used as pheromones differ across the genus (Arnaud et al. 2002), and are produced predominantly by males, eliciting the strongest responses from females (Stevenson et al. 2017). Diet alters the chemical composition of secretions, but may not alter their efficacy (Ming and Lewis 2010). Defensive compounds such as benzoquinones have antimicrobial properties (Prendeville and Stevens 2002) and show genetically controlled differential production across individuals (Yezerski et al. 2004). Due to the shared benefit of their action through density regulation, and the individual costs associated with their production, this can represent a social dilemma (Gokhale et al. 2017). The distinction between these two classes of secretions is not clear, with benzoquinones also shown to possess pheromone-like attractive properties (Verheggen et al. 2007). Understanding *Tribolium* chemobiology represents a fascinating future challenge, given the potential of secretions to influence individual, population and interspecies processes.

Considering its role as a pest, there has been much interest in the ability of *Tribolium* to utilise different food resources and the effect that these have on its life history. Early authors list a wide range of products on which *Tribolium* has been found, including flours of many grains, peas, beans, nuts, chocolate and several spices (e.g., Chittenden 1896). Grain preference was among the first tests performed by Chapman, the man credited with pioneering the use of *Tribolium* for experimental study, who found a preference for wheat flour, and an inability to feed on whole grains (Chapman 1918; Park 1934). Very many studies of responses to diet and diet quality in *Tribolium* have since been published, which show that natural or artificial diets result in large effects on a broad range of fitness parameters (e.g., Sinha 1966; Sokoloff et al. 1966; Wong and Lee 2011).

Finally, there is a limited body of research on ageing in *Tribolium. T. confusum* was the organism in which exposure to radiation was first shown to increase longevity (Davey 1917); this work was subsequently expanded on and replicated widely among insects (Ducoff 1986; Calabrese 2013). However, recent decades have seen *Tribolium* superseded as an invertebrate model of ageing by relatively short-lived alternatives in *Drosophila*, *C. elegans* and yeast (Kennedy 2008). Nonetheless, age-related changes have been shown in individual-level morphological, physiological, biochemical, behavioural and pathological traits (Soliman 1987). Ageing research in *Tribolium* has generated important insights into the link between development and ageing and their genetic basis and the evolution of senescence (Soliman and Lints 1975; Mertz 1975). Some more recent work has highlighted parental-age effects on development and stress tolerance (Halle et al. 2015) and a decline in metrics of movement with increasing age (Wexler et al. 2016). As a relatively long-lived arthropod model (adults can live for up to 4 years, Good 1936) *Tribolium* may have utility for understanding ageing in slower-senescing animals such as vertebrates.

Future directions and conclusions

We have highlighted some of the broad range of fields in which *Tribolium* has been successfully used as a research model. However, we believe that there is still much untapped potential from this organism for addressing several research areas, particularly the expansion of historically productive *Tribolium* fields in combination with genomic data. Studies of divergence can continue to exploit the genus' diversity and mating ecology, in combination with genomic techniques, to probe deeper into the process of species formation. Population dynamic and artificial selection responses can be investigated at the level of the entire genome, expanding the complexity of our understanding. Pest management can employ genomic information to increase the efficacy and specificity of its techniques, minimising the collateral damage while maximising benefits.

Importantly, *Tribolium* research can also incorporate work on climate change, which represents an enormous threat to biodiversity, with insects likely to be severely affected due to their short life cycles and temperature sensitivity (Bale et al. 2002). There is great scope for *Tribolium* as a model in which to study the effects of climate change on insects, for example by studying their physiological, ecological and evolutionary responses to experimental evolution under increased temperature. Many of the characteristics lending research utility to *Tribolium* (Box 1) also make it highly suitable as a research-led teaching resource (Hoste 1968), a role in which we feel it has been underutilised in the past.

Tribolium beetles possess many attributes that have made them a desirable study organism through a long history and breadth of application. They have contributed to many important past discoveries, and continue to be employed in addressing fundamental questions across fields in evolution and ecology. The utility of *Tribolium* spans levels of organisation, and a great responsiveness to genetic manipulation (Brown et al. 2009) promises to extend their relevance far into the genomic age. The ever-growing research infrastructure, and ability to integrate knowledge from across fields, makes *Tribolium* a valuable model system to complement the established invertebrate models of *Drosophila* and *C. elegans*.

Acknowledgements Thanks go to Mark McMullan, David Richardson and three anonymous reviewers for valuable comments that helped improve the manuscript. This research was funded by a BBSRC NRP DTP funded PhD studentship to MDP and a BBSRC Future Leader fellowship to LGS (BB/N011759/1).

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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APPENDIX TWO

Published version of chapter three:

Pointer MD, Spurgin LG, Gage MJG *et al.* (2023). Genetic architecture of dispersal behaviour in the post-harvest pest and model organism *Tribolium castaneum*. *Heredity* **131**, 253–262 https://doi.org/10.1038/s41437-023-00641-6

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ARTICLE OPEN

Genetic architecture of dispersal behaviour in the post-harvest pest and model organism *Tribolium castaneum*

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Dispersal behaviour is an important aspect of the life-history of animals. However, the genetic architecture of dispersal-related traits is often obscure or unknown, even in well studied species. *Tribolium castaneum* is a globally significant post-harvest pest and established model organism, yet studies of its dispersal have shown ambiguous results and the genetic basis of this behaviour remains unresolved. We combine experimental evolution and agent-based modelling to investigate the number of loci underlying dispersal in *T. castaneum*, and whether the trait is sex-linked. Our findings demonstrate rapid evolution of dispersal behaviour under selection. We find no evidence of sex-biases in the dispersal behaviour of the offspring of crosses, supporting an autosomal genetic basis of the trait. Moreover, simulated data approximates experimental data under simulated scenarios where the dispersal trait is controlled by one or few loci, but not many loci. Levels of dispersal in experimentally inbred lines, compared with simulations, indicate that a single locus model is not well supported. Taken together, these lines of evidence support an oligogenic architecture underlying dispersal in *Tribolium castaneum*. These results have implications for applied pest management and for our understanding of the evolution of dispersal in the coleoptera, the world's most species-rich order.

Heredity (2023) 131:253–262; https://doi.org/10.1038/s41437-023-00641-6

INTRODUCTION

Dispersal is important in the ecology and evolution of many species (Ronce 2007) and plays a key role in species' ability to cope with habitat fragmentation and anthropogenic climate change (Travis et al. 2013). Conversely, dispersal also drives inadvertent introductions and invasions of non-native species (Renault et al. 2018). Knowledge of the movement of pests, and its genetic basis, will also allow us to better preserve biodiversity, forecast outbreaks, and to design management and control strategies (Jeger 1999). The consequences of dispersal can be considerable for the organism; as a result of moving to different abiotic, biotic, and/or reproductive environments, individuals may experience extensive differences in fitness, but there are also effects at higher levels of organisation (i.e. at the population or species level compared to at the individual level; Clobert et al. 2012). As such, individual dispersal decisions combine to shape the distribution of individuals and populations and thus govern the area a given species occupies, defining the limits of range expansions and shifts (Kokko and López-Sepulcre 2006). Dispersal is also a key determinant of metapopulation persistence in fragmented, disturbed, or unstable environments, where it can allow recolonisation to balance local extinction (Eriksson et al. 2014). Dispersal also mediates metapopulation structure, determining migration rates and gene flow, which in turn influence evolutionary trajectories of dispersal and on-dispersal traits (Suárez et al. 2022). In both cases, the genetic architecture of the focal trait is an important factor, for example the number of controlling loci can affect the rate of dispersal evolution (Weiss-Lehman and Shaw 2022) and epistatic variance in any trait can be converted to additive variance by drift during founding events, or vice versa as a result of later additional gene flow into the population (Wade and Goodnight 1998; Hill 2017).

A genetic basis to dispersal has been shown in a wide variety of animal species, however, the identity, number, and mode of action of the genes underlying dispersal vary widely (Saastamoinen et al. 2018). Large-effect loci underlying dispersal have been identified in various species (e.g. Trefilov et al. 2000; Fidler et al. 2007; Krackow and König 2008; Edelsparre et al. 2014). Contrastingly, a powerful study of dispersal utilised the *Drosophila* Genetic Reference Panel and found 192 genes associated with variation in locomotion (Jordan et al. 2012). This finding agrees with the view that polygenic architectures generally underlie complex quantitative traits (e.g. Husby et al. 2015; Santure et al. 2015), such as dispersal. However, the genetic basis of dispersal remains obscure in most species.

Tribolium castaneum is a significant pest of stored food products (El-Aziz 2011), responsible for a portion of the ~10% of total grain lost to insects during storage (Boxall 2001), and therefore their control is of biological and economic importance. This species is also an important laboratory model across a range of disciplines, from evolutionary ecology to development (Denell 2008; Pointer et al. 2021). Additionally, as Coleopterans, they are members of the most species-rich order and, being an early diverging lineage in the phylogeny of metamorphosing insects, *T. castaneum* is considered highly representative of other insect species (Brown et al. 2003; Stork et al. 2015). Understanding the genetic causes and consequences of dispersal in *Tribolium* could have significant pest control benefits, as well as adding to our knowledge of how

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Received: 21 February 2023 Revised: 19 July 2023 Accepted: 19 July 2023
Published online: 29 July 2023

populations evolve during range expansion (Weiss-Lehman and Shaw 2022) and how insects may respond to habitat suitability shifts under climate change.

Several studies on *Tribolium* have demonstrated a genetic basis to dispersal through responses to artificial selection (Schurr and Bolduan 1967; Ogden 1970a, b; Ritte and Lavie 1977; Korona 1991; Ruckman and Blackmon 2020); though many of these measured dispersal only in single-sex groups. The rapid response to selection observed across these experiments, has led some authors to postulate that the trait is controlled by very few loci (Ogden 1970a; Ritte and Lavie 1977). For example, evidence of differential dispersal in the sexes between reciprocal crosses, have led to suggestion that dispersal is controlled by a single, sex-linked locus (Ritte and Lavie 1977). In contrast, line cross analysis on a similar set of crosses has provided evidence that epistatic interactions across loci dominate additive effects in dispersal adaptation (Ruckman and Blackmon 2020). Despite considerable effort spent investigating dispersal in *Tribolium* little is known definitively; findings are contradictory (Ritte and Lavie 1977; Ruckman and Blackmon 2020), and many studies are only modestly replicated (Ritte and Lavie 1977), and/or were conducted before the availability of modern computational and molecular genetic techniques.

Here, we combine quantitative genetic, population genetic, and computational approaches—comparing results from replicated experiments and agent-based simulations—to understand the genetic basis of *Tribolium* dispersal. Dispersal is known to consist of three distinct phases, emigration, transit, and immigration (Ronce 2007). While we acknowledge that the focus of this study and previous studies is technically emigration, local movement tendency has been linked to longer distance dispersal by flight (Zirkle et al. 1988) and we use the term dispersal for consistency with the existing *Tribolium* literature. Further, our experimental setup requires individuals to traverse an empty resource patch before they are deemed to have dispersed, making the movement trait studied here more emigration-like than previous work in the system (e.g. Ritte and Lavie 1977). Specifically, by comparing the results of artificial selection for dispersal to agent-based simulations modelling artificial selection under a range of genetic architectures we investigate the genetic basis of the trait. Next, by comparing the dispersal behaviour of experimental crosses to crosses simulated under either sex-linked or non-sex-linked architectures, we explicitly test the hypothesis of a single sex-linked dispersal locus proposed by Ritte and Lavie (1977). If this holds true we predict that simulations under a single-locus architecture will best fit the observations from experimental selection, and that behaviour of males and females will differ in the F1 generation of experimental and simulations under sex-linked architectures but not under non-sex-linked architectures. Lastly, we compare dispersal in experimentally inbred beetle lines and inbreeding simulations, gathering further evidence on the genetic basis of dispersal in this system.

MATERIALS AND METHODS

Beetles and husbandry

Beetles used were of the Krakow super-strain (KSS), bred to combine global *Tribolium castaneum* genetic variation (Laskowski et al. 2015). Stock populations were maintained at Ne ≈ 300 within 1.2 L plastic containers covered with lids into which 70 × 70 mm windows of fine mesh had been inserted for ventilation. Populations were kept on a 'standard fodder' medium of 90% organic wheat flour and 10% brewer's yeast at 30°C, 60% relative humidity, and a 12:12 light-dark cycle (light from 8 am–8 pm) at the University of East Anglia. These are the same conditions under which the stock populations have been kept for ~12 years. The standard husbandry cycle consisted of two phases; during the oviposition phase adult (12+/−3 days post-eclosion) beetles chosen to parent the next generation were removed from their populations and placed into fresh fodder for seven days of mating and egg-laying. Following this period, adults were sieved from the fodder and discarded, beginning the 35-day development phase—during which time eggs in the fodder developed through the larval and pupal stages to become adults. By preventing any interaction between sexually mature adults and offspring reduces the risk of negative density-dependent effects, removes the opportunity for intergenerational interactions, such as egg-cannibalism, and allows accurate tracking of passing generations.

Experimental methods—dispersal phenotyping assay

To investigate the genetic basis of dispersal behaviour and its response to selection we established selection lines by breeding from individuals based on their behavioural phenotypes, as determined using dispersal assays. Dispersal arenas were constructed that consisted of two square, 1.2 L plastic containers with removable lids, connected by a length of rigid PVC tubing with 8 mm internal diameter (Fig. 1A). When 200 ml of fodder was placed into container A and made level, the surface of the fodder intersected the opening of the tube. Stoppers made from baked Fimo polymer clay were used to block either end of the tube when required. On the first day of the assay the tube was blocked and 200 ml of fodder was placed into container A, made level and topped with oats to aid traction. Next, 200 adult beetles of mixed sex (12+/−3 days post eclosion) were added to container A and given a 2 h acclimation period. After this time, the tube was unblocked, beginning a 20 h dispersal period. The walls of

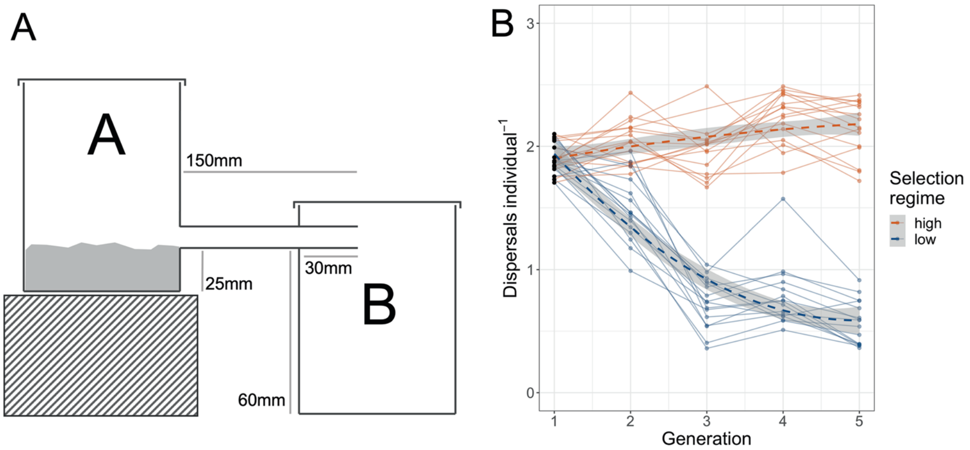

Fig. 1 Artificial selection on dispersal. A Experimental arena setup used to assay the dispersal behaviour of experimental *Tribolium castaneum* populations and provide a basis on which to artificially select individuals displaying high and low dispersal propensity. **B** Mean dispersals per individual (of a maximum of three) for each *T. castaneum* selection line across four generations of selection on high and low dispersal (generation 1 represents behaviour in the stock population). Orange and blue represent high and low selection regimes respectively. Solid lines connect repeated measurements from the same selection line, while dashed lines show predictions generated by a GLM fitting the interaction of selection regime and generation modelled as a second order polynomial.

both containers were smooth and prevented climbing, so individuals that fell from the tube to the floor of container B were unable to return to container A. Beetles in container B at the end of the period were considered to have dispersed. We utilised two types of dispersal assay, differing in the number of dispersal opportunities they afforded: a 1-opportunity assay, (described above) and a 3-opportunity assay, which provided greater resolution at which to measure behaviour. During the latter, following the first dispersal period, individuals from container B were marked with a dot of paint on the dorsal thorax, using a paint marker pen (Posca, 0.7 mm). The contents of container A were separated by sieving. The fodder was replaced in container A, flattened and topped with the same oats. The original 200 individuals from a single replicate, retrieved from container A or B, were returned to container A for a second round of dispersal. This process was repeated twice so each individual had three opportunities to disperse, and its number of dispersals recorded. To mitigate effects of unavoidable small differences in arena construction, lines were assigned randomly to arenas each generation. Processing each line was time-consuming so, in order to guard against time-of-day effects, lines were divided evenly between two temporal blocks (lines 1–8 for both treatments in block 1 and lines 9–16 in block 2). Some component of the measured behaviour may be due to learning across dispersal opportunities within generations, however, as any effect of learning would be consistent across generations we expect the overall impact to be minimal.

Experimental methods—breeding dispersal lines by artificial selection

Initial 3-opportunity dispersal assays were conducted and the selection criteria were three dispersals for high-dispersal lines and zero dispersals for low-dispersal lines. Assays were conducted on 16 replicate groups of stock beetles, with individuals meeting the selection criteria used to found 16 high- and 16 low-dispersal lines respectively. Where possible, 30 individuals were taken to produce the next generation in each replicate of each treatment, otherwise as many as met the selection criteria. This was only ever <30 in the initial generation; of the 32 created lines, the minimum population size was 21 (mean = 27.56, SD = 3.35). In each of generations 2–5, dispersal behaviour in high- and low-dispersal lines was retested with 3-opportunity assays. From individuals meeting the selection criteria of the relevant treatment, 30 were randomly selected to produce the next generation, all other individuals were discarded.

To maximise the effectiveness of selection, it was desirable that all offspring were parented by individuals meeting the selection criteria of the relevant regime, *i.e.* from the group of 30 parental individuals. However, during the assay, individuals selected to produce the next generation could potentially mate with others not from this group, for example, females selected to propagate the high dispersal regime could have been inseminated by non-highly-dispersing males. To mitigate this, we isolated the parental group of 30 individuals for 72 h of intragroup mating, allowing the strong last male precedence displayed by *Tribolium* (Lewis and Austad 1990) to minimise the number of offspring sired by non-group males. After this time, fodder potentially containing non-group eggs was discarded and beetles were transferred to fresh fodder for 7 days of oviposition. Following oviposition, eggs were left to develop in the fodder, adults were removed, frozen, and sexed by the presence of odiferous glands on male upper forelegs (Hinton 1942). Sexing allowed us to ensure that sex ratios were not overly skewed among reproductive groups, and to evaluate sex bias in dispersal behaviour itself. Using this procedure 16 high-dispersal and 16 low-dispersal lines were selected over 5 generations. Hereafter, referred to as 1–16H and 1–16 L respectively.

After generation 5, a 1-opportunity assay was used every other generation (to gen 15) to monitor dispersal propensity and select 30 individuals to produce the next generation. In non-assayed generations, 100 individuals were randomly selected to propagate each line. Selection lines were housed within 250 ml PVC containers with a circular base of 34 mm radius and 100 mm in height, round (20 mm radius) windows of fine metal mesh were inserted in the screw-on lids.

Experimental methods—phenotyping crosses within and between dispersal treatments

To investigate whether dispersal was under the control of a single, sex-linked locus we performed reciprocal crosses within and between selection lines and assayed the dispersal behaviour of offspring. High and low dispersal lines were paired according to their number (1H with 1 L, *etc.*) and four types of cross (within-line and reciprocal between-line crosses; Fig. S1) were performed on each pair. We began by obtaining virgin individuals, taken as pupae from the relevant populations on day 20 of the development phase and raised to adulthood in single-sex groups in 57 ml (61 mm diameter, 27 mm height) plastic containers with hinged lids and ventilation holes. Once mature, males were marked with a coloured dot on the dorsal thorax using a paint marker (0.7 mm; Uniposca, www.posca.com), a method shown not to alter behaviour (Sales et al. 2018). At 12+/−3 days post-eclosion, a single male and female were paired in a 5 ml vial on 0.5 ml of fodder for 48 h of mating opportunity, before males were discarded and females transferred to oviposit on fresh fodder for seven days in 57 ml containers. Each type of cross between a single pair of individuals was replicated five times for each pair of lines. After the females were removed following oviposition, egg-containing fodder from the five replicate crosses was combined so that offspring development took place in non-sibling groups. Each female oviposited on 10 ml of fodder, to maintain the per-female fodder volume from the selection lines, and maintain equal developmental population density. From these new populations, pupae were sexed and kept in single-sex groups until males could be marked post-eclosion. At 8+−3 days post-eclosion 100 males and 100 females were combined to form a cohort of 200 beetles with a 1:1 sex ratio, four days later this population entered a 1-opportunity dispersal assay.

Experimental methods—breeding and phenotyping inbred lines

We established highly inbred lines by repeated sib–sib mating to remove genetic variation within each line. Intense inbreeding will increase homozygosity, potentially fixing alleles at each locus. If dispersal is a single-locus trait, highly inbred lines should fix either the high or low dispersal allele, with probabilities proportional to the starting allele frequencies, and accordingly display either high or low dispersal behaviour, equivalent to that seen in artificially selected lines. By comparing the dispersal behaviour of these lines to predictions generated by simulation under different assumed genetic architectures we have another way to evaluate the genetic basis of this trait. To create the inbred lines, 110 KSS males and 110 KSS females were paired 1:1 (using the same method as for the previous experiment) and used to found 110 lines. Each generation for 10 generations, three male and three female pupae were sexed from each line at 20 days following the end of the oviposition period and left to eclose as virgins in single-sex single-line groups. Resulting adults were paired to create three replicate single-pair matings, coded A, B, and C—to guard against lines going extinct through failure to mate. Pupae used to parent the following generation were always taken from replicate A, unless it was extinct whereon B was used, etc. The fecundity of individual females is reduced by inbreeding depression (Fernández et al. 1995), so to obtain the 200 individuals required for a dispersal assay an additional generation was bred, from a group of 30 beetles per line. As this breeding immediately followed a single pair mating, these 30 individuals were siblings, meaning a total of 11 generations of inbreeding were conducted, following which 64 lines of the initial 110 were left extant. Dispersal behaviour of inbred lines was then measured using a 1-opportunity dispersal assay.

Simulation methods—agent-based simulation to model artificial selection on dispersal

A population genetic simulation of the dispersal selection experiment was created in R (ver.2021.09.1—R Core Team 2020). This agent-based simulation assumed combinations of several genetic (sex-linkage state, dominance value, trait heritability, starting dispersal allele frequency) and demographic parameter values, while incorporating some stochasticity in how they affected simulated populations. By running simulations across the available parameter space we were able to compare simulated data with experimental observations and make qualitative estimates of unknown parameters within our experimental populations (for details see supplementary material).

To estimate the contribution of dominance, heritability, and starting allele frequency to dispersal outcomes, we used the simulation model to perform a parameter scan across a range of values, so that we might compare the response to selection of dispersal behaviour in simulated populations to that observed in experimental populations. Parameter scans comprised a model run for each of 100 different combinations of dominance, heritability, and starting dispersal allele frequency values (Table S1). Scenarios containing allele frequencies of zero and one, or a heritability of zero, were excluded because evolution is not possible under these conditions.

To investigate the number of genes that might underlie dispersal, we developed versions of the simulation model that each assumed a different trait architecture, where dispersal was controlled by 1, 3, 5, and 10 additive, unlinked, biallelic loci, each inherited as described above, with no recombination. For architectures with multiple loci, an individual's dispersal probability was the mean of probabilities calculated independently for each locus. Parameter scans were carried out for each architecture, where each consisted of 50 independent repeats (each equivalent to one high and one low line from the experimental selection) and simulated populations were tracked across 4 generations of selection, to mirror the experimental procedure. For the single locus model, we studied an additional case where the trait was X-linked. *Tribolium castaneum* follows the XX/XY sex-determination system (Juan and Petitpierre 1991). Here females received two alleles, which combined to determine their phenotype, as described above, but males received only one dispersal-determining allele and a Y, which did not contribute to the dispersal phenotype.

Simulation methods—phenotyping crosses within and between dispersal treatments

The simulation model was extended to mirror the crosses performed with experimental beetle lines. This allowed us to generate predictions of the dispersal behaviour of offspring for comparison with results from experimental crosses. For each of the sex-linked and non-sex-linked versions of the single-locus architecture, we began by taking the parameter scan outputs from scenarios that best approximated the experimental data—for both architectures this was $h = 0.6$, $d = 0.5$, $A = 0.8$ (see results). These outputs contained simulated individuals that were the result of four generations of selection. We then simulated an additional mating, in the same way as before except, where during selection females were mated to males that matched their phenotype, here females were assigned to one of four cross treatments and mated to males with corresponding phenotypes. These cross types were: within high selection regime (Hf-Hm); high selection regime female x low selection regime male (Hf-Lm); low selection regime female × high selection regime male (Hm-Lf); within low selection regime (Lf-Lm; Fig. S1). Their simulated offspring were then put through a simulated 1-opportunity dispersal assay, where binary dispersal outcomes were assigned probabilistically according to individual genotype, dominance and heritability.

Simulation methods—simulating and phenotyping inbred lines

This simulation was adapted from the dispersal selection simulation, and simulated 10 generations of inbreeding through individual sibling-sibling matings and one additional generation parented by 30 individuals. Following inbreeding, a 1-opportunity dispersal assay was simulated on inbred lines, in the same way as described for the dispersal selection model. This was done separately for 250 replicates of each trait architecture (single-, 3-, 5- and 10-locus), using all sets of parameters values for each that gave highest agreement with experimental results during the parameter scan ($R^2 > 0.8$). In this way we generated predictions about the dispersal behaviour of individuals of highly inbred lines, under different assumptions regarding the genetic control of the trait.

Statistical methods

All data wrangling and analysis were performed in R ver.2021.09.1 (R Core Team 2020) and data-wrangling largely used the Tidyverse packages (Wickham et al. 2019). Generalised linear mixed models (GLMMs) were fitted using 'Lme4' (Bates et al. 2015) and Satterthwaite method p-values were obtained using 'lmerTest' (Kuznetsova et al. 2017; Luke 2017).

Statistical methods used for experimental data

To investigate the effectiveness of selection on dispersal behaviour, we employed a GLMM. The response variable was the mean number of dispersals per beetle (maximum of 3) for each selection line each generation. Selection regime and block were entered as fixed effects, alongside generation fitted as a second-order polynomial, and the interaction of generation and selection regime. Line ID was fitted as a random factor. Additionally, a GLMM was applied separately to high-selection data to test for changes in dispersal propensity over generations. This model was fitted twice, first with generation as a linear predictor, then as a second-order polynomial.

We tested for sex bias in dispersal behaviour in the initial generation using chi-squared tests on sex ratios of 3-time and 0-time emigrants summed across all replicates. We also analysed the sex of emigrants using generalised linear models (GLMs) with a binomial error distribution and a logit link function; proportion of males was the response variable and sample sizes were entered as weightings (Wilson and Hardy 2002). The model was fitted separately to data on numbers of 0- and 3-time dispersers, with the effect of generation, fitted as a linear fixed effect, on sex bias in the high selection regime and the low selection regime.

To compare the dispersal behaviour of male and female offspring from different types of crosses between dispersal lines, we employed a GLMM with number of dispersals as the response variable. Because the density of organisms is known to affect dispersal rates (e.g. Ogden 1970b), assay-level means of disperser numbers were preferred to individual-level outcomes to avoid non-independence within replicate populations. A first model testing the individual effects of cross type and sex contained these as fixed predictors, along with block, while line ID was entered as a random predictor. A second model contained the interaction of cross type and sex, along with block as fixed predictors and line ID as a random factor. Hartigan's dip test (Hartigan and Hartigan 1985) was used to test for multimodality in the distribution of dispersal propensity of inbred lines, with p-values obtained by 10,000 Monte Carlo simulations using R package 'diptest' (Maechler 2021).

Statistical methods used for simulated data

To obtain information on the likely genetic architecture, starting allele frequency, dominance and heritability in experimental populations, we compared results from empirical selection to results of simulations. We did this by starting with the parameter estimates generated by the statistical model applied to the experimental data (above). We then fit those parameter estimates to the output of each simulated scenario to generate residuals. From these we manually computed R^2 values, to assess how well each simulated scenario approximated the experimental data. This analysis was performed for each genetic architecture and for autosomal and sex-linked versions of the single-locus simulation. To assess whether the sex-linked architecture led to sex-biased dispersal, we computed the difference in dispersal tendency of males and females for each scenario under both single-locus simulation and compared these visually using a heatmap of parameter space. Sex difference in dispersal was computed as the mean dispersal tendency in females across replicates subtracted from the same metric computed for males.

On each output from simulated crosses between selection lines, for sex-linked and non-sex-linked single-locus architectures we employed GLMMs as for the experimental cross data (see above). However, fitting these directly resulted in singularity as the random effect of line accounted for none of the variance in the data, we therefore removed this and fitted the same fixed effects as a GLM. We also fitted the statistical model generated using the experimental dataset to the simulated output from each architecture, using predicted values to obtain residuals and calculated an R^2 to compare the fits.

Hartigan's dip tests (as above) were used to test for multimodality of distributions of (i) mean allele frequencies within lines within each genetic architecture, and (ii) of numbers of dispersers in each line. Initially, this was done using 250 replicate simulated lines, but for architectures showing significant multimodality these 250 replicates were randomly subsampled down to 64 to match the number of experimental inbred lines and retested.

RESULTS

Experimental results—dispersal in artificially selected lines

Dispersal behaviour differed between selection regimes after only one generation of selection, as evident from the non-overlapping 95% CIs of model predictions (Fig. 1B). This was a result of dispersal falling in the low selection regime lines until generation 3, then remained low, while dispersal in the high selection lines showed a small but significant increase when generation was fitted as a linear predictor (GLMM, $\beta = 0.07$, SE = 0.01, $p < 0.001$). A significant interaction between selection regime and generation was found when the effect of generation was modelled as a quadratic (Table 1). Individual variation in dispersal propensity was present in all generations of both treatments, but a shift towards higher and lower propensities was observed in high and low lines respectively. For a comparison of results from 1- and 3-opportunity assays see supplementary material.

Table 1. A linear mixed model of the response to selection in experimental *T. castaneum* populations artificially selected for high and low dispersal behaviour.

	Estimate	SE	*p*
(Intercept)	2.16	0.04	<0.001
Generation	2.21	0.3	<0.001
Generation^2	−0.75	0.3	0.013
Selection regime	−1.1	0.04	<0.001
Block	−0.06	0.04	0.146
Generation x Selection regime	−5.64	0.42	<0.001
Generation^2 x Selection regime	6.26	0.42	<0.001

With mean dispersals per individual as the dependent variable, block was fitted as a fixed effect to account for potential variation in experimental conditions, generation was fitted as a second order polynomial and 'high' is the reference category for selection regime. As a random effect we modelled a random intercept of line ID (Var<0.01, SD = 0.05).

Within the low dispersal regime, we observed a male bias among dispersers ($\chi^2 = 26.79$, df=1, $p < 0.001$, $n = 344$; Fig. S2), which decreased across generations ($\beta = 0.10$, SE = 0.03, $p < 0.001$). The proportion of males among dispersers in high dispersal lines was lower than expected ($\chi^2 = 5.14$, df = 1, $p = 0.02$, $n = 467$; Fig. S2).

Experimental results—phenotyping crosses within and between dispersal treatments

When testing the individual fixed effects of whether cross type and sex predicted dispersal, we found a significant effect of cross type (Fig. 2; Table 2). Within-high regime crosses showed significantly higher dispersal than all other crosses, while dispersal from within-low regime crosses was significantly lower than all other cross types. Both reciprocal between regime crosses showed dispersal propensities intermediate to the within-regime crosses but did not different significantly from each other. No effect of sex on dispersal was found. No significant interaction was seen between cross type and sex (Table 2).

Experimental results—dispersal phenotypes of inbred lines

Inbred lines displayed a range of dispersal propensities, from 0–188/200 beetles dispersing (Fig. 3, row A), with a distribution not differing from unimodal (D = 0.04, $p = 0.72$).

Simulated results—comparison of simulated to experimental selection on dispersal

Considering only non-sex-linked architectures, which varied in the number of underlying loci, 20 of 400 scenarios had R^2 values of $> = 0.80$, representing a good fit of the experimental model to the simulated data. Of these, 15 scenarios came from single-locus simulations, 11 from 3-locus, seven from 5-locus and one from a 10-locus simulation. Architectures varied in the parameter space over which they could simulate a good fit to the experimental data (Fig. 4), with less functional parameter space observed with increasing number of loci. Individual scenarios with good fit were observed across a wide range of parameter values.

Heritability values (h) required for good model fits were $h \geq 0.6$ under single- and 3-locus architectures (Fig. 4). Good model fits generally required higher heritability values when dominance (d) was low, especially at higher numbers of controlling loci. Within each architecture, fits were poorest at low starting dispersal allele frequency (A = 0.2), though good fits were seen across the rest of the range of this parameter ($A = 0.4$, $A = 0.8$). Dominance (d) showed a strong negative interaction with starting dispersal allele frequency (A), with higher values of dominance required when dispersal allele frequency was low.

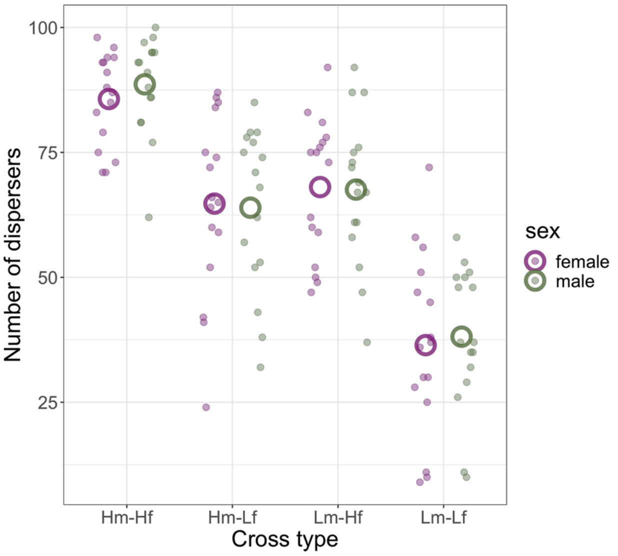

Fig. 2 Number of dispersers from assayed mixed-sex populations of 200 *T. castaneum* offspring from crosses within and between dispersal selection regimes. On the X-axis, 'H' and 'L' represent the selection regime and 'm' and 'f' the sex of parents, e.g. Hm-Lf indicates that the male parent came from a high selection line and the female parent from a low selection line. Data for males and females are shown in purple and green respectively. Small filled points show experimental measures from individual crosses, while large hollow points show predictions of a GLMM fitting the effect of the interaction of sex and cross type on number of dispersers, while controlling for line ID.

Table 2. Mixed models testing the dispersal behaviour of offspring of reciprocal crosses within and between *T. castaneum* lines experimentally selected for high and low dispersal propensity.

	Estimate	*SE*	*p*
(Intercept)	63.93	3.33	<0.001
Cross type [Hm-Lf]	(reference)		
Cross type [Hm-Hf]	22.81	2.47	<0.001
Cross type [Lm-Hf]	3.47	2.47	0.162
Cross type [Lm-Lf]	−21.16	2.47	<0.001
Sex	0.83	1.74	0.636
Cross type [Hm-Lf] x sex [female]	(reference)		
Cross type [Hm-Hf] x sex	3.75	4.98	0.453
Cross type [Lm-Hf] x sex	0.31	4.98	0.95
Cross type [Lm-Lf] x sex	2.5	4.98	0.62

Above the dashed line results are from a model fitting the individual effects of predictors, below the line results are from an equivalent model fitting the interaction between sex and cross type, with 'Hm-Lf' as the reference category. In both, we modelled the random intercept of line ID (model 1: Var = 116.26, SD = 10.78; model 2: Var = 116.26, SD = 10.78).

Considering the sex-linked single-locus model, the functional parameter space and parameter values of maximum fits were almost identical to the non-sex-linked version. Sex biases in dispersal were observed to be greater in sex-linked than non-sex-linked simulations under some parameter scenarios, though these differences were not of large magnitude. In high dispersal lines simulated with a sex-linked architecture, high dominance values ($d = 1$) resulted in greater dispersal in females than in males, with this pattern being strongest at low starting dispersal allele frequency and becoming minimal at the highest tested starting dispersal allele frequency ($A = 0.8$). In low selection lines, the inverse

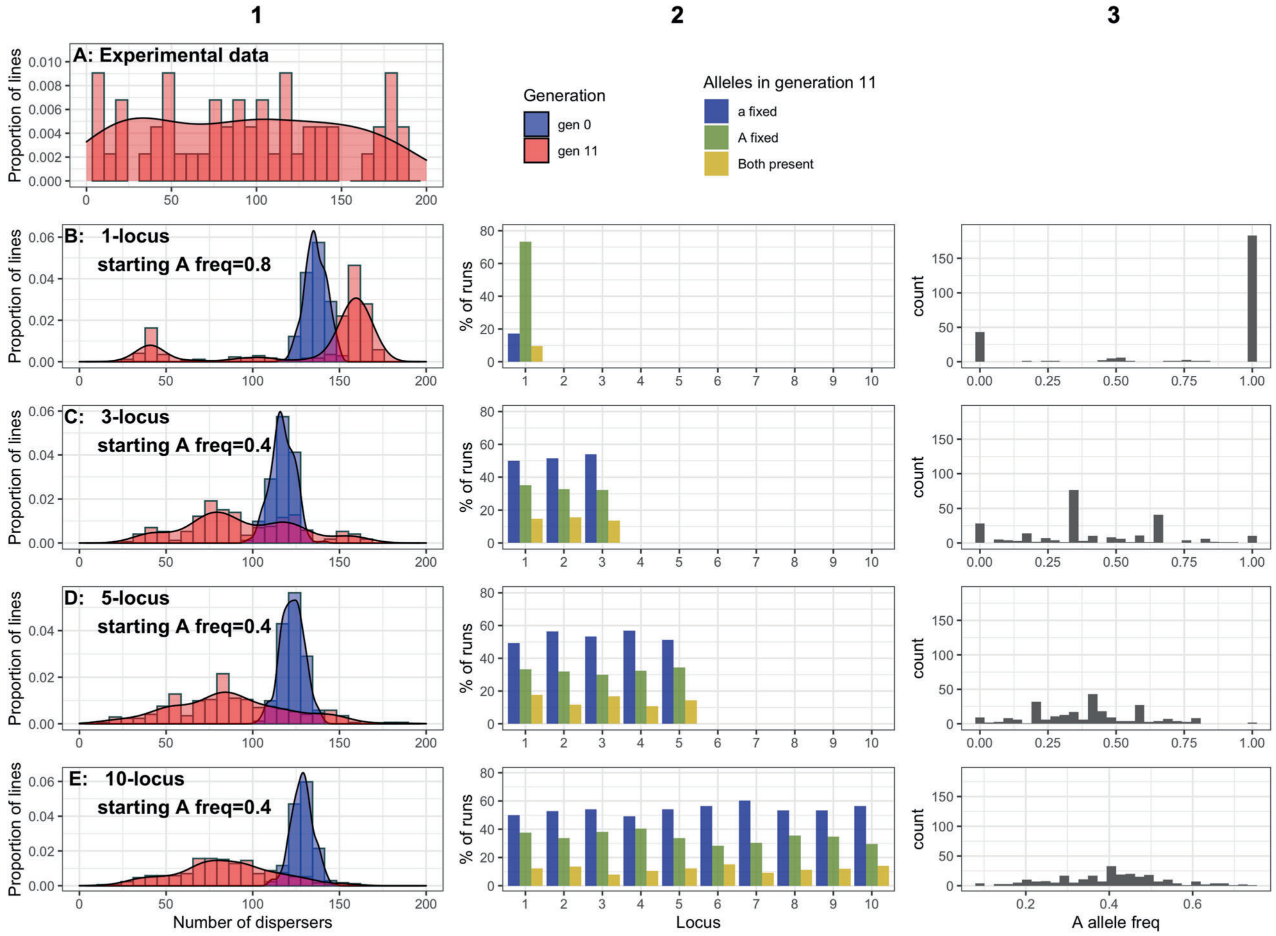

Fig. 3 Combined data from experimental (column 1, row A) and simulated (all other panels) evolution experiments selecting on dispersal behaviour. Column 1: (**A**) Experimental results showing the number of dispersers from each of 64 lines of *T. castaneum* following 11 generations of extreme inbreeding and (**B**–**E**) data from agent-based simulations designed to model the same inbreeding design and dispersal assay as used experimentally. Numbers of dispersers are shown prior to (blue) and following (red) 11 generations of inbreeding. Sample sizes for simulations were 250 independent populations for each genetic architecture, each modelling the trait as controlled by either 1, 3, 5 or 10 additive biallelic loci (rows B-E respectively). For each simulated architecture, starting dispersal allele frequencies were 0.8, 0.4, 0.4 and 0.4 in these architectures respectively, this and other parameters were selected as those maximising agreement with experimental results during a parameter scan (Fig. 4). For simulated data, additional columns display: 2) Allele outcomes at each locus at generation 11, i.e. whether either allele had become fixed, or both alleles remained in the population. 3) The distribution of allele frequencies averaged across loci for each individual simulated line.

pattern was observed, with the lowest dominance values having male-biased dispersal, most pronounced at the maximum starting dispersal allele frequency and minimal at $A = 0.2$. In contrast, the non-sex-linked scenarios showed small stochastic variations across parameter space in both high and low selection lines.

Simulated crosses between selection lines generated divergent outcomes between sex-linked and non-sex-linked single-locus architectures (Table 3). Under both architectures, the offspring of within-high and within-low regime crosses showed high and low dispersal behaviour respectively, in both sexes. In contrast, behaviour of offspring of between regime crosses differed between architectures: as expected, both sexes showed intermediate behaviour under a non-sex-linked architecture, as did the female offspring under sex-linkage; but male offspring from this architecture inherited the behavioural phenotype of their mother's regime. This pattern was reflected by the model, where a significant interaction was seen between cross type and sex under the sex-linked, but not non-sex-linked architecture (Table 3). Applying the mixed model fitting the interaction of cross type and sex, generated using experimental data, to the output from simulating each architecture resulted in better fit to the non-sex-linked data ($R^2 = 0.63$) than to the sex-linked data ($R^2 = 0.50$).

When simulating inbreeding with different numbers of loci, the patterns of alleles remaining in generation 11 were always similar across loci within each architecture. Patterns were also similar across architectures with the same starting allele frequency, with the frequency of an allele leading to its being fixed more often (Fig. 3, rows B-E, column 2).

Simulated results—dispersal of simulated inbred lines

Within the simulated inbred lines the distribution of dispersal at generation 0 was unimodal with mean values of 100–150 dispersers under all architectures (Fig. 3; column 1). By generation 11 the distribution displays two obvious modes when modelled with a single-locus architecture. These observations were confirmed with dip tests for multimodality, indicating that the single-locus simulation showed a significantly non-unimodal distribution of numbers of dispersers under the parameter set that gave the greatest agreement with results of experimental selection ($D = 0.06$, $p < 0.001$). This remained true under all other single-locus scenarios which resulted in high agreement (R^2 of >0.8; $D < 0.14$, $p < 0.001$). As expected, the same pattern was observed in allele frequencies; both the best performing ($D = 0.09$, $p < 0.001$) and all other high performing parameter sets

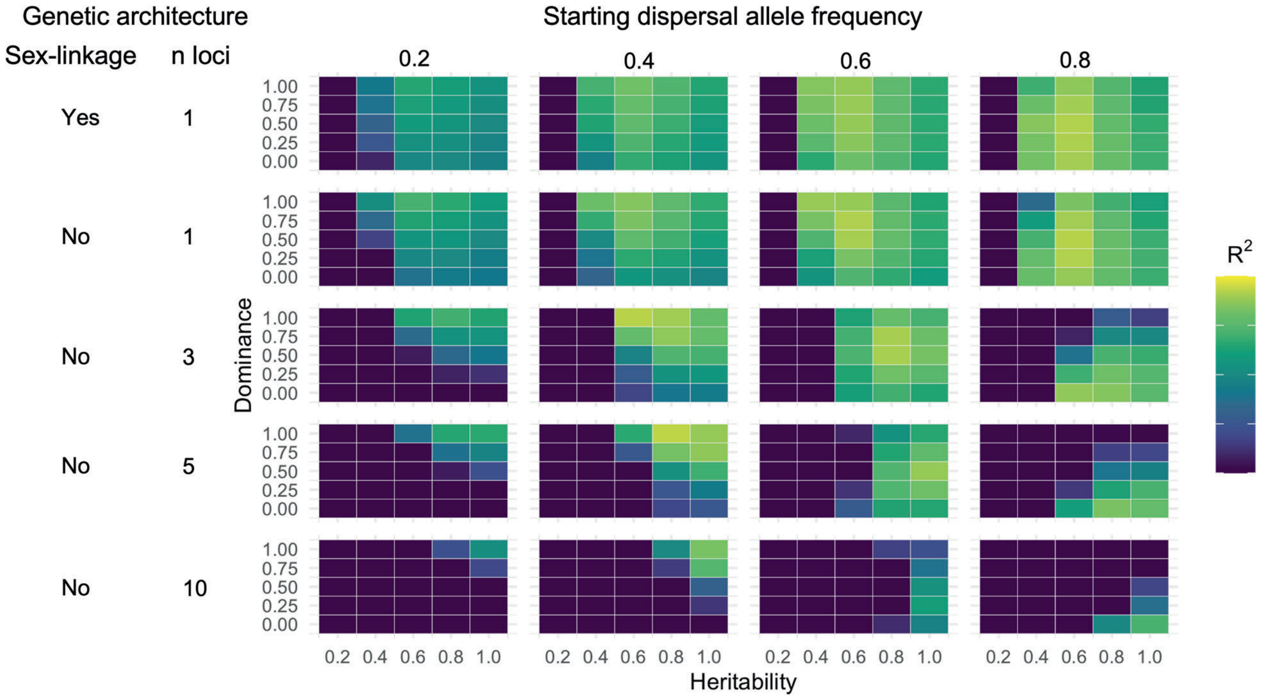

Fig. 4 Outputs from parameter scans using an agent-based simulation designed to model an emigration selection experiment using *T. castaneum* under different trait architectures. Colours represent R^2 values assessing the fit of a model defined on experimental data, and applied to simulated data across scenarios comprising combinations of dominance (d), trait heritability (h) and starting dispersal allele frequency (A), across a sex-linked single-locus, and unsex-linked single-, 3-, 5- and 10 locus architectures.

Table 3. General linear models of the dispersal behaviour of offspring of reciprocal crosses within and between simulated lines selected for high and low dispersal propensity, under sex-linked and unsex-linked genetic architectures.

	Sex-linked	**Unsex-linked**				
	Est	**SE**	***p***	**Est**	**SE**	***p***
(Intercept)	34.46	1.37	<0.001	51.04	0.69	<0.001
Cross type [HmLf]	(reference)					
Cross type [HmHf]	45.58	1.73	<0.001	28.33	0.87	<0.001
Cross type [LmHf]	30.26	1.73	<0.001	−0.66	0.87	0.447
Cross type [LmLf]	−14.94	1.73	<0.001	−30.19	0.87	<0.001
Sex	0.48	1.26	0.695	−0.15	0.61	0.807
Cross type [HmLf] x [female]	(reference)					
Cross type [HmHf] x male	29.32	1.71	<0.001	−2.62	1.73	0.13
Cross type [LmHf] x male	59.96	1.71	<0.001	1.8	1.73	0.298
Cross type [LmLf] x male	30.08	1.71	<0.001	0.54	1.73	0.755

Above the dashed line results are from a model fitting the individual effects of predictors, below the line results are from an equivalent model fitting the interaction between sex and cross type, with ‘HmLf’ as the reference category (model 1, sex-linked: Var = 116.26, SD = 10.78; model 2: Var = 116.26, SD = 10.78).

($R^2 > 0.8$) showed significantly non-unimodal distributions ($D < 0.18$, $p < 0.001$).

Under the 3-, 5- and 10-locus architectures the distribution of dispersal outcomes in generation 11 was shifted to the left but retained an apparently unimodal distribution. However, many scenarios under the 3- and 5-locus architectures showed statistical multimodality, even after adjustment for multiple comparisons (3-locus: $0.03 < D < 0.09$, $0.4<p < 0.001$; 5-locus: $0.02 < D < 0.04$, $0.02<p < 0.99$). All scenarios under these architectures also showed multimodality of allele frequency distributions ($D < 0.13$, $p < 0.001$). When the trait was controlled by 10 loci, neither the number of dispersers ($D = 0.02$, $p = 0.86$), nor the underlying allele frequency showed significantly multimodal distributions ($D = 0.03$, $p = 0.12$). Across all architectures, peaks in disperser numbers appear to correspond to peaks in dispersal allele frequency (Fig. 3, columns C and D).

When single-locus simulation results were subsampled down to experimental sample size ($n = 64$) the bimodal pattern remained strong, though the pattern was no longer significantly different from unimodality (Hartigan’s dip test; $D = 0.06$, $p = 0.07$), but did show multimodal allele frequency distribution ($D = 0.07$, $p < 0.05$).

DISCUSSION

Tribolium beetles are significant agricultural pests and important model organisms, whose life history is characterised by bouts of dispersal and colonisation (Dawson 1977), yet the genetic basis of dispersal behaviour in this species is unresolved. Here, we demonstrate rapid evolution of dispersal behaviour under strong selection, mostly as a response to selection for low dispersal but also as a significant increase in dispersal in high selection lines. We find no evidence of sex biases in dispersal in offspring of crosses, suggesting that the trait is not sex-linked. Simulations show that feasible parameter space over which simulated data can approximate experimental data exists under scenarios where the dispersal trait is controlled by one or few loci, but not many loci. However, levels of dispersal in experimentally inbred lines as compared with simulations, indicate that a single locus model is not well supported. Considered together, we suggest that these findings support an oligogenic architecture underlying dispersal in *Tribolium castaneum*.

Past work on *Tribolium* has provided evidence of a genetic basis to dispersal behaviour, which our results corroborate (Schurr and Bolduan 1967; Ogden 1970a, 1970b; Ritte and Lavie 1977; Korona 1991; Ruckman and Blackmon 2020). We saw a rapid response to selection on dispersal, creating extreme divergence between the behaviour of high and low dispersal selection regimes over just two generations in our *T. castaneum* lines. Such a result clearly demonstrates that variation for the trait was present in our stock *T. castaneum* population, despite more than ten years in the laboratory. This raises the question of how variation for dispersal behaviour is maintained in laboratory populations with no ability to disperse, especially since dispersal appears to trade-off with fecundity (Zirkle et al. 1988). It may be that positive correlates of dispersal observed in this species (e.g. shorter development time; Zirkle et al. 1988) are sufficient to keep dispersal phenotypes common. Interestingly, a greater reduction was observed in dispersal in low selection lines than the increase seen in high dispersal lines, indicating that dispersal tendency was already high in the original population. This is in contrast to previous studies which have shown lower initial rates and larger increases in dispersal in high selection lines (Ritte and Lavie 1977; Ruckman and Blackmon 2020). One explanation for this may be differences in methodology; our approach, with assays conducted on mixed-sex groups having had prior opportunity to mate, is more ecologically realistic than methods measuring dispersal in sibling groups (Ritte and Lavie 1977) and single-sex groups of virgins (Ruckman and Blackmon 2020), where movement may be driven by mate-searching. This observation may be explained by differences in the lines used; our KSS populations, having been bred to combine global *T. castaneum* diversity, are likely diverse relative to other strains and perhaps better capture the high level of dispersal present in wild populations existing in patches of ephemeral habitat, even though the strains used in both other studies were recently collected from the wild. Results are also consistent with differences in husbandry practices between laboratories (personal comm. Blackmon), our laboratory may have inadvertently selected for increased dispersal in stock populations prior to the study, perhaps by retrieving breeding individuals from the surface of the fodder to parent each subsequent generation. If this is the case, it suggests a relationship between dispersal and use of different strata of the fodder, which may warrant further investigation.

Our results, comparing experimental selection to selection simulated over a range of genetic parameters and architectures, broadly support an oligogenic architecture of dispersal. Models involving one, three and five, and to a lesser degree 10, additive loci were capable of agreeing with our empirical results under at least one combination of heritability, dominance and starting dispersal allele frequency. Therefore our findings suggest that if additive variance predominates in the genetic determination of dispersal, few loci are major contributors to the trait. However, we find no evidence that there needs to be only a single locus to account for the observed rapid change in behaviour under selection. Indeed, results from simulations and experiments using inbred lines provide tentative evidence that the trait involves more loci. Theoretically, under architectures with fewer interacting loci, fixation of alleles resulting from inbreeding has a larger impact on the focal trait. We observed this in simulations, where under a single locus the fixation of dispersal alleles was detectable as multimodality in the dispersal distribution. Importantly, the experimental results did not show multimodality in their dispersal distribution, suggesting that the trait may involve more than one locus. This finding is counter to the conclusions of Ritte and Lavie (1977), who postulated that *Tribolium* dispersal was controlled by a single locus. Recent theoretical work suggests that rapid adaptation can also occur with complex genetic architectures, including highly polygenic ones (Jain and Stephan 2017a, 2017b), and that the evolution of dispersal may be more rapid under the control of larger number of loci (Weiss-Lehman and Shaw 2022); counter to the prevailing wisdom that selective sweeps on large-effect loci are the predominant mode of rapid responses to selection (Messer and Petrov 2013). Such rapid evolution of dispersal can be important, for example, during range expansion; there is theoretical evidence that evolving increased dispersal at the range front can prevent the accumulation of expansion load (Peischl and Gilbert 2020). As a species that relies on ephemeral habitat (Dawson 1977), and has undergone extensive natural and human-mediated range expansion, this process may well have been important in the evolutionary history of *T. castaneum*. Oligogenic architectures of dispersal traits have been found in other insect taxa, notably in the Glanville Fritillary butterfly (*Melitaea cinxia*), where an epistatic interaction between two genes influences metapopulation dynamics through variation in dispersal (Hanski 2011; Niitepõld and Saastamoinen 2017).

We found that offspring of crosses between high and low dispersal lines demonstrated intermediate dispersal behaviour. This is consistent with some prior work (Ritte and Lavie 1977), but contrasts with one study which found that the dispersal of between treatment crosses was higher than in their high dispersal lines (Ruckman and Blackmon 2020). This elevated dispersal behaviour of cross offspring contributed heavily to their conclusion that epistatic variance outweighs additive variance in dispersal. This difference could, again, be due to their use of virgin single-sex groups, as opposed to mixed-sex groups, in dispersal assays, though further experiments would be needed to determine this. We were able to approximate through simulation both the response to selection and behaviour of the offspring of crosses observed in experiments, using only simple additive interactions between loci, without invoking epistasis. This is not to say that epistasis could not also account for the observed data, which we did not test, but that our observations are consistent with a simple additive architecture.

When we crossed experimental lines selected for high and low dispersal, offspring displayed the pattern expected under a non-sex-linked genetic architecture. Further, we included a single-locus sex-linked architecture among those which we simulated selection on dispersal. Experimental results agreed more closely with simulated crosses performed under an assumption of non-sex-linkage than with sex-linkage. These findings suggest that the dispersal trait on which we have selected is not controlled by a single sex-linked locus, as posited by Ritte and Lavie (1977).

Sex-biased dispersal is common in nature (Trochet et al. 2016), and sex-linkage of dispersal genes is likely important to its evolution (Brom et al. 2018). In our simulations, we saw sex-biased dispersal in only a small subset of scenarios of single loci sex-linked architecture. Complete dominance of either allele resulted in sex-biased dispersal, with females dispersing more when the dispersal allele was dominant and males dispersing more when the non-dispersal allele was dominant, as expected in an XX/XY system. When dominant alleles are rare, the strength of this effect is increased, explaining the interaction we observed between sex-biased dispersal and starting allele frequency in these simulations. Experimentally, while we saw no sex bias in the dispersal of high selection lines, we saw evidence of a weak female bias in low selection lines—a pattern not seen in our simulations, suggesting a cause not captured by our model. Similar results have been obtained by other studies of mixed-sex populations (Ogden 1970b; Ziegler 1977), suggesting that the male-biased dispersal seen in groups of siblings (Ritte and Lavie 1977) or single-sex virgins (Prus 1963; Ruckman and Blackmon 2020), is the result of either the mating-status, population sex ratio or relatedness. All of these are known to be important mediators of individual dispersal in other systems (Clobert et al. 2012), and in the case of relatedness, in *Tribolium* larvae (Jasieński et al. 1988). In particular mating status has recently been shown to alter dispersal decisions in the northern tamarisk beetle, *Diorhabda carinulata* (Clark et al. 2022). The contrast between our results and those using virgin or mated single-sex groups of *Tribolium* (Ritte and Lavie 1977; Ruckman and Blackmon 2020) highlights the importance of mating status and/or social environment in this system, and the ability of individuals to perceive and alter behaviour in response. Social environment is known to modify developmental life-history traits in *Tribolium* (Ellen et al. 2016), however, tests of mating status on flight have shown mixed results (Perez-Mendoza et al. 2011).

Our results support the view that dispersal in *Tribolium* is under the control of a small number of loci. A logical next step would be to investigate where these genes are and how they act to determine the phenotype. That *Tribolium* dispersal appears to be oligogenic (rather than polygenic) means that a genome-wide association approach may be able to detect loci across lines selected for differential dispersal. Known genes with large effects on dispersal in insects commonly have broad physiological, metabolic or neurological functions and are important beyond their effects on movement (Goossens et al. 2020); and it will be interesting to discover how genes are acting to control dispersal in *Tribolium*. It would also be instructive to explore whether dispersers and non-dispersers differ in measures such as leg length, activity and movement pattern. For example, a difference in dispersal but not leg length or movement pattern might suggest a physiological rather than a morphological or neurological basis. Such findings could then be thought of in relation to any candidate genes identified using genomics. *Tribolium* is a leading model system for RNA interference (Klingler and Bucher 2022), and candidate dispersal genes would be attractive targets for knockouts which, if effective, could present a valuable method of controlling this prolific pest.

Dispersal is an important aspect of the life history of many species, and its study in taxa such as pests and invaders could bring significant agroeconomic and biosecurity benefits. However, as a complex behaviour is challenging to study. We provide evidence that dispersal in the pest insect *T. castaneum* is under oligogenic control, opening up the possibility of identifying the loci involved using molecular genomics.

DATA AVAILABILITY

All data files, simulation scripts and analysis scripts associated with this article are available in Dryad (https://doi.org/10.5061/dryad.c866t1gcb).

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ACKNOWLEDGEMENTS

We are grateful to George West for his part in creating the inbred lines used in this study, and to three anonymous reviewers for constructive comments on the manuscript. This research was supported by a Biotechnology and Biological Sciences Research Council (BBSRC) studentship to MDP (BB/M011216/1), through the Norwich Research Park Doctoral Training Partnership, a BBSRC Future Leader fellowship to LGS (BB/N011759/1).

AUTHOR CONTRIBUTIONS

The study was conceived by LGS, MDP with input from MJG, and the experimental procedure designed by LGS and MDP. MDP carried out all experiments, data analysis and wrote the manuscript with input from DSR. The manuscript was then reviewed by all authors but MJG, before revision by MDP and DSR.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41437-023-00641-6.

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Supplementary material

Simulation methods

We simulated starting populations of 200 individuals, equal to that of experimental assays, and assigned genotypes probabilistically using specified starting allele frequencies (in runs using a sex-linked architecture the probability of receiving a Y as the second allele was equal to the sex ratio). Individuals were each assigned binary dispersal outcomes for each dispersal opportunity of the simulated assay, according to their genotype. The following equations were used to calculate the contribution of a single locus to the probability of dispersing given specified values of dispersal trait heritability (h) and dominance (d), which were then used to draw outcomes from a Bernoulli distribution. For an aa genotype this was:

$$\frac{1-h}{2} \tag{1}$$

for an Aa genotype;

$$\frac{2hd-h+1}{2} \tag{2}$$

And for an AA genotype;

$$\frac{h+1}{2} \tag{3}$$

Figure S3 shows how the probability of dispersal varied for each genotype across the possible combinations of h and d.

Following the format of the dispersal selection experiment, in the model 30 individuals were chosen at random from those that emigrated zero or three times to act as parents of the subsequent generation of low and high selection regime lines respectively. From this point, the model simulated and tracked low and high lines as discrete populations. Within each selection regime, each parental female was mated with between one and three males, with equal probability. The number of offspring produced by each female was chosen from a normal distribution with a mean of 100 and standard deviation of 25 (approximating typical values for *T.castaneum,* eg. (Vasudeva *et al.*, 2021). At each simulated locus independently, each of these offspring was assigned its first allele randomly chosen from the female parent and its second allele from one of that female’s mates with equal probability. These new individuals were then assigned dispersal outcomes as above, and the cycle of reproduction and dispersal assays repeated over a total of 5 generations.

Comparison of 3-opportunity and 1-opportunity dispersal assays

To ensure the continuity of inferences between 1- and 3-opportunity dispersal assays, we used dispersal data from generation 5 and fitted the same model to both 1- and 3-opportunity data and compared the results. First a model was fitted to the full 3-opportunity dataset, then the same model was separately fitted to the same dataset filtered to just the first of three dispersal opportunities, to represent a 1-opportunity assay. Initially we applied the same GLMM to the 1-opportunity data as has been described in the main analysis, however with the lower amount of data these models had singular fit (the variance explained by the random effect was zero). We therefore simplified the effects structure by dropping the random effect of selection line and fitted a GLM to both datasets - incorporating the response variable, with selection regime and block ID as fixed effects.

Data from both 1- and 3-opportunity dispersal assays were capable of showing that individuals from the high emigration selection regime were significantly more likely to emigrate than low regime beetles. The greater resolution of data over three dispersal opportunities led to a larger effect of selection regime using these data (GLM, $\beta=-1.58$, SE=0.06, $p<0.001$) relative to the data over a single dispersal opportunity (GLM, $\beta=-0.62$, SE=0.02, $p<0.001$).

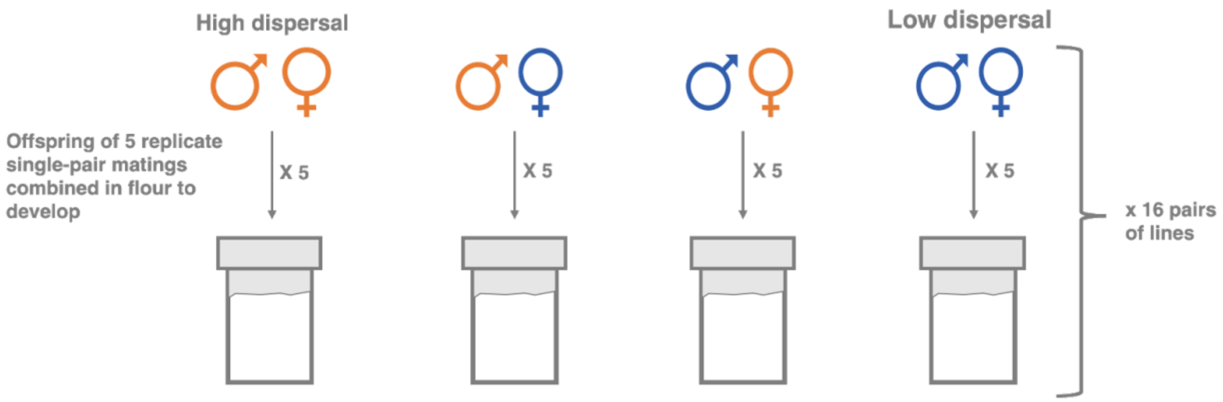

Figure S1. Representation of the method used to obtain offspring for dispersal assays of reciprocal crosses between pairs of high- and low-dispersal selection lines of *T.castaneum*. For each of 16 pairs of lines, each of 4 cross types was replicated with 5 single-pair matings, with egg-containing fodder combined to develop as a population.

Table S1. The genetic architectures simulated, and the range and increments used for each of the parameters heritability (h), dominance (d) and starting allele frequency (A). One combination of these parameters is termed a 'scenario'. The model was run using all 100 unique scenarios for each of the 5 genetic architectures tested.

Genetic architecture		Simulated scenarios: a unique combination of h, d and A		
		Range [increment] of simulated parameters		
Number of loci	Sex-linked	Heritability (h)	Dominance (d)	Starting allele frequency (A)
1	Yes	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]
1	No	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]
3	No	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]
5	No	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]
10	No	0.2 - 1 [0.2]	0.2 - 1 [0.2]	0.2 - 0.8 [0.2]

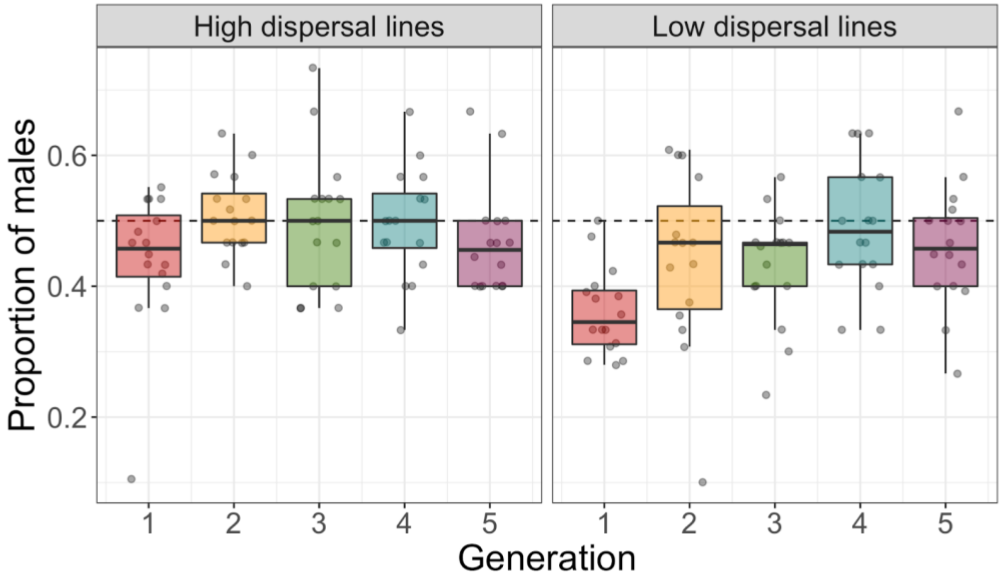

Figure S2. Sex ratio within groups of 30. *Tribolium castaneum* beetles selected to parent the next generation of high and low dispersal lines during an artificial selection experiment. The dashed line represents an equal sex ratio.

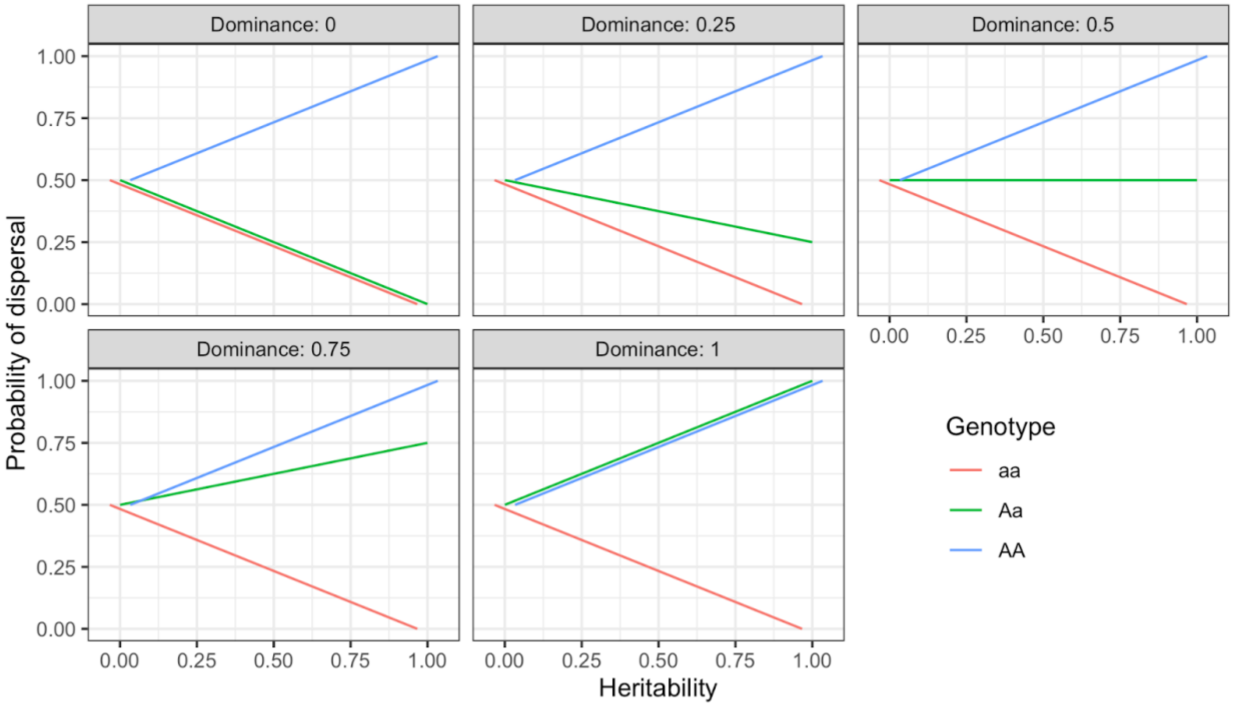

Figure S3. Dispersal probabilities of simulated individuals having each possible single-locus biallelic genotype, at combinations of heritability, and dominance (panels). Where lines occupy the same plot space they have been shifted so that both are visible, i.e. blue and red lines show reflective symmetry across the line y=0.5.

APPENDIX THREE

Published version of chapter four:

Pointer MD, Spurgin LG, Vasudeva R *et al.* (2024) Traits Underlying Experimentally Evolved Dispersal Behavior in *Tribolium castaneum*. *J Insect Behav* 37, 220–232. https://doi.org/10.1007/s10905-024-09862-x

J Insect Behav (2024) 37:220–232
https://doi.org/10.1007/s10905-024-09862-x

RESEARCH

Traits Underlying Experimentally Evolved Dispersal Behavior in *Tribolium castaneum*

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Received: 26 November 2023 / Revised: 27 August 2024 / Accepted: 12 September 2024 / Published online: 27 September 2024
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Abstract Dispersal is an important behavior in many animals, with profound effects on individual fitness and the evolutionary trajectories of populations. This is especially true within taxa with particular life-history strategies, for example those that exploit ephemeral habitat. Further, dispersal is commonly seen to be part of behavioral syndromes - suites of traits that covary across behavioral contexts. The red flour beetle, *Tribolium castaneum* (Coleoptera, Tenebrionidae), is a major post-harvest crop pest responsible for large losses through the infestation of stored grain. In this system dispersal is known to have a strong genetic basis and differential artificial selection on dispersal traits produces strong phenotypic divergence. However, it is unknown which traits are able to rapidly evolve to produce these results, or which behavioral components underlie differences in dispersal. Using replicate lines of *T. castaneum* previously selected for divergent dispersal behavior, we test for correlated activity and movement patterns, morphology and substrate surface use. We find robustly repeatable associations between the dispersal phenotype and higher activity, straighter paths, larger body size (but not relative leg length) and increased tendency to remain at the surface of fodder. Together our results suggest that dispersal is part of a syndrome of traits in *T. castaneum*, and must be treated as such when considering the evolution of dispersal in this system, and in attempting to predict and control its spread.

Keywords Artificial selection · Boldness · Dispersal · Emigration · Experimental evolution · Flour beetle · Tenebrionidae · *Tribolium*

Introduction

Dispersal is a life-history trait with great importance in the ecology and evolution of many species, and across different levels of organisation (Clobert et al. 2012). For the individual, the fitness consequences of relocation to a new environment can be enormous (Clobert et al. 2012), while individual dispersal outcomes aggregate to determine species' ranges and metapopulation structures through effects on gene flow (Kokko and Lopez-Sepulcre 2006; Ronce 2007). Consequently dispersal is a key parameter underlying evolutionary trajectories and metapopulation

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10905-024-09862-x.

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persistence in fragmented and unstable habitats (Suarez et al. 2022; Eriksson et al. 2014).

Dispersal is also central to several problems in contemporary biology. Greater insight into dispersal processes will hopefully enable us to better understand and predict species' ability to cope with anthropogenic changes to climates and landscapes (Travis et al. 2013). This will also make us better prepared to combat the introduction and spread of non-native species, a major driver of biodiversity loss (Renault et al. 2018). Research into the factors underlying intraspecific variation in dispersal, and the traits associated with dispersal strategies will also allow the integration of evolutionary theory into pest management (Mazzi and Dorn 2012); improved knowledge of the movement of pests will allow us to better forecast outbreaks, design pest management strategies and improve global food security (Jeger 1999).

A genetic basis of dispersal-related traits has been shown in a range of species (Saastamoinen et al. 2018), though the mechanistic basis of effects on dispersal are more difficult to study and often remain unknown. The genetic architecture of dispersal is usually thought to be polygenic (e.g. Jordan et al. 2012); however, genes with large effects on dispersal have been identified across taxa; and can be broadly separated into those with either metabolic (Niitepõld and Saastamoinen 2017) or neurophysiological (Trefilov 2000; Fidler et al. 2007; Krackow and Konig 2008; Sokolowski 1980; Anreiter and Sokolowski 2019) effects on movement. Neurophysiological variation can underlie suites of traits within an individual, leading to consistent and correlated responses that differ among individuals, called personality, typically studied in terms of traits such as activity and boldness (Roche et al. 2016). Non-behavioral traits can also be part of syndromes, for example genetic differences in morphology can be correlated with, or directly affect, dispersal. Classically, a wing-polyphenism in the pea aphid *Acyrthosiphon pisum* is under the control of a single sex-linked locus determining the presence/absence of wings (Caillaud et al. 2002), however, in other species morphological differences associated with movement can be more quantitative. Within species, overall body size often covaries with dispersal, though the picture is complex and the direction of the relationship is dependent on the system (Bowler and Benton 2005). In many cases the key morphological trait may be the size or shape of a specific functional structure, such as a leg or pelvis (Losos 1990; Hudson et al. 2016).

The red flour beetle *Tribolium castaneum* (Coleoptera, Tenebrionidae) is a globally significant post-harvest agricultural pest and an established model organism (Boxall 2001; El-Aziz 2011; Pointer et al. 2021). Dispersal is an important aspect of *Tribolium* ecology (Dawson 1977) and dispersal within and between food storage facilities frustrates efforts to control their impact as pests (Semeao et al. 2013). *Tribolium castaneum* moves by both walking and flight, but flies under only certain environmental conditions (Drury et al. 2016), is only very rarely seen to fly inside and is caught outside in traps targeting walking individuals (Semeao et al. 2013). Therefore, while longer dispersals are likely by flight (Ridley et al. 2011), walking is the most common mode of dispersal. A body of previous work in this system has used artificial selection or experimental evolution to demonstrate strong genetic control of locomotive dispersal and rapidly generate large phenotypic differences in dispersal propensity between lines (E.g. Prus (1966); Ogden 1970a); Ritte and Lavie (1977); Korona (1991); Melbourne and Hastings (2009); Weiss-Lehman et al. (2017); Ochocki and Miller (2017); Ruckman and Blackmon 2020; Arnold et al. 2023; Pointer et al. 2023). Correlations between dispersal and other life history traits have been found (Ritte and Lavie 1978; Lavie 1981; Zirkle et al. 1988; Pointer et al. 2024), however, the traits providing the proximate mechanisms underlying differences in dispersal have received little investigation outside of morphology (Arnold et al. 2017, 2023). Even here, results are equivocal, with leg length seen to vary positively with movement ability (Arnold et al. 2017), negatively with dispersal (Arnold et al. 2023) and variously with walking distance (Matsumura and Miyatake 2018, 2019; Matsumura et al. 2019). Hence, it is not currently known how dispersal is evolving in these populations, or which traits are able to respond to novel rapid selection on dispersal. Further, lines subject to negative selection for dispersal in Pointer et al. (2023) had the greatest phenotypic response, showing almost no dispersal propensity after five generations of selection. Identifying traits responsible for a loss of dispersal may be of particular interest in fields where the spread of organisms may be problematic, such as invasion biology or pest management.

Here we use lines of *T. castaneum* previously selected for differential dispersal (Pointer et al. 2023) to investigate associations between dispersal and other traits to understand the proximate mechanism leading to differences in dispersal tendency, and determine whether dispersal in this system is part of a behavioral syndrome. Specifically we test for differences in activity and movement patterns, morphology, and use of the surface of the habitat medium, that could result in the observed differences in levels of dispersal.

Methods

Beetles and Dispersal Propensity

The *Tribolium castaneum* beetles used in this study were from 44 experimental lines: 16 high dispersal lines and 16 low dispersal lines (referred to collectively as dispersal regimes) and 12 unselected control lines from the same original Krakow super-strain (KSS) stock (Laskowski et al. 2015), maintained under the same conditions as the selection lines for 5 generations prior to experiments. Full details of the selection experiment and husbandry procedures can be found in Pointer et al. (2023) but in brief, high (1–16 H) and low (1–16 L) dispersal lines were generated by five generations of divergent artificial selection, using a dispersal assay. In this assay, each individual was given three opportunities to “disperse” from a mixed-sex group of 200 conspecifics (i.e., leave a patch of suitable habitat (120 × 120 × 200 mm container filled to 50 mm with a 9:1 mixture of organic wheat flour and brewer’s yeast, and topped with oats for traction); cross a short distance of unsuitable habitat (150 mm of plastic tubing) and not return – see Fig. S1). Individuals that dispersed three times out of the three opportunities were considered to display a dispersive phenotype, and individuals that dispersed zero times from the three opportunities were considered to display a not-dispersive phenotype. Individuals of each of these phenotypic extremes were bred to produce the subsequent generation, while intermediate phenotypes were discarded. After a single generation of selection, mean dispersal phenotypes (measured as the mean number of dispersal events per individual out of three opportunities) between the treatments were significantly different. After five generations of selection, dispersal phenotypes were strongly divergent, and the distributions of dispersal phenotypes between the two treatments were non-overlapping (Pointer et al. 2023). Thereafter, in order to reduce experimental effort, selection was applied only in even numbered generations up to generation 16. In generation 17 we conducted an assay - using the above procedure as above - on populations of 200 beetles randomly selected from each experimental line. Individuals were marked after each dispersal opportunity and dispersal propensity in each line was scored as the mean number of realised dispersals per individual (out of the three opportunities).

Activity and Movement Pattern

For an individual to disperse in an assay, it had to encounter the opening of the tube to the second container of the dispersal arena (Fig. S1). More active individuals would be more likely to encounter the opening and therefore disperse more often than less active individuals. Thus it is possible that differential levels of locomotor activity and/or movement are driving differential dispersal in our selection lines, so we developed an activity assay to test for this difference.

Activity arenas were constructed by cutting around the sides of a 1.2 L plastic tub 20 mm from its bottom, to remove the base. This was then attached to a large white clay tile wrapped in laboratory tissue paper, using hot glue on the outer surface (Fig. S2A). This created a square area within which beetles were able to grip and could move freely, but could not escape by climbing the smooth walls of the tub. A set of twelve arenas were arranged such that each was positioned directly below a video camera (models: Sony HDRCX115E; Sony HDRCX190E; Sony HDRCX405) mounted to a horizontal board 300 mm above (Fig. S2B). Arenas were lit by LED strips attached to the underside of the board. Pupae from each of the 44 experimental lines were sexed and sorted to form test populations of 10 individuals at 1:1 sex ratio, as mating status has been shown to alter movement pattern (Wexler et al. 2017). At this stage the experiment was blinded, line identities were replaced with three figure codes to mitigate unconscious bias during data collection and analysis. Two temporal blocks were used, block 1 consisted of high dispersal lines 1–8, low dispersal lines 1–8 and control lines 1–6, block 2 consisted of high dispersal lines 9–16, low dispersal lines 9–16 and control lines 7–12. At 7 ± 1 days post-eclosion, each test population was placed into an experimental arena, given 10 min acclimation time, then recorded for 10 min at 25 frames per second. Assays were conducted either in the afternoon (1400–1600), or in the evening (1700–1900) in order to include the time of day when activity is known to peak in this species (Rafter et al. 2019). The timing of assays was randomised across all treatments. Event logging software BORIS (Friard and Gamba, 2016) was used to manually record the time of any escapes from activity arenas for each video.

Surface Affinity

Over the course of the selection experiment, we observed anecdotally that fewer beetles were present on the fodder surface in replicates of low dispersal selection lines than high dispersal lines. It is possible that individual decisions on whether to remain on the fodder surface are driving differences in dispersal (as only individuals on the surface encounter the dispersal tube). To test this we assayed the surface affinity of populations under the same conditions experienced during dispersal assays. Arenas were identical to pot A of the dispersal arenas used during artificial selection for dispersal (Fig. S1), but lacking the opening of the tube leading to a second pot. Test populations were placed onto the surface of the fodder in an arena. After 2 h, photographs were taken of the surface of the fodder and the number of beetles remaining on the surface of the fodder in each replicate was determined. This may have been an underestimate as some individuals were likely obscured by the oats, but gave a minimum value.

To determine if surface affinity is under genetic control we conducted selection on this trait over a single generation. The experimental setup was exactly as described above, but in addition the arena contained a horizontal slot through which a thin plastic separator could be pulled, partitioning the arena contents (Fig. S2). The slot was positioned such that, when the arena contained 250 ml of fodder and the separator was pulled across, fodder within 8 mm of the fodder surface was above the partition, and fodder more than 8 mm from the fodder surface was below the partition. Early trials suggested that an 8 mm distance provided the best separation of surface from not-surface individuals. Populations of 200 unselected stock beetles were placed into arenas, and after two hours the separator was pulled across and the beetles in each partition sieved from their fodder. Thirty individuals from each group of “surface” and “not-surface” beetles were then isolated together for three days, before being transferred to fresh fodder to oviposit. The adults were removed after 7 days and the eggs left to develop to adulthood. At 12 ± 3 days post-eclosion the offspring were assayed using the same procedure (above) as their parents to ascertain their surface affinity. The timing of assays was controlled, as above, by randomising all replicate trials across periods before and during the peak of activity.

Morphology

Beetles from the 44 experimental lines were collected 12 ± 3 days post eclosion and frozen at -80 C until thawed for dissection. The left elytrum and rear left leg were dissected out from 780 individual beetles, 15 of each sex from each of lines 1–10 L, 1–10 H and 1–6 C. Dissections were carried out under an Olympus SZX9 microscope using fine tip watchmaker’s forceps, in 30 µl insect saline solution on a clean glass slide. Body parts were imaged under 4X using a dark field phase contrast microscope and an Olympus BX41 camera through GX capture v8.5 software. A 1 mm calibration slide was used between sessions. ImageJ software (Schneider et al. 2012) was used to measure the length of the elytrum, femur, tibia and first tarsus segment, along with femur width at the widest point. *Tribolium* leg segments shown some curvature; however, we were interested in their linear extent as the determinant of stride-length. For individuals where an accurate measurement could not be made from the photograph, no measurement was taken. To standardise the procedure as far as possible, all dissection and photography was carried out by RV, using the outer surfaces of the right leg, and all measurements were taken by MDP. For a subset of 90 individuals measurements were repeated four days after the last initial measurements for analysis of repeatability.

Statistical Methods

All data wrangling and analyses were performed in R (ver.4.3.1; R core team 2023). Mixed models were fitted using package ‘lme4’ (Bates et al. 2015) with p-values added with ‘lmerTest’ (Kuznetsova et al. 2017). Summary statistics are presented as means ± standard error throughout.

To test for a difference in dispersal propensity we fitted a linear model (Table 1). The response variable was a population-level measure of the mean number of dispersals per individual, this was used as the density dependence of dispersal behavior (e.g. Ogden 1970b) means that individual dispersal events are non-independent.

Movement tracking was performed on 110 10 min (15000 frame) video clips using a machine learning algorithm in the software package Loopy (http://loopbio.com/loopy/; settings are provided in Table S1). Additionally, eight 400 frame clips were human-annotated to ground-truth the model. While individuals were tracked, due to the difficulty associated with tracking individuals moving over each other, tracks were aggregated to give a measure of activity at the population-level for each replicate. Location data from tracking software was used to derive three metrics of activity for each population.

1) As a replicate-level measure of activity, path lengths were calculated from location data as the total distance traveled, in pixels, by each individual per second (details provided in Supplementary methods). Pearson’s correlation was very high between path distances calculated from human-annotated clips and the same clips tracked with the machine learning model providing confidence in the tracking model ($n = 8$, rho = 0.99, 95%CIs = 0.98, 0.99).
2) Sinuosity of beetle paths was calculated per replicate, as a measure of movement tortuosity, according to the method of (Benhamou 2004); details provided in Supplementary methods).
3) Edge affinity was computed for each replicate as the proportion of recorded beetle locations that were within 10 mm of an edge of the arena, with edges of the arena defined as the maximum and minimum X and Y locations recorded across the whole replicate recording.

During data exploration, all records from a single camera (11) in block 2 were identified as extreme outliers and omitted from the analysis. The same linear mixed effects model (GLMM) structure was used to model the three movement metrics, path length, sinuosity and edge affinity (Table 1). The sinuosity model was fitted twice, once for each of two rediscretisation distances ($p = 10$ and $p = 20$), a parameter used to control for non-independence of consecutive turns (see Supplementary methods), results presented are from models using data where $P = 10$, however using $P = 20$ did not qualitatively change the results and these are presented in Supplementary results.

The surface affinity of populations and the genetic control of surface affinity were each analyzed with GLMMs (Table 1). Repeatability of morphological data was assessed by calculating Spearman’s rank correlation between repeated measurement taken from the same individual. As all morphological variables were highly correlated ($r > 0.6$), we condensed the information using factor analysis, on a correlation matrix using

Table 1 Model structure used in each test of a specific movement variable

Test	Independent variable	Fixed factor/s	Random factor/s
Dispersal	Mean dispersals per individual	Dispersal regime	
Path length	Path length	Dispersal regime	Block ID, Line ID, camera ID
Sinuosity	Sinuosity	Dispersal regime	Line ID, camera ID
Edge affinity	Edge affinity	Dispersal regime	Line ID, camera ID
Surface affinity - populations	Number on surface	Dispersal regime	Block
Genetic control of surface affinity	Proportion on surface	Selection regime	Block
Overall body size	Morphological PC1	Dispersal regime*sex	Line ID
Relative leg length	Leg length (femur + tibia)	Dispersal regime *sex, morphological PC1	Line ID

Models containing only fixed factors were fitted as GLMs, those containing random factors were fitted as GLMMs. Interactions are shown with the ‘*’ symbol, representing an interaction term alongside each of the interacting terms added individually the function *prcomp* from the R package 'stats' (R Core Team 2023). However, factor analysis cannot deal with missing data, of which there was a high proportion for femur length due to residual thorax tissue preventing accurate measurement; we therefore took forward only records for which all metrics had been quantified (n = 330). A single principal component (PC1) accounted for 74% of the total variation (Fig. S4). Each variable contributed roughly equally to PC1 (23–26%) and all were negatively correlated (Table S2), we therefore flipped the sign of PC1 to make the interpretation more intuitive. The remaining PCs each captured $\leq 10\%$ of the total variation and were less biologically interpretable. We therefore took forward only PC1 into further analyses, as a proxy for overall body size. We used GLMMs to test separately for overall size differences (represented by PC1) between selection regimes and sexes (Table 1) and for a difference in leg length relative to overall body size (Table 1). Where interaction terms were non-significant they were removed and models refit to test the independent effects of fixed factors.

Results

Dispersal Propensity

Beetles from low dispersal lines (0.70 ± 0.06) dispersed significantly less than those from high dispersal lines (2.44 ± 0.04; LM, β=-1.74, se = 0.07, $p < 0.001$; Fig. 1) and significantly less than unselected control lines (1.94 ± 0.06; LM, β=-1.23, se = 0.08, $p < 0.001$; Fig. 1). High dispersal lines dispersed more than control lines, with the magnitude of the difference being greater than that between low dispersal lines and controls (LM; β = 0.51, se = 0.08, $p < 0.001$; Fig. 1).

Activity and Movement Pattern

Average distances (pixels second^{-1}) traveled by beetles from low dispersal lines (111 ± 1.46) were shorter than those traveled by either high dispersal (129 ± 1.76; GLMM: β = -17.94, SE = 3.14, $p < 0.0001$; Fig. 2A) or control (125 ± 1.6; β=-14.24, SE = 3.35, $p < 0.001$) regimes, which did not differ from each other (β = 3.70, SE = 3.40, $p = 0.28$).

Beetles from low dispersal lines moved with more sinuous paths than did beetles from high dispersal lines ($P = 10$, GLMM: $P = 10$, β = 0.01, SE = 0.003, $p < 0.001$; Fig. 2B), and also moved more sinuously than control lines ($P = 10$, β = 0.009, SE = 0.003, $p = 0.01$). The control treatment sinuosity was intermediate between high and low lines, but did not differ from high lines ($P = 10$, β=-0.002, SE = 0.003, $p = 0.48$).

Edge use in low dispersal lines (0.50 ± 0.10) was not significantly higher than in high dispersal lines (0.47 ± 0.11; β = 0.03, SE = 0.02, $p = 0.06$; Fig. 2C) or controls (0.47 ± 0.09; β = 0.03, SE = 0.02, $p = 0.15$). Edge affinity of high dispersal lines did not differ significantly from that of controls (β=-0.006, SE = 0.02, $p = 0.76$).

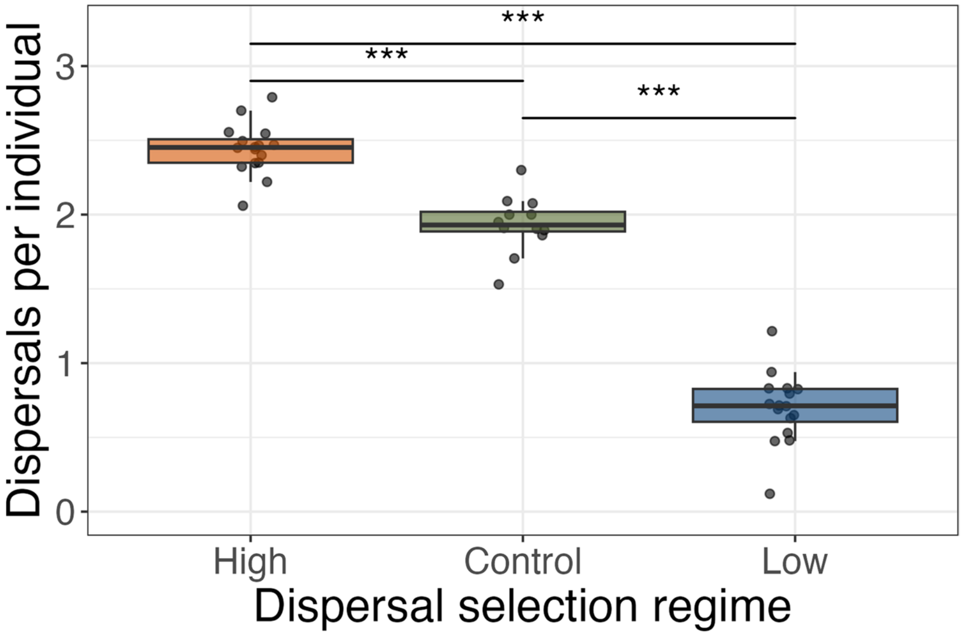

Fig. 1 Mean number of dispersals per individual (out of a maximum of three) in populations of 200 *Tribolium castaneum* flour beetles, taken from lines artificially selected for high ($n = 16$) or low ($n = 16$) levels of dispersal behavior or from unselected control lines ($n = 12$)

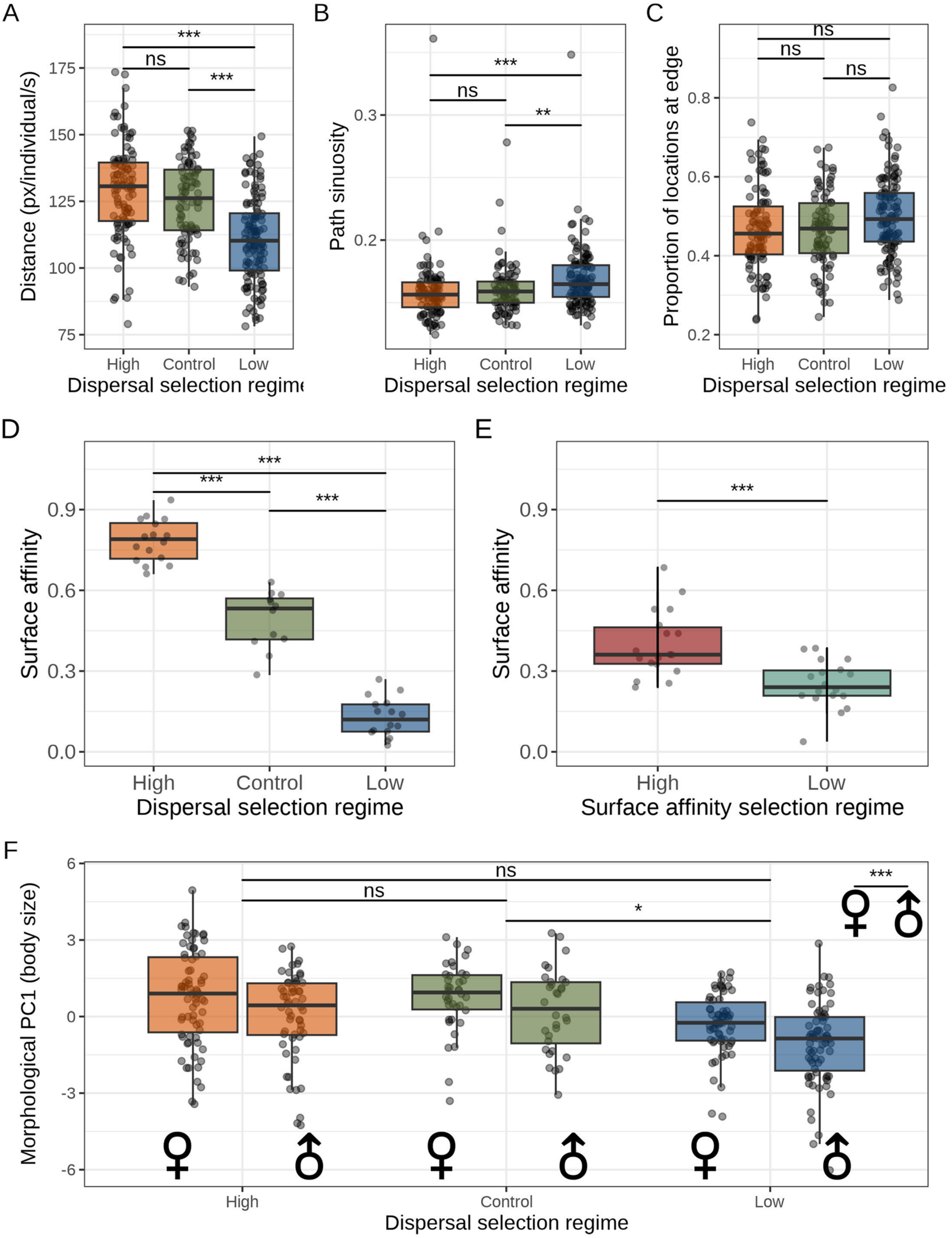

Fig. 2 Variation in traits across selection lines. Activity and movement traits in dispersal selection lines are shown as (**A**) tracked path length; **B** Path sinuosity; **C** Edge use. Surface affinity (proportion of 200 beetles remaining on the surface after 2 h) in lines artificially selected for (**D**) dispersal propensity over nine generations and (**E**) surface affinity over a single generation. **F** Body size represented by morphological PC1 in dispersal selection lines. Boxes display interquartile ranges and bold lines show medians

Surface Affinity

The surface affinity of high dispersal lines (157.00 ± 3.48) was greater than that of low dispersal lines (57.90 ± 6.60, GLMM; $\beta = 131.25$, SE = 5.96, $p < 0.001$; Fig. 2D) and controls (99.2 ± 9.33, GLMM; $\beta = 58.63$, SE = 6.43, $p < 0.001$). Low dispersal lines had lower surface affinity than did controls (β=-72.63, SE = 6.43, $p < 0.001$).

The offspring of unselected stock parents collected from the fodder surface were found on the surface significantly more than the offspring of parents collected from below the surface of the fodder (GLMM; $\beta = 0.15$, SE = 0.03, $P < 0.001$, Fig. 2E).

Morphology

Measurements of elytra length, femur length, femur width and tibia length were all highly repeatable (Spearman's rank correlation, $p < 0.001$; Table S2). Length of the first tarsus segment had substantially lower repeatability ($N = 55$, $r = 0.73$, $p < 0.001$) and was dropped from further analyses.

Sex was a strong predictor of PC1, with males being smaller than females (-0.42 ± 0.14 versus 0.39 ± 0.12 respectively; GLMM; $\beta = 0.82$, SE = 0.16, $p < 0.001$; Fig. 2F). PC1 was significantly lower in the low selection lines (-0.70 ± 0.13) than in controls (0.45 ± 0.16; GLMM, β=-1.10, SE = 0.52, $p = 0.049$), but did not differ either between high dispersal lines (0.52 ± 0.18) and controls (β=-0.32, SE = 05.2, $p = 0.55$), or between low and high dispersal lines (GLMM; β=-0.79, SE = 0.4, $p = 0.09$). There was no significant interaction effect between selection regime and sex on PC1 (GLMM; all $p > 0.07$). When controlling for PC1 as a measure of body size, leg length did not differ significantly between any combination of selection regime and control lines (GLMM; all $p > 0.42$), nor between males and females ($p = 0.07$), nor was any interaction significant between the effects of selection regime and sex (GLMM; all $p > 0.68$).

Discussion

We found that *Tribolium castaneum* populations selected for divergent dispersal behavior differ in levels of activity and movement pattern, use of the substrate surface and body size. The consistency of these effects across many independently evolving replicate lines (see Fig. 2) indicates that the coevolution of these traits is robustly repeatable. The tested traits are commonly seen as part of dispersal syndromes in other taxa, suggesting that dispersal in *Tribolium* should be considered in the context of a broad life-history strategy.

We observed that artificial selection on dispersal propensity generated significantly different levels of path sinuosity and activity in lines of *T. castaneum*. Previous studies in *Tribolium* have shown that movement distance is heritable, generating large differences in the trait under divergent artificial selection (Matsumura and Miyatake 2015), but have not assayed the dispersal propensity in these populations. Our finding agrees with many other studies across animals showing that dispersal is associated with exploratory activity (Krackow 2003; Cote et al. 2010b). The picture is similar for sinuosity, with exploratory individuals in many species moving on straighter paths (Brown et al. 2014; Klarevas-Irby et al. 2021), as we have shown for *T. castaneum*. Further, such activity and movement traits are commonly seen as personality traits and observed as components of behavioral syndromes, suites of traits that covary and show consistency within individuals (Wolf and Weissing 2012). In addition, we observe large variation within treatments of all activity and movement traits. High variance in movement traits, including dispersal itself, has also been seen as a feature of populations where dispersal is evolving (Melbourne and Hastings 2009; Ochocki and Miller 2017; Weiss-Lehman et al. 2017). However, we do not see similarly high variance in our measure of dispersal. Therefore, it may be that each movement trait is contributing only a small amount to the large overall difference in realised dispersal. It is also possible that the population-level approach we took to measuring activity and the AI tracking methodology introduced measurement error and inflated variances. If true, this may have prevented us from detecting differences in movement traits between high dispersal lines and controls, where the magnitude of the dispersal differences is less than between low dispersal lines and controls (reasons why dispersal may have responded more strongly to negative selection are discussed in more detail in Pointer et al. 2023).

For organisms living within a three-dimensional matrix - such as dead wood and grain masses, the ancestral and contemporary habitats of *T. castaneum* (Dawson 1977) - presence at the fodder surface increases risk. Therefore, an individual's willingness to expose itself to this risk may well be indicative of boldness. Boldness - broadly defined as risk-taking (Sloan Wilson et al. 1994) - is another prominent animal personality trait, which shows considerable individual variation in a broad range of taxa, from humans to cockroaches (Sloan Wilson et al. 1994; Stanley et al. 2017). Our results show that individuals from high dispersal lines spend more time at the fodder surface than those from low dispersal lines, suggesting a higher level of boldness. Dispersal is known to correlate with boldness across animal taxa, with important implications for invasiveness (Cote et al. 2010a; Myles-Gonzalez et al. 2015) and are thought to be mediated through common physiology and/or endocrinology (Cote et al. 2010a). This study examined surface use, as a measure of boldness, in the absence of predators. As predation represents one of the main risks to individuals, it may be that the presence of predators would modify surface use, and thus change, or reveal more about variation in, individual boldness. In the cockroach *Gromphadorhina portentosa*, past predator interaction reduces boldness (but not activity; McDermott et al. 2014). In contrast, boldness and predator avoidance represent separate axes of behavior in the ground beetle *Nebria brevicollis* (Labaude et al. 2018). Measuring surface use in the presence versus absence of a predator would resolve which of these patterns is followed in *Tribolium*, and even whether the response to predation risk varies across dispersal phenotypes. Additionally, time of day might play a role in mediating boldness and movement traits. Our measures here were averages across the on- and off-peak activity periods for *Tribolium* (Rafter et al. 2019), but future work performing separate tests across these times would reveal if dispersers show differential circadian cycles of activity than not-dispersers, however this was beyond the scope of the current project.

In addition to the effects of behavior, animal movement patterns also often differ as a result of quantitative individual differences in morphology. For example, sprint speed in lizards is dependent upon leg length (Losos 1990). Current evidence for associations between morphology and movement in *Tribolium* is not simple to interpret. Greater leg length, but not body size, was related to increased movement ability when tested within a single generation (Arnold et al. 2017), suggesting a simple mechanistic relationship between morphology and movement ability. Recent work measuring body size and femur length following artificial selection on dispersal showed that body size and mass were inversely related to dispersal propensity (Arnold et al. 2023). In addition, both body size and leg length (controlling for body size) were correlated with walking distance in lines selected for death-feigning duration (Matsumura and Miyatake 2019). However, separate studies have shown the opposite relationship, with shorter leg length in males artificially selected for walking distance (Matsumura et al. 2019), and no relationship in females (Matsumura and Miyatake 2018). In the current study we did not test morphology directly against movement, but tested both in lines selected for differential dispersal propensity. We found that lines selected for low dispersal propensity have experienced a concomitant reduction in body size - so in that sense dispersers have longer legs - but that leg length did not differ relative to body size. Hence, our results agree with the general expectation that dispersive individuals are larger (Renault 2020), and with specific examples where invasive beetles are larger at the range front (Laparie et al. 2013; Yarwood et al. 2021). However, we find a contrast with Arnold et al. (2023), which at first appears to be very similar work to ours in terms of comparing morphology in dispersal lines. On closer examination, the dispersal assays employed in each study differ markedly, with the present study requiring walking, whereas Arnold et al. required individuals to climb to disperse. As Arnold et al. note, "smaller individuals are biomechanically and energetically better suited to dispersal by climbing", in contrast to dispersal by walking, which may explain the differential findings. Interestingly, Arnold et al. (2023) also find that the sexes respond differently to selection on dispersal when using a design that selects separately on males and females in isolation. Thus, in their study dispersal included mate-finding behavior. In contrast, we aimed to exclude mate-finding by selecting on dispersal in mixed-sex groups of beetles and found no difference in morphological response between males and females. In this light, our results support the suggestion of Arnold et al. that mate-finding, which drives higher dispersal in males in the absence of females (Prus 1966; Ogden 1970b), may determine sex differences in morphology. Overall, such patterns serve to highlight the complexity of studying dispersal, and support the thesis that measures of movement traits are sensitive to differences in experimental design, setup and arena settings, as found by a recent study using *Tribolium* (Scharf et al. 2023).

Body size is a trait commonly seen as part of dispersal syndromes - the suites of traits associated with dispersal - though the direction of the relationship between size and dispersal is dependent on the specifics of the system, and even on environmental conditions (Bowler and Benton 2005). Dispersal in *Tribolium* is usually thought of as a way for small individuals who may be poor competitors to avoid resource competition (Zirkle et al. 1988; Arnaud et al. 2005), so a larger body size of dispersers suggests that other factors might also be at work. Greater size of dispersers is observed in many animal species, including insects (Anholt 1990; Bowler and Benton 2005). Possibly larger size is mechanistically beneficial for efficient movement, or it may be that larger size/better condition is required to undertake movement (Bowler and Benton 2005).

Since dispersal is “any movement of individuals or propagules with potential consequences for gene flow across space” (Ronce 2007), rather than a behavior per se, individual activity can be defined as a tendency that contributes to dispersal behavior (Benton et al. 2012). High dispersal lines were more active and moved on straighter paths, and it is difficult to imagine that such behavior would not tend to be more dispersive than the converse. Moving with greater activity and in straighter lines would achieve a given dispersal distance in less time, with less energy expenditure and less exposure to risk. Surface use also seems straightforwardly related to dispersal; the first stage of dispersal is emigration; in order to disperse an individual must leave its initial location (Clobert et al. 2012), a necessary first step in dispersing to a new patch might be choosing not to burrow into the substrate but to remain on the surface. We also observed that surface use responded significantly to a single generation of selection, suggesting that this trait has a simple genetic basis. There is evidence for an oligogenic genetic architecture of dispersal in *Tribolium* (Pointer et al. 2023), and the tight correlation between dispersal and surface use may point to either a shared mechanism or causal relationship between these traits. As commonly associated personality-related traits, activity and boldness may be underpinned by shared neurophysiological pathways, such as those related to dopamine signalling (Silva et al. 2020; Wu and Seebacher 2022). Loci with neurophysiological effects on dispersal include those relating to dopamine signalling in birds and mammals (Fidler et al. 2007; Krackow and Konig 2008; Trefilov 2000). In insects, the *foraging* gene, coding for a signalling regulator, affects dispersal in the diptera, lepidoptera and orthoptera (Sokolowski 1980; Anreiter and Sokolowski 2019). Dopamine and other biogenic amines have been previously linked to behavior in *T. castaneum* (Miyatake et al. 2008; Nishi et al. 2010). It would be instructive to investigate molecular genomic variation between the lines used in this study and sites related to dopamine signalling seem to be reasonable a priori candidates based on our results.

Overall, our results suggest that dispersal is associated with a suite of traits in *T. castaneum*. The presence of a such a dispersal syndrome, or personality-dependent dispersal, is known from many taxa, from single-celled organisms to mammals, including many insects (Cote et al. 2010a; Clobert et al. 2012; Fronhofer et al. 2018; Renault 2020). Further, dispersal syndromes may be part of overarching pace-of-life syndromes linking personality to multiple behaviors (Réale et al. 2010). Some traits in a syndrome may directly assist dispersal, whereas others mitigate costs, although the line between these categories is often blurred (Cote et al. 2010a). The set of traits tested in this study: activity pattern; morphology; surface use, all covary with dispersal tendency in a direction that plausibly enhances the dispersal in high dispersal lines and/or mitigates the costs of dispersing. Deeper understanding of the dispersal syndrome in *T. castaneum* requires genomic study and multivariate analysis of a broad range traits, across a large set of lines. Ideally these lines would be isogenic to remove individual-level variation and enable characterisation of traits and genotypes in genetically identical, and genetically stable, populations across space and time. Dispersal interacts with many aspects of ecology and life-history, and traits of interest might include those related to development, reproduction, population dynamics and social environment, physiology, and senescence – all of which have been subjects of *Tribolium* research, but not in a framework capable of resolving their interrelatedness or genetic underpinnings. Knowledge of eco-evolutionary dynamics is key to understanding biogeography and changes in range (Wellenreuther et al. 2022), which is especially important for species with significant effects on the environment or human populations. Our findings suggest that suites of correlated traits enable species to respond to selection on dispersal; therefore, this context must be considered when investigating the limits of dispersal evolution, and in attempting to predict and control the spread of organisms such as invasive species, crops pests and disease vectors.

Acknowledgements AcknowledgmentsWe are grateful to members of the Tribolium lab at the University of East Anglia, in particular to Ryan Brock for technical help during video recording.

Author Contributions Michael Pointer conceptualised the work with David Richardson and Lewis Spurgin, who also acquired the funding. Michael Pointer developed the methodology with help from Lewis Spurgin and Ramakrishnan Vasudeva, and carried out investigation with help from Ramakrishnan Vasudeva. Michael Pointer conducted the analyses, prepared figures and drafted the original manuscript, and was assisted in reviewing and editing by Mark McMullan, Simon Butler and David Richardson. Project administration was done by David Richardson, who also provided supervision with Mark McMullan and Simon Butler.

Funding This research was supported by a Biotechnology and Biological Sciences Research Council studentship to MDP (BB/M011216/1), through the Norwich Research Park Doctoral Training Partnership, and a Future Leader fellowship to LGS (BB/N011759/1).

Data Availability The data files and analysis scripts used in this study are openly available from Mendeley data (https://doi.org/10.17632/zcb97xf8xt.1).

Declarations

Ethical Note This research consisted of non-invasive observations of natural behavior of insects and no ethical approval was required in the UK. Nevertheless, all experiments were carried out according to good scientific and ethical practice.

Competing Interests The authors declare no competing interests.

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Supplementary methods

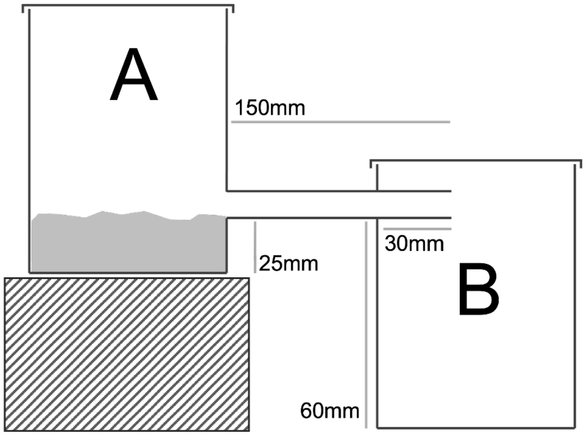

Figure S1. Experimental arena setup used to assay the dispersal behaviour of experimental *Tribolium castaneum* populations and provide a basis on which to artificially select individuals displaying high and low dispersal propensity.

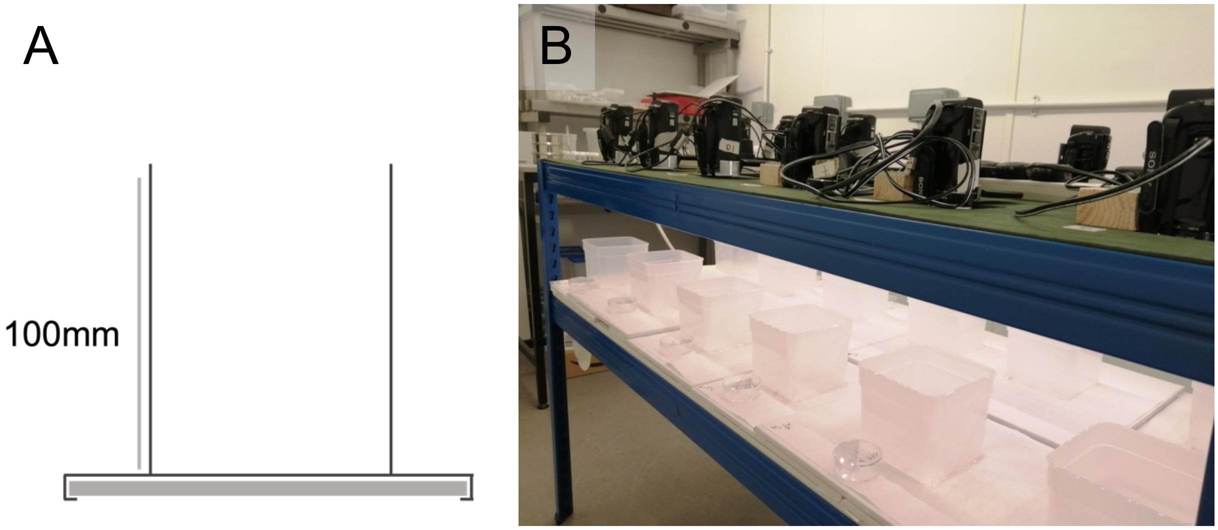

Figure S2. Experimental setup used to assay the movement behaviour of experimental *Tribolium castaneum* populations. A) Schematic of the arena, consisting of a plastic food container with the base removed, attached using hot glue to a ceramic tile wrapped in white laboratory tissue paper (shown in grey). B) How the arenas shown in A were arranged beneath a custom-made tabletop, to which 12 video cameras were mounted to film video clips.

Activity and movement pattern

Path lengths were calculated from location data for each detected object as:

$$\sum_{i=min}^{max} \sqrt{(x_{i-1} - x_i)^2 + (y_{i-1} - y_i)^2}$$

Where each i is a frame of the video and x and y are horizontal and vertical coordinate locations respectively, in pixels. These values were then summed across all objects from a single replicate to give a total path length for that population. Note that the tracking model struggles to follow individuals moving over each other, therefore an ‘object’ does not correspond to an individual beetle over the length of the recording, but a feature in the recording with locations assigned to it by the tracking model over a series of consecutive frames - as such the number of objects detected in a recording is many more than the number of individual beetles, because many objects may be different components of the same individual’s path.

To account for beetles escaping during the observation period, time of escapes was noted from videos and the sum taken of length of time each beetle was present to give a measure of the number of beetle-seconds represented in each video observation. Total path lengths were divided by beetle-seconds per replicate to give the measure of mean distance traveled per individual per second.

Sinuosity of beetle paths (S; a measure of straightness of travel) was calculated from location data as:

$$S = 2[P(\frac{1 - c^2 - s^2}{(1 - c)^2 + s^2} + b^2)]^{-0.5}$$

Where P = the physical distance between location readings, b = the coefficient of variation of P, c = the mean sine of all turning angles within a path, s=the mean cosine of all turning angles within a path (Benhamou, 2004). Prior to the calculation, object tracks were trimmed to the longest sequence where no location was within 1/35 of the width of the area from any edge - because encountering the edge would influence the path. Object tracks were then ‘rediscretised’ (given a standardised physical distance between recorded locations) of p - this accounts for spatial autocorrelation of turning angles in data with high measurement density (Benhamou 2004). Sinuosity was independently computed for each tracked object and a mean taken across all objects from a single video file.

Table S1. Values of parameters used when applying a trained keypoint pose predictor algorithm to track *Tribolium castaneum* beetles from pre-recorded videos in software LOOPY (LoopBio).

Parameter	**Value**
Score	0.5
Max detections per class	10

Min detection distance	8
Max tracking distance	22
Death threshold	10

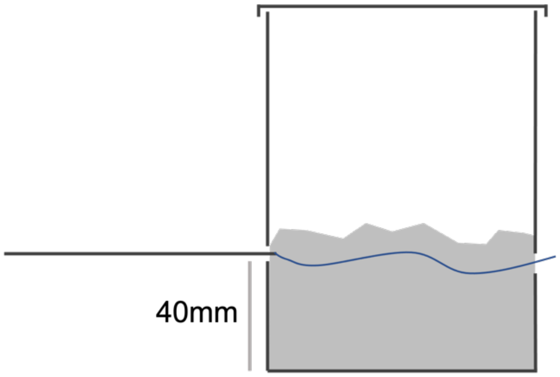

Figure S3. Experimental arena used to assay the surface affinity behaviour of experimental *Tribolium castaneum* populations and provide a basis on which to artificially select individuals displaying high and low surface affinity. By pulling a string (blue) threaded through small holes in the walls of the container, a stiff plastic separator could be pulled through a slit in the opposite wall. This completely isolated fodder and beetles within 8mm of the fodder surface from those below.

Supplementary results

Activity and movement pattern

Sinuosity models using the larger rediscretisation distance ($P=20$) produced qualitatively the same results as those using $P=10$, for completeness we give the full results for $P=20$ here. Beetles from low dispersal lines moved with more sinuous paths than did beetles from high dispersal lines ($P=20$, $\beta=0.007$, SE=0.002, $p<0.01$), and also mov**ed more sinuously than high dispersal lines ($P=20$, $\beta=0.005$, SE=0.003, $p=0.049$).** The control treatment sinuosity was intermediate between high and low lines, but did not differ from high lines ($P=20$, $\beta=-0.002$, SE=0.003, $p=0.42$).

Morphology

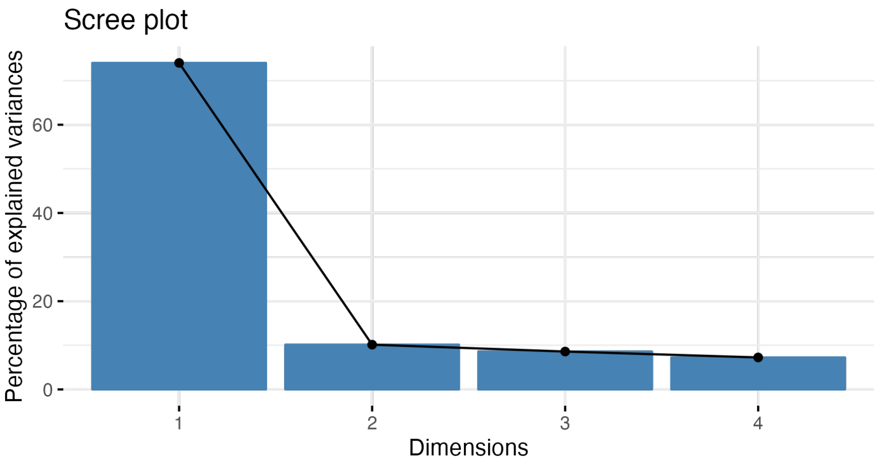

Figure S4. The variance explained by each principal component generated by factor analysis on morphological variables measured from *T. castaneum* individuals taken from replicate lines experimentally evolved for high (h), control (no selection; KSS) or low (l) levels of dispersal behaviour.

Table S2. Loadings for each principal component generated by factor analysis on morphological variables measured from *T. castaneum* individuals taken from replicate lines experimentally evolved for high (h), control (no selection; KSS) or low (l) levels of dispersal behaviour.

Variable	**PC1**	**PC2**	**PC3**	**PC4**
Elytra length	-0.5024428	-0.05924221	-0.8126212	0.2892894
Femur length	-0.5082749	0.20898609	0.5291783	0.6464919
Femur width	-0.4888609	-0.77011670	0.2402095	-0.3320160
Tibia width	-0.5002229	0.59977949	0.0437777	-0.6229969

Table S3. Repeatability of morphological measurements taken from *Tribolium castaneum* beetles artificially selected for dispersal propensity.

		Repeatability (Spearman's rank correlation)	
Variable	**N**	**rho**	**p**
Elytra length	90	0.99	<0.001
Femur length	51	0.98	<0.001
Femur width	82	0.91	<0.001
Tibia length	83	0.92	<0.001
First tarsus segment length	55	0.73	<0.001

APPENDIX FOUR

Published version of chapter five:

Pointer MD, Spurgin LG, McMullan M, Butler S, Richardson DS (2024) Life history correlations and trade-offs resulting from selection for dispersal in *Tribolium castaneum*. *Journal of Evolutionary Biology* 37, 748–757. https://doi.org/10.1093/jeb/voae041

Journal of Evolutionary Biology, 2024, **37**, 748–757
https://doi.org/10.1093/jeb/voae041
Advance access publication 24 April 2024
Research Article

JOURNAL OF
Evolutionary Biology
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Life history correlations and trade-offs resulting from selection for dispersal in *Tribolium castaneum*

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Abstract

Dispersal is an important facet of the life history of many organisms and is, therefore, subject to selective pressure but does not evolve in isolation. Across nature, there are examples of dispersal syndromes and life history strategies in which suites of traits coevolve and covary with dispersal in combinations that serve to maximize fitness in a given ecological context. The red rust flour beetle, *Tribolium castaneum*, is a model organism and globally significant post-harvest pest that relies on dispersal to reach new patches of ephemeral habitat. Dispersal behaviour in *Tribolium* has a strong genetic basis. However, a robust understanding of the relationship between dispersal and other life-history components, which could elucidate evolutionary processes and allow pest managers to control their spread and reduce the impact of infestation, is currently lacking. Here, we use highly replicated lines of *T. castaneum* previously artificially selected for divergent small-scale dispersal propensity to robustly test several important life history components: reproductive strategy, development time, and longevity. As predicted, we find that a suite of important changes as a result of our selection on dispersal: high dispersal propensity is associated with a lower number of longer mating attempts by males, lower investment in early life reproduction by females, slower development of later-laid offspring, and longer female life span. These findings indicate that correlated intraspecific variation in dispersal and related traits may represent alternative life history strategies in *T. castaneum*. We therefore suggest that pest management efforts to mitigate the species' agro-economic impact should consider the eco-evolutionary dynamics within multiple life histories. The benefits of doing so could be felt both through improved targeting of efforts to reduce spread and also in forecasting how the selection pressures applied through pest management are likely to affect pest evolution.

Keywords: dispersal, dispersal syndrome, emigration, experimental evolution, flour beetle, life history, *Tribolium*

Introduction

Dispersal is the movement of individuals with the potential to induce gene flow and, therefore, plays a crucial role in evolutionary ecology (Ronce, 2007). It is an important element of the life history of many organisms, with effects ranging from the individual level to the spatial dynamics of metapopulations (Matthysen, 2012; Benton & Bowler, 2012). A greater understanding of the ecology and evolution of dispersal will help in addressing several key problems in contemporary biology, including species' responses to climate change, spatial functioning of populations under habitat fragmentation, and the spread of invasive species (Travis et al., 2013; Renault et al., 2018).

Dispersal shows associations with many other traits across a broad range of taxa, and these suites of correlated phenotypes can be termed dispersal syndromes. Where there is individual variation in dispersal within species, high and low dispersal may represent different life history strategies (Clobert et al., 2012). As movement is energetically costly, it might be assumed that correlations between dispersal and other traits may be negative simply because the portion of any resource expended on dispersal is unavailable to be invested elsewhere. In reality, however, the picture is far more complex, and the presence, strength, and direction of relationships, as well as the traits involved, are highly context dependent (Bonte & Dahirel, 2017) and vary even with the individual components of dispersal (Jervis et al., 2007). The concerted evolution of suites of traits serves to mitigate the significant costs of dispersal and maximize individual fitness within the specific ecology of different systems (Bonte et al., 2012). Dispersal syndromes, and the underlying correlations and trade-offs in phenotypes associated with dispersal, have attracted much attention (Ronce & Clobert, 2012) as they provide insight into the multivariate nature of selection on life history traits and the constraints on their evolution (Bonte et al., 2012; Clobert et al., 2012). Most notably, dispersal consistently shows a relationship between fecundity and survival across taxonomic orders; however, even these correlations are far from universal, depending on a range of factors, including locomotion strategy and mode of thermoregulation (Stevens et al., 2014). The majority of research has focussed on vertebrates where individual tracking is possible (e.g., Cote & Clobert, 2012) and on wing-dimorphic insects where the dispersal phenotypes are discontinuous (Zera & Denno, 1997; Guerra, 2011). Less has been done on wing-monomorphic insects where dispersal phenotypes are more difficult to quantify. Among these wing-monomorphic insects, the majority of studies are on species where flight is the major mode of

Received October 10, 2023; revised March 14, 2024; accepted April 23, 2024

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movement, and therefore, differences in energetic output between dispersal phenotypes are likely to be much more important than in systems where dispersal is by walking. Studies are required within more diverse systems to enable the recognition of more general patterns and to identify the extent to which causal factors and evolutionary consequences are dependent on the evolutionary ecology of the individual taxa (Bonte & Dahirel, 2017; Ronce & Clobert, 2012).

The red flour beetle, *Tribolium castaneum*, is a globally significant post-harvest crop pest (El-Aziz, 2011) long used as a model system in evolutionary and population biology (Pointer et al., 2021). The species is long-lived for a small beetle, having a short egg, larval, and pupal stages (<5, <20, and <5 days, respectively, under near optimal conditions) followed by a long adult stage of up to 4 years (Good, 1936; Pointer et al., 2021). Historically, *Tribolium* were free-living, likely beneath the bark of trees or in rotting wood, where they were secondary colonizers characterized by rapid population growth, intense intraspecific competition, and ready dispersal (Dawson, 1977). At an unknown time in its history, the species switched to being predominantly human-commensal, infesting a wide variety of stored food products (Dawson, 1977). While they are capable of long-distance movement by flight, *Tribolium* beetles fly only in high temperatures (~>25 °C; Drury et al., 2016), and therefore, dispersal movements by flight are likely limited to outdoor movements in the tropics. In contrast, dispersals in the species' introduced, temperate range, or within artificially cooled food storage facilities, are likely to be predominantly by walking. Recent work in this system has shown variation in life history traits in natural populations (Matsumura et al., 2023), also that local dispersal (specifically emigration, but we keep the term dispersal for consistency with the existing literature) in *Tribolium* has a strong genetic basis, and that the architecture of the trait may be relatively simple (Pointer et al., 2023). Furthermore, dispersal propensity is correlated with several functionally dispersal-promoting traits (activity, movement pattern, affinity for the surface of the medium; Pointer et al. in review) related to activity and boldness, generally considered to be important dimensions of animal personality (Roche et al., 2016). As an established model system, the evolutionary ecology of *Tribolium* is relatively well understood (Pointer et al., 2020), yet despite the importance of dispersal to the species and to human pest management (Dawson, 1977; Jeger, 1999), little is known about a possible dispersal syndrome among life history traits. Such knowledge could help to predict the evolutionary consequences of management practices (Karlsson Green et al., 2020). The only previous studies used poorly replicated selection lines, selected under suboptimal conditions (Lavie & Ritte, 1978; Zirkle et al., 1988 using lines from Ritte & Lavie, 1977), or focused on correlations with walking motivation (distance walked by an individual in 30 min in the absence of food or conspecifics; e.g., Matsumura & Miyatake, 2019, using lines from Matsumura & Miyatake, 2015). As both the propensity to leave the fodder and directionality of movement are important aspects of walking dispersal in *Tribolium* (Korona, 1991; Pointer et al., in review), walking motivation is a single component of dispersal and a potential dispersal-enhancing trait, rather than dispersal per se, whereas the dispersal selection assay we employ explicitly captures all these components of dispersal behaviour.

Here, we use highly replicated lines of *T. castaneum* previously artificially selected for high and low levels of local dispersal propensity to investigate correlations between dispersal and several life history traits. The first trait we evaluate is male mating success. In wing-dimorphic insects, dispersal is known to show a negative association with mate acquisition traits (Guerra, 2011). Evidence for a similar trade-off in *Tribolium* has come from studying walking motivation lines (e.g., Matsumura et al., 2019). We expand on this previous work by using lines selected for a trait that better reflects dispersal and using mating assays within an ecologically realistic social environment, incorporating female choice and male–male competition, which are important aspects of reproductive biology in this system (Fedina & Lewis, 2008). If dispersal trades off with an investment in competitive ability, we expect to see highly dispersive males achieving fewer matings than less dispersive males.

We also evaluate female reproduction using a measure of fecundity. Fecundity is often seen to covary with dispersal, but the direction of the relationship is dependent on the cause (Stevens et al., 2014; Campos-Candela et al., 2019). The release from competition experienced by successful dispersers often means that they are able to invest more in reproduction (Burton et al. 2010). In contrast, the oogenesis-flight syndrome known from flying insects results from energetic constraint, meaning that dispersive individuals invest less in reproduction, particularly in early life (Johnson, 1969; Harshman & Zera, 2007; Tigreros & Davidowitz, 2019; Asplen, 2020). For this reason, we also include a temporal component, comparing fecundity before and after peak dispersal. Given the importance of intraspecific competition in *Tribolium* (Dawson, 1977), we expect population dynamic processes to dominate and, therefore, expect to observe increased female fecundity in highly dispersive lines, especially following the time of peak dispersal.

Another trait often seen to covary with dispersal is development time (Guerra, 2011). Associations between dispersal and development time in insects vary widely in strength and direction (Guerra, 2011), though these studies are overwhelmingly focussed on wing-dimorphic insects (but see Goodwyn & Fujisaki, 2007 in water-striders, Heteroptera: Gerridae). Such species are adapted to very different ecological conditions than the boom-bust cycles experienced by *Tribolium* populations, with bust phases characterized by cycles of very high intraspecific competition (including prolific larval and adult cannibalism of eggs and pupae [Stevens, 1989] and scarce resources [Dawson, 1977]). Under such conditions, a reversed dispersal-development pattern might be expected, in which a high-competition environment selects for rapid development to mitigate the risk of cannibalism by larvae to later-hatching eggs, with this pressure being relaxed among the offspring of dispersers in less competitive environments. Following this logic leads us to expect that a release from competition gained through dispersal will result in dispersive phenotypes being associated with slower development.

Finally, we examine longevity. As for fecundity, if energetic constraints influence life span, we might expect to see a negative relationship between dispersal and longevity. However, we expect high dispersal lines to have longer life spans, in line with the relationship observed among terrestrial animals, where greater longevity is thought to allow more time for a suitable reproductive habitat to be located (Stevens et al., 2014). This may be particularly strong considering the release from intense competition potentially afforded by successful dispersal in this system. Overall, if a dispersal syndrome

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exists in this system, we expect to see significant correlations between dispersal phenotype and many of the studied traits. We discuss our results in the context of a dispersal syndrome in *T. castaneum*, the relevance to both the evolution of this and similar species, and pest management aiming to control this prolific pest.

Materials and methods

Beetles and dispersal propensity

The *T. castaneum* beetles used in this study were from 44 experimental lines: 16 high dispersal lines, 16 low dispersal lines previously bred from an outbred stock population and 12 unselected control lines from the same original Krakow super-strain stock (Laskowski et al., 2015), maintained under the same conditions as the selection lines for five generations prior to experiments. High and low dispersal lines were bred under divergent artificial selection over five generations, using a dispersal assay in which each individual was given three opportunities to "disperse" from a mixed-sex group of 200 conspecifics (i.e., leave a patch of suitable habitat (120 mm × 120 mm × 200 mm container filled to 50 mm with a 9:1 mixture of organic wheat flour and brewer's yeast, and tops with oats for traction); cross a short distance of unsuitable habitat (150 mm of plastic tubing) and not return). Individuals that dispersed three times out of the three opportunities were considered to display a dispersive phenotype, and individuals that dispersed zero times from the three opportunities were considered to display a non-dispersive phenotype. Individuals of each of these extreme phenotypes were bred to produce the subsequent generation, while intermediate phenotypes were discarded. Further details of the selection experiment and of husbandry procedures can be found in Pointer et al. (2023). After a single generation of selection, mean dispersal phenotypes (measured as the mean number of dispersals per individual out of three opportunities) between the treatments were significantly different. After five generations of selection, dispersal phenotypes were strongly divergent, and the distributions of dispersal phenotypes between the two treatments were non-overlapping (Pointer et al., 2023). The lines were split into two temporal blocks to manage workload: block one consisted of high dispersal lines 1–8, low dispersal lines 1–8, and control lines 1–6, and block two consisted of high dispersal lines 9–16, low dispersal lines 9–16 and control lines 7–12. Individuals used for all experiments were taken from the same generation as those in which dispersal was quantified in Pointer et al. (in review), where dispersal was found to be still strongly divergent and non-overlapping between treatments (dispersals per individual out of a maximum of three, low dispersal lines = 0.70 ± 0.05; high dispersal lines = 2.44 ± 0.07). All experiments in this study used the 32 selected lines; in experiments where we were less constrained by limits imposed by experimental effort, we also incorporated 12 unselected control lines. Throughout all experiments, populations were kept on a fodder mix of 90% organic white bread flour and 10% brewer yeast; environmental conditions were maintained at 30 °C and 60% relative humidity—hereafter referred to as "standard conditions." This research consisted of non-invasive observations of the natural behaviour of insects, and no ethical approval was required in the United Kingdom. Nevertheless, all experiments were carried out according to good scientific and ethical practice.

Male mating behaviour

Mating behaviour was scored from 305 video recordings. Full details are given in Pointer et al. (in review), but briefly, test populations of 10 individuals at a 1:1 sex ratio were taken from the 44 experimental lines in 2 temporal blocks. Each assay consisted of recording one test population for 10 min within a 120 mm × 120 mm arena, which was smooth-sided to prevent climbing. The number of mountings and total duration of mounting within a 10-min window was scored for each replicate using event logging software BORIS (Friard & Gamba, 2016). It was impossible to entirely prevent escapes during recording; therefore, some replicates represent fewer beetles for part of the assay. To account for this, trait measures for each replicate were adjusted by the number of "beetle-seconds" described by that replicate (trait measure divided by the sum of the length of time each individual was present in the assay arena). This results in small values but represents the most accurate way to present the data.

Female reproductive output

The 32 dispersal selection lines were split into 2 temporal blocks. Twenty-two virgin adults of each sex were obtained per line by sexing as pupae and raising in single-sex groups of 20. Males were marked with a dot of paint on the dorsal thorax using a method that was shown not to affect reproductive output (Sales, 2018). Males and females were paired in vials for 48 hr of mating opportunity, after which the males were discarded. At 4 ± 2 days post-eclosion, females were transferred into pots to oviposit for 8 days, then transferred to a fresh pot for an additional 8 days of oviposition (hereafter termed oviposition periods). These timings were chosen to encompass the known dispersal peak of *T. castaneum*, which occurs at ~10 days post-eclosion (Ziegler, 1976). In the second temporal block of lines, the first 8 hr of oviposition were in a third pot to obtain individuals whose laying time was more precisely known to allow measurement of development time (below); however, offspring from the first 8 hr and the rest of the 8 days were combined in evaluating reproductive output. Oviposition pots were kept under standard conditions for 35 days after the removal of the female before being frozen until adult offspring could be counted. Offspring were allowed to develop in excess of fodder to eliminate any effect of density or resource limitation. A female's reproductive output was taken as the number of adult offspring resulting from a given period of oviposition. Replicates producing no offspring were removed from the analysis as they are far more likely to represent male reproductive failure rather than a lack of female fecundity, which is the variable of interest here. This is because rates of female infertility are extremely low in this system relative to those of males (Pai, 2001; Matsumura et al., 2023).

Development time

During the reproductive fitness assay, females from lines in the second temporal block (176 females from each dispersal regime) oviposited in a separate pot for the first 8 hr to obtain a cohort whose laying time was more precisely known. The offspring developing in these pots were observed every 24 hr, and each pupation day was recorded. We use pupation time as a measure of development to allow direct comparison with existing work (Zirkle et al., 1988).

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Longevity

Within the 2 temporal blocks, 10 males and 10 females from each of 44 experimental lines were sexed as pupae and raised in single-sex sex groups until 6 ± 3 days post-eclosion. At this point, each virgin individual was placed into a separate vial with 3-ml fodder. Each individual was observed every 14 days until day 462, with the time of deaths recorded until all individuals were dead.

Statistical methods

All data wrangling and analyses were performed in R (ver.4.3.1; R Core Team, 2021). Mixed models were fitted using package "*lme4*" (Bates et al., 2015) with *p*-values added with "*lmerTest*" (Kuznetsova et al., 2017). Model validation used the check_model function from the "*performance*" package (Lüdecke et al., 2021) and/or simulated residuals with DHARMa (Hartig, 2022). Most experiments included temporal block as a control variable. This could not be modelled as a random factor in GLMMs as it has only two levels, and random effects with few levels are known to produce imprecise estimates (Hodges, 2013). We had no reason to believe that block would have any effect on the traits under study, and the study design balanced dispersal treatments across the two blocks. Therefore, to simplify the main models in each analysis, we first checked that temporal block was not a significant predictor of the dependent variable (see Supplementary Material) and did not include it in the main models.

Both total reproductive output and timing of reproduction were modelled with LMMs (Table 1). To test the timing of reproduction, we initially modelled the difference in reproductive output between oviposition periods one and two; however, high overdispersion in these models prevented an adequate investigation. We therefore focussed on reproductive output in the first oviposition period, as early life reproduction is likely to be the most relevant for dispersal trade-offs (Zera & Denno, 1997).

Neither development time nor longevity data conformed to the proportional hazards assumption of Cox PH models. Seeing as the pupation rate among offspring in the development analysis was 100%, and no individuals needed to be censored from the dataset, we modelled time to pupation using a GLM with a Poisson error distribution (Table 1). Initially, we included female ID as a random factor to account for the fact that multiple individuals had the same mother; however, it was removed as it accounted for 0 variance in development time.

As Cox models were not appropriate, we modelled longevity using event history analysis (Keiding, 2014), which is qualitatively similar but without the assumption of proportional hazards. Rather than considering the time until some event (death), this approach considers the risk of an event occurring; hence, longevity is evaluated as a mortality risk. Accordingly, we modelled event (death) risk per time period using a binomial distribution; at each observation, individuals were assigned "0" if the event had not occurred and assigned "1" once the event took place. Repeated measures from each individual were included in the model via a random effect of individual ID. Line ID was initially included as a random effect but was removed as it added a large amount of complexity to the model structure without significantly improving model performance. Longevity models were also fitted for each sex independently, using the same structure but excluding sex as a fixed factor. The structure of GLMMs used to conduct these event history analyses is given in Table 1.

Results

Male mating behaviour

The number of mating attempts (mountings per individual per second, adjusted to account for escapes) observed in low dispersal lines (mean ± SE = 7.13e−4 ± 3.01e−5) was greater than that in either high dispersal (5.10e−4 ± 3.17e−5, GLMM: $\beta = 2.01\text{e}{-4}$, SE = 6.23e−5, $p < 0.01$; Figure 1Ai) or control lines (4.81e−4 ± 2.74e−5, GLMM: $\beta = 2.38\text{e}{-4}$, SE = 6.65e−5, $p < 0.001$). High dispersal lines did not differ from controls in their number of mating attempts ($\beta = 3.49\text{e}{-5}$, SE = 6.76e−5, $p = 0.61$). The total duration of mating (seconds per individual, adjusted to account for escapes) was not different between high dispersal lines (mean ± SE = 2.1e−2 ± 2.3e−3) and low dispersal lines (2.5e−2 ± 1.9e−3, GLMM: −3.9e−3, SE = 3.5e−3, $p = 0.27$; Figure 1Aii), between high dispersal lines and controls (2.0e−2 ± 2.2e−3, GLMM: $\beta = 9.2\text{e}{-4}$, SE = 3.8e−3, $p = 0.8$), or between low dispersal lines and controls (GLMM: $\beta = 4.8\text{e}{-3}$, SE = 3.7e−3, $p = 0.20$).

Reproductive output

Early life reproduction was significantly different between high and low selection lines, with high dispersal lines laying significantly fewer eggs in the first oviposition period (98.60 ± 1.74) than low dispersal lines (122.58 ± 2.08; LMM, $\beta = -23.89$, SE = 4.43, $p < 0.001$; Figure 1Bi). However, the total reproductive output across two oviposition periods did not differ between pairs from high (mean ± SE = 191.1 ± 3.9)

Table 1. Structure of models used to fit the effect of artificial selection for dispersal on several life history traits in replicate lines of *Tribolium castaneum*.

Trait	Dependent variable	Fixed factor/s	Random factor/s
Number of matings by males	Number of matings (per individual per second)	Dispersal regime	Line ID
Duration of mating by males	Mating duration (per individual per second)	Dispersal regime	Line ID
Total reproductive fitness	Total reproductive output	Dispersal regime	Line ID
Timing of reproductive fitness	Reproductive output in first oviposition period	Dispersal regime	Line ID
Development time	Pupation time	Dispersal regime × oviposition period	
Longevity	Alive (0)/dead (1)	Dispersal regime × sex	Individual ID, time

Note. The first column gives the biological trait of interest to a part of the analysis, and the second column gives the measured included as the dependent variable in the model, including standardization or codings.

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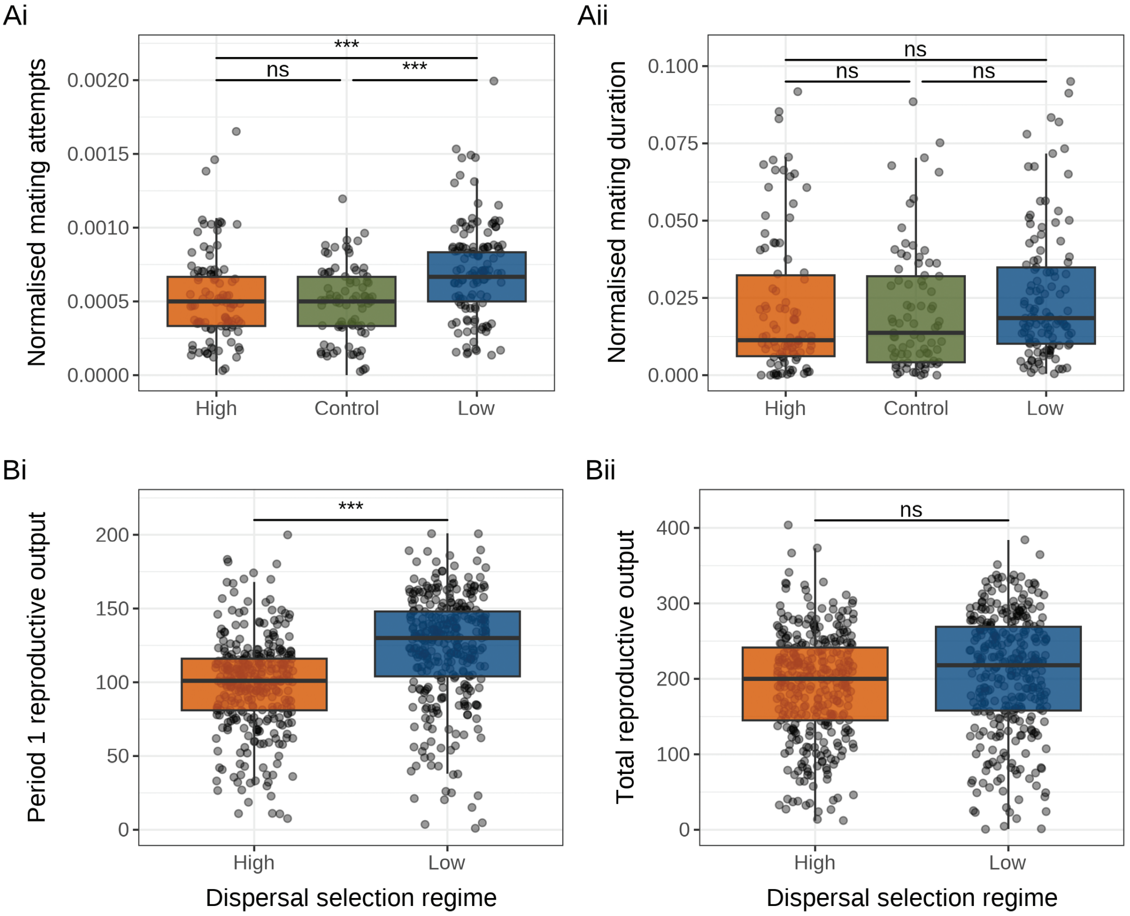

Figure 1. Reproductive traits in experimental lines of *Tribolium castaneum* selected for high or low dispersal behaviour and unselected controls. (A) Mating traits scored from 10-min video recordings of mixed-sex populations of ten individuals, showing (i) the number of mating attempts by males (normalized to be expressed per beetle per second) and (ii) the total mating duration (per beetle per second). These data are normalized by the number of "beetle-seconds" represented in replicate recordings to account for uneven representation due to escapes (trait measure divided by the sum of the number of seconds each beetle was present in the assay arena). (B) Reproductive output (number of adult offspring produced) of individual females, (i) in total across two 8-day periods of oviposition, roughly equating to before and after the peak of dispersal, and (ii) reproductive output in the first 8-day period of oviposition. Points are semi-transparent, so dark points indicate multiple overlapping points. ***A significance level of <0.001. "ns" indicates a non-significant difference.

and low dispersal lines (207.3 ± 4.4, GLMM: $\beta = 16.00$, SE = 8.73, $p = 0.077$; Figure 1Bii).

Development time

The pupation time of high dispersal lines (mean ± SE = 23.92 ± 0.08) was not significantly different from that of low dispersal lines (23.45 ± 0.08; Figure 2A; Table 2). Pupation time was significantly higher in the second oviposition period (24.44 ± 0.09) than the first (23.05 ± 0.07; Figure 2A; Table 2). There was also a significant interaction between the dispersal and oviposition period, with high dispersal lines in the second oviposition period having significantly lower pupation probability than expected from the additive effects of high dispersal and period 2 alone (Figure 2A; Table 2).

Longevity

High dispersal lines (median life span in days [mld] = 252) had significantly lower mortality risk than either low dispersal lines (mld = 224; Table 3) or controls (mld = 224; Table 3). Male mortality risk (mld = 294) was significantly lower than that of females (mld = 224; Table 3). There was a significant interaction between the dispersal regime and sex, with males from high dispersal and control regimes having greater mortality risk than expected under the additive effects of sex and dispersal regime alone (Table 3). Low dispersal males had lower mortality risk than predicted by a purely additive model. Post hoc tests individually for each sex showed that the difference in mortality risk between dispersal phenotypes was absent in males, where high dispersers (mld = 308) did not have significantly longer life spans than either control (mld = 252; GLMM mortality risk, $\beta = 0.30$, SE = 0.23, $p = 0.186$; Figure 2Bi) or low dispersal lines (mld = 308; GLMM mortality risk, $\beta = -0.12$, SE = 0.16, $p = 0.433$; Figure 2Bi). Instead, the overall difference was driven by a significant difference in females, where high dispersers (mld = 252) had longer life spans than control (mld = 210; GLMM mortality risk, $\beta = 0.41$, SE = 0.20, $p = 0.043$; Figure 2Bii) and low dispersal lines (mld = 210; GLMM mortality risk, $\beta = 0.58$, SE = 0.14, $p < 0.001$; Figure 2Bii).

Discussion

Our results show that aspects of mating behaviour, reproductive timing, development time, and longevity are covaried, with the tendency to initiate local dispersal by walking in *Tribolium castaneum*. Specifically, high dispersal propensity

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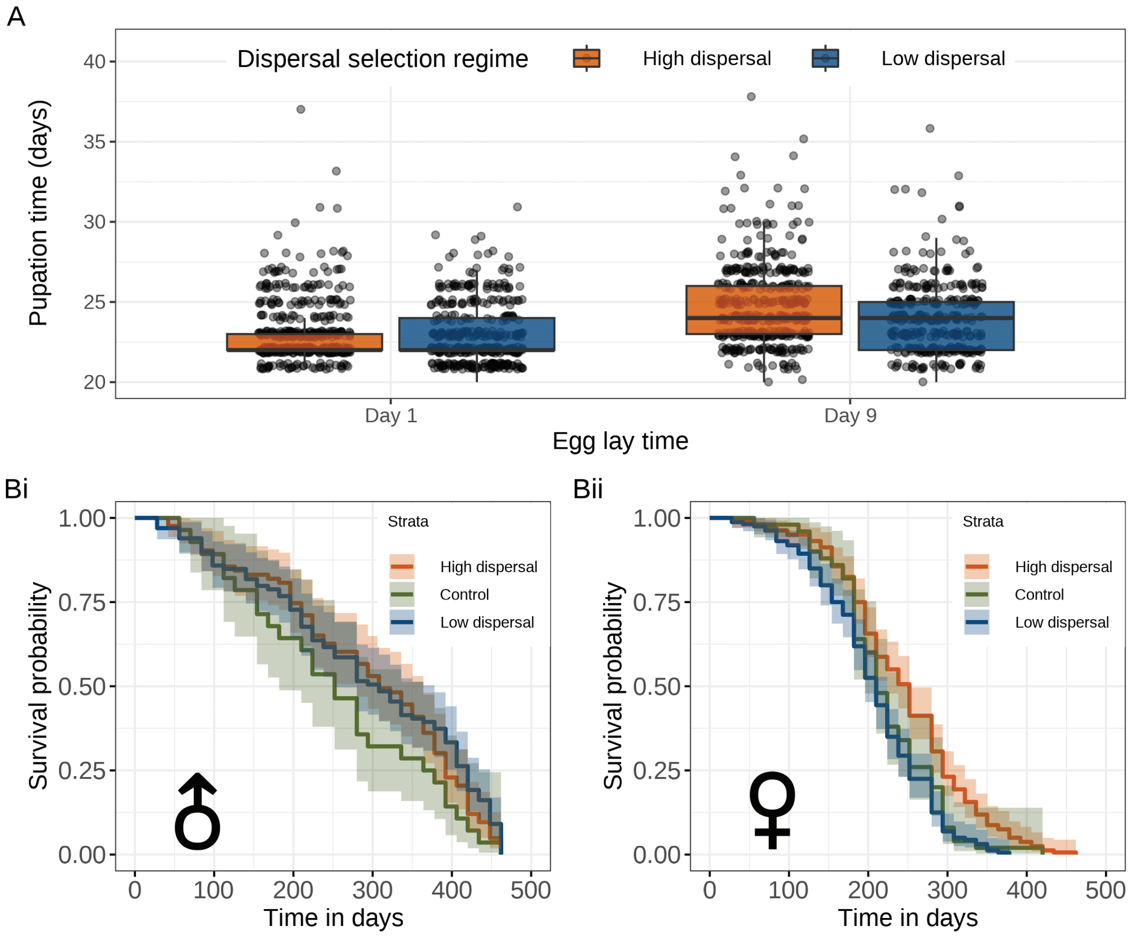

Figure 2. Life history traits in experimental lines of *Tribolium castaneum* selected for high or low dispersal behaviour. (A) Pupation time (days since the egg was laid) of offspring laid on either day 4 ± 2 or 13 ± 2 of adult life span (day 1 of oviposition period 1 and day 1 of oviposition period 2—these time points correspond to before and after the peak of dispersal in *Tribolium*), across dispersal regimes. (B) Survival probability across dispersal regimes for (i) males and (ii) females. Points are semi-transparent, so dark points indicate multiple overlapping points.

Table 2. GLM modelling the pupation time (days since the egg was laid) of offspring laid on either day 4 ± 2 or 13 ± 2 of adult life span across replicate lines selected for either high or low dispersal propensity.

	β	SE	*p*
Intercept	23.12	0.10	<0.001
Dispersal regime (low)	−0.14	0.15	0.34
Oviposition period (2)	1.66	0.15	<0.001
Dispersal regime (low): Period (2)	−0.64	0.22	0.004

Note. These time points correspond to day 1 of oviposition period 1 and day 1 of oviposition period 2, which are chosen to represent before and after the peak of dispersal in unselected *Tribolium* populations.

is significantly associated with fewer mating attempts, lower investment in early-life reproduction, slower development of later-laid offspring, and longer female life span.

Reproductive traits are commonly seen to vary with dispersal, and there are many possible reasons why mating behavior and dispersal might coevolve. For example, an individual might alter its reproductive environment through dispersal, changing the level of male–male competition or sexual antagonism it experiences. Alternatively, resources may need to be directed away from reproductive effort in preparation for dispersal. Correlations in both directions exist between reproductive traits and dispersal among insects, e.g., mating frequency is higher for dispersive Glanville fritillary butterflies (*Melitaea cinxia*, Bonte and Saastamoinen 2012) but lower for more dispersive long-winged male crickets (*Velarifictorus* spp., Zeng & Zhu, 2012; Zhao et al., 2017). In *T. castaneum*, we observed fewer matings overall among the high dispersal phenotype. While total mating duration was equal across the high and low dispersal phenotypes, we observed fewer matings among the high dispersers, suggesting that each high dispersal line mating is of longer duration. There are a number of plausible explanations for this observation; similar patterns are seen in other beetle species, where the balance of mating duration and remating frequency is adjusted in response to investment in other physical or behavioural traits (Simmonds & Kotiaho, 2007; Yamane et al., 2010). More specifically, the longer mating duration could, for example, be driven by prolonged mate-guarding in these lines (Fedina & Lewis, 2008), potentially as a result of uncertainty about the mating environment in a post-dispersal habitat. Alternatively, greater investment in peri-copulatory mechanisms might compensate for poorer sperm competition of dispersers (Matsumura et al., 2019). This represents an interesting opportunity for further work to investigate questions such as whether sperm competitiveness differs with dispersal, whether the mating behaviour

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Table 3. GLMM uses event history analysis to model longevity as mortality risk across time using a binomial distribution in lines of *Tribolium castaneum* artificially selected for dispersal propensity.

	Log odds	SE	*z*	Pr(>|*z*|)
(Intercept)	−2.58	0.37	−6.89	<0.001
Low dispersal	0.51	0.12	4.13	<0.001
Unselected dispersal control	0.35	0.17	1.98	0.047
Male	−0.72	0.15	−4.77	<0.001
Unselected dispersal control: Male	0.03	0.29	0.12	0.908
Low dispersal: Male	−0.63	0.20	3.09	0.002
(Intercept)	−2.07	0.38	−5.46	<0.001
High dispersal	−0.50	0.13	−3.91	<0.001
Unselected dispersal control	−0.16	0.18	−0.85	0.369
High dispersal: Male	0.63	0.20	3.10	<0.01
Unselected dispersal control: Male	0.69	0.30	2.33	0.024

Note. Data above the dashed line are from a model in which "high dispersal" was the reference category, below the dashed line "low dispersal" was the reference.

of males differs before and after the age of peak dispersal, and whether there is an effect of social environment on each reproductive strategy.

With regard to the reproductive output of females, in the present study, we saw no overall difference in the number of eggs laid between dispersal phenotypes, which aligns with the finding that reproductive output did not differ in *Tribolium* lines divergently selected specifically for walking distance (Matsumura & Miyatake, 2018). However, that study did not have a temporal component, whereas in the present study, we saw less investment in early reproduction in high dispersal lines. "Early" and "late" here, while both relatively early in the long lifetime of *Tribolium*, roughly coincide with before and after the age of peak dispersal, which occurs at ~10 days post-eclosion (Ziegler, 1976). Two possible, though not mutually exclusive, explanations for this observation are that (i) high investment in movement early in life reduces reproductive investment through energy constraint, and (ii) dispersers delay reproductive investment until after dispersal "in expectation" of finding a more favourable environment. Existing examples of delay in reproduction appear to be limited to flying insects and are attributed to energetic constraints (e.g., Zera & Zhao, 2006). However, in this case, the fact that total reproductive output is equal over both oviposition periods seems to lend support to the latter explanation. Similar findings have come from rhesus macaques (*Macaca mulatta*), where reproductive timing and dispersal are associated with the serotonin transporter gene genotype (Trefilov et al., 2000). This gene has been linked to aggressive behaviour and risk-taking, traits commonly seen as important elements of animal personality and behavioural syndromes (Roche et al., 2016). More research is required to uncover whether either i, ii, or both of these causes are driving reproductive timing differences between dispersal phenotypes, as well as the genetic and physiological mechanisms.

In our experiments focussed on development time, we observed no average difference in development time with dispersal phenotype over the whole laying period, in contrast to a previous study using *Tribolium*, which showed slower development of dispersers (Lavie & Ritte, 1978). However, we found that eggs laid later developed more slowly, and this effect was strongest in high dispersal lines. It is likely that this pattern is driven by the effects of cannibalism acting at different strengths across different temporal and spatial scenarios. Evidence is provided by a study in which populations selected for increased population size also showed increased cannibalism and shorter development times (Wade, 1979). A similar pattern has been seen in invasive cane toad populations (*Rhinella marina*), where tadpoles in high-density populations evolved cannibalistic feeding behaviour and, in response, reduced development time (DeVore et al., 2021). However, while these examples are suggestive, the situation in *Tribolium* is very complex, and to confirm a causative role of cannibalism, more information is needed on the relative strengths of different types of cannibalism (larvae eating eggs, larvae eating pupae, adults eating eggs, adults eating pupae) in different dispersal contexts. Interestingly, our results show some agreement with an experimental evolution study in *Tribolium*, where dispersal and other traits were allowed to coevolve as a population expanded across a landscape of connected habitat patches (Weiss-Lehman et al., 2017). Here, populations at the range front evolved high dispersal and low intrinsic growth rates. Our finding is that development time increases post-dispersal in dispersive lines, which might contribute to low growth rates in range-front populations.

We found that highly dispersive females had greater longevity than females from low dispersal lines but saw no difference among males. This result was opposite to that found in the only previous comparable study (Lavie, 1981; further discussed below). Among insects, most data on dispersal and longevity suggest that highly dispersive individuals have shorter life spans (Hanski et al., 2006; Gu et al., 2006). However, as with other traits, those studies on insects overwhelmingly focus on species where flight is the major mode of movement and differences in energetic output between dispersal phenotypes are likely to be much more important than in *Tribolium*. Indeed, the *Tribolium* dispersal phenotype has been shown previously to lack any association with a higher metabolic rate (Arnold et al., 2017), which might be expected to lead to early mortality (Lints, 1989). Our own experimental lines show differences in dispersal-enhancing traits that would not be expected to require higher metabolic output, such as straightness of movement and disinclination to burrow (Pointer et al., in review). Rather, our findings fit better with the most common relationship between these traits more broadly across terrestrial animals, where longevity is higher in dispersers, potentially allowing a longer time in which to locate a suitable habitat patch (Stevens et al., 2014). This may be additionally explained by competitive pressures in *Tribolium*, where the particularly intense competition experienced by residents selects for competitive ability over the life span, and escape from this competition could allow longer life in dispersers. In other systems, highly competitive environments are shown to select for higher metabolic rates (Hack, 1997; Pettersen et al., 2020). That the effect is seen only in females is interesting. Male *T. castaneum* ordinarily live far longer than females, so the observed effect served to equalize the life span of the sexes in high dispersal lines. Possibly, female longevity is suppressed more than male longevity under high competition, for example, by the known effect of male harassment (e.g., Attia et al., 2023), and release from competition allows them to approach the greater life span of males.

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Previous investigations into life history correlations with dispersal in *Tribolium* have either utilized poorly replicated selection lines generated under potentially unreliable methodologies (Ritte & Lavie, 1977; Lavie & Ritte, 1978; Lavie, 1981; Zirkle et al., 1988) or tested for associations with walking motivation as a proxy for dispersal, rather than dispersal per se (Matsumura & Miyatake, 2015, 2018). As well as serving as a robust, explicit test of a dispersal syndrome in *Tribolium*, we can compare the results of this study to previous work to assess the consistency of effects across independent studies using this system. Overall, our results conflict with those of earlier tests of life history traits using dispersal lines derived from Ritte and Lavie (1977), which found greater fecundity, faster development, and shorter life span among dispersers (Lavie & Ritte, 1978; Lavie, 1981; Zirkle et al., 1988). Some issues in the original selection experiment may contribute to these differences, as sample sizes were small (3 replicates per treatment vs. 16 in the present study), dispersal assays were conducted on groups of full siblings, and the strength of selection was highly inconsistent between generations (Ritte & Lavie, 1977). Nevertheless, it seems surprising that these methodological differences could flip the direction of relationships between traits. The present study finds greater agreement with work on lines selected for walking motivation by Matsumura and Miyatake (2015), who have subsequently shown that individuals with higher walking motivation have shorter matings and equal fecundity to those with lower walking motivation (Matsumura & Miyatake, 2018; Matsumura et al., 2019). In combination, data from these studies indicate that life history correlations in *Tribolium* are not necessarily robust to variations in methods used to generate and measure dispersal phenotypes. Consequently, the effects of all possible contributing factors must be considered when designing artificial selection regimes. We consider this study the most rigorous effort to date addressing these questions in the *Tribolium* system.

Taken together, our results suggest that correlations between life history traits and dispersal in *Tribolium* are qualitatively different from insects whose principal dispersal mode is by flight, where bioenergetic constraints are responsible for trade-offs. Instead, we suggest that the coevolution of life history and dispersal in *Tribolium* is shaped by dispersal, facilitating escape from intense intraspecific competition that characterizes the species' ecology (Dawson, 1977). It is important to note here that our investigations focus on emigration and walking movement as the mode of travel likely most relevant within temperature-controlled food storage facilities. It is possible that experimental selection on flight might uncover a set of correlations and trade-offs more akin to those seen in flying insects, but this is beyond the scope of the current study.

The observation of associations between traits inevitably raises questions about the mechanistic basis of such patterns. Genetic correlations between life history traits may be due to linkage-disequilibrium between causal loci or because of pleiotropy with key genes underlying multiple traits. Characterizing correlations in our *T. castaneum* lines opens up the potential for investigating the links between traits using molecular genomics. Current theory suggests that the genetic basis of *Tribolium* dispersal is relatively simple (Ritte & Lavie, 1977; Pointer et al., 2023) or at least involves only a few genes of large effect. Major effect loci implicated in dispersal and life history variation in insects are known (Saastamoinen et al., 2018), the best-studied example being the Pgi locus in the Glanville Fritillary butterfly (Klemme & Hanski, 2009; Niitepõld & Saastamoinen, 2017). However, the most comprehensive studies are in *Drosophila* and have identified hundreds of genes differentially expressed in common between lines independently artificially selected for aggression, mating behaviour and locomotor activity (Jordan et al., 2007). Resolving the genetic basis of dispersal and its association with life history variation may be highly instructive in considering how these traits evolve and how they may be managed.

Overall, our findings suggest that correlated intraspecific variation in local dispersal and related traits may represent alternative life history strategies in *T. castaneum*. We therefore suggest that pest management efforts to mitigate the species' agro-economic impact should consider the eco-evolutionary dynamics within multiple life histories. The benefits of doing so could be felt both through improved targeting of efforts to reduce spread and also in forecasting how the selection pressures applied through pest management are likely to affect pest evolution.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

Raw data files used in this study can be found on Dryad 10.5061/dryad.j6q573nn8 Representative image files can be found on Zenodo at https://doi.org/10.5281/zenodo.10852855.

Author contributions

Michael Pointer (Conceptualization [equal], Formal analysis [lead], Investigation [lead], Methodology [lead], Project administration [equal], Validation [equal], Visualization [lead], Writing—original draft [lead], Writing—review & editing [lead]), Lewis Spurgin (Conceptualization [equal], Investigation [supporting], Supervision [supporting], Writing—review & editing [supporting]), Mark McMullan (Conceptualization [supporting], Supervision [supporting], Writing—review & editing [supporting]), Simon Butler (Conceptualization [supporting], Supervision [supporting], Writing—review & editing [supporting]), and David Richardson (Conceptualization [supporting], Formal analysis [supporting], Investigation [supporting], Project administration [equal], Supervision [lead], Writing—review & editing [equal])

Funding

This research was supported by a Biotechnology and Biological Sciences Research Council (BBSRC) studentship to MDP (BB/M011216/1) through the Norwich Research Park Doctoral Training Partnership and a BBSRC Future Leader fellowship to LGS (BB/N011759/1).

Conflicts of interest

None declared.

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Supplementary material

The effect of temporal block on variables of interest.
Temporal block was not a significant predictor of mating duration (LM: β=-2.22e-3, SE=2.45e-3, p=0.365). Nor did block have a significant effect on the number of mating attempts (LM: β=-5.67e-5, SE=3.70e-5 , p=0.13). A GLM fitting the effect of block on total reproductive output were non-significant (β=-8.13, SE=5.91, p=0.170). An equivalent model fitted to just data from oviposition period one was also non-significant (β=5.03, SE=2.86, p=0.079). Considering longevity, a binomial GLMM fitting the effect of temporal block on time of death for showed no significant effect of block (β=-0.11, SE=0.09, p=0.233). Neither did a similar model fitted to only male data demonstrate a significant effect of block on time of death (β=-0.09, SE=0.15, p=0.551), and the same result was found for females (GLMM: β=-0.19, SE=0.11, p=0.090).

APPENDIX FIVE

Chapter six – supplementary material

Supplementary methods

Library preparation, performed at the Earlham institute

A total of 1ng of DNA was combined with 0.9 µl of Tagment DNA buffer, 0.1 µl Tagment enzyme TDE1 and 2 µl nuclease free water in a reaction volume of 5 µl and incubated for 10 minutes at 55˚C. Following the initial incubation, 5 µl of custom barcoded P5 and P7 compatible primers (2µM), 4 µl 5x Kapa Robust 2G reaction buffer B, 0.4 µl 10mM dNTPs, 0.1 µl Kapa Robust 2G enzyme (Sigma Aldrich: KK5005) and 5.5 µl water were mixed, giving a total PCR volume of 20 µl. The DNA was enriched with 14 cycles of PCR (72˚C for 3 minutes, 98°C for 3 minutes, 14 cycles of: 95°C for 10 seconds, 62°C for 30 seconds, 72°C for 3 minutes, final hold at 4 ˚C). Post PCR, the DNA was cleaned up with 1.25x volume of KAPA Pure Beads from Roche (07983298001) utilising the Tecan Fluent 780 liquid handling platform and final libraries were eluted in EB. The size distribution of each library was determined using the Perkin Elmer GX Touch DNA High Sensitivity assay (DNA High Sensitivity Reagent Kit CLS760672), and a smear analysis on a 450-650bp size range was performed, to equimolar pool the libraries. The pool of libraries were then subjected to size selection on a Blue Pippin 1.5% agarose cassette (R2 marker) from SAGE Science (BDF1510) recovering library molecules between 450-650bp. The final pool was quantified by q-PCR and sequenced on one lane of a 300 cycle Illumina NovaSeq 6000 S4 Reagent Kit v1.5 (Illumina 20028312). For this run the library was diluted down to 0.5 nM using EB (10mM Tris pH8.0) in a volume of 30ul before spiking in 1% Illumina phiX Control v3. This was denatured by adding 7ul 0.2N NaOH and incubating at room temperature for 8 mins, after which it was neutralised by adding 8ul 400mM tris pH 8.0. A master mix of DPX1, DPX2, and DPX3 from an Xp 4-lane kit v1.5 (Illumina 20043131) was made and 105ul added to the denatured pool leaving 150ul at a concentration of 100 pM 130ul of which was loaded onto a NovaSeq S4 flow cell using the NovaSeq Xp Flow Cell Dock. The flow cell was then loaded onto the NovaSeq 6000 along with an NovaSeq 6000 S4 cluster cartridge, buffer cartridge, and 300 cycle SBS cartridge. The NovaSeq had NVCS v1.7.5 and RTA v3.4.4 and was set up to sequence 150bp PE reads. The data was demultiplexed and converted to fastq using bcl2fastq2.

BayPass

The omega dataset, used to define the background allele frequency matrix, contained a subset of putatively neutral, high-confidence, highly representative, independent SNPs. Putatively neutral sites were obtained by filtering to those lying more than 10kb from exonic regions. Exon locations were extracted from the Ensembl annotation (Tribolium_castaneum.Tcas5.2.59.gff3.gz) and any SNP within these or 10kb in both directions (bedtools slop -b 10000; (Quinlan and Hall 2010) were excluded from the SNP vcf (bcftools -R). Next, sites were filtered to those that remained variant once KSS control samples were removed. We then filtered to sites on the linkage-group-level scaffolds, before removing rare variants (bcftools -- view MAF<0.04). BayPass is robust to missing data, but not when computing the allele frequency matrix (Ahrens et al. 2018; Gautier 2015), which requires low missingness and independent loci (Lotterhos 2019). Therefore, to ensure SNPs were representative of every population, we removed any sites missing in >2 individuals in any single population (vcftools –max-missing-count 2). To increase independence, variants were stringently filtered for LD, informed by our linkage estimation (bcftools +prune -m 0.3 -w 50kb).

The dataset used in BayPass analysis runs consisted of the SNP vcf, filtered to sites that were variant in dispersal selection treatments, on linkage-group-level scaffolds and filtered for MAF, but no additional missingness filter or LD prune was applied. Once filtered omega and analysis datasets were finalised in VCF format, the allele frequencies at these sites were converted to the population allele count format required by BayPass in a custom shell script (using vcftools –counts2). This allele count file of the omega dataset was then provided to a BayPass run (--gfile), using default settings, to generate the background matrix. The generated matrix (--omegafile) was then fed back to BayPass in a separate run, along with the allele count file of the analysis dataset (--gfile), and the treatment of each population (-contrastfile) to obtain the C_2 contrast statistic for each SNP. For these analytical runs, SNPs were filtered with the same minor allele frequency and linkage prune parameters as above, leaving 184,842 sites.

The obtained C_2 estimates were calibrated using pseudo-observed datasets. We used the *simulate.baypass* function from BayPass_utils.R; https://github.com/andbeck/BayPass/blob/master/baypass_utils.R) to simulate allele counts for a set of 100,000 SNPs sampled from the allele frequency correlation distribution defined by the omega matrix, computed from our empirical populations. We ran BayPass on this simulated data, supplying the same accompanying files and parameters as for empirical runs. The 0.999 quantile of C2 estimates derived from this ‘neutral simulation’ was then used to define an outlier threshold for the empirical BayPass output.

Table S1. Details of sequencing per individual, following the removal of duplicates (Picard v2.26.2 RemoveDuplicates; Broad Institute, 2019) and quality filtering (MAPQ >25 SAMtools view)

duplicates_removed			duplicates_removed_filtered		
number of reads	primary mappings	mean coverage	number of reads	primary mappings	mean coverage
12,213,204	11,684,170	10.16	9,040,962	8,995,909	7.94
11,181,508	10,728,659	9.32	8,276,169	8,236,634	7.26
10,425,395	9,977,670	8.69	7,919,440	7,881,689	6.96
12,104,960	11,537,101	10.03	9,134,284	9,089,122	8.02
13,771,468	13,180,772	11.46	10,277,445	10,224,109	9.03
10,120,377	9,734,410	8.51	7,291,604	7,258,510	6.45
10,362,134	9,913,406	8.56	7,858,414	7,819,982	6.87
10,611,604	10,166,845	8.79	7,852,622	7,813,241	6.87
9,826,285	9,417,418	8.16	7,405,197	7,368,977	6.47
13,005,436	12,421,980	10.80	9,564,395	9,511,943	8.41
13,212,085	12,640,549	10.96	9,750,140	9,697,072	8.57
14,565,958	13,807,107	12.06	10,954,229	10,892,994	9.65
11,890,264	11,398,020	9.92	8,955,488	8,910,523	7.88
11,518,764	11,025,793	9.57	8,778,140	8,735,137	7.69
14,091,513	13,419,350	11.63	10,281,156	10,222,152	9.00
14,237,868	13,573,229	11.81	10,472,325	10,411,939	9.20
16,489,244	15,826,742	13.74	12,727,019	12,678,179	11.13
14,365,689	13,787,213	12.07	10,924,327	10,884,351	9.67
16,073,839	15,405,970	13.34	12,329,458	12,266,262	10.79
15,609,648	14,894,910	12.94	11,674,119	11,608,965	10.25
12,247,155	11,729,231	10.11	9,031,087	8,986,659	7.89
10,907,012	10,448,871	9.05	8,141,347	8,100,188	7.14
11,973,966	11,482,501	9.95	9,093,261	9,048,379	7.96
17,252,361	16,463,403	14.32	12,938,383	12,868,991	11.37
10,806,857	10,324,558	8.99	8,141,634	8,103,577	7.15
10,913,659	10,463,709	9.12	8,117,116	8,077,124	7.14
11,069,282	10,581,127	9.19	8,370,271	8,330,026	7.34
1,057,722	1,016,880	0.89	796,404	792,759	0.70
10,960,006	10,436,905	9.07	8,327,421	8,286,340	7.30
10,981,086	10,489,461	9.13	8,294,628	8,255,154	7.29

12,021,087	11,544,331	10.06	9,258,694	9,213,373	8.14
11,070,232	10,594,834	9.18	8,350,263	8,308,174	7.32
12,418,797	11,887,244	10.32	9,427,808	9,380,081	8.28
13,371,881	12,182,829	10.62	9,423,498	9,375,222	8.27
12,719,940	12,110,720	10.53	9,494,796	9,446,322	8.34
12,619,027	12,060,310	10.52	9,474,340	9,421,495	8.34
16,028,913	15,163,897	13.16	11,704,238	11,643,016	10.27
13,500,176	12,809,054	11.14	10,101,092	10,048,328	8.87
14,291,205	13,623,022	11.82	10,663,374	10,606,599	9.36
17,755,447	16,619,115	14.47	13,373,131	13,296,167	11.76
12,005,315	11,484,854	9.98	9,165,177	9,119,988	8.04
11,716,639	11,253,652	9.74	8,835,546	8,791,169	7.74
15,333,333	14,649,239	12.76	11,602,942	11,539,121	10.21
13,313,445	12,623,097	11.00	9,711,915	9,656,200	8.52
15,087,784	14,297,701	12.43	10,882,153	10,816,183	9.54
12,804,168	12,165,298	10.57	9,463,249	9,413,764	8.31
12,198,603	11,706,831	10.14	9,276,316	9,232,191	8.12
12,605,109	12,052,758	10.48	9,607,231	9,556,346	8.44
10,703,491	10,252,231	8.90	7,996,967	7,958,579	7.00
6,078,228	5,839,512	5.07	4,615,696	4,594,964	4.04
10,759,204	10,287,491	8.93	8,163,087	8,122,809	7.16
11,061,631	10,607,875	9.21	8,188,561	8,145,636	7.20
12,401,619	11,800,748	10.26	9,371,629	9,325,778	8.22
11,921,755	11,393,328	9.87	8,998,119	8,951,803	7.89
12,924,225	12,350,467	10.77	9,733,120	9,684,583	8.55
11,775,684	11,301,921	9.82	9,018,313	8,975,114	7.91
10,749,449	10,147,512	8.84	7,319,953	7,271,745	6.43
12,436,081	11,893,275	10.36	9,198,505	9,149,475	8.08
13,731,246	13,134,502	11.43	10,199,151	10,147,924	8.95
13,069,182	12,535,235	10.89	9,923,707	9,872,736	8.71
18,600,212	17,760,696	15.38	13,836,700	13,765,302	12.13
11,088,508	10,576,009	9.18	8,014,385	7,972,980	7.03
12,378,789	11,867,465	10.31	9,099,520	9,053,167	7.99
15,067,549	14,404,874	12.52	11,304,402	11,242,903	9.92
12,515,418	11,980,431	10.42	9,569,913	9,520,858	8.40
143,986	139,136	0.12	106,865	106,340	0.09
17,412,721	16,501,457	14.26	12,917,937	12,843,221	11.30

12,997,996	12,331,202	10.68	9,640,572	9,588,392	8.46
14,996,072	14,279,609	12.36	11,094,544	11,032,388	9.71
11,026,120	10,430,449	9.08	7,536,229	7,485,955	6.61
12,536,912	12,023,216	10.44	9,441,331	9,393,191	8.30
6,863,454	6,432,839	5.59	4,384,393	4,358,401	3.84
10,848,382	10,398,937	9.01	8,094,965	8,054,858	7.09
13,518,050	12,956,962	11.24	10,244,752	10,193,611	8.98
12,435,544	11,828,492	10.18	9,235,973	9,189,532	8.03
11,529,839	11,094,695	9.57	8,944,844	8,901,671	7.82
13,201,448	12,585,846	10.95	10,077,513	10,023,660	8.86
11,839,473	11,376,272	9.86	8,908,763	8,860,102	7.82
6,860,487	6,459,367	5.61	4,478,265	4,451,818	3.92
10,363,606	9,809,624	8.56	7,021,531	6,977,921	6.17
10,657,720	10,236,527	8.90	8,051,251	8,011,587	7.07
9,198,269	8,795,421	7.67	6,691,839	6,655,571	5.89
12,225,244	11,639,493	10.09	9,023,817	8,974,299	7.92
11,662,428	11,102,277	9.62	8,693,353	8,648,384	7.61
12,989,393	12,206,757	10.63	9,662,274	9,612,292	8.50
12,176,438	11,664,612	10.13	9,502,782	9,456,105	8.34
11,715,316	11,216,265	9.73	8,658,038	8,611,329	7.59
14,533,788	13,752,973	11.96	10,602,094	10,538,536	9.31
12,138,605	11,566,883	10.06	8,781,736	8,731,146	7.72
12,035,817	11,481,361	9.96	8,788,926	8,740,613	7.70
17,710,426	16,914,030	14.73	13,996,814	13,935,690	12.32
11,778,630	11,279,862	9.77	8,819,860	8,774,196	7.74
11,987,904	11,427,213	9.87	8,943,690	8,899,459	7.82
10,448,610	9,977,120	8.65	7,898,648	7,861,215	6.92
10,431,838	9,906,286	8.56	7,764,456	7,728,708	6.79
13,256,545	12,562,557	10.97	9,851,467	9,798,684	8.67
13,983,395	13,287,316	11.57	10,308,770	10,259,002	9.06
12,985,922	12,396,095	10.77	9,592,485	9,540,314	8.43
12,326,905	11,773,978	10.19	9,248,871	9,203,403	8.10
12,446,359	11,879,732	10.34	9,233,086	9,180,766	8.12
11,647,756	11,040,817	9.55	8,523,194	8,477,193	7.46
13,178,610	11,896,797	10.36	9,271,263	9,225,165	8.15
11,483,934	10,932,251	9.53	8,969,076	8,926,151	7.87
13,938,041	13,336,158	11.62	10,656,851	10,603,350	9.36

11,526,261	10,998,601	9.52	8,682,078	8,638,080	7.60
15,745,062	15,053,383	13.08	12,173,699	12,112,129	10.67
9,965,923	9,545,106	8.25	7,478,586	7,442,718	6.54
11,653,929	11,168,018	9.72	9,060,087	9,018,093	7.95
11,635,455	11,058,525	9.60	8,342,893	8,300,311	7.33
11,646,297	11,148,438	9.66	8,400,207	8,358,608	7.37
10,748,614	10,327,897	8.99	8,036,443	7,997,274	7.07
10,568,382	10,105,762	8.75	7,889,680	7,850,830	6.91
11,841,622	11,382,107	9.85	8,943,548	8,898,921	7.83
11,691,136	11,146,667	9.63	9,041,574	9,001,370	7.88
14,493,451	13,823,722	12.04	10,868,519	10,807,667	9.56
12,616,232	12,069,098	10.47	9,599,745	9,547,188	8.41
11,732,242	11,062,372	9.65	8,187,354	8,138,527	7.19
10,275,603	9,853,420	8.52	7,725,688	7,687,177	6.76
10,953,576	10,518,955	9.12	8,255,795	8,213,949	7.23
3,995,031	3,697,482	3.22	2,412,314	2,395,717	2.12
11,211,632	10,691,895	9.29	8,208,648	8,169,133	7.21
13,572,589	12,967,546	11.23	10,225,148	10,173,895	8.94
6,627,616	6,360,941	5.50	5,008,835	4,984,721	4.38
11,764,013	11,258,119	9.76	8,827,600	8,782,088	7.75
2,401,470	2,315,754	2.00	1,769,941	1,762,209	1.54
9,797,113	9,352,026	8.10	7,228,974	7,192,540	6.34
10,422,469	9,931,609	8.66	7,552,629	7,508,231	6.63
13,908,357	13,335,312	11.63	10,753,356	10,700,615	9.45
15,789,591	15,073,326	13.12	12,161,493	12,097,619	10.68
8,840,784	8,332,742	7.26	6,259,122	6,225,006	5.49
10,674,812	10,236,848	8.87	8,156,722	8,117,549	7.16
12,436,324	11,840,569	10.33	9,496,676	9,450,143	8.36
13,006,242	12,338,133	10.73	9,418,446	9,368,446	8.28
10,865,991	10,360,084	8.96	7,903,591	7,866,419	6.92
13,261,267	12,720,129	11.08	10,012,708	9,962,362	8.81
11,664,035	11,209,448	9.68	9,063,344	9,020,820	7.92
12,683,898	12,176,713	10.47	9,731,101	9,683,103	8.48
10,678,514	10,207,393	8.81	7,946,350	7,905,669	6.95
7,708,253	7,249,250	6.33	5,319,777	5,282,599	4.67
14,849,629	14,175,720	12.33	11,110,038	11,048,787	9.76
12,027,830	11,362,881	9.93	8,535,693	8,478,971	7.51

5,974,983	5,466,743	4.76	3,928,522	3,901,771	3.45
10,650,776	10,024,119	8.71	7,372,998	7,328,256	6.46
7,740,489	7,291,414	6.35	5,198,751	5,165,999	4.56
13,370,489	12,814,664	11.18	10,125,245	10,075,324	8.92
9,583,975	9,125,635	7.95	7,005,108	6,969,916	6.15
13,197,317	12,613,463	10.99	10,148,285	10,098,677	8.92
12,568,291	12,018,260	10.44	9,529,089	9,480,901	8.37
11,249,891	10,782,325	9.37	8,614,940	8,568,829	7.58
1,736,551	1,668,610	1.45	1,305,802	1,299,531	1.15
9,231,628	8,427,334	7.33	6,238,597	6,199,001	5.47
8,926,888	8,336,881	7.28	6,165,457	6,128,511	5.42
10,345,263	9,458,038	8.26	6,990,260	6,943,576	6.14
8,821,857	8,064,309	7.03	6,082,909	6,048,965	5.34
12,339,102	11,612,150	10.13	9,508,367	9,463,085	8.36
12,285,187	11,718,564	10.18	9,318,477	9,271,055	8.20
12,819,912	11,807,600	10.24	9,229,736	9,184,223	8.08
11,740,190	11,196,315	9.72	8,565,136	8,520,003	7.52
11,614,810	11,001,944	9.61	8,434,074	8,389,894	7.43
12,668,585	12,146,536	10.58	9,606,041	9,559,066	8.44
12,678,357	12,164,523	10.59	9,716,856	9,670,572	8.54
12,328,811	11,774,281	10.22	9,226,320	9,176,416	8.09
7,041,324	6,513,036	5.67	4,697,232	4,666,611	4.12
15,045,607	14,395,726	12.52	11,348,006	11,286,125	9.97
13,195,352	12,635,660	10.98	9,929,082	9,875,805	8.72
18,166,834	17,357,578	15.07	13,478,294	13,399,500	11.83
8,424,272	7,870,832	6.84	5,557,744	5,521,432	4.87
11,463,552	10,912,475	9.48	8,430,562	8,387,832	7.40
11,415,722	10,968,617	9.48	8,472,252	8,432,846	7.41
10,721,502	10,261,297	8.86	7,781,361	7,743,483	6.80
15,107,716	14,450,804	12.58	11,286,415	11,228,296	9.92
13,663,370	13,064,589	11.37	10,360,553	10,308,389	9.11
11,781,373	11,303,029	9.80	9,083,395	9,038,823	7.96
12,501,391	11,991,405	10.45	9,567,471	9,518,160	8.43
9,770,541	9,207,740	8.02	6,746,995	6,705,439	5.92
11,988,940	11,372,905	9.85	8,996,775	8,949,682	7.89
9,731,719	9,237,748	8.03	6,807,607	6,767,841	5.97
10,395,554	9,903,040	8.64	7,551,366	7,507,251	6.64

11,302,886	10,785,665	9.38	8,442,426	8,396,587	7.42
11,615,392	10,720,150	9.36	8,458,386	8,413,783	7.45
13,157,419	12,505,316	10.93	9,719,942	9,663,082	8.56
12,204,241	11,702,517	10.17	9,317,466	9,268,767	8.18
11,451,896	10,981,278	9.54	8,541,072	8,499,124	7.51
12,686,698	12,108,515	10.43	9,059,172	9,006,911	7.90
14,941,999	14,248,144	12.40	10,911,894	10,843,063	9.59
11,308,660	10,836,539	9.39	8,223,433	8,178,383	7.21
17,894,228	17,010,556	14.80	13,386,922	13,313,236	11.77
13,046,978	12,442,626	10.80	9,794,650	9,743,912	8.59
13,957,245	13,302,052	11.51	10,241,180	10,186,956	8.97
12,763,549	12,159,370	10.46	9,431,722	9,384,352	8.19
12,136,799	11,620,696	10.08	9,037,989	8,992,687	7.92
11,949,027	11,428,810	9.83	9,132,246	9,087,070	7.92
11,702,614	11,175,617	9.70	8,725,690	8,682,521	7.68
13,558,019	12,973,543	11.19	9,951,575	9,901,140	8.72
15,295,590	14,615,585	12.69	11,337,881	11,277,026	9.98
14,674,025	13,910,357	12.09	10,592,556	10,535,523	9.29
10,697,649	10,233,396	8.83	7,869,539	7,831,672	6.89
13,476,442	12,932,853	11.17	10,154,149	10,103,914	8.91
12,667,435	11,690,972	10.18	8,019,580	7,956,418	7.04
11,779,772	11,001,816	9.59	7,877,723	7,826,141	6.92
11,432,243	10,941,301	9.51	8,612,114	8,571,886	7.57
9,323,612	8,845,182	7.70	6,392,825	6,354,846	5.61
9,359,940	8,862,664	7.73	6,524,433	6,485,245	5.73
11,419,220	10,879,901	9.49	8,159,339	8,112,511	7.17

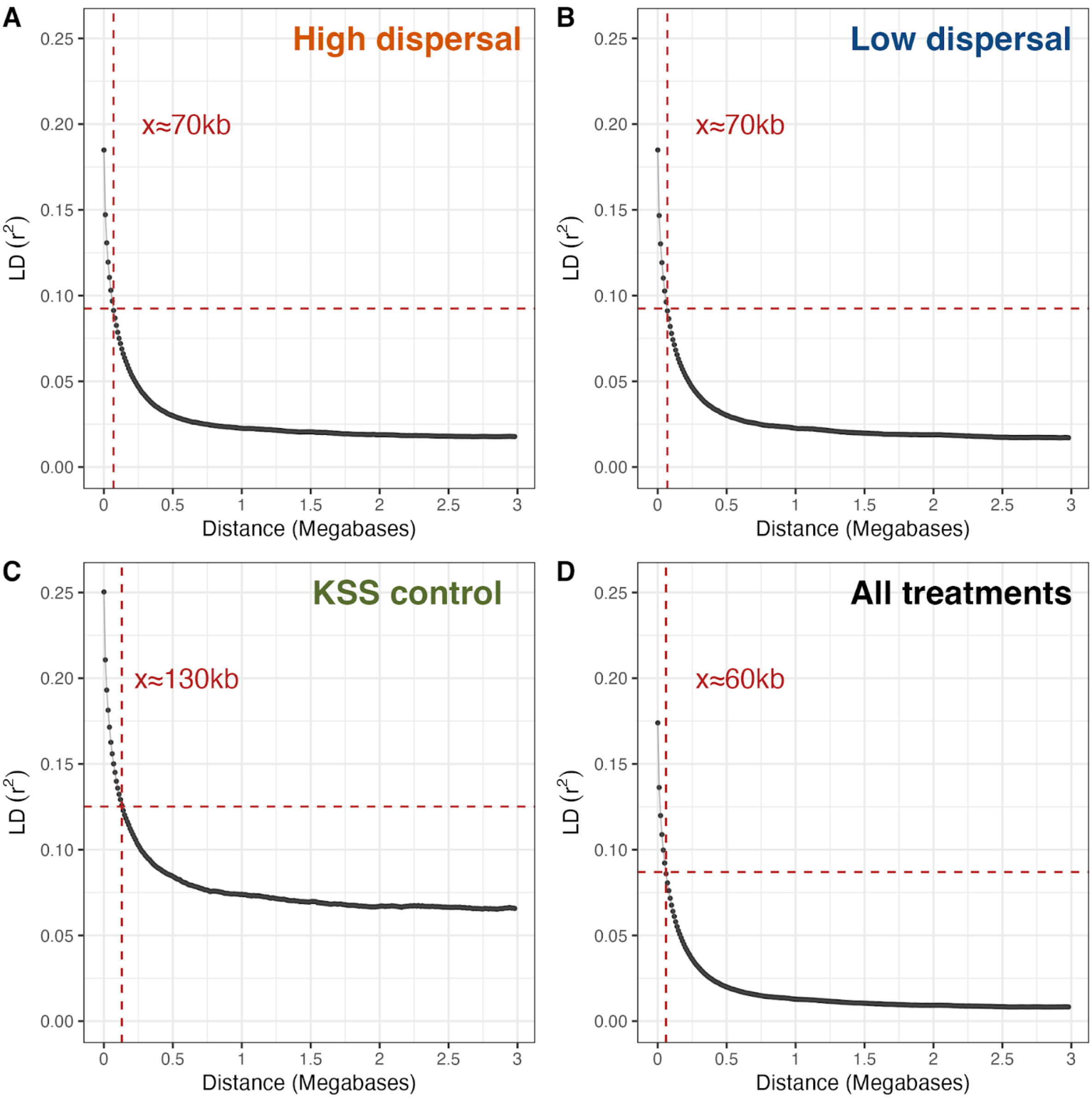


Figure S1. Linkage decay estimated from genetic samples derived from an experiment exposing *Tribolium castaneum* to artificial selection for dispersal propensity. Panels show each dispersal treatment and the ancestral line considered separately (A-C), and considering all samples as a single population (D). The value of x represented in red shows the genomic distance over which the linkage halves from the maximum estimated value, rounded to the nearest 10kb. Note that sample sizes differ across panels/treatments and that the pattern we observe is consistent with the estimates of linkage reducing with increasing sample size (in A n=

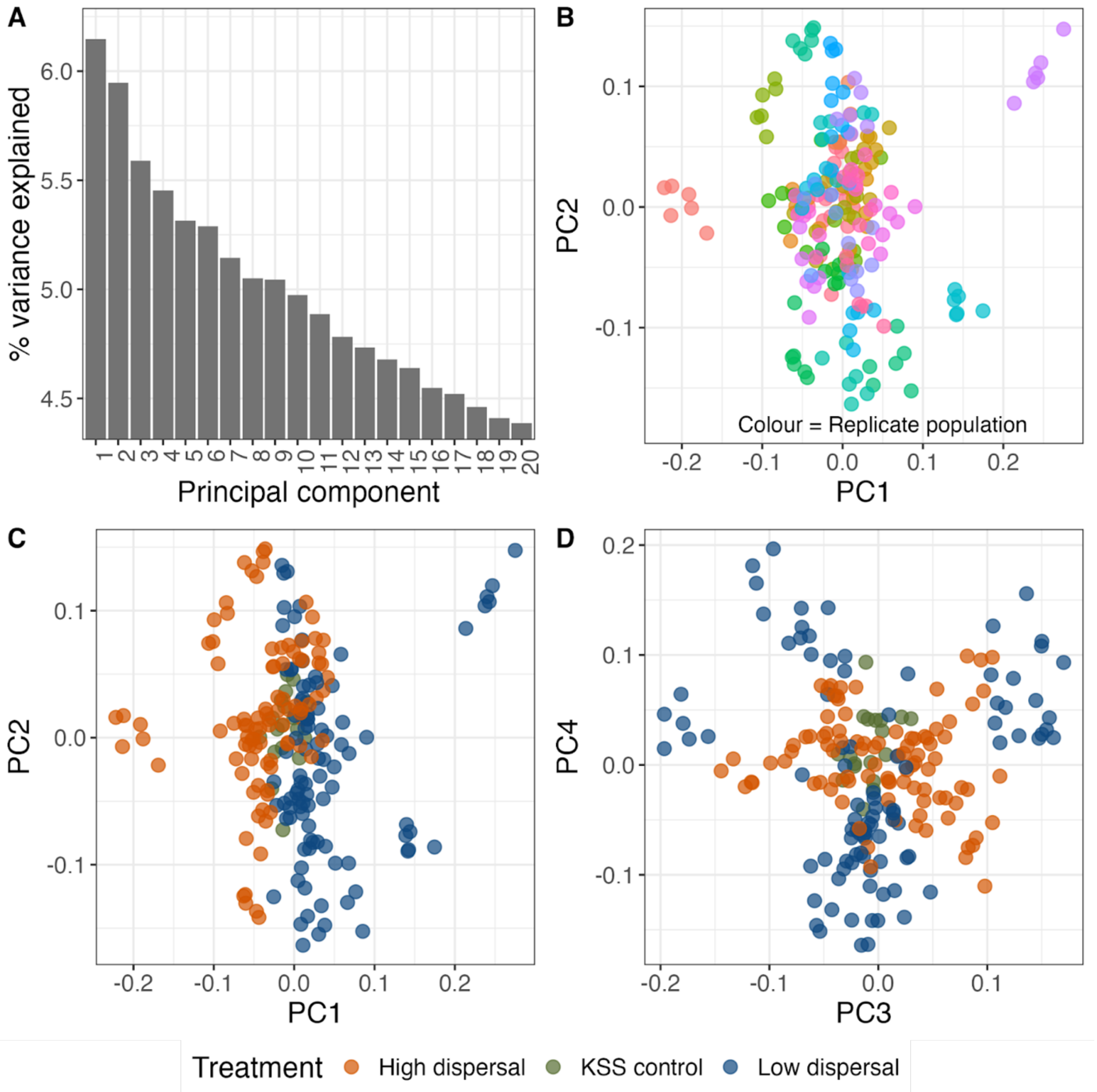


Figure S2. Principal component analysis (PCA) on genetic samples derived from an experiment exposing *Tribolium castaneum* to artificial selection for dispersal propensity. Panels show, **A**) the explanatory power of each PC, **B**) strong clustering of samples by their population of origin (colour), **C**) how PC1 largely represents dispersal selection treatment and **C&D**) how samples spread out from the core of the parameter space occupied by the ancestral KSS population. Interestingly, low dispersal samples appear to be less similar to KSS control samples than high dispersal samples are, in both directions, on both PC3 and PC4.

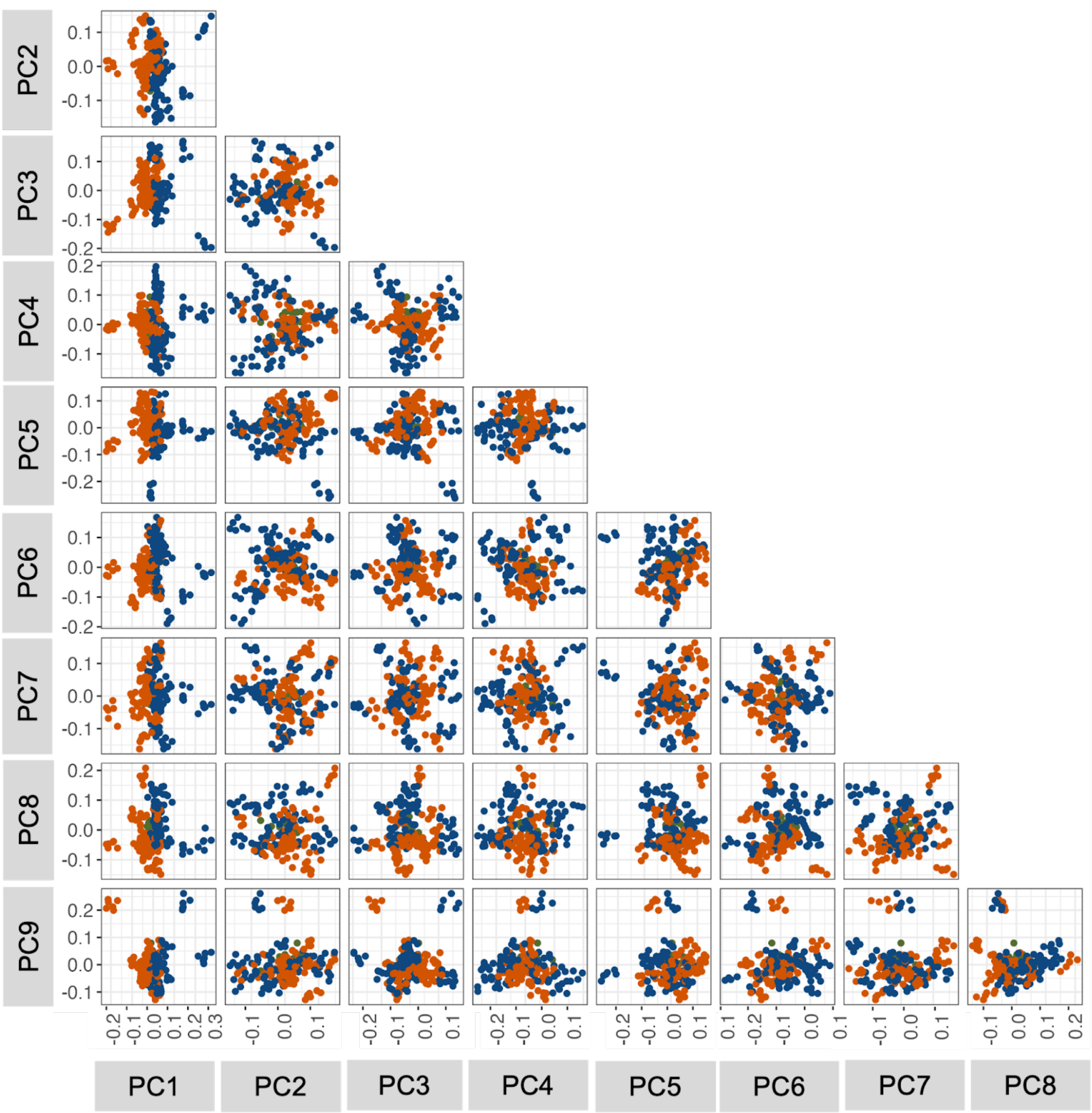


Figure S3. Matrix of PCs explaining >5% of total variance in a PCA analysis of genomic data derived from an experiment exposing *Tribolium castaneum* to artificial selection for dispersal propensity. PC1 appears to capture most of the difference between dispersal selection regimes.

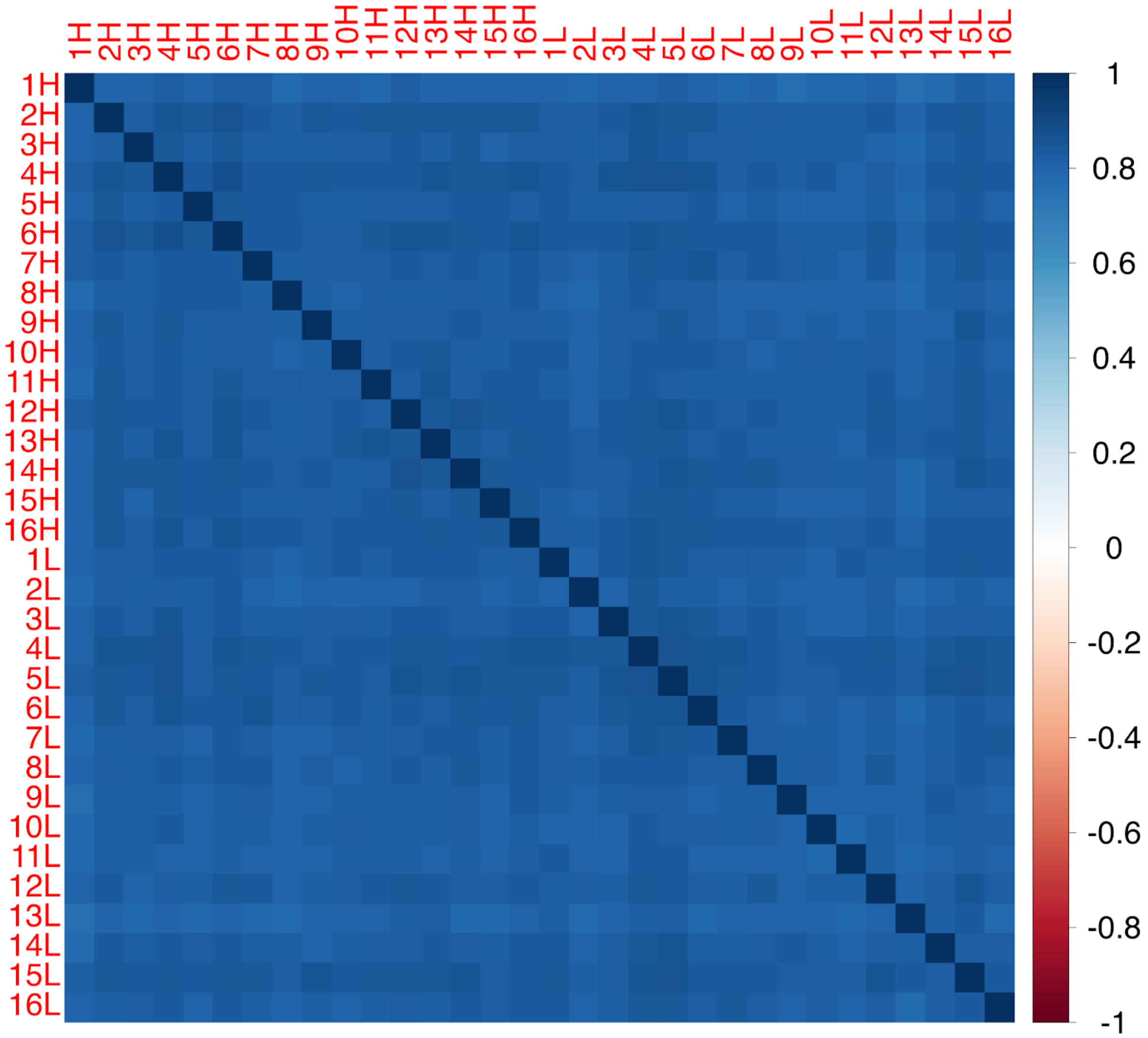
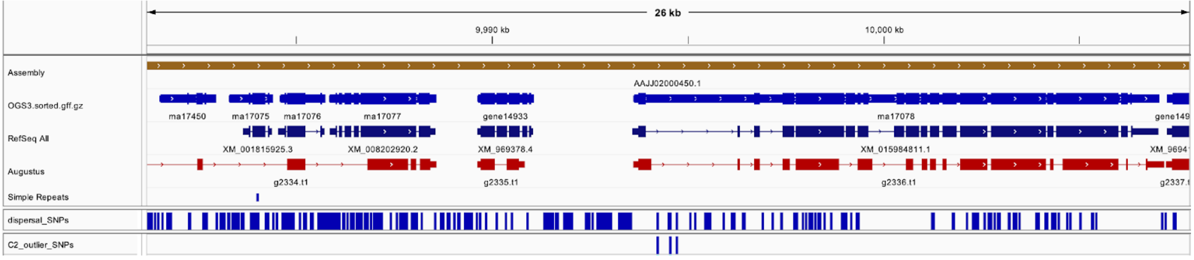


Figure S4. Visualisation of the background allele frequency matrix (omega) generated by BayPass, using a set of genome-wide SNPs derived from an experiment exposing *Tribolium castaneum* to artificial selection for dispersal propensity. Each row and column represents a single, independently evolving high dispersal (n=16) or low dispersal (n=16) population, with the colour of each square showing the correlation in allele frequencies between population pairs.

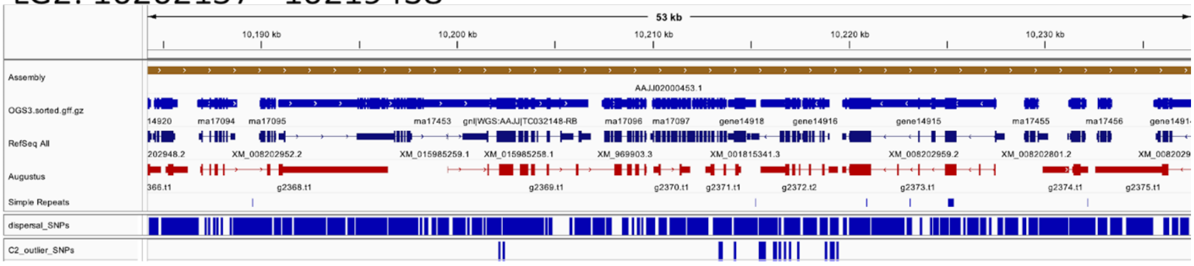
A)

LG2: 9994244 - 9994716



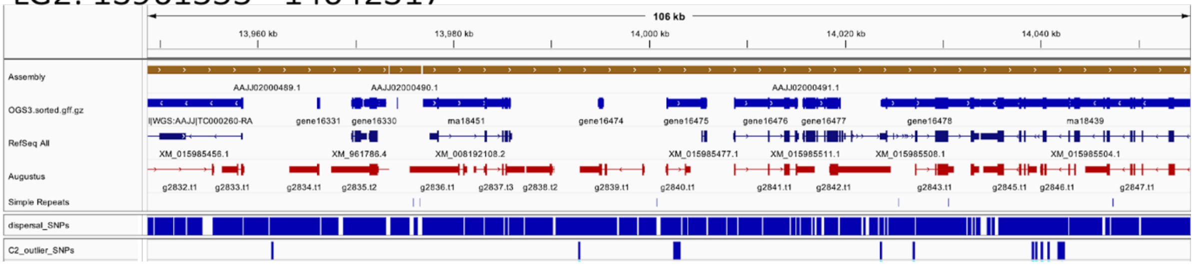
B)

LG2: 10202157 - 10219438



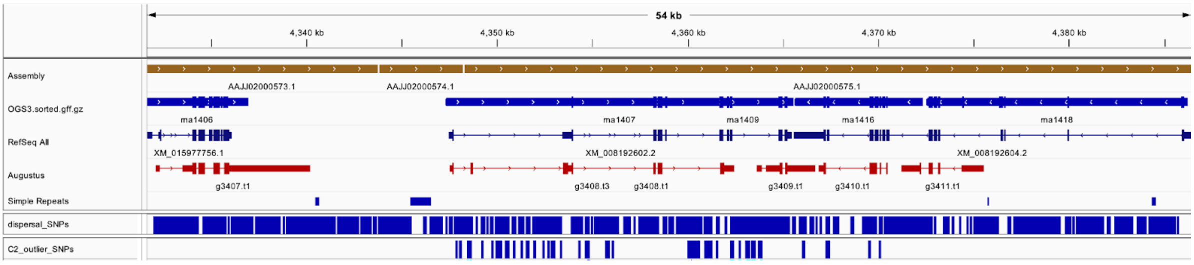
C)

LG2: 13961555 - 14042317



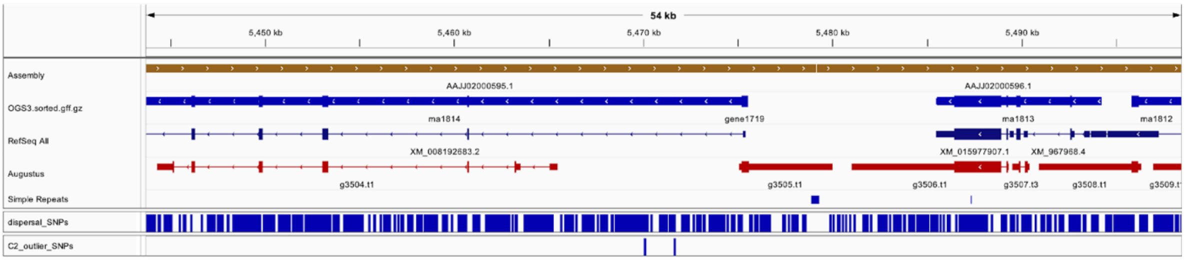
D)

LG3: 4347867 - 4370105



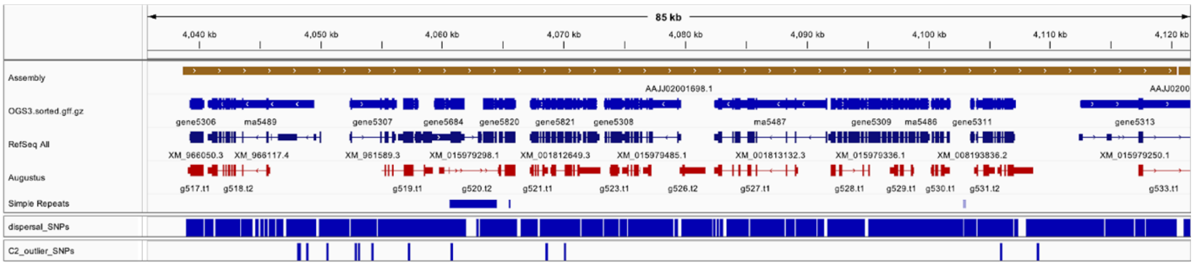
E)

LG3: 5470109 - 5471688



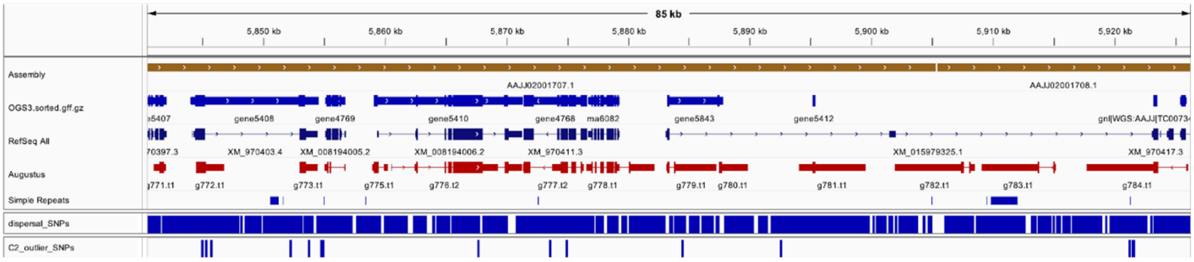
F)

LG4: 4048154 - 4109033



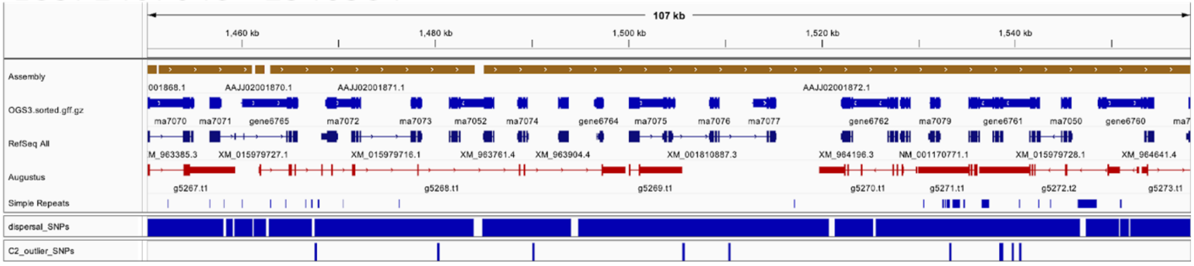
G)

LG4: 5845018 - 5921563



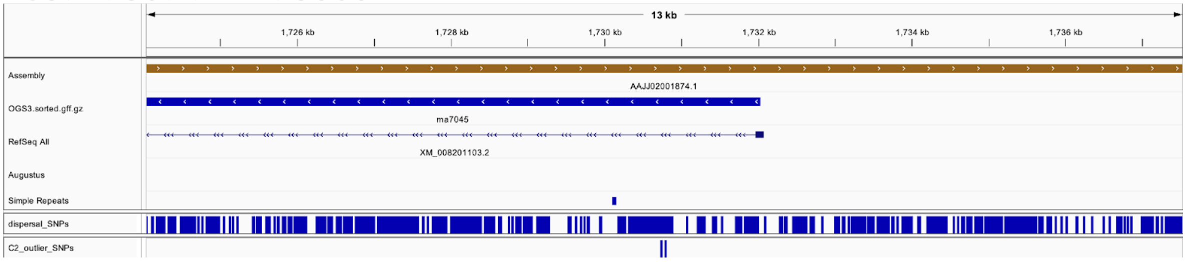
H)

LG5: 1467648 - 1540584



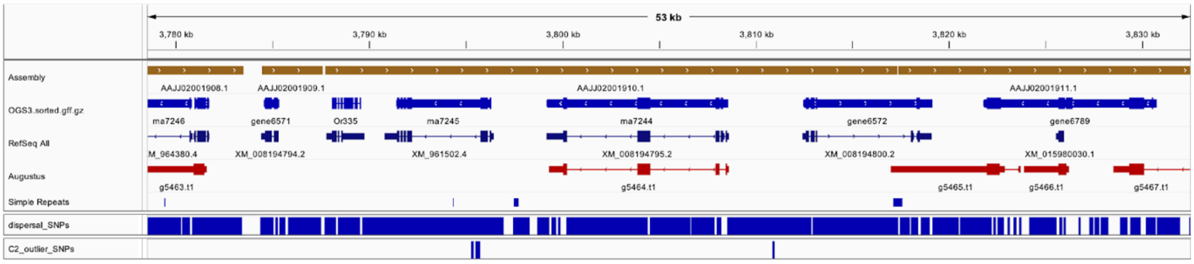
I)

LG5: 1730741 - 1730802



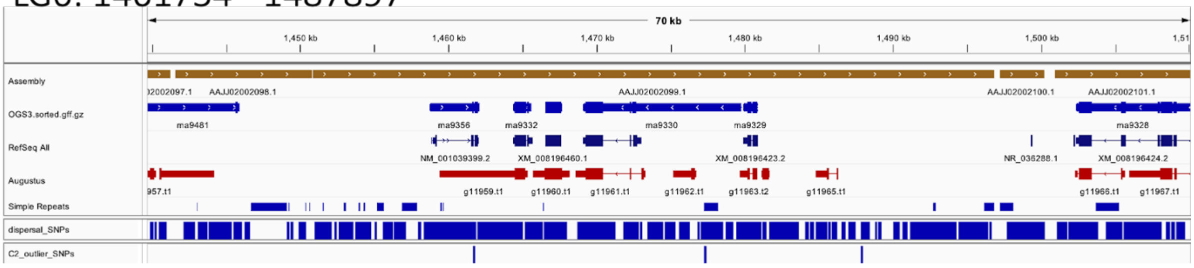
J)

LG5: 3795349 - 3810931



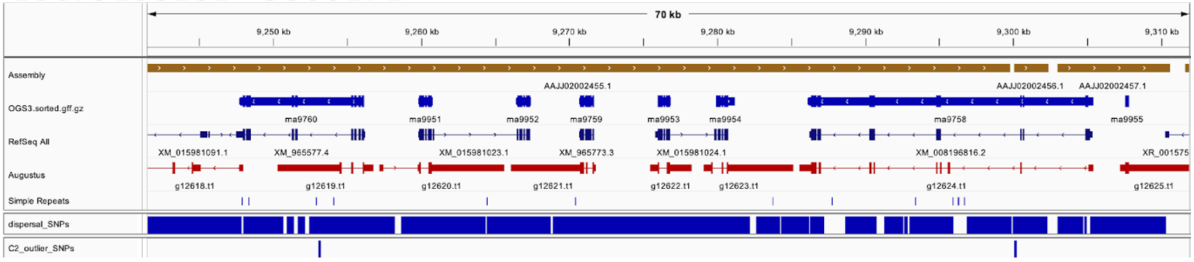
K)

LG6: 1461754 - 1487897



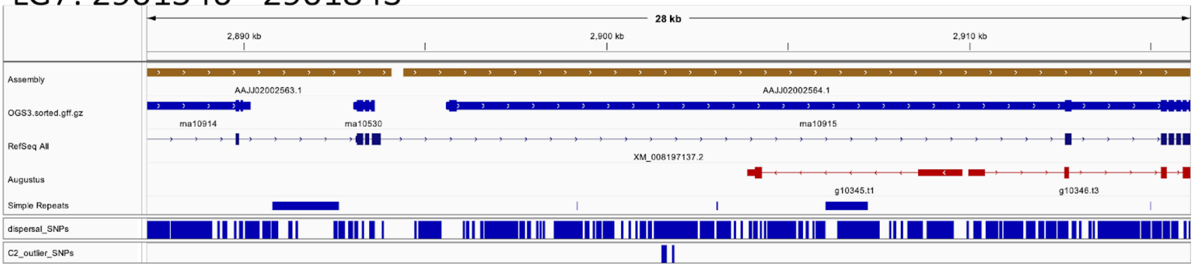
L)

LG6: 9253132 - 9300171



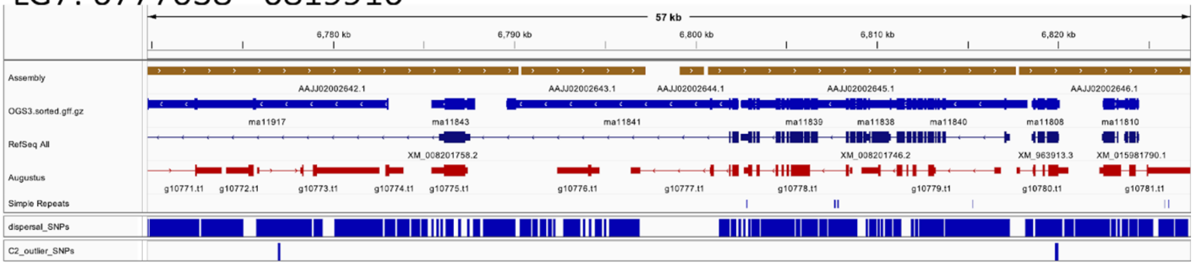
M)

LG7: 2901546 - 2901843



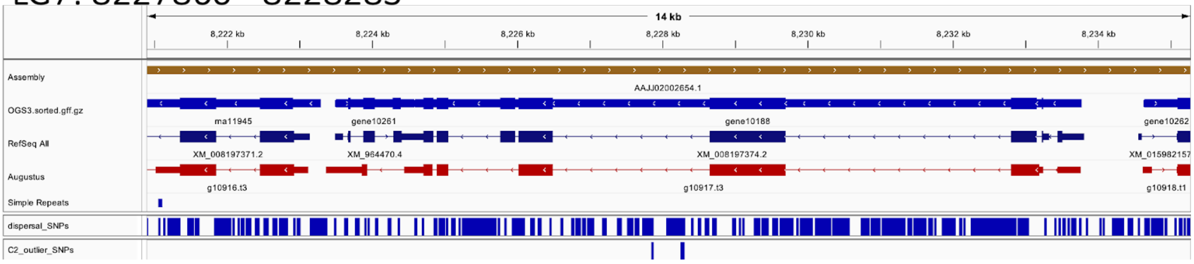
N)

LG7: 6777038 - 6819916



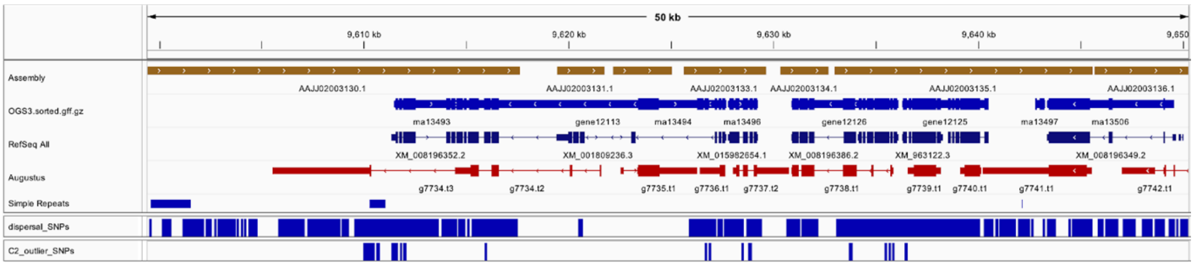
O)

LG7: 8227860 - 8228283



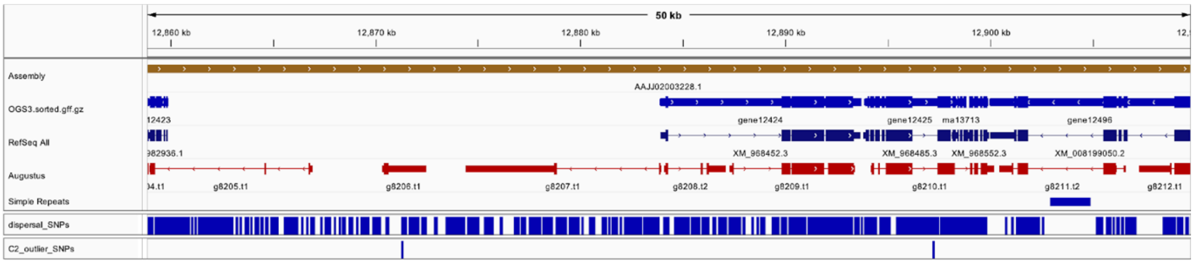
P)

LG8: 9610013 - 9636502



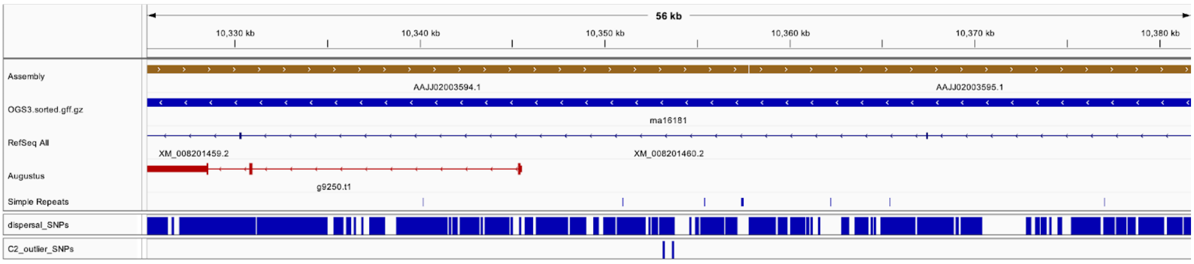
Q)

LG8: 12871312 - 12897244



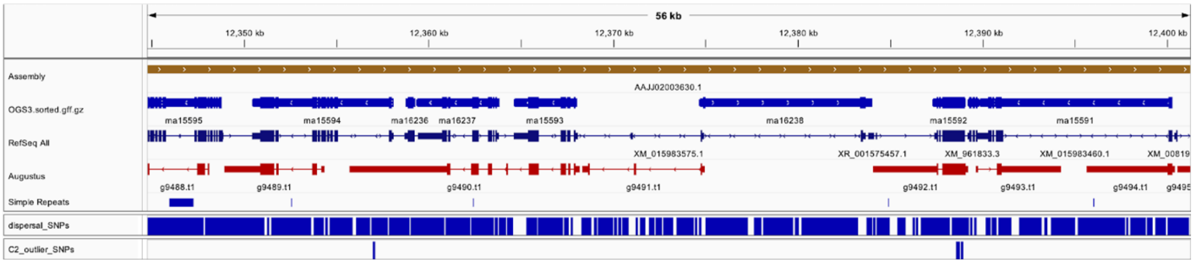
R)

LG9: 10353204 - 10353689



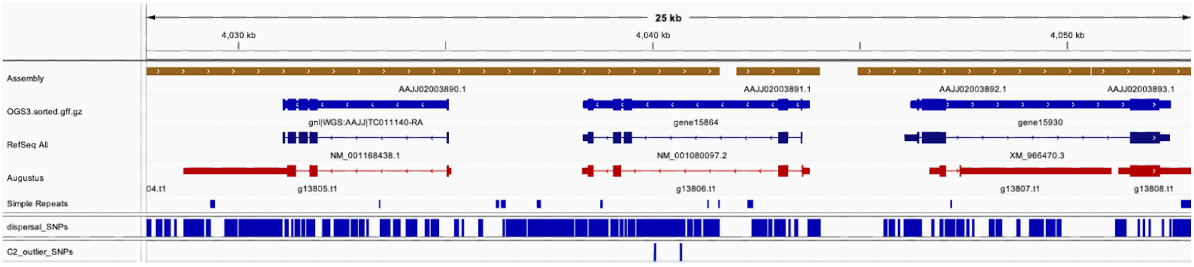
S)

LG9: 12357074 - 12388871



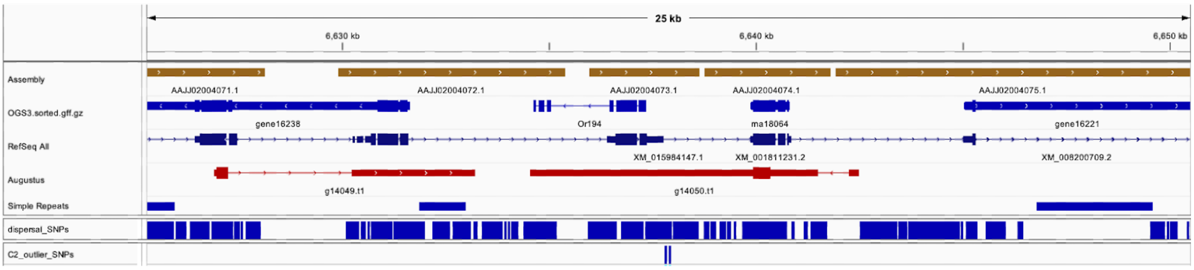
T)

LG10: 4040062 - 4040694



U)

LG10: 6637835 - 6637917



V)

LGX: 1559420 - 1559524

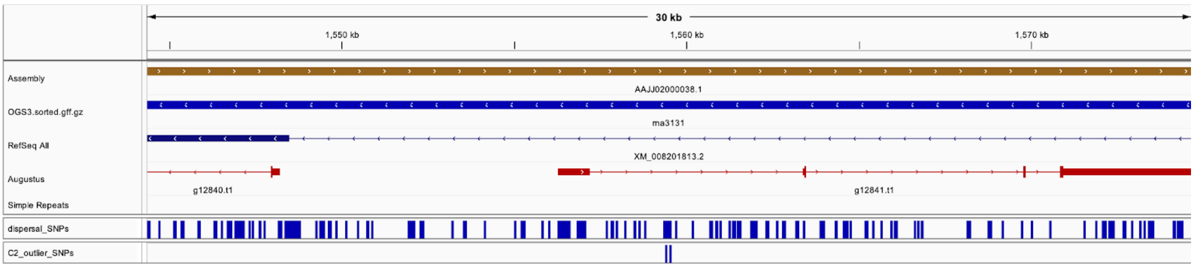


Figure S5. Each of 22 candidate regions identified by BayPass C2 outlier analysis, visualised with Integrative Genomics Viewer (Robinson et al. 2023). Panels A-V each show a single region, arranged by genomic position, and display, from top to bottom: assembled regions of the Tcas5.2 genome; OGS3 gene annotations; Ensembl RefSeqall gene annotations; Augustus gene annotations; the locations of all called SNPs across all populations in the dataset; and the locations of significant outlier SNPs from BayPass C2 outlier analysis.

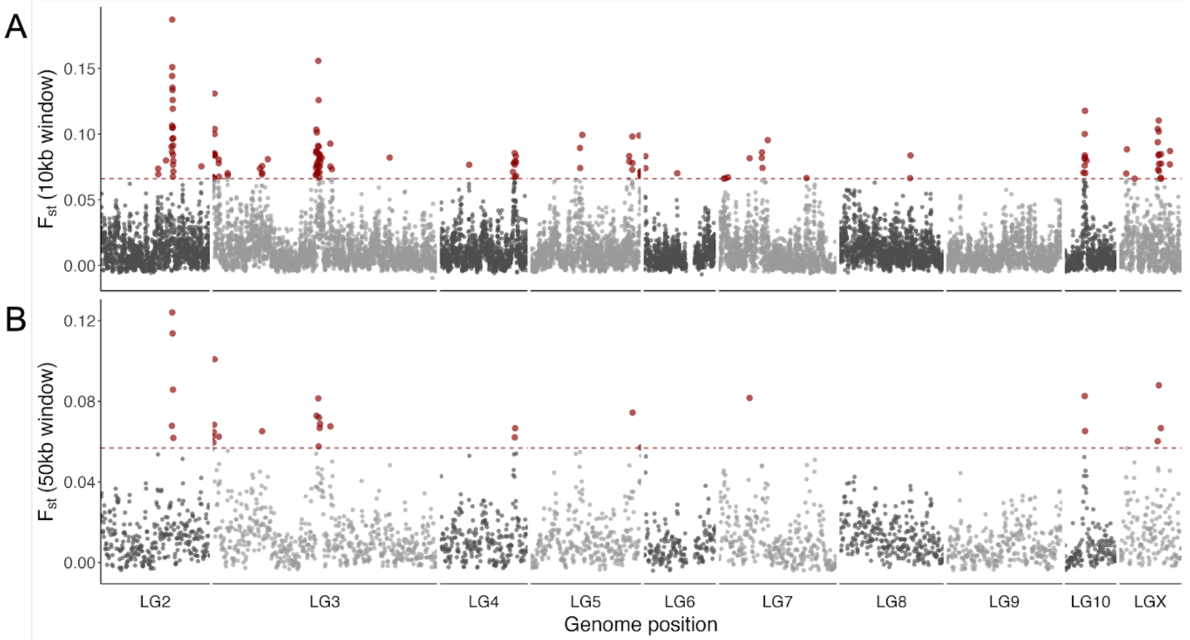


Figure S6. Windowed mean F_{ST} between all individuals from each dispersal selection regime considered as a single population (i.e. Combining the 16 replicate lines in each treatment). Red points are in excess of the 0.99 quantile of F_{ST} values genomewide. Window size was **A**) 10kb, **B**) 50kb. Results were similar across window sizes; all windows identified as outliers by the 50kb analysis were also identified using 10kb windows, and the top five 50kb window peaks contained the top five 10kb window peaks.

Table S2. Genes identified as associated with dispersal behaviour in lines of *Tribolium castaneum* artificially selected for divergent dispersal propensity. All genes listed were found to be within candidate regions (C2), also indicated is whether each was within an outlier F_{ST} window (F_{ST}). Genes are ordered by the number of analyses supporting them, then by their genomic position. Information is provided from any Drosophila homologs listed in iBeetleBase and retrieved from FlyBase.

Genomic location (LG:POS)	**Gene ID**	**C_2**	**F_{ST}**	**Flybase homolog ID**	**Flybase homolog name**	**Putative function**
2:9993610-10007042	TC001033	✓	✓	FBgn0043884	Multiple ankyrin repeats single KH domain	Mediator of receptor tyrosine kinase (RTK) signaling, required for flight muscle sarcomere formation
2:10190871-10206697	TC032148	✓		FBgn0266579	Tau	Microtubule associated protein; human ortholog implicated neuronal degradation
2:10215491-10217569	TC000523	✓		-	-	-
2:14001779-14005903	TC001290	✓		-	-	-
3:4347291-4365573	TC003405	✓		FBgn0024315	Picot	Predicted inorganic phosphate cotransporter
4:4059326-4060956	TC030194	✓		-	-	-
4:4060704-4061868	TC030193	✓		-	-	-
4:5859118-5871357	TC008156	✓		FBgn0259146	Fire dancer	Encodes a protein involved in heat resistance
4:5871397-5876636	TC007344	✓		FBgn0026015	Topoisomerase 3β	DNA binding; transcription and translation regulation

4:5883188-5887835	TC032826	✓		-	-	-
5:3791386-3796339	TC013804	✓		FBgn0000120	Arrestin 1	Photoreceptor maintenance and smell perception
7:2895583-2925437	TC033574	✓		FBgn0085390	Diacyl glycerol kinase	ATP-dependent diacylglycerol kinase activity; calcium ion binding. May be involved in muscle function and regulating neuron signalling
9:10278308-10422001	TC034367	✓		FBgn0038881	Argus	Autophagosome-lysosome fusion. mature autophagosome degradation. Loss of its autophagy function results in sleep loss
9:12387287-12389095	TC011868	✓		-	-	-
9:12350445-12358135	TC011872	✓		FBgn0250755	-	Negative regulation of fatty acid biosynthetic process
2:10207381-10209696	TC001051	✓		FBgn0038666	Smu1 spliceosomal factor	Encodes a spliceosomal protein required for normal neuromuscular junction development and function.
2:10209941-10211960	TC001052	✓		FBgn0038047	-	Predicted to enable DNA-binding transcription factor activity
2:10211972-10213759	TC000525	✓		-	-	-
2:10213821-10215269	TC000524	✓		FBgn0028662	Vacuolar H+ ATPase PPA1 subunit 1	Encodes a protein involved in tracheal terminal branching
2:10217617-10219009	TC000522	✓		FBgn0250732	GST-containing FLYWCH zinc-finger protein	Predicted glutathione transferase activity
2:13966054-13966404	TC000259	✓		-	-	-
2:13969569-13970791	TC001287	✓		FBgn0035980	Mitochondrial ribosome recycling factor 1	Predicted to enable ribosomal large subunit binding activity

2:13970858-13973137	TC000258	✓		FBgn0032492	Proteasome alpha6 subunit, Testis-specific	Encodes a testes-specific subunit of the 26S proteasome; spermatogenesis
				FBgn0250843	Proteasome alpha6 subunit	Proteasome has an ATP-dependent proteolytic activity' adult lifespan
2:13974218-13974362	TC001288	✓		FBgn0027660	Bloated tubules	Encodes a member of the neurotransmitter symporter family
2:13976786-13985962	TC034566	✓		FBgn0036274	-	Enable DNA-binding transcription factor; neuron differentiation; regulation of transcription
2:13994799-13995468	TC000256	✓		-	-	-
2:14008656-14015259	TC001291	✓		FBgn0052105	LIM homeobox transcription factor 1 alpha	Enable DNA-binding transcription factor; neuron differentiation; regulation of transcription
2:14015621-14016244	TC000255	✓		FBgn0036277	-	RNA binding activity; regulation of alternative mRNA splicing
2:14016336-14019511	TC001292	✓		FBgn0041147	Imaginal discs arrested	Encodes ubiquitin ligase that regulates mitotic metaphase/anaphase transition
2:14033838-14073163	TC032248	✓		FBgn0261698	Slowpoke 2	Encodes a channel involved in potassium ion transmembrane transport
				FBgn0262514	Vacuolar H+ ATPase PPA1 subunit 2	Predicted to contribute to proton-transporting ATPase activity, rotational mechanism
2:14023666-14033821	TC001293	✓		FBgn0031407	-	-
		✓		FBgn0054049	-	-
		✓		FBgn0031412	-	Involved in sexual reproduction
		✓		FBgn0051482	-	-
		✓		FBgn0051286	-	-

3:4365581-4372318	TC032364	✓		FBgn0004784	Inactivation no afterpotential C	Encodes an eye-specific protein kinase C (PKC) involved in visual signaling; female gonad development; response to light
3:5432673-5475367	TC002977	✓		FBgn0035756	Unc-13-4A	Predicted to be active in neurotransmitter secretory vesicle
		✓		FBgn0029727	-	Transmembrane transporter activity; monoatomic anion transport
4:4040766-4049514	TC007470	✓		FBgn0264494	-	Predicted to enable ATPase-coupled transmembrane transporter activity
4:4052386-4056270	TC008024	✓		FBgn0043841	Virus-induced RNA 1	Induced by viral infection, used as a marker of the induction of an antiviral response
4:4056806-4058067	TC030196	✓		-	-	-
4:4063386-4066090	TC032800	✓		-	-	-
4:4063396-4064561	TC030192	✓		-	-	-
4:4067233-4071547	TC032801	✓		FBgn0262738	No receptor potential A	Phototransduction; photoentrainment; locomotor behaviour
4:4071553-4072791	TC030191	✓		-	-	-
4:4073343-4074953	TC008028	✓		FBgn0003965	Vermilion	Heme-dependent dioxygenase, required during larval growth to control the level of potentially harmful free tryptophan; eye pigmentation
4:4074968-4079671	TC007467	✓		FBgn0031220	-	Predicted to enable ATPase-coupled transmembrane transporter activity
4:4082376-4091666	TC007466	✓		FBgn0288229	-	Enable ATPase-coupled transmembrane transporter activity.

4:4091939-4098733	TC008029	✓		FBgn0000463	Delta	Regulates cell fate decisions and cell proliferation; leg/wing/antenna development; photoreceptor development; Notch signalling
4:4098749-4100066	TC007465	✓		FBgn0032727	Betaine-homocysteine S-methyltransferase	Enable S-adenosylmethionine-homocysteine S-methyltransferase activity
				FBgn0032726	-	Enable S-adenosylmethionine-homocysteine S-methyltransferase activity
4:4100198-4101823	TC008030	✓		FBgn0019957	NADH dehydrogenase (ubiquinone) 42 kDa subunit	Encodes a subunit of complex I of the mitochondrial electron transport chain
4:4103408-4103809	TC008031	✓		FBgn0037579	Cytochrome c oxidase subunit 7A-like	Involved in mitochondrial respirasome assembly and regulation of oxidative phosphorylation
				FBgn0085201	Cytochrome c oxidase subunit 7A-like 2	Involved in mitochondrial respirasome assembly and regulation of oxidative phosphorylation
				FBgn0040529	Cytochrome c oxidase subunit 7A	Component of the cytochrome c oxidase, the last enzyme in the mitochondrial electron transport chain which drives oxidative phosphorylation
4:4103878-4107194	TC008032	✓		-	-	-
4:5843998-5854519	TC008154	✓		FBgn0001404	Egghead	Glycosphingolipid biosynthesis; germ cell development
4:5855090-5856776	TC007345	✓		-	-	-
4:5859049-5859492	TC008155	✓		-	-	-
4:5876728-5879344	TC008157	✓		FBgn0003520	Staufen	Encodes a double-stranded RNA binding protein involved in mRNA localization; long-term memory; oogenesis

4:5895151-5895408	TC008159	✓		-	-	-
5:1468679-1472342	TC010933	✓		FBgn0250815	Jonah 65Aiv	Enables serine hydrolase activity; innate immune respponse
				FBgn0036264		Serine-type endopeptidase activity; innate immune response
				FBgn0052383	Sphinx1	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
				FBgn0052382	Sphinx2	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
5:1477499-1478648	TC010934	✓		FBgn0250815	Jonah 65Aiv	Enables serine hydrolase activity; innate immune respponse
				FBgn0036264		Serine-type endopeptidase activity; innate immune response
				FBgn0052383	Sphinx1	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
				FBgn0052382	Sphinx2	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
5:1481411-1486042	TC010910	✓		FBgn0250815	Jonah 65Aiv	Enables serine hydrolase activity; innate immune respponse
				FBgn0036264		Serine-type endopeptidase activity; innate immune response
				FBgn0052383	Sphinx1	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
				FBgn0052382	Sphinx2	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
5:1488476-1489657	TC010935	✓		250815	Jonah 65Aiv	Enables serine hydrolase activity; innate immune respponse
				36264		Serine-type endopeptidase activity; innate immune response

				52383	Sphinx1	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
				52382	Sphinx2	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
5:1492716-1493874	TC010908	✓		250815	Jonah 65Aiv	Enables serine hydrolase activity; innate immune respponse
				36264		Serine-type endopeptidase activity; innate immune response
				52383	Sphinx1	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
				52382	Sphinx2	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
5:1496434-1497448	TC033014	✓		-	-	-
5:1500054-1504630	TC010936	✓		250815	Jonah 65Aiv	Enables serine hydrolase activity; innate immune respponse
				36264		Serine-type endopeptidase activity; innate immune response
				52383	Sphinx1	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
				52382	Sphinx2	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
5:1503565-1504914	TC033013	✓		-	-	-
5:1508306-1509471	TC010937	✓		-	-	-
5:1512846-1515333	TC010938	✓		-	-	-

5:1521986-1527854	TC033012	✓		250815	Jonah 65Aiv	Enables serine hydrolase activity; innate immune respponse
				36264		Serine-type endopeptidase activity; innate immune response
				52383	Sphinx1	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
				52382	Sphinx2	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
5:1528136-1529203	TC010939	✓		250815	Jonah 65Aiv	Enables serine hydrolase activity; innate immune respponse
				36264		Serine-type endopeptidase activity; innate immune response
				52383	Sphinx1	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
				52382	Sphinx2	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
5:1531166-1532316	TC010940	✓		-	Serine protease P121	May be involved in wound healing
5:1535151-1542504	TC033011	✓		FBgn0250815	Jonah 65Aiv	Enables serine hydrolase activity; innate immune respponse
				FBgn0036264		Serine-type endopeptidase activity; innate immune response
				FBgn0052383	Sphinx1	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
				FBgn0052382	Sphinx2	Serine protease, strongly expressed in the male accessory gland; sexual reproduction
5:1710318-1732029	TC010898	✓		FBgn0261258	Regeneration	Carbohydrate binding; tissue regeneration

5:3799189-3808589	TC013803	✓		10583	Dreadlocks	Adapter protein inking cell surface receptor tyrosine phosphorylation to downstream signaling pathways and effectors; protein binding; insulin receptor signalling
6:1458748-1462133	TC015494	✓		FBgn0023214	ETS-domain lacking	Egfr signaling pathway regulation; sensory organ development; nuclear export
6:1464348-1465586	TC015211	✓		33653	-	Predicted to be involved in social behavior
6:1466477-1467676	TC015210	✓		29851	-	-
6:1469091-1479796	TC015209	✓		FBgn0034025	Polypeptide N-Acetylgalactosaminyltransferase 1	Catalyst of oligosaccharide biosynthesis
6:1479915-1480926	TC015208	✓		29851	-	-
6:9247748-9256186	TC014879	✓		FBgn0012051	Calpain-A	Encodes a calcium-dependent modulatory protease; adult lifespan; muscle development; larval locomotion
				FBgn0025866	Calpain-B	Calcium-regulated non-lysosomal thiol-protease
				FBgn0260450	Calpain-C	Encodes a calcium-dependent cysteine protease
6:9259798-9260781	TC015841	✓		39203	Juvenile hormone binding protein 12	-
				38850	Juvenile hormone binding protein 15	-
6:9266428-9267445	TC015842	✓		39203	Juvenile hormone binding protein 12	-
6:9270702-9271646	TC014878	✓		39203	Juvenile hormone binding protein 12	-
6:9275948-9276894	TC015843	✓		39203	Juvenile hormone binding protein 12	-
6:9279888-9281209	TC015844	✓		39203	Juvenile hormone binding protein 12	-

6:9286120-9305396	TC014877	✓		FBgn0034909	Pippin	Transmembrane transport of sugars
7:6750458-6783030	TC033673	✓		FBgn0083946	Lost boys	Key component of the nexin-dynein regulatory complex; sperm motility; sperm storage
7:6785383-6787610	TC016383	✓		50401	Distal antenna-young	Chromatin-binding protein required in spermatocytes for a normal gene expression profile
				39283	Distal antenna-related	Transcription factor with a role in the retinal determination (RD) network; eye/antenna development; nervous system formation
7:6787398-6787895	TC016382	✓		-	-	-
7:6789568-6802399	TC016381	✓		-	-	-
7:6802465-6818299	TC016380	✓		44452	Autophagy-related 2	Encodes a protein known to be required for autophagy; wound healing
7:6818541-6820106	TC016342	✓		FBgn0010803	Tryptophanyl-tRNA synthetase	Tryptophan-tRNA ligase activity; ATP binding. Dendrite morphogenesis
7:8224581-8233768	TC009038	✓		FBgn0086784	Stambha A	Synaptic vesicle endocytosis; phototransduction; and synaptic vesicle exocytosis
8:9611455-9615202	TC015261	✓		FBgn0003510	Serendipity α	cellularization of the syncytial blastoderm embryo
				FBgn0033348	Spitting Image	Encodes an stabilizing protein in the early embryo
8:9615206-9627710	TC030897	✓		-	-	-
8:9623376-9626906	TC015262	✓		-	-	-

8:9627795-9629240	TC015264	✓		FBgn0036856	Methyltransferase like 5	Methylates the 6th position of adenine in 18S rRNA
8:9627856-9628159	TC015263	✓		259720	-	Predicted to be involved in DNA repair
8:9630888-9636133	TC033340	✓		FBgn0000594	Esterase P	Enables carboxylesterase activity
				FBgn0000592	Esterase 6	Carboxylesterase; odorant sensing; pheromone response; courtship behaviour, egg-laying behaviour, sexual reproduction
8:9636275-9640528	TC033339	✓		FBgn0000594	Esterase P	Enables carboxylesterase activity
				FBgn0000592	Esterase 6	Carboxylesterase; odorant sensing; pheromone response; courtship behaviour, egg-laying behaviour, sexual reproduction
8:12883884-12893699	TC006687	✓		-	-	-
8:12893824-12898338	TC006688	✓		FBgn0040268	Topoisomerase 3alpha	Encodes a type IA topoisomerase involved in junction dissolution during homologous recombination
9:12358722-12359295	TC034415	✓		-	-	-
9:12359354-12363845	TC034416	✓		31213	Galectin	Encodes a galactoside binding protein involved in synaptic target recognition
				31214	-	Predicted to enable carbohydrate binding activity and galactoside binding activity
9:12364626-12368045	TC011870	✓		1297	Kayak	Transcription factor involved in multiple biological processes; germ cell / eye development / response to wounding / locomotion / locomotor rhythm

9:12374634-12384015	TC034417	✓		-	-	-
9:12374736-12383773	TC034887	✓		-	-	-
10:4038317-4043813	TC011139	✓		FBgn0027600	Obstructor-B	Chitin binding activity
X:1533167-1577906	TC004127	✓		-	-	Involved in nervous system development

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