Investigation of dispersal ecology and evolution using laboratory populations of *Drosophila melanogaster*

A thesis submitted in partial fulfilment

of the requirements for the degree of

Doctor of Philosophy

by

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20153395

INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH PUNE

2020

Dedicated to my parents,

Smt Pushpa Mishra

and

Shri Abhay Narayan Mishra

CERTIFICATE

Certified that the work incorporated in thesis titled 'Investigation of dispersal ecology and evolution using laboratory populations of *Drosophila melanogaster*', submitted by Abhishek Mishra was carried out by the candidate, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other university or institution.

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DECLARATION

I declare that this written submission represents my ideas in my own words and where others ideas have been included, I have adequately cited and referred to original sources. I also declare that I have adhered to all principles of academic honesty and integrity and I have not misinterpreted or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above can cause disciplinary action by the institute and evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Date: June 29, 2020

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ACKNOWLEDGEMENTS

"It is good to have an end to journey toward; but it is the journey that matters, in the end." – Ursula K. Le Guin (*The Left Hand of Darkness*)

This thesis is about dispersal, i.e. the journey from a source to a destination, a metaphor as perfect as any for PhD. And as the quote says above, it is indeed the journey that matters. I was incredibly fortunate to have a number of amazing people with me during this journey, whose support I wish to acknowledge.

"The mediocre teacher tells. The good teacher explains. The superior teacher demonstrates. The great teacher inspires." – William Arthur Ward

I would start by thanking a great teacher, my PhD supervisor and mentor, Prof. Sutirth Dey. I have learned a lot from him, not just in science, but also about various aspects of life. I am truly grateful to him for the freedom and guidance during all my projects, and taking chances on ideas that seemed too far-fetched, convoluted, or even confusingly vague when I first presented them to him. Non-academically as well, I would highlight two aspects that made him a great mentor: a) maintaining a lab space that is cohesive, fun-filled, and open to free exchange of ideas, and b) keeping us updated about the non-scientific (the so-called 'soft') skills needed to grow as a researcher. He remains an amazing conveyor of ideas, both in the classroom and the lab, and his writing and speaking skills are something I will keep learning from. Also, he was never 'too busy' to address the most trivial doubts or have the most casual conversations. Finally, he always ensured that our interactions were friendly and informal, which meant that I never had to worry about the prospect of expressing something that went against his ideas or arguments at the moment.

Next, I am thankful to my Research Advisory Committee members, Prof. Amitabh Joshi and Dr. MS Madhusudhan, who provided timely guidance and helpful suggestions to me during this journey. Even though neither of them works on dispersal biology, they always welcomed the discussion of any project ideas and provided insightful critique.

"It is one of the blessings of old friends that you can afford to be stupid with them." – Ralph Waldo Emerson

A scientific research lab can be an intense place. Odd working hours, whiteboards full of project ideas and assay schedules, and consistent smell of some or the other culture media – these are only a few features of our ever-bustling Population Biology Lab. But the awesome people in the lab made it a thoroughly fun place to work, discuss and generally hang around. They ensured that the odd working hours had fun ambient music and the whiteboards had amazing doodles and sketches. This camaraderie extended well beyond the lab, as we planned excursions, lab parties and all other kinds of general lab outings. As I highlight a few people below, I likely missed many others, but to all my past and present lab-mates, I express my gratitude.

Sudipta Tung has been an amazing senior to work with, and his way of approaching things in life and science has had a profound effect on me. I could approach him with any idea, doubt or musing, and those conversations were always fun and insightful. I also want to thank the first PhD student of the lab, Shraddha Karve, who remains a great friend and an inspiration. Many thanks also to my PhD batch-mate, Selveshwari S, for our discussions and her amazing cooking during parties at her apartment. I have also cherished my time with another lab senior, Yashraj Chavan, who was always ready and enthusiastic for the exchange of both scientific and extra-curricular ideas. Fellow PhD students, Akshay Malwade and Vibishan B, have both been inspirational in several ways. Finally, Partha Pratim Chakraborty and Radha Barve, during their time in the lab as project fellows, made valuable contributions to my work and helped share a significant load of fly maintenance.

"In learning you will teach, and in teaching you will learn." – Phil Collins

The mention of lab colleagues would be incomplete without the many undergrad and MS students I worked with. In addition to their valuable help in various experiments, they taught me a lot about science and life in general, and maintained a lively atmosphere in the lab. I would thank Shreenidhi, Sruti, Aamir, and Sahana for their extended stay in the lab and helping me with a large chunk of projects. I also thank the next cohort of undergrads, i.e. Aarcha, Shraddha Lall, Gayathri, and Santhosh, for all their help and enthusiasm during the later projects of my PhD. Finally, I thank Kasturi, Saumil, Sayantan, Subhashree, Ruchitha, Harsha, and Poornima, who, along with many others mentioned above, helped proofread the

chapters in this thesis.

“The two most beautiful words in the English language are 'cheque enclosed.” – Dorothy Parker

Research and life require money to sustain, and I thank the Council of Scientific and Industrial Research (CSIR) for the Junior and Senior Research Fellowships during my PhD. I also thank the Biology Department at IISER Pune for the intra-mural and infrastructure support for carrying out these projects. Finally, travel grants from British Ecological Society (BES) and European Society for Evolutionary Biology (ESEB) made it possible for me to travel to international conferences and present parts of my thesis work.

“I sustain myself with the love of family.” – Maya Angelou

Any words of gratitude towards my parents are insufficient. I thank them for their incredible support and the constant love and guidance over all the years of my life. They continue to remain an inexhaustible source of inspiration and affection for me.

CONTENTS

SYNOPSIS

Thesis title: Investigation of dispersal ecology and evolution using laboratory populations of *Drosophila melanogaster*

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Chapter 1. Introduction

Biological dispersal, resulting from movement of individuals across space, shapes several ecological and evolutionary phenomena (Bowler & Benton 2005; Clobert *et al.* 2012). While generally advantageous at the population level, dispersal is often associated with high costs at the individual level (Bonte *et al.* 2012). The net result is that dispersal is a complex phenomenon, shaped by the interaction between individual variation and several environmental factors (Matthysen 2012).

In this thesis, I investigate both population- and individual-level patterns of dispersal using microcosm experiments with laboratory populations of *D. melanogaster*. Under highly controlled environmental conditions, I was able to follow a bottom-up approach for studying the precise effect of various biotic and abiotic factors on dispersal patterns. In this introductory chapter, I highlight the various gaps in our understanding of dispersal ecology and evolution, and describe an outline of the work presented in subsequent chapters.

Chapter 2. Pre-dispersal context and presence of mates modulate density dependence and sex bias of dispersal

Density-dependent dispersal (DDD) has been observed across taxa, and is expected to affect phenomena such as population dynamics, biological invasions, range expansions, and community assembly (Namba 1980; Aars & Ims 2000; Amarasekare 2004; Travis *et al.* 2009). However, little is known about whether the patterns of DDD

are robust to changes in the environment. For example, the environmental context could affect the physiology of organisms, which in turn could alter their DDD. Similarly, in sexually reproducing organisms, males and females might be differentially affected by the environment, with possible changes in their dispersal properties (Gilroy & Lockwood 2012; Trochet *et al.* 2016). To investigate these issues, I performed three independent experiments using laboratory populations of *Drosophila melanogaster*, which tested the effects of pre-dispersal context, sex of the dispersers and presence of mates on DDD. A two-patch dispersal setup was used to estimate the dispersal propensity and temporal dispersal profile of adult fruit flies. Comparing the data from two different pre-dispersal contexts (variable and uniform pre-dispersal adult densities), I found that longer pre-dispersal exposure to higher densities led to stronger negative DDD in both males and females. Surprisingly, this change in DDD strength was accompanied by a switch in the direction of sex-biased dispersal: from female-biased dispersal at a low density to male-biased dispersal at a high density. Moreover, I found that patterns of both density dependence and sex bias were contingent upon the interaction of males and females, as neither sex exhibited DDD in the absence of the other. Taken together, these results suggest that DDD and sex-biased dispersal can be labile and be driven by the environmental context.

The contents of this chapter have been published as the following research article:

***Mishra, A.**, Tung, S., Sruti, V. S., Sadiq, M. A., Srivathsa, S., & Dey, S. (2018). Pre-dispersal context and presence of opposite sex modulate density dependence and sex bias of dispersal. Oikos, 127(11), 1596-1604.*

Chapter 3. Mate-finding dispersal reduces local mate limitation and sex bias in dispersal

Sex-biased dispersal (SBD) often skews the local sex ratio in a population. This can result in a shortage of mates for individuals of the less-dispersive sex. Such mate limitation can lead to Allee effects in populations that are small or undergoing range expansion, consequently affecting their survival, growth, stability and invasion speed (Taylor & Hastings 2005; Gascoigne *et al.* 2009; Meier *et al.* 2011). Theory predicts that mate shortage can lead to either an increase or a decrease in the dispersal of the less-dispersive sex (Shaw & Kokko 2014; Fromhage *et al.* 2016). However, neither of these predictions have been empirically validated. To investigate how SBD-induced mate limitation affects dispersal of the less-dispersive

sex, I used *Drosophila melanogaster* populations with varying dispersal propensities. To rule out any mate-independent density effects, I examined the behavioural plasticity of dispersal in presence of mates as well as same-sex individuals with differential dispersal capabilities. In the presence of high-dispersive mates, the dispersal of both male and female individuals was significantly increased. However, the magnitude of this increase was much larger in males than in females, indicating that the former show greater mate-finding dispersal. Moreover, the dispersal of either sex did not change when dispersing alongside high- or low-dispersive individuals of the same sex. This suggested that the observed plasticity in dispersal was indeed due to mate-finding dispersal, and not mate-independent density effects. Strong mate-finding dispersal, as observed here, diminishes the magnitude of sex bias in dispersal. This can modulate the evolutionary processes that shape range expansions and invasions, depending on the size of the population. In small populations, mate-finding dispersal can ameliorate Allee effects. However, in large populations, it can dilute the effects of spatial sorting.

The contents of this chapter have been published as the following research article:

***Mishra, A.**, Tung, S., Sruti, V. S., Srivathsa, S., & Dey, S. (2020). Mate-finding dispersal reduces local mate limitation and sex bias in dispersal. Journal of Animal Ecology, 89(9), 2089-2098.*

Chapter 4. Dispersal evolution via spatial sorting diminishes the density dependence in dispersal

Despite its ecological importance, empirical evidence for the evolution of DDD remains extremely scarce. This is especially relevant because rapid evolution of dispersal traits has now been empirically confirmed in several taxa (Fronhofer *et al.* 2014; Williams *et al.* 2016; Weiss-Lehman *et al.* 2017; Tung *et al.* 2018). Changes in DDD of dispersing populations could help clarify not only the role of DDD in dispersal evolution, but also the possible pattern of subsequent range expansion (Travis *et al.* 2009; Altwegg *et al.* 2013). Here, I investigate the relationship between dispersal evolution and DDD using a long-term experimental evolution study (~75 generations) on *Drosophila melanogaster*. I compared the DDD patterns of four dispersal-selected populations and their non-selected controls. The control populations showed negative DDD, which was stronger in females than in males. In contrast, the dispersal-selected populations showed density-independent dispersal, where neither males nor females exhibited DDD. These results are contrary to the

expectations from previous studies, which predict that dispersal evolution at range edges leads to stronger negative DDD patterns (Travis *et al.* 2009; Fronhofer *et al.* 2017). I discuss the possible reasons for this divergence from earlier predictions and its implications for spatial ecology and evolution.

The contents of this chapter have been submitted as the following research article:

***Mishra, A.**, Chakraborty, P.P., & Dey, S. (2020) Dispersal evolution via spatial sorting diminishes the density dependence in dispersal. Evolution, 10.1111/evo.14070.*

Chapter 5. Sex differences in dispersal syndrome are modulated by environment and evolution

Dispersal syndromes (i.e. suites of phenotypic correlates of dispersal) are potentially important determinants of local adaptation in populations (Ronce & Clobert 2012). Species that exhibit sexual dimorphism in their life history or behaviour may exhibit sex-specific differences in their dispersal syndromes. Unfortunately, there is little empirical evidence of sex differences in dispersal syndromes and how they respond to environmental change or dispersal evolution. I investigated these issues using two same-generation studies and a long-term (>70 generations) selection experiment on laboratory populations of *Drosophila melanogaster*. There was a marked difference between the dispersal syndromes of males and females, the extent of which was modulated by nutrition availability. Moreover, dispersal evolution via spatial sorting reversed the direction of *dispersal* × *sex* interaction in one trait (desiccation resistance), while eliminating the sex difference in another trait (body size). Thus, I show that sex differences obtained through same-generation trait-associations ('ecological dispersal syndromes') are likely environment-dependent. Moreover, even under constant environments, they are not good predictors of the sex differences in 'evolutionary dispersal syndrome' (i.e. trait-associations shaped during dispersal evolution). These findings have implications for local adaptation in the context of sex-biased dispersal and habitat-matching, as well as for the use of dispersal syndromes as a proxy of dispersal (Stevens *et al.* 2013).

The contents of this chapter have been published as the following research article:

Mishra, A., Tung, S.*, Shreenidhi, P. M., Aamir Sadiq, M., Shree Sruti, V. R., Chakraborty, P. P., & Dey, S. (2018). Sex differences in dispersal syndrome are modulated by environment and evolution. Philosophical Transactions of the Royal Society B: Biological Sciences, 373(1757), 20170428. *Equal contribution*

Chapter 6. Desiccation stress as a cause and a cost of dispersal in *Drosophila melanogaster*

Environmental stress is one of the important causes of biological dispersal, potentially driving the dispersal of organisms away from a given area (Matthysen 2012). In contrast, for the individuals undertaking movement, the process of dispersal itself can incur stress and/or increase susceptibility to stress (Bonte *et al.* 2012). Therefore, in principle, stress can serve as both a cause and a cost of dispersal. Desiccation stress is an environmentally relevant stress faced by many organisms, known to shape their population dynamics and distribution (Kellermann *et al.* 2009; Rajpurohit *et al.* 2013). It is also a part of the dispersal syndrome in some organisms, including *Drosophila melanogaster*. However, the potentially contrasting roles of desiccation stress as a cause and a cost of dispersal have not been investigated. Furthermore, while desiccation stress often affects organisms in a sex-biased manner, it is not known whether the desiccation-dispersal relationship varies between males and females. I studied the role of desiccation stress as a cause and cost of dispersal using *D. melanogaster* adults using two-patch dispersal setups. Using a series of experiments, where I modulated the degree of desiccation stress faced by flies as well as the provision of rest following a dispersal event, I investigated whether: (a) dispersers are the individuals that are more susceptible to desiccation stress, (b) dispersers pay a cost in terms of reduced resistance to desiccation stress, (c) dispersal evolution alters the desiccation cost of dispersal, and (d) females pay a reproductive cost of dispersal. The data showed that desiccation stress served as a significant cause of dispersal in both sexes. Further investigation revealed an increase in male and female dispersal propensity with increasing desiccation duration. Next, I found a male-biased cost of dispersal in terms of reduced desiccation resistance. This trend was preserved in dispersal-selected and non-selected controls as well, where the desiccation cost of dispersal in females was very low compared with males. Finally, I found that the females instead paid a significant reproductive cost of dispersal. These results highlight the complex relationship between desiccation stress and dispersal, whereby desiccation resistance can show both a positive and a negative association with dispersal. Furthermore, the sex differences observed in these trait associations may translate into differences in movement patterns, thereby giving rise to sex-biased dispersal.

This chapter is being written up as the following research article:

Mishra, A, *Tung, S, Sruti, VRS, Shreenidhi, PM, & Dey, S. Desiccation stress as a cause and cost of dispersal in Drosophila melanogaster. (in prep)*

Chapter 7. Conclusions

Here, I highlight the salient findings from the above chapters, discuss their implications, and propose potential avenues for future research.

Apart from the research articles mentioned above, I am also associated with the following publications and manuscripts under preparation:

1. ***Mishra, A,*** *Thadi, A, & Dey, S. Reduced immunity and starvation resistance in Drosophila melanogaster populations selected for higher dispersal. (in prep)*
2. ***Mishra, A,*** *Lall, S, Barve, R, Thadi, A, Gayathri, K, & Dey, S. Use of nutritional geometry to assess the ecological dispersal syndrome in Drosophila melanogaster. (in prep)*
3. *Tung, S,* ***Mishra, A,*** *Gogna, N, Sadiq, MA, Shreenidhi, PM, Sruti, VRS, Dorai, K, & Dey, S (2018). Evolution of dispersal syndrome and its corresponding metabolomic changes. Evolution, 72, 1890-1903.*
4. *Tung, S,* ***Mishra, A,*** *Shreenidhi, PM, Sadiq, MA, Joshi, S, Sruti, VRS, & Dey, S (2018). Simultaneous evolution of multiple dispersal components and kernel. Oikos, 127, 34-44.*
5. *Tung, S,* ***Mishra, A,*** *& Dey, S (2016). Simultaneous enhancement of multiple stability properties using two-parameter control methods in Drosophila melanogaster. Ecological Complexity, 26, 128-136.*
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CHAPTER 1

Introduction

Living organisms often move from one place to another. Even the relatively sessile taxa such as plants and fungi have evolved mechanisms that allow their propagules to cover long distances across space. In more motile species, individuals often exhibit movement in various forms, including foraging, migration, and dispersal. These movement patterns, in turn, determine how species are distributed across space at a given time. Dispersal in particular, commonly defined as 'the movement of individuals or propagules across space with a potential for gene flow' (Ronce 2007), affects a range of biogeographic phenomena, including population dynamics, range expansions, community assembly, and biological invasions (Bowler & Benton 2005; Clobert *et al.* 2009; Lowe & McPeek 2014). Furthermore, acting as the first line of defence against changing habitats, dispersal is a major component of a species' responses to anthropogenic climate change (Best *et al.* 2007; Buckley *et al.* 2013; Travis *et al.* 2013). Increasing instances of habitat destruction and fragmentation have meant that several at-risk species are forced to abandon their domains and move out in search of more hospitable areas (Boeye *et al.* 2013; Buckley *et al.* 2013).

With these academic and real-world implications, it is not surprising that there are several past and ongoing efforts to understand the process of biological dispersal. Empirical studies have catalogued the patterns of movement across numerous taxa, both in natural habitats and under semi-natural or controlled settings. Similarly, modelling studies continue to use theoretical and computational approaches to fit the empirical data and generate predictions for future movements. Despite this rich and growing body of literature, there remain several challenges to understanding dispersal. *Inter alia*, these challenges stem from the fact that the modes and adaptations for dispersal vary quite significantly across taxa (Clobert *et al.* 2012). Even within a given population of a certain species, dispersal traits often exhibit wide variation among individuals, as the act of dispersal often involves multiple phenotypic components. Some examples of these components include: assessment of the immediate habitat and the bodily resources, decision-making based on these assessments, behavioural initiations that allow for emigration from the current habitat, expenditure of energy during the movement, and assessment of a new habitat and decision-making regarding immigration and settling. In other words, not only is dispersal heavily influenced by a myriad of external environmental factors, but also due to differences in the body condition of individuals (Bowler & Benton 2005; Matthysen 2012).

In view of the above, several studies have tried to untangle these complexities and identify factors that could explain a substantial part of the variation in dispersal. As

a result, we now know of some factors that are frequently associated with the movement patterns across taxa. These factors, or 'causes' of dispersal, can often be categorized in several ways. In one such broad, dichotomist classification, Clobert et al. (Clobert *et al.* 2009; Clobert *et al.* 2012) proposed a distinction between 'context-dependent dispersal' and 'phenotype-dependent dispersal'. The former refers to the cases where variation in dispersal is caused by the environment external to the dispersers, whereas the latter refers to the cases where individual phenotypes largely predict the variation in dispersal. Of course, many a times, the distinction between these two types may not be very clear, as the factors external and internal to the organisms can interact to drive the variation in movement. Nevertheless, this conceptual dichotomy of context- vs. phenotype-dependent dispersal can serve as a useful framework for studying dispersal properties at a population- vs. individual-level, respectively.

One of the most common factors that modulate dispersal at the population-level is the local density of conspecific individuals. The resultant form of context-dependent dispersal, termed 'density-dependent dispersal' (henceforth, DDD), is defined as a change in per-capita dispersal with a change in the population density. In the cases where movement increases with a rise in population density, the phenomenon is known as positive DDD, denoting a positive association between density and dispersal (e.g. Aars & Ims 2000; De Meester & Bonte 2010; Bitume *et al.* 2013; Lutz *et al.* 2015). Classically the most commonly observed form of DDD (Matthysen 2005; Harman *et al.* 2020), positive DDD is often explained by elevated intraspecific competition for resources, or as a mechanism of inbreeding avoidance (Hamilton & May 1977; Travis *et al.* 1999). In contrast, the per-capita movement in some taxa is known to decrease with increasing population density, marking a negative association between density and dispersal (e.g. Andreassen & Ims 2001b; Baguette *et al.* 2011; Pennekamp *et al.* 2014). Dubbed as negative DDD, this phenomenon is mostly explained by the presence of Allee effects, favourable social interactions, or simply as an indication that the current habitat is suitable (Bowler & Benton 2005; Matthysen 2005; Harman *et al.* 2020). In addition to the positive or negative DDD, some species are known to exhibit non-linear DDD, where the relationship between density and dispersal changes between low- and high-density ranges. Overall, DDD is thought to be closely related to the biology of the species, including its density-dependent fitness and degree of sociality or territoriality (Trewhella *et al.* 1988; Andreassen & Ims 2001b; Matthysen 2005; De Meester & Bonte 2010). However, there exist instances where different studies on the same species have observed contrasting patterns of DDD. For instance, Kussari et al. (1996) observed a negative

DDD in Glanville fritillary butterflies, whereas a positive DDD was later reported by Enfjäll and Leimar (2005) in the same species. Similarly, both negative (Einum & Nislow 2005) and positive DDD (Einum *et al.* 2006) have been reported for the Atlantic salmon. These examples suggest that the type of DDD exhibited by a species is perhaps not an invariant, but could be a function of other factors like environment or age of individuals. Given that the type of DDD exhibited by a population is known to influence its dynamics, fitness, and invasiveness (Namba 1980; Aars & Ims 2000; Amarasekare 2004; Travis *et al.* 2009), there is a need to understand how DDD is shaped differently by different environments. Finally, an under-appreciated topic of research in the DDD literature has been sex-specific differences. Why and how males and females exhibit differences in their DDD patterns is a topic of interest to population and conservation genetics, as well as the broader area of 'sex-biased dispersal'.

Sex-biased dispersal (SBD), as the name suggests, refers to an instance where the males and females of a given taxon differ in some aspect of their movement. Some common examples of SBD include: lions (Packer & Pusey 1987), spotted hyenas (Höner *et al.* 2007), and great white shark (Pardini *et al.* 2001), where males show a higher dispersal than females. Conversely, several avian species exhibit female-biased dispersal (reviewed in Clarke *et al.* 1997). The classical hypothesis in this context says that the direction of SBD in a given species is dictated by the type of its mating system (monogamous vs. polygamous, polygynous vs. polyandrous, etc.) (Greenwood 1980). However, with a wealth of SBD data collected from across taxa, it is increasingly clear that the relationship between mating type and SBD is much more nuanced. For instance, a review of the empirical literature concluded that sexual dimorphism and extent of parental care are stronger determinants of SBD in a species (Trochet *et al.* 2016). On the other hand, a review of SBD-related theoretical literature led Li and Kokko (2019) to propose that the relative order of mating and dispersal can lead to differences in SBD, even across species with identical mating systems. However, as we make progress in our understanding of SBD at the population-level, there is also a need to focus on the sex-specific adaptations to dispersal. The idea here is that the pervasive sex differences, in the physiological, life-history, and behavioural traits of sexually dimorphic species, are likely to feature in their adaptations to dispersal as well. If so, these sex-specific adaptations in a given species can potentially shape or interact with the SBD patterns of that taxon. However, very few studies have investigated sex-specific adaptations to dispersal (Legrand *et al.* 2016). This is despite the fact that individual trait associations to

movement represent a key component of identifying and studying phenotype-dependent dispersal.

A key conceptualization in the phenotype-dependent dispersal literature is that of a 'dispersal syndrome', defined by Ronce and Clobert (2012) as the suite of morphological, life-history, behavioural, or physiological traits strongly associated with dispersal. Identification of such syndromes is expected to aid not only the understanding of individual adaptations to movement, but also in potential prediction of future movement patterns (Stevens *et al.* 2013). However, these relatively ambitious goals are undermined by the fact that these phenotypic trait associations with dispersal can arise in a variety of ways. For instance, traits may show a positive association with dispersal due to their useful role in some aspect of dispersal or because of linked selection (Cote *et al.* 2010; Ronce & Clobert 2012). Similarly, negative associations with dispersal may arise due to genetic trade-offs or physiological costs of dispersal (Bonte *et al.* 2012; Ronce & Clobert 2012). Therefore, these trait correlations may be specific to factors such as the study habitat, population history, and the assay conditions. In other words, there is a very real possibility that these discovered associations may not carry over to another habitat or a different population of the same species. In the absence of systematic investigations of these syndromes under controlled conditions, the strength of their association with dispersal, and consequently their utility for real-world applications, remains questionable.

Finally, an added layer of complexity for these dispersal-associated phenomena is that of evolution. While it remained a question of considerable debate until a decade ago (reviewed in Lowe & McPeek 2014), a host of recent empirical studies have now established that evolutionary changes in the dispersal of a population are both common and rapid (e.g. Fronhofer *et al.* 2014; Williams *et al.* 2016; Weiss-Lehman *et al.* 2017; Tung *et al.* 2018b). These evolutionary changes in dispersal patterns often result from some or the other form of 'spatial selection', where the selection pressures faced by individuals differ with their relative spatial location within the range of a population (Phillips *et al.* 2010; Williams *et al.* 2019). With growing instances of habitat loss and fragmentation for many natural populations, individuals are expected to face a high selection pressure for movement (Bonte *et al.* 2010; Boeye *et al.* 2013; Travis *et al.* 2013), likely leading to numerous instances of spatial selection. In such a scenario, it stands to reason that such selection might also modulate DDD, SBD and dispersal syndromes, all of which had largely been studied in an ecological context until now. In particular, this could undermine the utility of

dispersal syndromes as proxies for prediction of future dispersal patterns (Ronce & Clobert 2012; Stevens *et al.* 2013), if the dispersal trait-associations show a significant change with evolution. Therefore, investigating the evolutionary changes in these phenomena would lead to a more complete understanding and better predictions of dispersal.

In this thesis, I use microcosm experiments, with *Drosophila melanogaster* as a model organism, to investigate the above issues in ecological as well as evolutionary contexts. In addition to its usual benefits such as short generation time, ease of laboratory rearing, and sexual dimorphism, *D. melanogaster* also offers a tremendous advantage in terms of the extensive extant literature on its life history, behaviour and physiology (Prasad & Joshi 2003; Rose *et al.* 2004). Chapters 2–4 in this thesis deal with the population-level phenomena related to dispersal (i.e. DDD and SBD), whereas Chapters 5 and 6 focus on the individual-level causes and consequences of dispersal (i.e. dispersal syndromes).

Chapter 2 details a series of experiments on the ecological aspects of DDD and SBD. Not only is the empirical data on the robustness of DDD and SBD quite limited, the environmental factors modulating DDD and SBD have been rarely investigated. Using a series of experiments with carefully controlled environmental differences, I examined the robustness of DDD and SBD patterns, as well as the potential factors that modulate them. I found remarkable changes in the DDD and SBD patterns of the same populations, even with seemingly minor environmental alterations. Comparing the data across these experiments, I was able to establish that pre-dispersal context and presence of mates can be two significant determinants of DDD and SBD.

Chapter 3 focuses on the phenomenon known as 'mate-finding dispersal'. Following a SBD event, populations often experience a skewed sex ratio. This can lead to an acute shortage of mates for one of the sexes, which is incidentally thought to be the most common cause of Allee effects in small populations (Taylor & Hastings 2005; Gascoigne *et al.* 2009; Berec *et al.* 2018). How do individuals of the more abundant sex deal with this kind of mate limitation? The extant theory proposes two contrasting adaptations for such mate limitation (Shaw & Kokko 2014; Fromhage *et al.* 2016). The first is mate-finding dispersal, wherein the less-dispersive sex increases its movement to locate mates. The second way is through physiological adaptations that counter the impact of mate limitation, allowing the less-dispersive sex to safely suppress its movement further, in a bid to re-allocate body resources into

reproduction. Despite the intuitive appeal of both these contrasting hypothesis, neither (i.e. increased or decreased dispersal of the less-dispersive sex) had been empirically demonstrated to date. Using artificially selected fly populations with varying dispersal propensities in the laboratory (Tung *et al.* 2018b), I directly tested the dispersal response of the less-dispersive sex in the aftermath of an SBD event. With appropriate controls, I was able to demonstrate significant mate-finding dispersal in both sexes. As a result, the dispersal of the less-dispersive sex, as well as that of the whole population, was dramatically increased even if only one sex initially exhibited high dispersal.

Chapter 4 extends the focus on DDD and SBD in an evolutionary context. Empirical evidences for evolutionary changes in DDD and SBD have been extremely rare. This is perhaps because most evolutionary studies on dispersal have been limited to very few generations. To investigate evolutionary changes in DDD and SBD, I used the aforementioned dispersal-selected populations from a long-term evolutionary experiment in the lab (~75 generations of selection at the time of this study) (Tung *et al.* 2018b). Comparing the dispersal-evolved populations with their controls, I was able to test directly the extant hypotheses regarding evolutionary changes in DDD and SBD during dispersal evolution (Travis *et al.* 2009; Fronhofer *et al.* 2017; Weiss-Lehman *et al.* 2017). This study provided the first empirical demonstration of an evolutionary change in SBD, even under identical environmental conditions. Furthermore, contrary to the theoretical predictions from literature, we observed a complete loss of negative DDD in populations undergoing dispersal evolution.

Shifting the focus to the organismal level, **Chapter 5** examines dispersal syndromes in an ecological and evolutionary context. This study had a twofold aim of uncovering sex differences in dispersal syndromes, and assessing the robustness of dispersal syndromes in the face of ecological and evolutionary changes. I conducted a series of experiments to assess the association of three life-history and behavioural traits with dispersal in males and females. Not only did I find substantial sex differences in dispersal syndromes, I also found these associations to be subject to significant changes under altered nutrition levels and evolution. Interestingly, for one of the traits (desiccation resistance), the correlation with dispersal switched from positive to negative after dispersal, even under identical environmental conditions.

Chapter 6 deals with the relationship between desiccation stress and dispersal. As noted in Chapter 5 above, I had observed both a positive and a negative association

of desiccation stress with dispersal. A likely explanation for this could be the putative role of desiccation as both a cause and a cost of dispersal. As a common environmental abiotic stress (Black & Pritchard 2002; Holmstrup *et al.* 2002; Kranner *et al.* 2008; Holzinger & Karsten 2013), desiccation is likely a major driver of dispersal in many taxa. On the other hand, desiccation stress is also something dispersing individuals may experience during the transfer phase of their movement. Therefore, I set out to examine whether desiccation stress indeed serves as a cause and/or a cost of dispersal for *D. melanogaster*. Using a set of four experiments, I show both the cause and the cost aspect of desiccation-dispersal relationship. Furthermore, I found appreciable sex differences in these associations, which could help understand sex-specific adaptations for dispersal.

Finally, in **Chapter 7** I summarize the salient results from this thesis. I also discuss some eco-evolutionary implications of these findings, while presenting possible avenues for future research in these areas. Most of the chapters in this thesis are slightly modified versions of published (Chapters 2, 3, 5), submitted (Chapter 4), or under-preparation manuscripts (Chapter 6).

CHAPTER 2

Pre-dispersal context and presence of mates modulate density dependence and sex bias of dispersal

Highlights

- Strong negative density-dependent dispersal (DDD) observed in both male and female flies
- DDD patterns were altered by the pre-dispersal environmental context
- Sex differences in DDD patterns led to a switch in the direction of sex-biased dispersal
- Interaction between sexes was crucial for DDD; neither sex showed DDD on its own

Published as and adapted from:
Mishra, A., Tung, S., Sruti, V. S., Sadiq, M. A., Srivathsa, S., & Dey, S. (2018). Pre-dispersal context and presence of opposite sex modulate density dependence and sex bias of dispersal. *Oikos*, *127*(11), 1596-1604.

1 INTRODUCTION

Dispersal is a complex process which is caused and modulated by a large number of factors such as landscape structure, resource availability and individual phenotypes (reviewed in Matthysen 2012). One factor that has been often implicated as a cause of variation in dispersal is population density (Matthysen 2005), and density-dependent dispersal (DDD) in turn has been shown to have major ecological and evolutionary implications. For instance, DDD is expected to have pronounced effects on the synchrony and dynamics of spatially structured populations (Amarasekare 2004; Lecomte *et al.* 2004), which is of particular significance in the context of endangered species (Rouquette & Thompson 2007). Furthermore, DDD is shown to have population genetic consequences (Aars & Ims 2000), indicating that it could play a role, along with other plastic dispersal behaviours, in macro-evolutionary processes such as speciation (Arendt 2015).

Not surprisingly therefore, density-dependence of dispersal has been thoroughly investigated both theoretically and empirically. Theoretical investigations have focused on the effects of DDD on population distribution (Namba 1980), synchrony (Liu *et al.* 2016), and source-sink dynamics (Amarasekare 2004), as well as the conditions under which DDD is expected to evolve (Travis *et al.* 1999; Metz & Gyllenberg 2001; Poethke & Hovestadt 2002). Empirical studies across multiple taxa show that increased intraspecific competition is expected to drive higher emigration at high densities, leading to positive DDD (De Meester & Bonte 2010; Lutz *et al.* 2015). In contrast, some other organisms show a reduced dispersal with increasing population density, either due to reduced availability of mates and resources (Lambin 1994), or social costs (Hestbeck 1982). In spite of this wealth of studies on DDD, an issue that remains relatively unexplored is the robustness of DDD patterns under different environments.

In most studies, the current density (i.e., the density observed at the beginning of the dispersal phase) is taken into account while quantifying DDD (e.g. Bitume *et al.* 2013; Schultz *et al.* 2017), thus neglecting the history of the population. However, dispersal can be affected by the physiology of the dispersers (Zera & Denno 1997), which in turn is expected to be a function of the pre-dispersal context. Therefore, intuitively speaking, it is possible that DDD patterns can themselves be modulated by the pre-dispersal context faced by the organisms. Here, we define pre-dispersal context as the environment faced by the individuals prior to the dispersal phase (*sensu* 'context-dependent dispersal' in Clobert *et al.* 2012). Unfortunately, the effects of pre-dispersal context on the manifestation of DDD remain relatively poorly understood,

partly because of the difficulties in obtaining such information about dispersing populations (although see Andreassen & Ims 2001a; Betini *et al.* 2015).

An additional source of complication arises when the pre-dispersal context affects males and females differently, which might result in a differential impact on their dispersal traits (Hovestadt *et al.* 2014a). It is already known that males and females can have different DDD patterns (e.g. Albrectsen & Nachman 2001; Lutz *et al.* 2015). Thus, the inherent differences in the DDD responses of the two sexes could interact with the pre-dispersal context to yield a stronger or weaker overall DDD in the population. This could even result in the emergence of sex-biased dispersal due to differential DDD, a phenomenon that has been acknowledged (Trochet *et al.* 2016) but never empirically demonstrated. Finally, because dispersal is affected by the local sex ratio (Trochet *et al.* 2013), population composition in terms of the number of members of the opposite sex could potentially modulate the patterns of both DDD and sex-biased dispersal.

Here we investigate some of these issues related to the robustness of DDD patterns using laboratory populations of *Drosophila melanogaster* under controlled environmental conditions. Specifically, we asked whether DDD is affected by a) pre-dispersal adult density, b) sex of the dispersers, and c) presence of mates. We find that both pre-dispersal population density and interaction between the sexes greatly affect DDD. In addition, the direction of sex-biased dispersal is shown to undergo a significant and complete reversal, via an interaction between the pre-dispersal and dispersal densities. We discuss potential reasons for the observed patterns and discuss how these results can affect several ecological and evolutionary processes.

2 MATERIAL AND METHODS

2.1 Fly population

All flies used in this study were collected from a large, outbred (breeding size ~2400 individuals) laboratory population of *D. melanogaster* (namely DB_4), maintained at uniform environmental conditions of 25 °C temperature and 24-h light (Sah *et al.* 2013).

2.2 Dispersal setup and assay

D. melanogaster has been used extensively to study the process of dispersal under natural (Wallace 1975; Roff 1977) and laboratory conditions (Betini *et al.* 2015; Tung *et al.* 2018b). Following an earlier protocol (Tung *et al.* 2018b), we used a two-patch *source-path-destination* setup to study dispersal (Fig. 1). The source was a 100-mL conical flask, which led into a 2-m long, transparent plastic tube (inner diameter ~1 cm) that served as the path. The other end of the path opened into a 250-mL plastic bottle that served as the destination. This end of the path protruded ~3 cm into the destination, which prevented the flies that had reached the destination from going back into the path. The flies were introduced into the source and allowed to disperse for 4 h. The bottle that served as the destination was replaced with another empty bottle every 30 minutes during these 4 h, with minimum possible disturbance to the rest of the setup. The number and sex of dispersers at each of these 30-minute intervals were recorded.

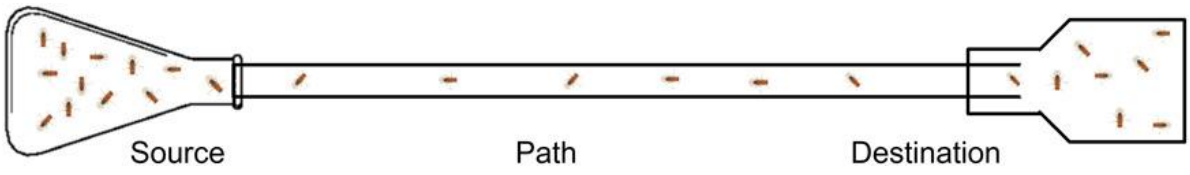

Fig. 1 Schematic diagram of the two-patch dispersal setup. The *source* was a 100-mL glass flask (total volume ~135 mL). The *path* was a transparent plastic tube with inner diameter ~1 cm. The *destination* was a 250-mL plastic bottle. For dispersal assay, adult flies were introduced into the *source*, which then dispersed through the *path* into the *destination*. The entire setup was maintained under controlled, uniform laboratory conditions (constant light, 25 °C temperature).

2.3 Culturing flies for the experiments

We performed three separate experiments to investigate density dependence and sex differences in dispersal. To eliminate any confounding effects of habitat quality, the entire dispersal setup was devoid of food and moisture, with the number of individuals in the source being the only difference across treatments during the dispersal assay. It is well known that age (Hastings 1992), kin competition (Gandon 1999) and inbreeding avoidance (Charlesworth & Charlesworth 1987) can affect the patterns of dispersal. We avoided these confounding factors by appropriate rearing of the flies as mentioned below.

Two cages with ~2400 adult flies each, derived from the DB_4 population (section 2.1) were maintained. To collect eggs for a particular day of the assay, one of the two cages was supplied with live yeast plate for ~24 h. Fresh banana-jaggery medium was then supplied to the flies, allowing the females to oviposit, for 12 h. After this period, ~50 eggs each were collected into forty 35-mL plastic vials containing ~6 mL of banana-jaggery medium. Following this, a plate with live yeast paste was provided to the flies again, to enable another round of egg collection. The same procedure was followed for the other population cage as well, with a constant difference of 24 h between the cages. Therefore, it was possible to collect eggs every day, alternatively from two cages of the same population, for 10 days. The large breeding population size (~2400) in each cage ensured that the flies were not inbred, and random sampling from a large number of eggs during the collection reduced the chances of kin being sampled together. Moreover, as flies from a single set of collected eggs were used for the dispersal assay on a particular day, they were all of the same age (12^{th} day from egg collection) at the time of assay.

2.4 Three DDD Experiments

All experiments were performed on 12-day (from egg-lay) old flies. All experiments used the same four dispersal density treatments (defined as the density at the beginning of dispersal assay), namely 60, 120, 240 and 480 individuals. The lowest density (60 individuals) is the approximate per-vial adult density during the routine maintenance of the DB_4 flies (Sah *et al.* 2013).

2.4.1 Experiment 1: Mixed-sex dispersal with variable pre-dispersal densities across treatments

One day before the dispersal assay (i.e., on day 11 post egg-collection), the adult flies were separated by sex under light CO_2 anaesthesia. The adults were then randomly assigned to the four dispersal density treatments (60, 120, 240 and 480) with a strict 1:1 sex ratio, i.e., the density treatment of 60 consisted of 30 males and 30 females, and so on. These flies were then transferred into separate 100-mL conical glass flasks (identical to the source in dispersal setup) containing ~35 mL of banana-jaggery medium. After 21 h, the dispersal behaviour of these flies was measured as described in section 2.2 (Fig. 2A). The population density and composition during this 21-h maintenance period comprised the pre-dispersal context. Since the flies were housed under strictly controlled laboratory conditions, there were almost no deaths during the 21-h holding period. In the rare cases where 1-2 flies died, they were not replaced. This experiment happened over 9 consecutive days with a fresh set of flies on each day, thus yielding 9 independent replicates per dispersal density. One replicate of each dispersal density was assayed on each day and a total of 8,100 flies (9 days × 900 flies/day) were used in this experiment. Flies of both sexes were present in a given dispersal setup, and the pre-dispersal density (i.e., the density during anaesthesia-recovery period) was different for the four treatments.

2.4.2 Experiment 2: Mixed-sex dispersal with a uniform pre-dispersal density across treatments

This experiment was identical to Experiment 1 in all respects, except for the fact that the pre-dispersal density for all the flies was held equal. For this, at the time of separation by sex under CO_2 anaesthesia, the flies were randomly assigned to 15 groups of 60 individuals with 1:1 sex ratio. Each set of 60 flies was transferred into a 35-mL plastic vial, containing ~3 mL of banana-jaggery medium. Twenty-one hours later, at the time of dispersal setup, flies from an appropriate number of vials were mixed together to yield the density treatments (i.e., 1×60, 2×60, 4×60 and 8×60) and transferred into the corresponding source flasks (Fig. 2B). Thus, in this experiment, both sexes were assayed together, and a uniform pre-dispersal density was maintained across the four dispersal treatments.

2.4.3 Experiment 3: Uni-sex dispersal with a uniform pre-dispersal density across treatments

This experiment was identical to Experiment 2 in all aspects, except that the males and females dispersed in the absence of the opposite sex and there were 10 replicates per density per sex. In other words, the males and females were introduced into separate dispersal setups for all four density treatments (Fig. 2C) and a total of 18,000 flies (2 sexes × 10 days × 900 flies/day/sex = 18,000 flies) were used to obtain the data. Thus, in this experiment, the two sexes were assayed independently, and the pre-dispersal density was uniform across the treatments.

In total, across the three experiments, 34,200 flies were scored for their dispersal behaviour.

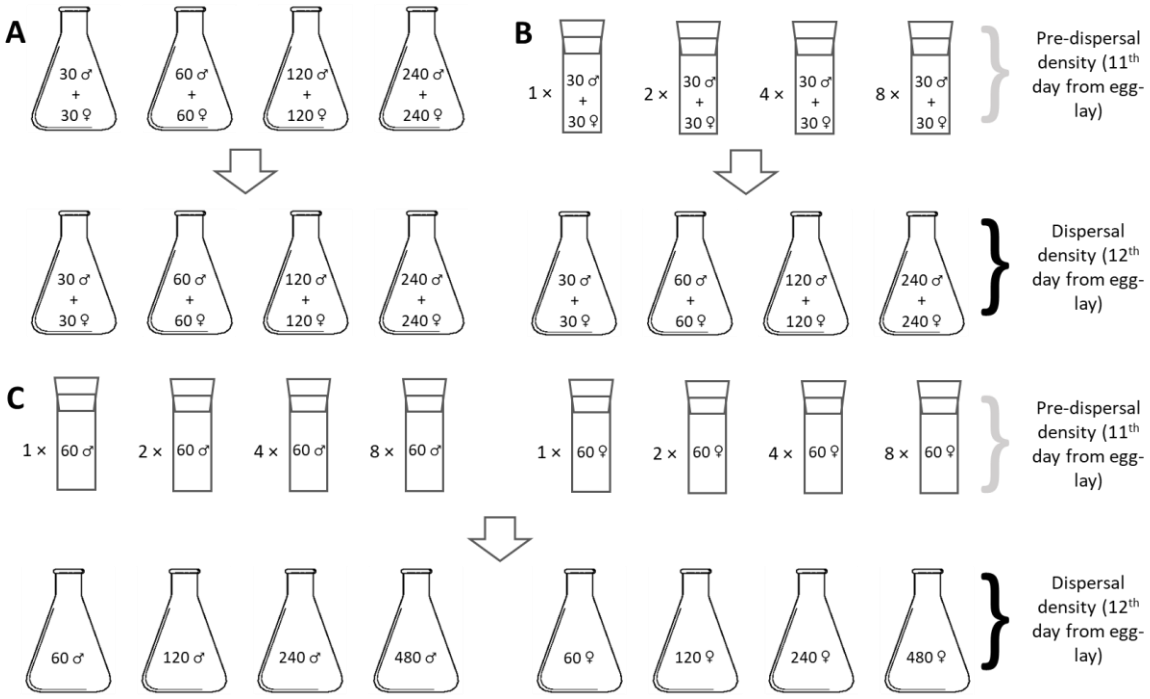

Fig. 2 Experimental design. (A) Experiment 1 (4 treatments; 9 replicates per treatment) **(B)** Experiment 2 (4 treatments; 9 replicates per treatment) **(C)** Experiment 3 (8 treatments; 10 replicates per treatment)

2.5 Dispersal traits

For each experiment, the observations included the number and sex of flies that reached the destination during each of the 30-minute intervals until the end of dispersal assay (4 h). We also counted the flies that emigrated from the source but

did not reach the destination, i.e., the flies found within the path tube at the end of dispersal assay.

Using these data, we estimated the dispersal propensity, i.e., the proportion of flies that initiated dispersal from the source (Friedenberg 2003) as follows:

$$Dispersal\ Propensity = \frac{\sum_i n_i + n_p}{N}$$

where N is the total number of flies introduced in the setup, n_i is the number of flies that reached the destination during the i^{th} time interval and n_p is the number of flies found within the path at the end of dispersal assay.

In addition, we obtained the overall temporal profile of dispersers reaching the destination. For each dispersal setup, we divided the number of dispersers that reached the destination in each 30-minute interval by the final number of successful dispersers.

2.6 Statistical analyses

As stated above, each experiment was performed over multiple consecutive days, with a fresh set of age-matched flies every day. One replicate each for the four dispersal densities was assayed on each day. Thus, *day* was included in the analyses as a random blocking factor, to account for any day-specific micro-environmental variations. Dispersal propensity Data from each experiment were analysed using a three-way mixed-model ANOVA, with *density* (60, 120, 240 and 480) and *sex* (male and female) as the fixed factors, and *day* (1-9 for the first two experiments and 1-10 for the third experiment) as a random factor. For analysing the temporal profiles of dispersers for each sex, individual two-way mixed-model ANOVAs were performed at each time point, with *density* as the fixed factor crossed with *day* (random factor), followed by sequential Holm-Šidák correction to control the family-wise error rates (Abdi 2010). As all the data were in the form of fractions, they were arcsine-square root transformed prior to ANOVA (Zar 1999). Whenever a significant main effect was obtained, the pairwise differences were analysed using Tukey's HSD test. All ANOVAs were performed using STATISTICA® v5 (StatSoft. Inc. Tulsa, Oklahoma).

3 RESULTS

3.1 Pre-dispersal density modulates the effect of dispersal densities on dispersal propensity in both males and females

There was a significant *dispersal density* × *sex* interaction in both Experiment 1 (variable pre-dispersal densities) ($F_{3,24}$ = 10.86, p = 0.0001; Fig. 3A) and Experiment 2 (uniform pre-dispersal densities) ($F_{3,24}$ = 5.73, p = 0.004; Fig. 3B). Two observations can be made from a comparison of these two datasets (i.e., Figs. 3A and 3B). First, the number of statistically significant pairwise differences for a given sex was greater in Experiment 1 (Fig. 3A: three for males, five for females) than in Experiment 2 (Fig. 3B: none for males and three for females). This suggests that the DDD in both sexes was amplified by variable pre-dispersal densities. Second, females had a stronger negative DDD than the males, which is evident from the greater number of significant pairwise differences across a wider range of dispersal densities for females in both Figs. 3A and 3B (see Table 1 for the exact Tukey's p values and the associated effect sizes). In other words, while the stronger DDD in Experiment 1 (variable pre-dispersal densities) than in Experiment 2 (uniform pre-dispersal densities) was reflected in both sexes, the females always exhibited a more negative density-dependent response than the males. This suggested that there are sex-specific differences in DDD in *D. melanogaster*.

The difference in the strength of DDD between males and females affected the direction of sex-biased dispersal as well. In Experiment 1, significant sex-biased dispersal was seen only at the highest density (i.e., 480 individuals), with higher male dispersal than female dispersal (Fig. 3A). However, in Experiment 2, significant sex-biased dispersal was observed only at an intermediate density (i.e., 120 individuals), where a female-biased dispersal was observed (Fig. 3B; see Table 2 for the exact Tukey's p values and associated effect sizes). In other words, pre-dispersal density interacted with dispersal density to determine the direction of sex-biased dispersal.

3.2 Patterns of sex-biased dispersal are produced by interaction between the sexes

When males and females were assayed separately while maintaining a uniform pre-dispersal density (Experiment 3), the *dispersal density* × *sex* interaction was not significant ($F_{3,27}$ = 0.54, p = 0.66; Fig. 3C). Thus, males and females showed different dispersal propensities only when they dispersed together (Figs. 3A and 3B) and not when they dispersed on their own (Fig. 3C). Hence, we concluded that in *D.*

melanogaster, the presence of both sexes is a necessary condition for sex-biased dispersal to occur, at least under the densities and environmental conditions used in this experiment.

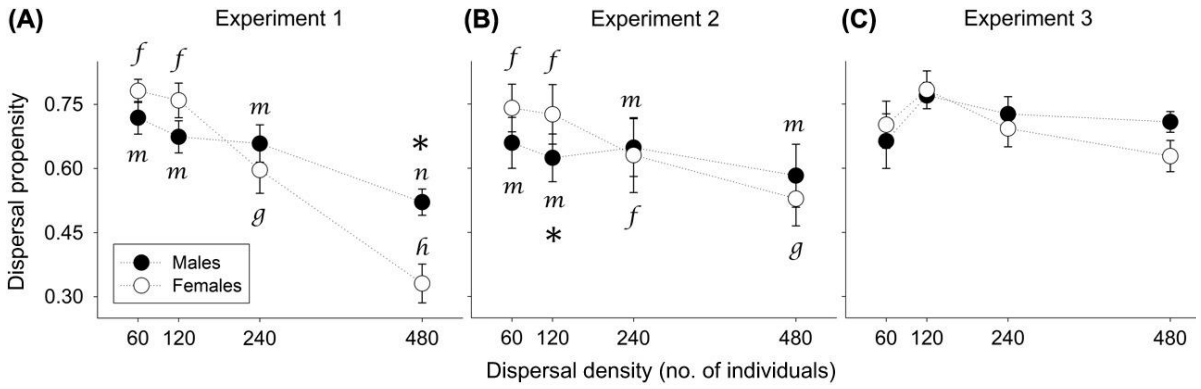

Fig. 3 Sex differences in density-dependent dispersal. (A) Mean dispersal propensity (± SE) of males (filled circles) and females (open circles) vs. dispersal density in Experiment 1 (mixed-sex dispersal with variable pre-dispersal densities across treatments). **(B)** Mean dispersal propensity (± SE) of males and females vs. dispersal density in Experiment 2 (mixed-sex dispersal with a uniform pre-dispersal density across treatments). **(C)** Mean dispersal propensity (± SE) of males and females vs. dispersal density in Experiment 3 (uni-sex dispersal with a uniform pre-dispersal density across treatments). For significant *dispersal density* × *sex* interactions, two kinds of pairwise comparisons are performed (using Tukey's HSD). First, for a given sex, the extent of density dependence is examined by comparing the propensity means across the four dispersal densities (significant differences denoted using different lower-case alphabets: starting with *m* for males and *f* for females; see Table 1 for the exact Tukey's p values and the associated effect sizes). Second, for each dispersal density, the male and female dispersal propensities were compared (significant differences denoted by asterisks; see Table 2 for the exact Tukey's p values and the associated effect sizes). Effect sizes for all the significant pairwise differences were high, except for the one between females at densities 240 and 480 in Experiment 2 (low) and the one between males and females at density 120 in Experiment 2 (medium).

3.3 Variable pre-dispersal densities and mixed-sex dispersal yield sex bias in the temporal patterns of dispersal

In Experiment 1, there was a clear difference in the temporal patterns of male and female dispersal among the density treatments. For the proportion of males completing dispersal per time interval, there were no observable differences across the treatment densities (Fig. 4A). In contrast, dispersal at the highest density treatment (i.e., 480) for the females was significantly different from all other treatments at four out of the eight time points (Fig. 4B, see Table 3 for the exact

Tukey's p values and the associated effect sizes). In terms of the shape of the temporal profile, the three lower densities (i.e., 60, 120 and 240) showed a distinct peak at 1 h, followed by a steady decrease over the next 1.5 h. (Fig. 4B). However, at the density of 480, the proportion of dispersers increased slightly over the first 1 h, but then did not change over the next 3 h. Thus, at densities lower than 480, the majority (>50%) of female dispersers completed dispersal within the first 1.5 h, whereas at 480 individuals, the dispersal was considerably delayed.

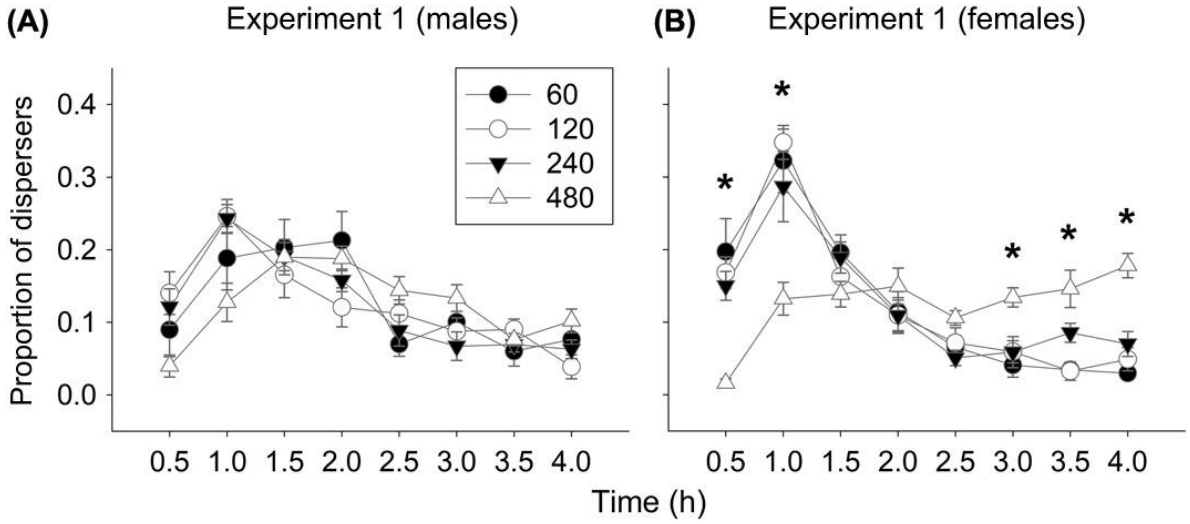

Fig. 4 Temporal profile of dispersers in Experiment 1 (mixed-sex dispersal with variable pre-dispersal densities across treatments). (A) Average proportion (± SE) of males reaching the destination vs. time. **(B)** Average proportion (± SE) of females reaching the destination vs. time. Asterisks indicate a significant main effect ($p < 0.05$) of density at a given time point. At all such points in **(B)**, treatment density 480 differed significantly from all other treatment densities, with large effect sizes; the only exception was 3.5 h, where treatment density 480 was not significantly different from treatment density 240 (see Table 3 for the exact p values and associated effect sizes).

In Experiments 2 and 3, for both males and females, there were very few time points at which the temporal profiles of dispersers differed across the four density treatments (Fig. 5, Table 3). Thus, it was difficult to claim that there were any effects of changing density on the temporal profile of dispersal in these two experiments.

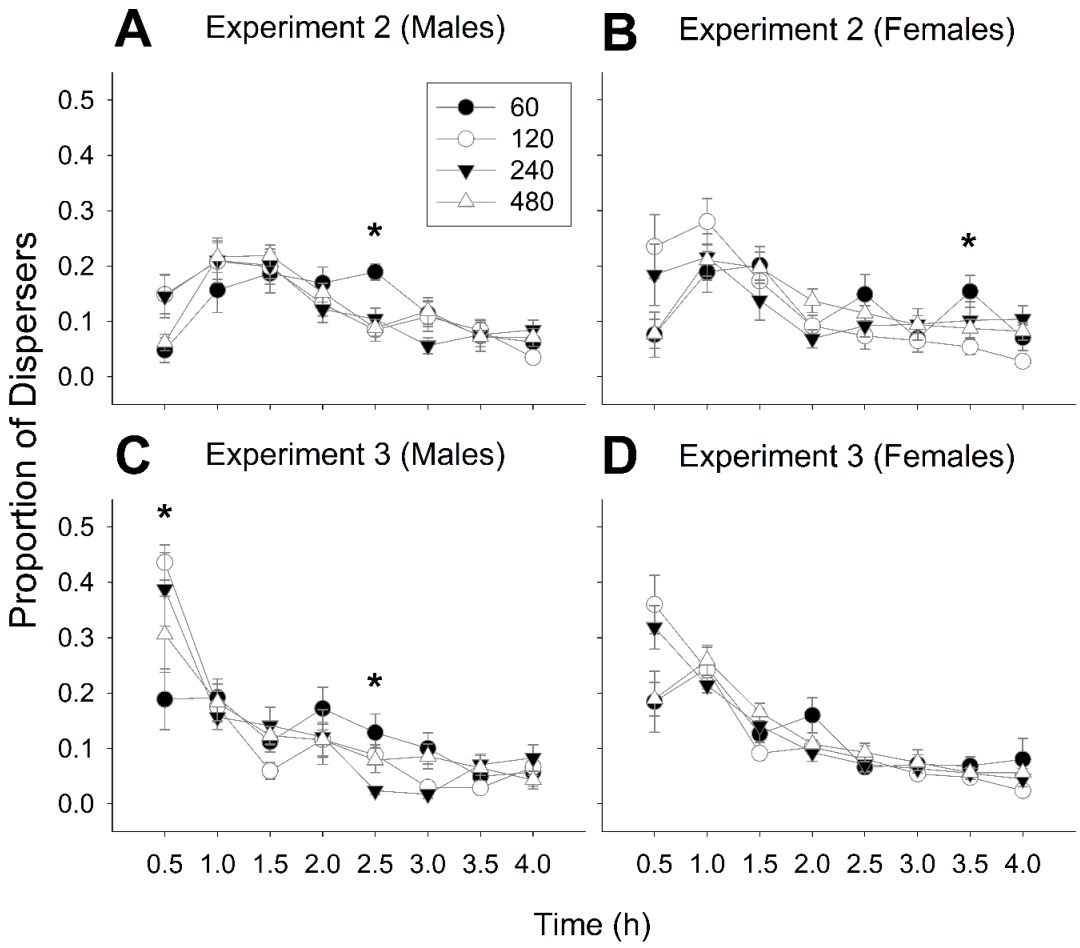

Fig. 5 Temporal profile of dispersers in Experiment 2 (mixed-sex dispersal with uniform pre-dispersal densities across treatments) and Experiment 3 (uni-sex dispersal with uniform pre-dispersal densities across treatments). Average proportion (± SE) of **(A)** males and **(B)** females reaching the destination vs. time in Experiment 2. Average proportion (± SE) of **(C)** males and **(D)** females reaching the destination vs. time in Experiment 3. Asterisks indicate a significant main effect ($p < 0.05$) of density at a given time point (see Table 3 for the exact Tukey's p values and the associated effect sizes).

Table 1. Within-sex pairwise differences for dispersal propensity in Experiment 1 (variable pre-dispersal densities) and Experiment 2 (uniform pre-dispersal density). For significant pairwise differences (Tukey's $p < 0.05$), effect sizes (Cohen's *d*) are computed.

* denotes a significant pairwise difference (Tukey's $p < 0.05$).

Pre-dispersal density	Sex	Densities	Tukey's p	Cohen's *d*	Effect size interpretation
Variable (Experiment 1)	Males	60 – 120	0.925705	-	-
		60 – 240	0.820068	-	-
		60 – 480	0.001099*	1.903589	Large
		120 – 240	0.999994	-	-
		120 – 480	0.019294*	1.490408	Large
		240 – 480	0.035166*	1.214002	Large
	Females	60 – 120	0.998385	-	-
		60 – 240	0.001293*	1.431496	Large
		60 – 480	<0.000001*	4.011698	Large
		120 – 240	0.005694*	1.13081	Large
		120 – 480	<0.000001*	3.319658	Large
		240 – 480	0.000020*	1.762668	Large
Uniform (Experiment 2)	Males	60 – 120	0.947242	-	-
		60 – 240	0.999894	-	-
		60 – 480	0.311063	-	-
		120 – 240	0.995785	-	-
		120 – 480	0.914312	-	-
		240 – 480	0.537093	-	-
	Females	60 – 120	1.000000	-	-
		60 – 240	0.101614	-	-
		60 – 480	0.000037*	1.184309	Large
		120 – 240	0.071835	-	-
		120 – 480	0.000025*	0.987034	Large
		240 – 480	0.045248*	0.443643	Small

Table 2. Sex-bias in dispersal propensity in Experiment 1 (variable pre-dispersal densities) and Experiment 2 (uniform pre-dispersal density). For significant pairwise differences (Tukey's $p < 0.05$), effect sizes (Cohen's *d*) are computed.

* denotes a significant pairwise difference (Tukey's $p < 0.05$).
m: males, f: females

Pre-dispersal density	Densities	Tukey's p	Cohen's *d*	Effect size interpretation
Variable (Experiment 1)	60m – 60f	0.628952	-	-
	120m – 120f	0.294901	-	-
	240m – 240f	0.739523	-	-
	480m – 480f	0.001862*	1.641389	Large
Uniform (Experiment 2)	60m – 60f	0.232469	-	-
	120m – 120f	0.016373*	0.538466	Medium
	240m – 240f	1.000000	-	-
	480m – 480f	0.820914	-	-

Table 3. List of significant pairwise differences in the temporal dispersal profiles for Experiments 1, 2 and 3. Data are presented for the time points which had a significant main effect of density after sequential Holm-Šidák correction, with Tukey's p value and effect size (Cohen's *d*) presented for the significantly different density pairs.

	Sex	Time-point	Holm–Šidák corrected p (main effect)	Densities	Tukey's p	Cohen's *d*	Effect size interpretation
Experiment 1	**Females**	0.5 h	0.000013	60 – 480	0.000008	2.05312	Large
				120 – 480	0.000011	3.5767	Large
				240 – 480	0.000033	3.5059	Large
		1 h	0.007382	60 – 480	0.007575	1.90575	Large
				120 – 480	0.002110	3.20866	Large
				240 – 480	0.033480	1.44887	Large
		3 h	0.008689	60 – 480	0.001007	2.03337	Large
				120 – 480	0.020908	1.59277	Large
				240 – 480	0.022451	1.58731	Large
		3.5 h	0.008977	60 – 480	0.009496	1.59809	Large
				120 – 480	0.003873	1.65852	Large
		4 h	0.000418	60 – 480	0.000053	3.19632	Large
				120 – 480	0.000670	2.42169	Large
				240 – 480	0.012975	2.13133	Large
Experiment 2	**Males**	2.5 h	0.02085717	60 – 120	0.002752	1.75208	Large
				60 – 240	0.040136	1.69398	Large
				60 – 480	0.011477	2.28395	Large
	Females	3.5 h	0.03614052	60 – 120	0.002179	1.55798	Large
Experiment 3	**Males**	0.5 h	0.018957196	60 – 120	0.001230	1.72326	Large
				60 – 240	0.001130	1.03045	Large
				60 – 480	0.041550	0.64396	Medium
		2.5 h	0.014798988	60 – 240	0.007548	1.75265	Large

4 DISCUSSION

4.1 Patterns of density-dependent dispersal are affected by the pre-dispersal density

When the flies were maintained at pre-dispersal densities equal to the respective dispersal densities (Experiment 1; Fig. 3A), there was a strong negative DDD for both males and females. However, when the flies experienced a uniform pre-dispersal density (Experiment 2; Fig. 3B), the negative effect of high density on dispersal was diminished. Thus, we show that even in an otherwise constant environment, something as transient as the density faced by the individuals for a brief period prior to the dispersal event can modulate the strength of DDD response. While several studies have noted the emergence of DDD in terms of resource availability at earlier life stages (reviewed in Benard & McCauley 2008; Matthysen 2012), our results highlight that seemingly innocuous short-term changes in the environmental context during the same life-stage can also have important consequences for DDD.

In the dispersal literature, negative DDD in asocial and non-territorial organisms has primarily been explained in terms of availability of food (Lambin 1994) and availability of mates (Kokko & Rankin 2006). However, similar larval rearing conditions and the absence of food and moisture in the dispersal setup ensured that there were no differences in terms of resources across the dispersal density treatments. The maintenance of a strict 1:1 sex ratio in Experiments 1 and 2 ensured that in these two experiments, there was no per-capita difference in terms of number of individuals of the opposite sex either. Thus, we explicitly controlled for the two most widely cited reasons for negative DDD and show that differences in adult pre-dispersal density can also be a potential cause for the same.

One reason for the observed difference in negative DDD could be the detrimental effects of adult crowding. In *D. melanogaster*, even brief periods of enhanced adult crowding can reduce female fecundity (Joshi *et al.* 1998) and adult longevity (Joshi & Mueller 1997), which suggests a physiological change in the condition of individuals at high densities. Thus, at higher pre-dispersal densities, the flies could have been under increased stress, which could have led to the stronger negative DDD in Experiment 1. We discuss this possibility in detail in section 4.3.

4.2 Sex differences in DDD and reversal in the direction of sex-biased dispersal

In both Experiment 1 and 2, the females showed a stronger negative DDD (as seen in the greater number of within-sex pairwise differences in Figs. 3A and 3B) than the males. Sex-specific differences in DDD are well known in the dispersal literature (Albrectsen & Nachman 2001; Lutz *et al.* 2015), and here we report the same observation in *D. melanogaster*. Furthermore, there was no significant sex bias in dispersal propensity in all but one density each for Experiments 1 and 2. Interestingly though, we found significant male-biased dispersal at a density of 480 individuals in Experiment 1 (denoted by * in Fig. 3A), but significant female-biased dispersal at a density of 120 in Experiment 2 (denoted by * in Fig. 3B). Although the modulation of sex-biased dispersal due to density-dependence is possible in principle (Trochet *et al.* 2016), to our knowledge, this is the first empirical demonstration of this phenomenon. We also report a complete reversal in the direction of sex-biased dispersal within the same population.

4.3 Asymmetric DDD in males and females is caused by interaction between the two sexes

Comparison of the results from Experiments 2 and 3 revealed that there is a difference in DDD only when the two sexes disperse together (Fig. 3B) and not when they disperse separately (Fig. 3C). To our knowledge, this is the first study that compares the DDD of males and females dispersing in presence and absence of each other. Sex-biased dispersal has been recorded in a wide variety of taxa, and a number of hypotheses have been proposed to explain its origin (reviewed in Trochet *et al.* 2016). However, the observation that sex-biased dispersal can emerge as a result of interaction between males and females at different densities, even when they have intrinsically similar dispersal patterns, is novel.

As discussed above (section 4.1), the negative DDD observed in Experiment 1 could be due to a change in the physiological condition of the individuals at high densities. The fact that the females showed a stronger negative DDD than the males (Figs. 3A and 3B) indicated that the females were affected more than the males. However, if that were the case, then one would also expect a significant *dispersal density × sex* interaction in Experiment 3, which was not seen (Fig. 3C). Taken together, these observations suggest that, at least for the conditions of the present study, the presence of mates was a necessary condition for the manifestation of sex differences in DDD.

The asymmetry in the negative DDD patterns of males and females is further supported by observations in Fig. 4. While dispersing in the presence of the opposite sex under variable pre-dispersal densities (i.e., Experiment 1), females at the highest density (480 individuals) show a very different temporal profile of dispersal compared with the other densities (Fig. 4B). On the other hand, the temporal profiles for the males are not affected by dispersal densities (Fig. 4A).

In *D. melanogaster*, the effects of mate-harm by males are well documented. It has been shown that lifetime female fecundity (Fowler & Partridge 1989; Carazo *et al.* 2014), egg-production rate (Pitnick & García–González 2002) and female longevity (Cohet & David 1976; Lew & Rice 2005) get affected due to mating and re-mating. Given that the frequency of such multiple matings is expected to be proportional to the local population density (Levine *et al.* 1980; Harshman *et al.* 1988), and the costs of each subsequent mating increase non-linearly (Kuijper *et al.* 2006), females at higher densities for long durations could be subjected to substantially greater mate harm. Moreover, female flies also experience non-mating costs of exposure to males, which has been shown to affect traits such as longevity (Partridge & Fowler 1990). Thus, it is possible that high adult density and the presence of males together could have reduced the female dispersal propensity in Experiment 1, leading to the observed overall negative DDD.

4.4 Implications

In our experiments, we set out to investigate context-dependent dispersal with respect to pre-dispersal density and the presence of opposite sex. The results indicated a strong effect of both these factors on the sex-biased DDD response. As discussed above, a likely explanation involves a differential effect of these conditions on the two sexes (i.e., females facing more stress in mixed-sex conditions). In other words, it means that context-dependent dispersal can manifest via a condition-dependent response (*sensu* Clobert *et al.* 2012) in dispersing populations. As patterns of DDD and sex-biased dispersal are, in turn, expected to modulate other eco-evolutionary phenomena, these results could have several potential implications, some of which are discussed below.

First, several studies have investigated the role of DDD on population dynamics (Amarasekare 2004; Ims & Andreassen 2005), biological invasions (Travis *et al.* 2009), climate change response (Best *et al.* 2007), range expansions (Altwegg *et al.* 2013) and community assembly (French & Travis 2001). However, context-dependent plasticity

in the patterns of DDD itself would mean that the results and predictions from such studies might not hold across different environmental scenarios. For instance, modulation of negative DDD by pre-dispersal density, as observed in our study (Figs. 3A and 3B), could lead to reduced spatial connectivity in fragmented populations, thereby reducing the chances of evolutionary rescue from extinctions (Andreassen & Ims 2001a; Schiffers *et al.* 2013). Thus, this result suggests that environment-dependent heterogeneity in dispersal patterns can affect key population-level processes, and needs to be explicitly accounted for in theoretical and empirical studies (Hawkes 2009).

Second, factors like sex ratio (Pérez-González & Carranza 2009) and breeding site availability (Arlt & Pärt 2008) have been hypothesized as potential reasons for variations in sex-biased dispersal. Our results show that even a seemingly innocuous factor, such as adult density for a short period prior to dispersal, can completely alter the pattern of sex-biased dispersal. Populations in natural environments are expected to experience much greater variations in abiotic and biotic factors, many of which could potentially affect the two sexes differently. This implies that sex bias in dispersal is perhaps even less of a robust phenomenon than what is normally believed, and extrapolations across populations (let alone species) should be made with extreme care. Furthermore, sex-biased dispersal is known to shape phenomena such as invasion success (Miller *et al.* 2011; Miller & Inouye 2013) and adaptive divergence (Fraser *et al.* 2004). However, these studies typically assume a constant pattern of sex bias, which is unlikely to be true under varying natural contexts. Thus, the implications of density- or environment-dependent switching of the direction of sex-biased dispersal would be an interesting area for future investigations.

Third, at the end of dispersal, biased settlement with respect to population composition in a new habitat is expected to promote population divergence (Arendt 2015). Therefore, density-dependent switching between male- and female-biased dispersal could manifest as strong founder effects in newly colonized patches at range fronts, potentially affecting the ecological and evolutionary processes that follow colonization (Ibrahim *et al.* 1996). Also, in addition to these direct and immediate founder effects, population composition in newly colonized patches can have indirect and extended effects on the future dispersal events following colonization (Le Corre & Kremer 1998). In other words, while environment can influence the composition of a dispersing population, the resulting skew in the composition, in turn, can be a potential determinant of the subsequent dispersal. It

is, therefore, timely to investigate the role of population composition, both as a consequence and as a cause of dispersal.

Finally, even though DDD is a population-level phenomenon (i.e., per capita increase or decrease in dispersal with density), its classical interpretations in active dispersal often involve an active information use and/or decision making by the individuals (reviewed in Matthysen 2005; Clobert *et al.* 2009). We show that DDD can also be potentially exhibited via physiological effects (here, likely male mate harm), even if there is no obvious active decision making involved. This is thus an instance of body-condition-dependent dispersal (Matthysen 2012), which can be effected through density-mediated processes. Further experiments involving direct measurements of the physiological conditions of the organisms under varying density levels would help corroborate these observations.

CHAPTER 3

Mate-finding dispersal reduces local mate limitation and sex bias in dispersal

Highlights

- First empirical evidence of mate-finding dispersal, following a skew in local sex ratio
- Males showed a much stronger mate-finding dispersal than females
- Predicted to be an even stronger effect in taxa without physiological adaptations for mate limitation
- Contrasting effects of mate-finding dispersal predicted for small and large populations

Published as and adapted from:
Mishra, A., Tung, S., Sruti, V. S., Srivathsa, S., & Dey, S. (2020). Mate-finding dispersal reduces local mate limitation and sex bias in dispersal. *Journal of Animal Ecology, 89*(9), 2089-2098.

1 INTRODUCTION

Dispersal, i.e. movement of organisms with potential consequences for gene flow (Ronce 2007), is a key life-history trait (Bonte & Dahirel 2017) that determines the temporal and spatial distribution of individuals. Dispersal has possible effects on population dynamics, biological invasions and community assembly (reviewed in Bowler & Benton 2005; Clobert *et al.* 2009; Lowe & McPeek 2014). Interestingly, in many sexual species, males and females can have very different dispersal properties. For example, males are known to exhibit higher dispersal in taxa such as lions (Packer & Pusey 1987), great white shark (Pardini *et al.* 2001) and spotted hyena (Höner *et al.* 2007). In contrast, many avian species typically exhibit a female bias in dispersal (reviewed in Clarke *et al.* 1997). Besides affecting the process of net dispersal itself, sex-biased dispersal (SBD) may have a pronounced bearing on phenomena such as adaptation to heterogeneous habitats (Kawecki 2003; Lopez *et al.* 2008), as well as invasions and range expansions (Miller & Inouye 2013). In sexually reproducing populations, the magnitude of SBD can also modulate the strength of Allee effects, which in turn may affect their survival, growth, stability and invasion speed (Taylor & Hastings 2005; Miller *et al.* 2011; Shaw & Kokko 2015). Given the academic as well as practical implications of these phenomena, SBD has been a major topic of investigation over the last several decades (reviewed in Pusey 1987; Clarke *et al.* 1997; Prugnolle & De Meeûs 2002; Lawson Handley & Perrin 2007).

A key question in the SBD literature has been the relationship between SBD and mating. Greenwood (1980) postulated that SBD occurs due to local competition for mates or other resources, and is further reinforced by the role of dispersal in inbreeding avoidance. As a result, he predicted that the direction of SBD is determined by the type of mating system (Greenwood 1980). The latter has often served as the conceptual framework for empirical studies on SBD across taxa (e.g. Ribble 1992; Langen 1996; Croft *et al.* 2003; Nagy *et al.* 2007; Pérez-Espona *et al.* 2010). However, a recent analysis of data across several species suggested that evolution of SBD is better explained by sexual dimorphism and parental care (Trochet *et al.* 2016). Similarly, in a review of theoretical literature on the topic, Li and Kokko (2019) reported that the association between SBD and mating may not be limited to the type of mating system. Instead, the relative order of mating and dispersal in a given species might be a more important factor in this context (Li & Kokko 2019). However, while these studies discuss the role of mating as a cause of SBD, the consequences of SBD on mating have remained relatively unexplored.

Among other things, SBD can lead to a skew in the local sex ratio of the population. This, in turn, can affect the movement of the less dispersive sex in different ways, depending on the exact nature of the selection pressure faced by them (Shaw & Kokko 2014). For example, dispersal acts as a mechanism to avoid inbreeding in many species (Greenwood *et al.* 1978; Pusey 1987; Lambin 1994). Under such circumstances, emigration of any one sex (say males) from the population could be sufficient for promoting mating between unrelated individuals, thus allowing the other sex (females in this example) to potentially reduce its investment in dispersal (Shaw & Kokko 2014; Fromhage *et al.* 2016). This would result in even lower dispersal by the less-dispersive sex, thus, widening the magnitude of sex bias in dispersal. However, once the difference in dispersal between the sexes becomes high, it can lead to mate limitation and a reduction in local mating opportunities (Meier *et al.* 2011; Miller *et al.* 2011; Miller & Inouye 2013). This could then lead to an increased pressure on the less-dispersive sex for a subsequent "mate-finding dispersal", thus reducing the magnitude of difference in SBD (Meier *et al.* 2011). Thus, there exists a way for both a decrease or an increase in the dispersal of the less-dispersive sex following an SBD event.

A straightforward way for studying this phenomenon is to examine and compare dispersal under varied sex ratios. However, empirical studies on dispersal under artificially skewed sex ratios have either failed to observe SBD at all (Trochet *et al.* 2013), or found that the sex bias in movement was independent of the sex ratio (Baines *et al.* 2017). Interestingly, these empirical studies investigated the effects of an existing skew in the sex ratio of a population. However, if one sex disperses more than the other does, then a skew in sex ratio can develop over the time course of dispersal, even if the population had roughly equal proportions of the two sexes to begin with. To the best of our knowledge, the effect of this kind of a skew on the less-dispersive sex has never been investigated empirically.

Here, we investigate the interplay between dispersal and mate availability using laboratory populations of *Drosophila melanogaster* under controlled environmental conditions. Specifically, we asked the following questions: (1) Is the movement of individuals affected by differential dispersal of mates? (2) Is the movement of individuals affected by differential dispersal of same-sex individuals? (3) Do males and females differ in their response in the above two scenarios? We focus on the fact that the sex ratio gets gradually skewed along the course of an SBD event, rather than abruptly, resulting in a time-dependent decrease in local mate availability. Therefore, we study the temporal dispersal profile of individuals in response to

high- vs. low-dispersive mates. Moreover, we also study the dispersal of flies in response to high vs. low dispersive individuals of the same sex, to control for a potential confound of sex-independent density-dependent response. For these experiments involving mixed-sex and same-sex dispersal, we used individuals from fly populations with different dispersal properties. We found that neither male nor female dispersal was affected by the dispersal pattern of individuals of the same sex. However, the dispersal of mates significantly affected movement, more so in males than in females. We discuss the potential reasons for the observed behavioural plasticity, and its implications for SBD as well as dispersal evolution.

2 MATERIALS AND METHODS

2.1 Fly populations

We used four different laboratory-maintained populations of *D. melanogaster* in this study (namely, DB_4, DBS, VB_4, and VBC_4). All these populations are outbred and maintained at large population sizes (breeding size ~2400 individuals) at uniform environmental conditions of 25 °C temperature, 24-h light and 80–90% humidity. The first population, DB_4, is a part of four population blocks (i.e. $DB_{1–4}$) that trace their ancestry to the IV populations that were wild caught in Amherst, Massachusetts, USA (Ives 1970). The maintenance regime of the DB populations is available elsewhere (Sah *et al.* 2013; Mishra *et al.* 2018b), and is mentioned here in brief. These populations (including DB_4) are maintained under a 21-day discrete generation cycle, where 60–80 eggs are collected from the adult population cage of previous generation into 40 transparent 35-mL vials with ~6 mL of banana-jaggery medium. On the 12th day after egg collection, the adults are transferred to a fresh plexi-glass cage that contains ~70 mL banana-jaggery medium in a 100-mm petri plate. The food plate is replaced with a fresh food plate every alternate day for the next six days, i.e. until the 18th day since egg collection. On the 18th day, the usual banana-jaggery food plate is supplemented with live yeast to boost the fecundity of female flies. On the 20th day, the flies are provided with a food plate for oviposition over a window of ~14 hours. These eggs are then randomly sampled and distributed over 40 vials (as mentioned above) to start the next generation of the corresponding population.

The second population, named DBS [DB Scarlet-eyed], comprises individuals with a scarlet eye-colour mutation. These flies were obtained from the ancestors of the DB populations that had been pooled together. At the time of this study, the DBS population was ~100 generations old, meaning that it had been roughly ~6 years since the initial screening and collection of these scarlet-eyed flies. The maintenance of DBS population is identical to that of the DB populations, as described above.

The other two populations, VB_4 and VBC_4, have been derived from DB_4 as a part of an ongoing dispersal-selection experiment. VB_4 undergoes selection for higher dispersal every generation, while VBC_4 serves as its corresponding control (Tung *et al.* 2018b). As a result of this selection, VB flies have evolved a higher dispersal propensity (see section 2.5 below) and locomotor activity than the corresponding VBC flies (Tung *et al.* 2018a; Tung *et al.* 2018b).

These populations have been previously used for studying context-dependent dispersal. For instance, it has been shown that density dependence and sex bias in

dispersal is affected by the pre-dispersal context and presence of mates (Mishra *et al.* 2018b). Similarly, it is known that the difference in the dispersal of VB and VBC populations is affected by the presence or absence of resources (food and water) (Tung *et al.* 2018b).

2.2 Dispersal setup and assay

We used two-patch *source-path-destination* setups (*sensu* Mishra *et al.* 2018a; Mishra *et al.* 2018b; Tung *et al.* 2018b) to study fly dispersal. A *source* container (100-mL conical glass flask) is connected to a 2-m long *path* (transparent plastic tube; inner diameter ~1 cm), the other end of which opens into a *destination* container (250-mL plastic bottle) (Fig. 1; Mishra *et al.* 2018b). For the dispersal assays in this study, we introduced flies into the *source* and allowed them to disperse for 2 h. During this period, we replaced the *destination* container with a fresh, empty container every 15 min, with minimum possible disturbance to the rest of the setup. The number, sex and eye colour (wherever applicable) of successful dispersers (flies found in the *destination* container) at each of these 15-min intervals were manually recorded. These data allowed us to compute the dispersal propensity and temporal profile of dispersers (see section 2.5 for more details).

This dispersal setup is based on extensive standardizations in the lab. Typically, the flies used in an experiment have *ad libitum* access to food and water (in the form of agar-based banana-jaggery medium) prior to the dispersal assay. Therefore, introducing them into an empty *source* container represents a change in the resource state of their environment. This mimics a situation where the local environment turns unfavourable or uninhabitable for a population, thereby promoting emigration from the *source* container.

2.3 Rearing flies for the experiments

To eliminate any confounding effects of habitat quality, the entire dispersal setup (section 2.2) was devoid of food and moisture, and the identity of individuals in the source was the only difference across treatments during the dispersal assay. As dispersal is known to be affected by factors such as age (Hastings 1992), density (Matthysen 2005), kin competition (Gandon 1999) and level of inbreeding (Charlesworth & Charlesworth 1987), we avoided these potential confounds through appropriate rearing of the flies as detailed below.

Two cages, with ~2400 adult flies each, were maintained for each of the populations used (DB_4, DBS, VB_4 and VBC_4). To collect eggs for a particular day of dispersal assay, one cage of each population was supplied with live yeast plate for ~24 h. Fresh banana-jaggery medium was then supplied to the flies, allowing the females to oviposit for 12 h. After this period, ~50 eggs each were collected into forty 35-mL plastic vials containing ~6 mL of banana-jaggery medium. Following this, a plate with live yeast paste was provided to the flies again, to enable another round of egg collection. The same procedure was followed for the other cage of a population as well, with a constant difference of 24 h between the cages. Therefore, it was possible to collect eggs every day, alternatively from two cages of the same population, for 7 days. The large breeding population size (~2400) in each cage ensured that the flies were not inbred, and random sampling from a large number of eggs during the collection reduced the chances of kin being sampled together. Moreover, as flies from a single set of collected eggs were used for the dispersal assay on a particular day, they were all of the same age (12^{th} day from egg collection) and likely mated at least once by the time of the assay.

2.4 Experimental design

Two experiments were performed to investigate the behavioural plasticity of male and female flies for dispersal. In Experiments 1 and 2, we examined the change in male and female dispersal under mixed-sex and same-sex conditions, respectively.

2.4.1 Experiment 1: Mixed-sex dispersal

This experiment aimed to discern how dispersal capability of one sex affects the dispersal of the other sex through differential mate-availability. Dispersal of males from the baseline population (DB_4) was measured in the presence of either dispersal-selected females (VB_4) or control females (VBC_4) that were not selected for dispersal. Similarly, dispersal of baseline females (DB_4) was examined in the presence of either more-dispersive (VB_4) or less-dispersive (VBC_4) males. Thus, this experiment had four treatments: [VB F+ DB M] and [VBC F + DB M], and [VB M + DB F] and [VBC M + DB F], where M stands for males and F denotes females (Fig. 1). The initial sex ratio in the source was 1:1 for each treatment, comprising 60 males and 60 females. Following an earlier protocol (Mishra *et al.* 2018b), we carried out the experiment over multiple consecutive days, with a fresh set of age-matched adult flies (12-day-old from the date of egg collection) every day. Twenty-one hours prior to the

dispersal assay on each day, a fresh batch of newly eclosed flies was separated by sex under light CO_2 anaesthesia and maintained in same-sex groups of 60 individuals. Right before the dispersal assay, 60 males and 60 females from the relevant populations were mixed to yield the corresponding treatments. The experiment ran for seven consecutive days, with one replicate/treatment on each day, resulting in seven independent replicates for each treatment. In total, 3,360 flies (2 sexes × 4 treatments × 7 replicates × 60 flies sex^{-1} $treatment^{-1}$ $replicate^{-1}$) were used for this experiment.

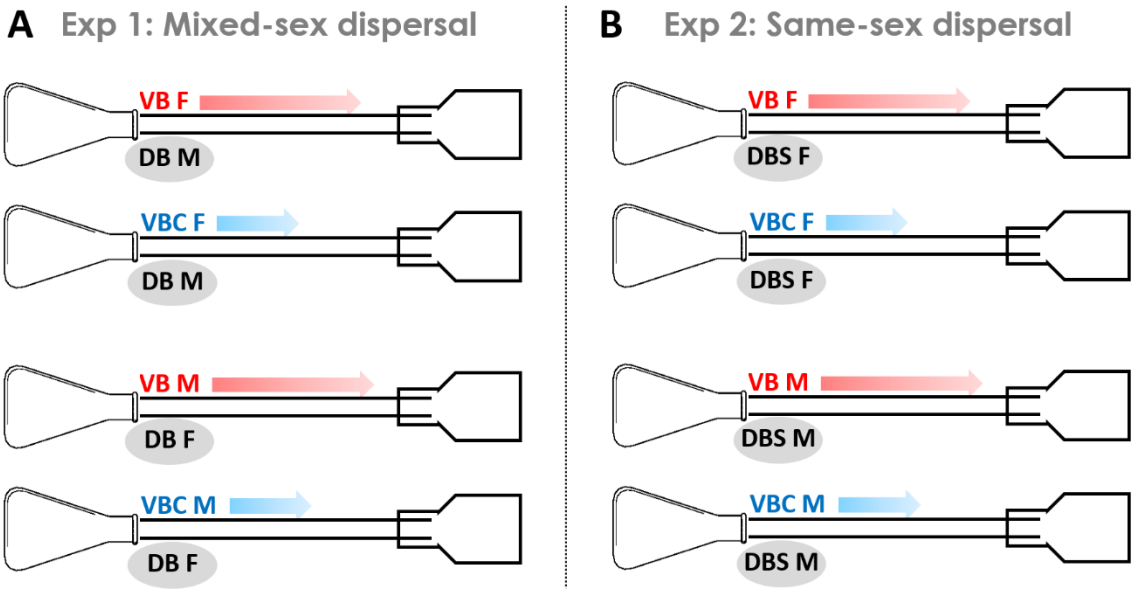

Fig. 1 Schematics of the experimental design. (A) In Experiment 1, we studied how the dispersal of one sex was affected by the dispersal of the other sex. The dispersal of DB flies (baseline population) was investigated in the presence of VB (dispersal-selected) vs. VBC (control) flies of the opposite sex. The arrow lengths denote the high-dispersive vs. low-dispersive nature of the VB vs. VBC individuals. (B) In Experiment 2, the behavioural plasticity of dispersal was tested in the context of differential dispersal by same-sex individuals. The dispersal of DBS (Scarlet-eyed baseline population) was investigated in the presence of VB (dispersal-selected) vs. VBC (control) flies of the same sex.

2.4.2 Experiment 2: Same-sex dispersal

In this experiment, we investigated whether dispersal of a sex (male or female) was affected by the dispersal of other individuals of the same sex. Similar to Experiment 1 (section 2.4.1), we compared the dispersal of baseline flies in the presence of dispersal-selected vs. control flies. However, since this experiment would comprise individuals of only one sex in each treatment, we used flies with a scarlet eye-colour mutation (population DBS) instead of DB_4, to distinguish between the baseline flies and VB or VBC flies. The rest of the protocol was identical to the one followed in

Experiment 1, and the four treatments in this case were: [VB F + DBS F] and [VBC F + DBS F], and [VB M + DB M] and [VBC M + DB M] (Fig. 1). As in Experiment 1, we used 3,360 flies (2 eye-colours × 4 treatments × 7 replicates × 60 flies eye colour^{-1} treatment^{-1} replicate^{-1}) for this experiment.

2.5 Dispersal traits

For every dispersal setup (replicate), we recorded the number and the sex (Experiment 1) or eye colour (Experiment 2) of flies that reached the *destination* during each 15-min interval of the dispersal assay (lasting 2 h). In addition, we also recorded these data for flies that emigrated from the source but did not reach the destination, i.e. the flies found within the *path* at the end of dispersal assay.

We then estimated the dispersal propensity, i.e. the proportion of flies that initiated dispersal (i.e. emigrated) from the source (Friedenberg 2003), as:

$$\text{Dispersal Propensity} = \frac{(\sum_i n_i) + n_p}{N}$$

where n_i is the number of flies that reached the destination during the i^{th} 15-min interval, n_p is the number of flies found within the path at the end of dispersal assay and N is the total number of flies introduced in the setup (here, 120). Therefore, the dispersal propensity can also be considered as the emigration probability from the source, a term that has been used in the literature in the past (e.g. Englund & Hambäck 2004). However, here we prefer to use dispersal propensity to refer to this property.

In addition, we also estimated the overall temporal distribution of dispersers reaching the destination (similar to Mishra *et al.* 2018b). For this, the number of dispersers that reached the destination during each 15-min interval was divided by the final number of successful dispersers.

2.6 Statistical analyses

As stated above, both Experiment 1 and Experiment 2 were performed over seven consecutive days, with one replicate of each treatment assayed every day. Therefore, we analysed this data in a randomized complete block design (RCBD) (Sokal & Rohlf 1981), with *replicate* as the random blocking factor.

In Experiment 1, the dispersal propensity of VB and VBC flies were first compared, to establish the difference in mate availability faced by DB flies while dispersing with VB or VBC individuals. For this, a three-way mixed model ANOVA was used, with sex (male/female) and *dispersal selection* (VB/VBC) as the fixed factors, and *replicate* (1–7) as a random factor. Next, the dispersal propensity data for DB individuals were analysed using a three-way mixed-model ANOVA, which had *sex* (male/female) and *mate dispersal* (VB/VBC) as the fixed factors, and *replicate* (1–7) as the random factor.

The analyses for Experiment 2 were similar to those of Experiment 1, except for one difference. As each treatment in Experiment 2 comprised only one sex, the three-way mixed model ANOVA for DBS individuals had *same-sex dispersal* (VB/VBC) as a fixed factor instead of *mate dispersal* (VB/VBC). The other fixed factor (*sex*) and the random factor (*replicate*) remained the same.

For analysing the temporal profiles of dispersers, separate two-way mixed-model ANOVAs were performed at each time point for both sexes, with *dispersal selection* as the fixed factor crossed with *replicate* (random factor). The family-wise error rates were then controlled using sequential Holm–Šidák correction (Abdi 2010).

As all the data were in the form of fractions, they were arcsine-square root transformed prior to the ANOVA (Zar 1999). The ANOVAs were performed using STATISTICA® v8 (StatSoft. Inc., Tulsa, Oklahoma). Cohen's *d* was used as a measure of effect size for pairs of groups, and the effect was interpreted as large, medium and small for $d \geq 0.8$, $0.8 > d \geq 0.5$ and $d < 0.5$, respectively (Cohen 1988).

3 RESULTS

3.1 Significant behavioural plasticity in dispersal for both sexes in the mixed-sex experiment

In Experiment 1, we investigated how the dispersal of males or females was affected by the movement of the opposite sex. For this, we first established the difference between the dispersal of VB (dispersal-selected) and VBC (control) individuals, to assess the extent of difference experienced by DB (baseline) individuals. This was followed by an analysis of DB dispersal across the four treatments, to examine the effect of dispersing with high- vs. low-dispersive mates.

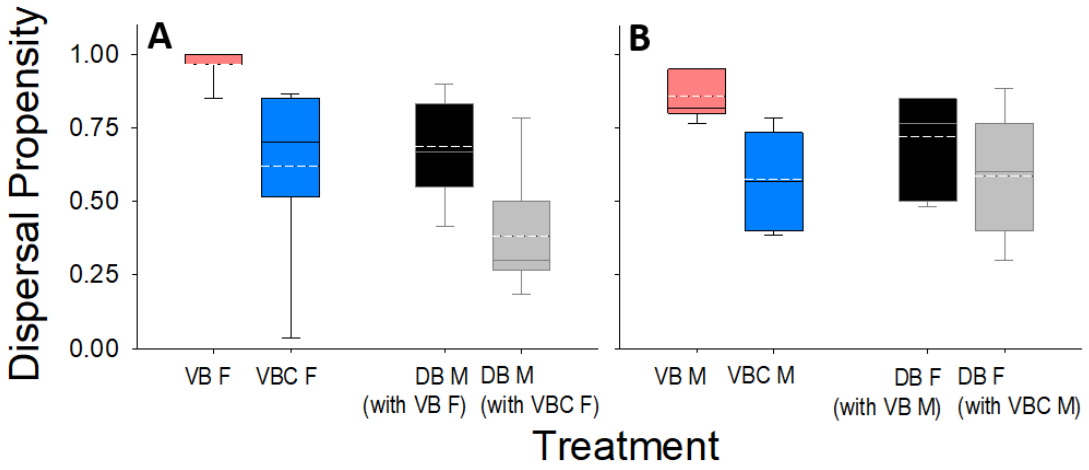

Fig. 2 Significant behavioural plasticity observed in mixed-sex dispersal (Experiment 1). Box-plots for the dispersal propensity of (A) VB (high-dispersive) vs. VBC (low-dispersive) females, and that of the DB (baseline) males dispersing with them, (B) VB (high-dispersive) vs. VBC (low-dispersive) males, and that of the DB (baseline) females dispersing with them. The edges of the box in each case denote the 25th and 75th percentiles, while the solid line and dashed line represent the median and the mean, respectively.

As expected from previous studies (Tung *et al.* 2018a; Tung *et al.* 2018b), *dispersal selection* had a significant main effect ($F_{1,18} = 47.84$, $P = 1.8\times10^{-6}$), with VB individuals being more dispersive than VBC individuals. In addition, there was a significant main effect of *sex* ($F_{1,18} = 4.58$, $P = 0.046$), as females had a higher dispersal propensity than the males. The *dispersal selection* × *sex* interaction, however, was not significant ($F_{1,18} = 2.57$, $P = 0.13$), indicating that the effect of dispersal selection was symmetric across males and females (Figs 2A and 2B). The difference between dispersal-selected and control flies was also apparent in their temporal dispersal profiles, as majority of VB dispersers completed the dispersal much faster than the VBC dispersers (Figs 3A and 3B). These differences meant that the DB flies would have faced markedly different temporal mate availability, depending on whether they

were dispersing with VB or VBC individuals of the opposite sex. The dispersal data of DB individuals were next analysed to capture the effects of this contrast in biotic environments.

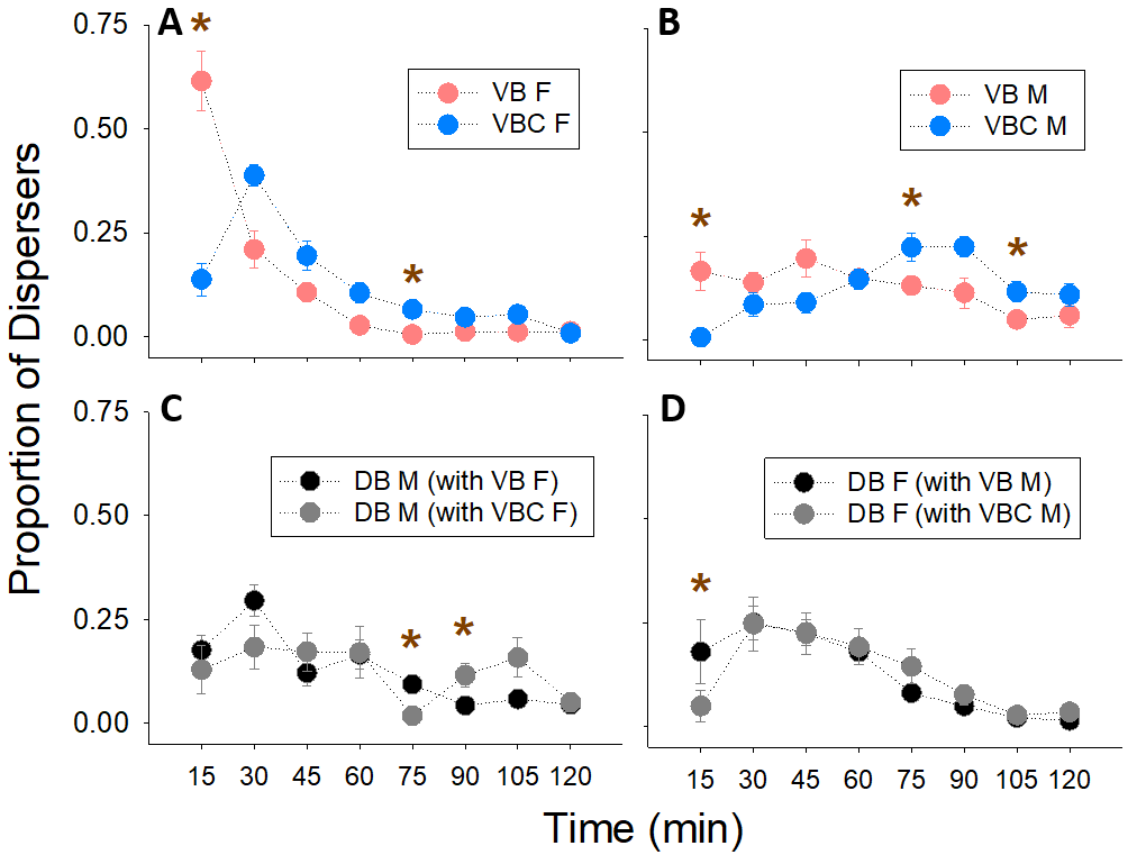

Fig. 3 Temporal dispersal profile in the mixed-sex scenario (Experiment 1). Proportion of individuals reaching the destination over the 2-h dispersal period, for (A) VB (high-dispersive) vs. VBC (low-dispersive) females, (B) VB (high-dispersive) vs. VBC (low-dispersive) males, (C) DB (baseline) males dispersing with VB vs. VBC females, and (D) DB (baseline) females dispersing with VB vs. VBC males. The asterisks (*) represent a significant difference (sequential Holm–Šidák-corrected p value < 0.05) between the two treatments in a panel for the indicated temporal bin. See Table 1 for the exact p values and the associated effect sizes.

We observed a significant effect of *mate identity* ($F_{1,18} = 17.65$, $P = 0.0005$) for DB individuals, as their dispersal propensity in the presence of VB mates was much higher than that with VBC mates (Figs 2A and 2B). There was a significant main effect of *sex* as well ($F_{1,18} = 5.18$, $P = 0.035$), with females showing higher overall dispersal than males. Furthermore, there was a non-significant *mate identity* × *sex* interaction ($F_{1,18} = 2.65$, $P = 0.12$). However, the effect size of this increase was larger in males than in females (males: d = 1.73 (large); females: d = 0.76 (medium)) (Figs 2A and 2B). This was also reflected in the temporal dispersal profiles of the DB

individuals, where DB males showed a greater change in the timing of their dispersal than DB females (Figs 3C and 3D). Thus, dispersal was found to be plastic in both sexes, as assessed in the presence of high- vs. low-dispersive mates, although the magnitude of this effect was larger in males.

3.2 No significant behavioural plasticity in dispersal for either sex in the same-sex experiment

Similar to Experiment 1, the dispersal of VB and VBC individuals was first compared across the four treatments in Experiment 2. Thereafter, data for DBS [DB Scarlet-eyed] individuals were analysed, to test if their dispersal was affected by high- vs. low-dispersive individuals of the same sex.

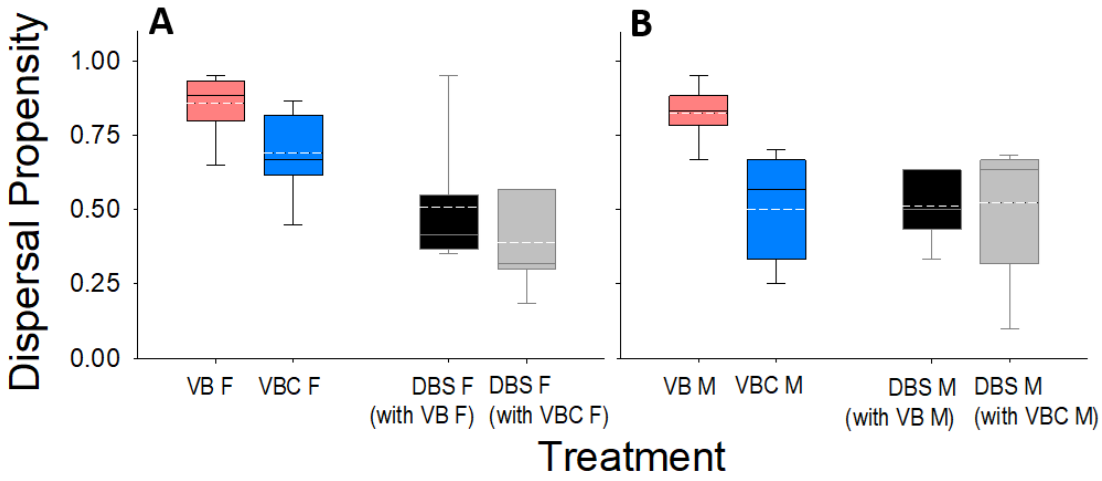

Fig. 4 No significant behavioural plasticity in same-sex dispersal (Experiment 2). Box-plots for the dispersal propensity of (A) VB (high-dispersive) vs. VBC (low-dispersive) females, and that of the DBS (scarlet-eyed baseline) females dispersing with them, (B) VB (high-dispersive) vs. VBC (low-dispersive) males, and that of the DBS (scarlet-eyed baseline) males dispersing with them. The edges of the box in each case denote the 25th and 75th percentiles, while the solid line and dashed line represent the median and the mean, respectively.

As observed in Experiment 1, there was a significant main effect of *dispersal selection* ($F_{1,18}$ = 23.63, P = 0.0001), as the dispersal propensity of VB individuals was higher than that of VBC individuals. There was also a significant effect of *sex* ($F_{1,18}$ = 4.67, P = 0.044), as the overall dispersal propensity was higher in females than in males. Finally, the *dispersal selection* × *sex* interaction was not significant ($F_{1,18}$ = 1.65, P = 0.22) (Figs 4A and 4B). However, difference in the temporal profile of VB and VBC dispersers was apparent only in males and not in females (Figs 5A and 5B).

For DBS flies, neither of the three effects, i.e. *same-sex dispersal* ($F_{1,18} = 0.78$, $P = 0.39$), *sex* ($F_{1,18} = 0.57$, $P = 0.46$) and *same-sex dispersal* × *sex* ($F_{1,6} = 0.89$, $P = 0.36$), were significant. This implied that dispersal of either sex was not affected by high- vs. low-dispersive individuals of the same sex. Congruent with this, the temporal profile of DBS males and females did not show much difference across treatments (Figs 5C and 5D).

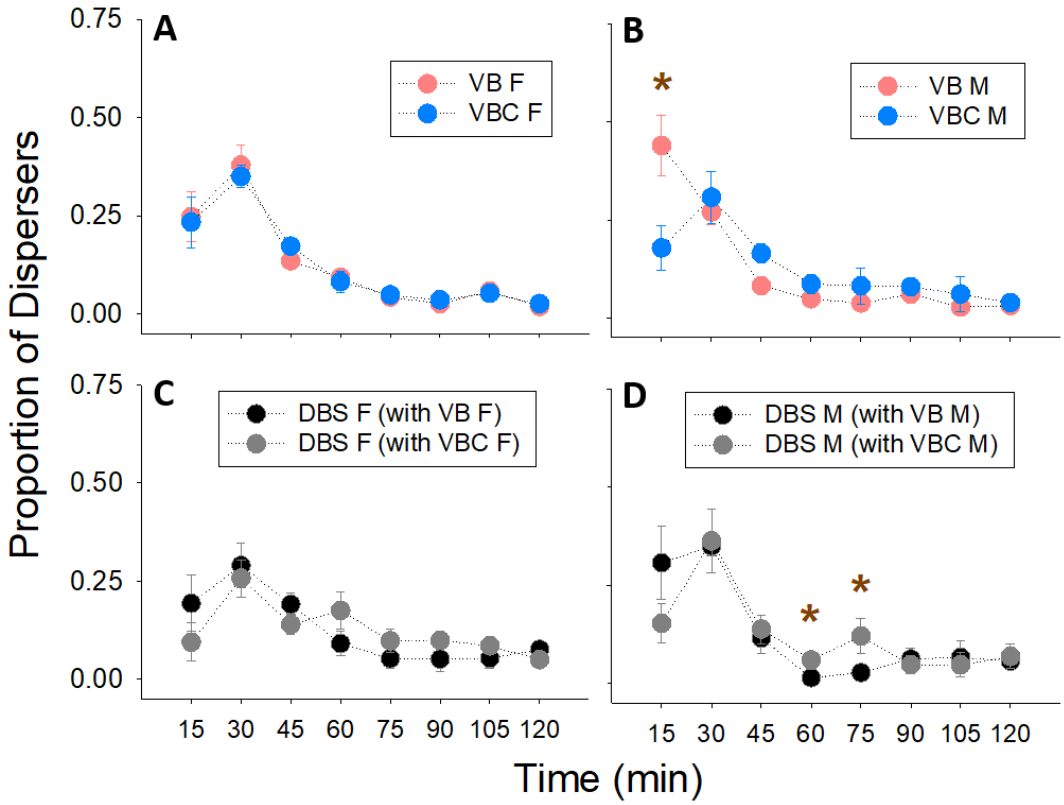

Fig. 5 Temporal dispersal profile in the same-sex scenario (Experiment 2). Proportion of individuals reaching the destination over the 2-h dispersal period, for (A) VB (high-dispersive) vs. VBC (low-dispersive) females, (B) VB (high-dispersive) vs. VBC (low-dispersive) males, (C) DBS (scarlet-eyed baseline) females dispersing with VB vs. VBC females, and (D) DBS (scarlet-eyed baseline) males dispersing with VB vs. VBC males. The asterisks (*) represent a significant difference (sequential Holm–Šidák-corrected p value < 0.05) between the two treatments in a panel for the indicated temporal bin. See Table 1 for the exact p values and the associated effect sizes.

Table 1. Significant differences in the temporal profile of populations in Experiment 1 (mixed-sex dispersal) and Experiment 2 (same-sex dispersal). Sequential Holm–Šidák-corrected p values are presented with the corresponding effect sizes (Cohen's *d*).

Experiment	Population(s)	Sex	Temporal bin	Seq Holm–Šidák corrected p value	Cohen's *d*	Effect size interpretation
Experiment 1 (Mixed-sex dispersal)	VB-VBC	Females	15 min	1.5×10^{-5}	3.13	Large
			90 min	0.031	2.10	Large
		Males	15 min	0.001	2.80	Large
			75 min	0.042	1.23	Large
			105 min	0.015	1.34	Large
	DB	Males	75 min	0.018	1.55	Large
			90 min	0.018	1.03	Large
		Females	15 min	0.043	0.91	Large
Experiment 2 (Same-sex dispersal)	VB-VBC	Males	15 min	0.008	1.52	Large
	DBS	Males	60 min	0.044	1.11	Large
			75 min	0.023	0.99	Large

4 DISCUSSION

4.1 Mate-finding dispersal observed in the presence of differential mate dispersal

The primary result of our study is that mate dispersal (and consequently, local mate availability) is a key proximate determinant of dispersal. In Experiment 1, we studied the dispersal of males and females of the baseline populations (DB) in the presence of high-dispersive (VB) and low-dispersive (VBC) mates. DB individuals showed significantly higher dispersal in the presence of high-dispersive mates than in the presence of low-dispersive mates (*cf.* Figs 2 and 4). While these results from Experiment 1 support the hypothesis that dispersal is influenced by different levels of mate dispersal, they do not constitute sufficient evidence for the same. This is because these results could simply be a manifestation of negative density-dependent dispersal, wherein per-capita dispersal increases with decreasing density (e.g. Baguette *et al.* 2011; Mishra *et al.* 2018b). In such a scenario, the observations could be explained without invoking the role of mates. This possibility was ruled out by Experiment 2, where mate availability was not a factor and we could test the effect of same-sex individuals on dispersal. In contrast to the results of Experiment 1, Experiment 2 showed that neither males nor females showed a difference in dispersal in the presence of same-sex individuals with different dispersal levels. Thus, taking together the results from Experiments 1 and 2, we concluded that mate dispersal, and not the dispersal of same-sex individuals, modulates dispersal in *D. melanogaster*.

While we used populations with differential dispersal propensity (i.e. VB and VBC) to achieve Sex-Biased Dispersal (SBD) and create differences in local mate availability, a few previous studies have tried using treatments with skewed sex ratios for the same effect. For instance, Trochet *et al.* (2013) used three different sex-ratio treatments to study dispersal in the butterfly, *Pieris brassicae*. Similarly, Baines *et al.* (2017) used treatments with equal, female-biased and male-biased sex-ratios to assess changes in SBD of a semiaquatic insect, *Notonecta undulata*. However, in both these studies, the authors reported that sex ratio treatments were not effective in producing or modulating SBD. The authors then hypothesized that other mechanisms, including competition and dispersal costs, were likely more important reasons for dispersal than local mate availability (Trochet *et al.* 2013; Baines *et al.* 2017). Since in our study, none of the treatments started with a skewed ratio, and rather, mate availability changed through time (as evidenced by the temporal dispersal profiles in Figs 3A and 3B), it is possible that active "mate-following" led to mate-finding dispersal.

As mate limitation is believed to be the most common cause of Allee effects in sexually reproducing species (Taylor & Hastings 2005; Berec *et al.* 2018), several theoretical studies have investigated the effects of mate limitation on population growth and spread, as well as the evolution of mate-finding dispersal. For instance, mate limitation and its effects are predicted to be especially pronounced in species with monogamous mating systems (Shaw & Kokko 2014; Shaw *et al.* 2018). However, we observed these effects in our *D. melanogaster* populations, which are not only polygamous, but their rearing and age were such that they had most likely mated by the time of the experiment (Section 2.3). As a result, the mate limitation faced by them was likely not very severe. Furthermore, adaptations other than mate finding, such as multiple matings, long mating window and sperm storage, are expected to mitigate mate limitation (Gascoigne *et al.* 2009; Shaw & Kokko 2014; Fromhage *et al.* 2016). All these traits are well documented in *Drosophila* (Fuerst *et al.* 1973; Pyle & Gromko 1978; Pitnick *et al.* 1999). Finally, this also means that the relative order of mating and dispersal, which is a central basis for many theoretical studies on mate-finding and sex-biased dispersal (Shaw & Kokko 2014; Li & Kokko 2019), may not be a very crucial factor for *Drosophila*, which have a long mating window and can potentially mate before, during or after dispersal. Therefore, mate-finding dispersal could be even more significant in those taxa where some or all of these traits are less pronounced (Fromhage *et al.* 2016).

4.2 Sex differences in mate-finding dispersal

Once mate-finding dispersal had been established, the next objective was to see if this behaviour was symmetric between males and females. Our results from Experiment 1 showed that the effect size of mate-finding dispersal was much larger for males than for females (section 3.1, *cf.* Fig. 2A and Fig. 2B). A straightforward explanation for this observation could be the differences in the temporal dispersal profile of VB vs. VBC males and females. As can be seen in Figs 3A and 3B, more than 60% dispersers among the VB females completed dispersal within the first 15 minutes. Coupled with the high dispersal propensity of VB females (Fig. 2A), this means that within the first few minutes of the experiment, the sex ratio in the *source* of [VB F + DB M] treatment deviated significantly from 1:1, leading to the DB males in this treatment facing a shortage of females. In contrast, it took 45 min for ~60% of the dispersers among the VB males to complete dispersal (Fig. 3B). As a result, the extent of mate shortage created by the dispersal of VB males was likely not as severe as that created by the dispersal of VB females. Another possible reason for the

greater mate-finding dispersal observed in DB males is that dispersal propensity of DB females was inherently higher than that of DB males. This can be observed by comparing the DB male data in the presence of low-dispersive (VBC) females in Fig. 2A with the DB female data in the presence of low-dispersive (VBC) males (Fig. 2B). As a result, the available scope for increase in dispersal was likely limited for females compared with males. This is possible because, in our setup, males were dispersing only to escape the desiccation and starvation stress, whereas females had a dual rationale for dispersal: escape from stress as well as the search for a suitable oviposition site. Finally, since our flies were likely already mated at least once, the males probably had more to gain from any extra matings than the females. In fact, extra matings could even lead to deleterious physiological consequences for the females, as evidenced by the extensive male mate-harm literature in *Drosophila* (Levine *et al.* 1980; Harshman *et al.* 1988; Kuijper *et al.* 2006). As a result, the higher inherent dispersal by DB females might also be a way to escape from excessive male mate harm (e.g. Byrne *et al.* 2008). Overall, these results are in line with the expectations of "male-biased mate searching" from earlier theoretical studies, which posit that females disperse primarily for resources, whereas males disperse primarily for mates (Meier *et al.* 2011; Hovestadt *et al.* 2014b; Fromhage *et al.* 2016).

Our current results are also relevant in the context of dispersal evolution. We had earlier reported that SBD did not evolve in the VB populations, even though a two-patch *source-path-destination* setup like ours could potentially select for female-biased dispersal (Tung *et al.* 2018b). This is because, in principle, the males could maximize their fitness by mating with as many females as possible before the dispersal run and avoid the dispersal costs altogether, whereas the females had no recourse but to complete the dispersal from *source* to *destination* to realize their fitness (Shaw & Kokko 2014; Tung *et al.* 2018b). Our current results demonstrate how strong mate-finding dispersal would have countered this asymmetry in the selection pressure between males and females, thus, hindering the evolution of SBD. This is also in line with the theoretical predictions that the two sexes should evolve similar dispersal kernels over time (Meier *et al.* 2011; Shaw & Kokko 2014). Therefore, our current results highlight the role of mate-finding dispersal in modulating phenomena such as the evolution of SBD.

4.3 Implications of our results

Our results revealed a strong effect of mate dispersal, more so in males than in females. To our knowledge, this is the first clear demonstration of how mate-finding dispersal, can counter the sex bias in dispersal. We discuss some implications of our results below.

First, SBD is known to be a major cause of differential mate availability (Meier *et al.* 2011; Miller *et al.* 2011; Miller & Inouye 2013). Here we show the effects of SBD on mate availability can be countered over a short time scale via mate-finding dispersal. Miller and Inouye (2013) hypothesized that demographic stochasticity can be a major factor that dampens the effects of SBD on mate availability. We show that a more deterministic factor could be demographic rescue via mate-finding dispersal. The extent of such dispersal-mediated demographic rescue, in turn, would depend on the degree of patch isolation and costs of dispersal (Gascoigne *et al.* 2009).

Second, mate limitation is especially important for small populations, particularly those found at invasion fronts and range boundaries. SBD can lead to skewed sex ratios at invasion fronts (Miller & Inouye 2013), with acute mate shortage resulting in strong Allee effects (Taylor & Hastings 2005; Contarini *et al.* 2009). As a result, it has been suggested that SBD makes invasions more variable (Miller & Inouye 2013). However, our results indicate that such variation would be limited in the presence of deterministic factors such as mate-finding dispersal. In fact, mate-search efficiency is predicted as a key parameter that determines population-level effects including growth and spatial spread (Shaw *et al.* 2018). Overall, we hypothesize that the effect of mate-finding dispersal on invasions would depend on population size. While mate-finding dispersal can rescue small populations from Allee effects, it might dilute the process of dispersal evolution via spatial sorting (Shine *et al.* 2011) in larger populations. This is because mate-finding dispersal is "context-dependent", as opposed to "phenotype-dependent" (*sensu* Clobert *et al.* 2009; Clobert *et al.* 2012), which would imply a low heritability of dispersal traits among the dispersers. If spatial sorting is diluted in this manner, it is expected to slow down the speed of expansions.

Third, sex differences have been reported in life-history/behavioural traits related to dispersal (Legrand *et al.* 2016; Mishra *et al.* 2018a). Sex bias in mate-finding dispersal can thus interact with these sex differences in life history or behaviour, to modulate the final distribution of individuals in spatially structured populations. *Inter alia*, such interactions can amplify/weaken the Allee effects experienced by the

population (Shaw & Kokko 2014). Thus, elucidating the mechanisms of sex bias in mate-finding dispersal (e.g. Shaw & Kokko 2014; Fromhage *et al.* 2016) would be an important factor in understanding the nature of these interactions and the consequent population-level effects.

CHAPTER 4

Dispersal evolution via spatial sorting diminishes the density dependence in dispersal

Highlights

- Strong negative density-dependent dispersal (DDD) observed in control and ancestral flies
- Dispersal-selected flies show a complete loss of DDD
- Sex differences in DDD patterns and resultant instances of sex-biased dispersal also reduced
- With previous results, suggests the evolution of context-independent dispersal

Archived as and adapted from:
Mishra, A., Chakraborty, P.P., & Dey, S. (2020) Dispersal evolution via spatial sorting diminishes the density dependence in dispersal. *Evolution*, 10.1111/evo.14070.

1 INTRODUCTION

Biological dispersal, an integral part of the life history in many taxa (Bonte & Dahirel 2017), is a major determinant of spatial distribution of living organisms. Dispersal patterns influence several ecological phenomena, including biological invasions, range expansions and community assembly (Bowler & Benton 2005; Clobert *et al.* 2009; Lowe & McPeek 2014). The specifics of a given dispersal event, in turn, are regulated by numerous biotic and abiotic factors (Bowler & Benton 2005; Matthysen 2012). One such factor that can be a prominent cause of variation in the dispersal patterns of many species is the local population density, leading to density-dependent dispersal (DDD) (reviewed in Matthysen 2005; Harman *et al.* 2020).

Population density can affect the movement of individuals in many ways. For instance, DDD is defined as positive when the per capita dispersal increases with increasing population density, often manifesting as greater proportional movement from dense regions to sparse regions (e.g. Aars & Ims 2000; De Meester & Bonte 2010; Bitume *et al.* 2013; Lutz *et al.* 2015). Similarly, negative DDD implies a reduction in per capita movement with increasing population density, resulting in greater aggregation in crowded regions and higher net emigration from sparse regions (e.g. Andreassen & Ims 2001b; Baguette *et al.* 2011; Pennekamp *et al.* 2014; Mishra *et al.* 2018b). In addition, recent empirical studies have also reported non-linear DDD, i.e. a combination of positive and negative DDD occurring at different density ranges. Here, U-shaped DDD involves negative DDD at low densities and positive DDD at high densities (e.g. Kim *et al.* 2009; Fronhofer *et al.* 2015; Maag *et al.* 2018), whereas hump-shaped DDD has positive DDD at low densities and negative DDD at high densities (e.g. Jacob *et al.* 2016; Chatelain & Mathieu 2017). The classical view has been that positive DDD is more prevalent than the other DDD patterns (Matthysen 2005). This was also supported by a recent literature review on density-dependent emigration, which identified positive DDD as the most common one, followed by negative DDD and non-linear DDD patterns (Harman *et al.* 2020).

Despite the wealth of experimental studies that have characterized DDD in various taxa, there remains a lack of empirical evidence for the ecological role played by DDD in a spatial context. This is particularly true for the cases where some or the other form of spatial selection (*sensu* Phillips *et al.* 2010; Williams *et al.* 2019) is involved. Over the past few years, empirical studies across a range of taxa have demonstrated that dispersal traits can evolve rapidly (e.g. Fronhofer *et al.* 2014; Williams *et al.* 2016; Weiss-Lehman *et al.* 2017; Tung *et al.* 2018b). If rapid dispersal evolution is indeed the norm, it stands to reason that context-dependent features of dispersal, such as DDD, could undergo evolutionary changes too. Furthermore,

DDD itself could modulate the spatial selection faced by expanding populations and their subsequent expansion.

In one of the first empirical studies on this topic, Simmons and Thomas (2004) used wild populations of bush crickets to show that the individuals at the range fronts exhibited a different DDD response (negative DDD) than the individuals at the range core (no DDD). Since then, the general consensus from studies has been that individuals at range edges are expected to evolve more negative DDD compared with those at range cores (Travis *et al.* 2009; Fronhofer *et al.* 2017; Weiss-Lehman *et al.* 2017). This prediction was empirically confirmed using the protist, *Tetrahymena thermophila* (Fronhofer *et al.* 2017). To our knowledge, this is the only empirical study that has demonstrated an evolutionary change in DDD. However, the fact that these results were observed in clonally reproducing *Tetrahymena* strains meant the lack of two things: a) initial standing genetic variation, and b) sexual reproduction, thereby precluding evolution via assortative mating of highly dispersive individuals (i.e. spatial sorting) (Shine *et al.* 2011). As these two features are central characteristics of dispersing populations in many species, there is a need for empirical investigation of DDD evolution in sexually reproducing populations with standing genetic variation. Moreover, it would allow the investigation of sex-specific changes, if any, during DDD evolution.

Here, we examined the evolutionary changes in DDD using large (breeding population size ~2400 individuals), laboratory-maintained populations of *Drosophila melanogaster*. We used four dispersal-selected populations from a long-running evolutionary experiment, along with their corresponding non-selected control populations. The selected populations underwent dispersal evolution every generation, with 75 generations (~3 years) of selection completed at the time of this study. By comparing the DDD patterns of dispersal-selected and control populations, we could test whether dispersal evolution strengthened or weakened the DDD response. Furthermore, we examined how the sex differences in DDD are affected by evolution. Our results showed a significant negative DDD in the control populations, similar to the ancestral populations. The dispersal-selected populations, however, revealed a complete loss of DDD, suggesting that dispersal evolution had significantly weakened the negative DDD pattern. This was also accompanied by a disappearance of sex differences in dispersal across the various densities. We discuss how our results compare to the previous predictions in this context, and the possible implications for dispersal biology.

2 METHODS

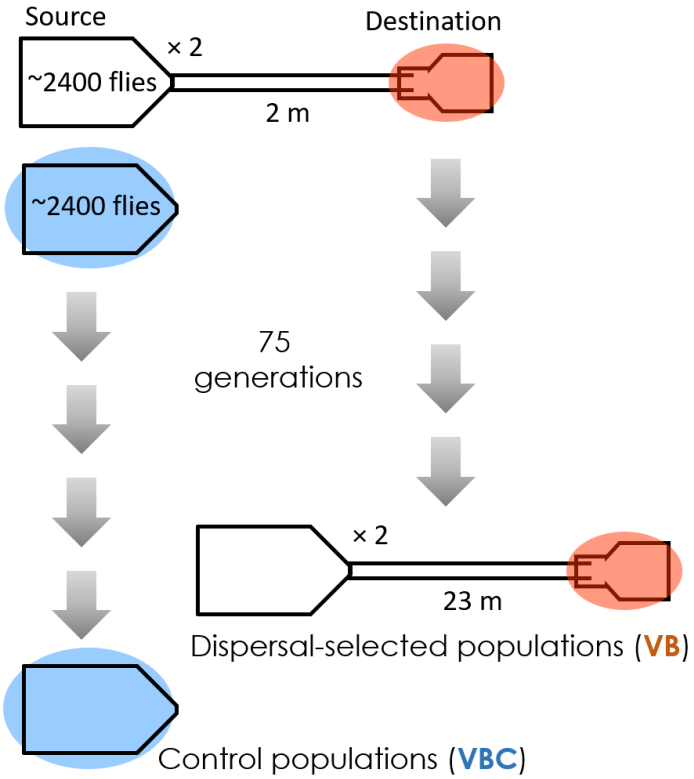

Fig. 1 Dispersal selection protocol. Each generation, flies from the four VB (dispersal-selected) populations were subjected to the above dispersal selection protocol. For this, ~2400 adult flies were introduced into the *source* container (1.5 L) of a dispersal setup, which opened into a *path* (plastic tube, ~1 cm diameter). The flies could thus emigrate from the *source* container into the *path*, which then led into the *destination* container (1.5 L). To maintain the population size across generations, two replicate dispersal setups (with ~2400 flies each) were used per population block (e.g. VB1), and the first 50% flies that completed dispersal were selected as the parental population for the next generation. The *path* length was increased intermittently across generations to mimic increasing habitat fragmentation, such that the *path* length was 2 m at generation 0, and 23 m at generation 75. The VBC (non-selected control) populations were maintained identically to the VB populations under the same conditions, but not subjected to the dispersal selection protocol.

2.1 Fly populations and dispersal selection

We used a total of eight laboratory-maintained populations of *D. melanogaster* in this study. Four of these populations (VB_{1-4}) had undergone selection for higher dispersal for ~75 generations at the time of the experiment. The other four populations (VBC_{1-4}) served as the corresponding controls for the VB populations, i.e. they were reared and maintained under similar conditions for the same number of generations, but not selected for higher dispersal. The subscripts for VB and VBC populations (i.e. 1–4) denote their ancestry, such that populations with the same subscript were derived

from the same ancestral population (e.g. VB_1 and VBC_1, and so on). The detailed maintenance and selection regime for these populations is available elsewhere (Tung *et al.* 2018a; Tung *et al.* 2018b). In brief, all eight populations were maintained at large populations sizes (~2400 individuals) to avoid inbreeding, under a 15-day discrete generation cycle. They had access to 24-h light and experienced a uniform temperature of 25 °C. Every generation, ~50% of the flies (i.e. those that successfully complete dispersal) were selected from the VB populations using replicate two-patch (*source-path-destination*) setups, whereas no such selection was imposed on the corresponding VBC populations (Fig. 1). The length of the path between the *source* and the *destination* was increased intermittently across generations, which mimicked increasing habitat fragmentation over time. As a result of the dispersal selection, VB populations evolved to have a higher dispersal propensity, ability and speed (Tung *et al.* 2018a; Mishra *et al.* 2020b).

2.2 Dispersal setup and assay

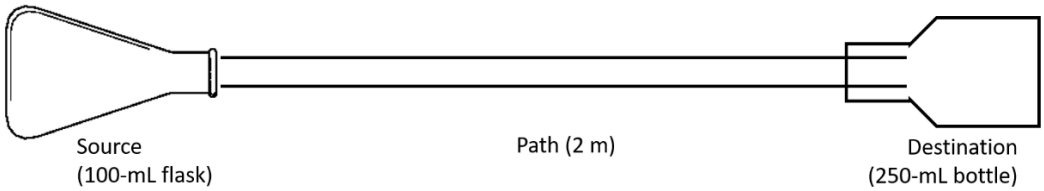

Fig. 2 Two-patch dispersal setup used in the study. Adult individuals of *Drosophila melanogaster* were introduced in the *source* container (100-mL conical glass flask), the opening of which was connected to the *path* (2-m long transparent plastic tube of internal diameter ~1 cm). The other end of the *path* opened into the *destination* container (250-mL plastic bottle), with a protrusion of ~3 cm to prevent backflow of successful dispersers into the *path*. During the dispersal assay, the *destination* container could be replaced periodically with a fresh container, to estimate the temporal profile of successful dispersers.

We used two-patch *source-path-destination* setups to study the dispersal of the flies. This setup was similar to the one used for selection of higher dispersers in VB populations (Tung *et al.* 2018b), with the only difference being in the size of the containers used as *source* and *destination*. Following an earlier protocol (Mishra *et al.* 2018b; Mishra *et al.* 2020b), we used 100-mL conical glass flasks as *source* containers and 250-mL plastic bottles as *destination* containers. The *source* and the *destination* were connected by a 2-m long *path* (transparent plastic tube; inner diameter ~1 cm) (Fig. 2). We introduced adult flies into the *source* and allowed them to disperse through the *path* into the *destination* for a period of 2 h. We replaced the *destination* container with a fresh, empty container every 15 min during this period, with minimum possible disturbance to the rest of the setup. The number and sex of

successful dispersers during each of these 15-min intervals were manually recorded, to estimate the dispersal propensity and temporal profile of dispersers (see section 2.5 for more details).

2.3 Culturing flies for the experiment

To minimize the contribution of non-genetic parental effects, we reared all VB and VBC populations for one generation under common conditions prior to the experiment. To eliminate any confounding effects of habitat quality during the dispersal assay, the dispersal setup (Section 2.2) was devoid of any food or moisture (similar to Mishra *et al.* 2018b; Mishra *et al.* 2020b). Finally, we generated the flies such that they were age-matched at the time of dispersal assay, and without any apparent confounds of kin- or inbreeding-related effects, as detailed below.

For a given population block (e.g. VB_1 and VBC_1), two cages each for the VB and VBC populations were prepared with ~2400 adult flies per cage. The parental generation of these flies had been reared under common conditions for one generation, to minimize the contribution of non-genetic parental effects (Section 2.3 in main text). To collect eggs for a particular day of the assay, one of the two cages for a given population (e.g. VB_1) was supplied with live yeast plate for ~24 h. Fresh banana-jaggery medium was then supplied to the flies, allowing the females to oviposit for 12 h. After this period, ~50 eggs each were collected into forty 35-mL plastic vials containing ~6 mL of banana-jaggery medium. Following this, a plate with live yeast paste was provided to the flies again, to enable another round of egg collection. The same procedure was followed for the second cage of the given population (VB_1, from the current example) as well, with a constant difference of 24 h between the cages. Therefore, it was possible to collect eggs, alternatively from two cages of the same population, for 4 days. Following this protocol for the VB and VBC populations of a given population block together, we ensured that we were able to get four independent replicates of VB and VBC flies per population block (Section 2.4 in main text). The large breeding population size (~2400) in each cage ensured that the flies were not inbred, and random sampling from a large number of eggs during their collection reduced the chances of kin being sampled together. Moreover, as flies from a single set of collected eggs were used for the dispersal assay on a particular day, they were all of the same age (12^{th} day from egg collection) at the time of their assay.

2.4 Experimental design

For comparing the pattern of DDD between VB (dispersal-selected) and VBC (control) populations, we assayed each of the four VB populations with its corresponding control population (i.e. VB_1 assayed with VBC_1, and so on). Four dispersal density treatments were used, namely 60, 120, 240 and 480 individuals per *source* container. We performed the experiment over multiple consecutive days, with a fresh set of age-matched (12-day-old from egg-lay) adult flies every day (Section 2.3). This way, all the density treatments for both VB and VBC populations could be assayed together every single day, allowing a complete replication of all the treatments each day. The four population blocks (i.e. 1–4) were assayed one after the other, wherein each block was assayed over 4 consecutive days. As a result, the entire experiment ran over the course of 16 days (4 blocks × 4 days, allowing us to obtain a total of 16 replicates (blocked by population block and day) for each density treatment of VB and VBC.

As mentioned above, 12-day-old (from egg-lay) adult flies were used for the dispersal assay. On day 11 of their age (21 h prior to the dispersal assay), the flies from the relevant populations were separated by sex under light CO_2 anaesthesia, and then randomly assigned to the four density treatments (60, 120, 240 and 480) with a strict 1:1 sex ratio. Thus, the density treatment of 60 individuals comprised 30 males and 30 females, and so on. The prepared sets of flies were then transferred into separate 100-mL conical glass flasks (identical to the *source* in the dispersal setup) containing ~35 mL of banana-jaggery medium. The next day (at 12^{th} day of age), these flies were assayed for their dispersal as described in Section 2.2 (Fig. 2). As we assayed one replicate of each density for VB and VBC populations per day, a total of 28,800 flies (2 populations × 4 blocks/population × 4 days/block × 900 flies/day) were used for this experiment.

2.5 Dispersal traits

For every dispersal setup (replicate), we counted the number of male and female flies that reached the destination during each of the 15-min intervals until the end of dispersal assay (2 h). Moreover, we recorded the number and sex of flies that emigrated from the *source* but did not reach the *destination*, i.e. those found within the *path* tube at the end of the dispersal assay.

These data allowed us to estimate the dispersal propensity, i.e. the proportion of flies that initiated dispersal from the *source* (Friedenberg 2003), as:

$$\text{Dispersal propensity} = \frac{(\sum_i n_i) + n_p}{N}$$

where n_i is the number of flies that reached *destination* during the i^{th} 15-min interval, n_p is the number of flies found in the *path* at the end of dispersal assay and N is the total number of flies introduced in the setup (i.e. 60, 120, 240 or 480).

In addition, we obtained the overall temporal profile of dispersers for each replicate. For this, we calculated the proportion of dispersers that reached *destination* during each 15-min interval, relative to the final number of successful dispersers (Mishra *et al.* 2018b; Mishra *et al.* 2020b).

2.6 Statistical analyses

As stated in section 2.4, the experiment involved four blocks of VB and VBC populations, each of which was assayed over 4 consecutive days, yielding 16 replicates in total for each density treatment. As one replicate for each of the four density treatments was assayed per day, *day* (1-4) was included as a random factor that was nested inside *block* (1-4), another random factor. This was done to account for any day-to-day microenvironmental variations. With the fixed factors of *dispersal selection* (VB and VBC), *density* (60, 120, 240 and 480) and *sex* (male and female), we had a balanced, full-factorial design for a five-way mixed-model ANOVA. For analysing the temporal profile of VB and VBC dispersers of each sex, we carried out separate three-way mixed-model ANOVAs for each 15-min interval, with *density* as a fixed factor and *day* nested inside *block*, both random factors. We then controlled the family-wise error rates using sequential Holm-Šidák correction (Abdi 2010).

As all the data were in the form of fractions, we used arcsine-square root transformation prior to the ANOVAs (Zar 1999), which were carried out using STATISTICA® v8 (StatSoft. Inc., Tulsa, Oklahoma). Cohen's d was used as a measure of effect size for pairwise differences, with the effect interpreted as large, medium and small for $d \geq 0.8$, $0.8 > d \geq 0.5$ and $d < 0.5$, respectively (Cohen 1988).

3 RESULTS

The ANOVA for the dispersal propensity data yielded a significant effect of *selection* ($F_{1,3} = 110.36$, $p = 0.002$), *selection* × *density* ($F_{3,9} = 7.03$, $p = 0.01$), as well as *selection* × *density* × *sex* ($F_{3,9} = 17.06$, $p = 0.0005$). We limit our interpretation to the final three-way interaction term, which suggests that not only was VB and VBC dispersal affected differently by density, but there were also sex-specific effects.

3.1 Negative and sex-biased DDD observed in control populations

VBC populations showed a negative DDD, where the dispersal propensity was lower at higher densities (Fig. 3A). Furthermore, the strength of negative DDD was stronger in VBC females than in VBC males (Fig. 3A, Table 1). As a result, a significant female-biased dispersal was observed at densities 60 and 120, whereas a significant male-biased dispersal was observed at the highest density, 480 (Fig. 3A, Table 2). Taken together, these results indicate a strong negative DDD in VBC populations, along with a sex-biased asymmetry: females showed a stronger negative DDD response than the males.

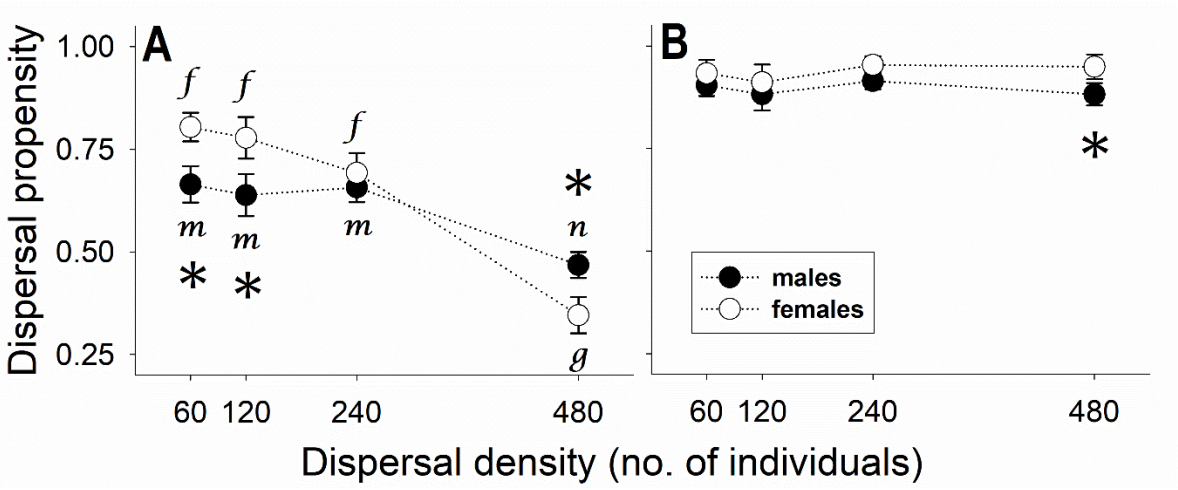

Fig. 3 Effect of dispersal evolution on density-dependent dispersal (DDD). Mean dispersal propensity (± SE) across the four density treatments for (A) VBC (control) and (B) VB (dispersal-selected) individuals. A strong negative DDD was observed for VBC individuals, but the VB individuals showed no DDD. Open circles denote the data for females, with lower-case letters starting from *f* representing the significant differences in female dispersal propensity across densities. Closed circles denote the data for males, with lower-case letters starting from *m* representing the significant differences in male dispersal propensity across densities. Asterisks (*) denote significant sex-biased dispersal ($p \leq 0.05$) at a given density. See Tables 1 and 2 for the exact p values and the associated effect sizes.

The effect of density for VBC populations was apparent in the temporal dispersal profile as well. Most of the dispersal (> 50%) occurred before the 1-hour mark in the first three density treatments (i.e. 60, 120, and 240 individuals), whereas a majority of dispersal took place after the 1-hour mark in the highest density treatment (480 individuals) (Figs. 4A and 4B). Taken together, this means that the dispersal propensity of VBC individuals was not only suppressed at higher density (Fig. 3A), but the dispersers also took longer to complete their journey from the *source* to the *destination* (Figs. 4A and 4B).

3.2 No DDD observed for either sex in dispersal-selected populations

In contrast to the results for VBC, we found that the VB populations did not show a DDD response at all. Neither males nor females showed a significant difference in their dispersal across the four treatment densities (Fig. 3B, Table 1). Moreover, only at the highest density (i.e. 480), we found a significant female-biased dispersal (Fig. 3B, Table 2), in contrast to the male-biased dispersal observed at this treatment in VBC populations (cf. Fig. 3A and 3B). The results show that the dispersal of both sexes was unaffected by density in VB populations, which also reduced the multiple instances of sex-biased dispersal observed in VBC populations.

In terms of the temporal dispersal profile as well, VB flies in all density treatments completed the dispersal much faster than the VBC flies (cf. Figs. 4A, 4B with Figs. 4C, 4D). As a result, within the first 30 minutes of the dispersal run, a majority of VB dispersers (> 50%) had completed their journey from the *source* to the *destination* (Figs. 4C and 4D). Taken together, the results suggest that both dispersal propensity and the speed of dispersal of VB populations were largely unaffected by the density treatments.

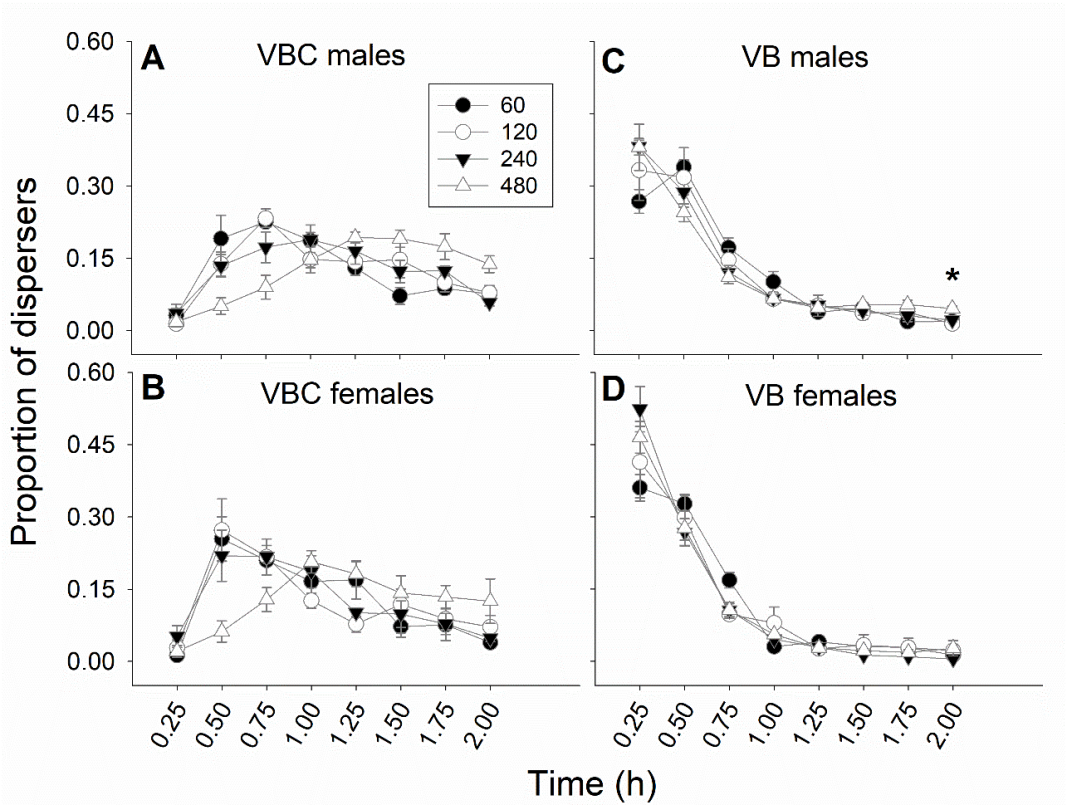

Fig. 4 Temporal profile of VB (dispersal-selected) and VBC (control) flies across the four density treatments. Average proportion (± SE) of individuals completing dispersal during the specified temporal bins for (A) VBC males, (B) VBC females, (C) VB males and (D) VB females. As can be observed, both male and female dispersers of VB populations completed the majority (> 50%) of dispersal within the first two temporal bins (i.e. by 0.5 h). In contrast, the VBC dispersers had a more staggered dispersal, thereby taking longer to complete 50% dispersal. Asterisk (*) denotes a significant difference ($p \leq 0.05$) among the density treatments for the relevant temporal bin, based on three-way ANOVA followed by sequential Holm-Šidák correction. See Table 3 for the exact p values and the associated effect sizes.

Table 1. Within-sex pairwise differences for dispersal propensity across the treatment densities for VBC (control) and VB (dispersal-selected populations). For significant pairwise differences (Tukey's $p \leq 0.05$), effect sizes (Cohen's *d*) are computed.

Pre-dispersal density	Sex	Densities	Tukey's p	Cohen's *d*	Effect size interpretation
VBC (Control)	Males	60 – 120	> 0.99	-	-
		60 – 240	> 0.99	-	-
		60 – 480	0.0032	1.209	Large
		120 – 240	> 0.99	-	-
		120 – 480	0.0083	0.954	Large
		240 – 480	0.0038	1.316	Large
	Females	60 – 120	> 0.99	-	-
		60 – 240	0.078	-	-
		60 – 480	2.01×10^{-6}	2.699	Large
		120 – 240	0.15	-	-
		120 – 480	2.52×10^{-6}	2.136	Large
		240 – 480	1.95×10^{-5}	1.760	Large
VB (Dispersal-selected)	Males	60 – 120	> 0.99	-	-
		60 – 240	> 0.99	-	-
		60 – 480	0.85	-	-
		120 – 240	> 0.99	-	-
		120 – 480	> 0.99	-	-
		240 – 480	0.88	-	-
	Females	60 – 120	0.99	-	-
		60 – 240	> 0.99	-	-
		60 – 480	> 0.99	-	-
		120 – 240	> 0.99	-	-
		120 – 480	0.98	-	-
		240 – 480	> 0.99	-	-

Table 2. Sex-bias in dispersal propensity for VBC (control) and VB (dispersal-selected populations). For significant pairwise differences (Tukey's $p \leq 0.05$), effect sizes (Cohen's *d*) are computed. m: males, f: females

Pre-dispersal density	Densities	Tukey's p	Cohen's *d*	Effect size interpretation
VBC (Control)	60m – 60f	0.010	0.906	Large
	120m – 120f	0.007	0.740	Medium
	240m – 240f	0.87	-	-
	480m – 480f	0.052	0.799	Medium
VB (Dispersal-selected)	60m – 60f	0.23	-	-
	120m – 120f	0.25	-	-
	240m – 240f	0.29	-	-
	480m – 480f	0.021	0.901	Large

Table 3. List of significant pairwise differences in the temporal dispersal profiles. Data are presented for the time points that had a significant main effect of density after Holm-Šidák sequential correction, with Tukey's p value and effect size (Cohen's *d*) presented for the density pairs with a significant difference.

Population	Sex	Time-point	Holm–Šidák corrected p (main effect)	Densities	Tukey's p	Cohen's *d*	Effect size interpretation
VB	**Males**	2 h	0.022	60 – 480	2.47×10^{-4}	1.44523	Large
				120 – 480	3.37×10^{-4}	1.67547	Large

4 DISCUSSION

4.1 Negative DDD in control populations

The non-selected control (VBC) populations showed a significant negative DDD, as the dispersal propensity showed a decreasing trend with increasing density (Fig. 3A). Furthermore, this response was significantly stronger in females than in males. Because of this asymmetry between the sexes, there was a switch in the direction of sex-biased dispersal: we observed a significant female-biased dispersal at low densities but a significant male-biased dispersal at high densities (Fig. 3A). Finally, the significant reduction in dispersal propensity was also accompanied by considerably delayed dispersal at the highest density (i.e. 480 individuals) (Figs. 4A and 4B). All these results are in line with those observed for the ancestral populations of VBC and VB flies, assayed under similar conditions (Mishra *et al.* 2018b). This consistency of results for ancestral and VBC populations confirms that the DDD response of these flies has remained relatively unchanged over the course of separate rearing for 75 generations (~3 years).

The DDD response of the dispersal-selected (VB) populations would thus be compared against this expectation of a negative DDD pattern. While empirical studies on the evolution of DDD are extremely rare (although see Fronhofer *et al.* 2017), the general prediction from studies is that populations undergoing rapid dispersal evolution should evolve higher dispersal rates at low densities. This is predicted to cause an abatement of positive DDD patterns and a likely emergence of negative DDD (Travis *et al.* 2009; Fronhofer *et al.* 2017; Weiss-Lehman *et al.* 2017). Since negative DDD was already seen in VBC populations, the next step was to see whether dispersal evolution in VB populations had indeed strengthened this negative DDD, as per the expectation from literature.

4.2 Absence of DDD in dispersal-selected populations

In contrast to the negative DDD observed in VBC populations, we observed a complete absence of DDD in VB populations. Both sexes in VB populations showed a consistently high dispersal propensity of >80% across the four densities (Fig. 3B). Therefore, our results directly contradict the hypothesis that dispersal evolution should lead to a more negative DDD response in expanding populations (Travis *et al.* 2009; Fronhofer *et al.* 2017; Weiss-Lehman *et al.* 2017). While there was an increase in dispersal propensity at low densities, as predicted by earlier studies, it was also accompanied by a much larger increase in the dispersal propensity at high densities (Fig. 3B). As a result, we obtained an almost flat DDD response, where a consistently high dispersal occurred irrespective of the density treatments.

In addition to the loss of negative DDD, the VB populations also narrowed the extent of sex differences in dispersal (*cf.* Figs. 3A and 3B). Sex-biased dispersal in VB populations was observed only at the highest density (i.e., 480), where a significant female-biased dispersal was observed. This is in direct contrast to the male-biased dispersal observed for VBC populations at the same density (*cf.* Figs. 3A and 3B). While the evolution of sex-biased dispersal remains a prominent topic of investigation, to our knowledge, this is the first empirical demonstration of an evolutionary change in sex-biased dispersal, that too under identical environmental conditions.

So what explains the change in DDD by dispersal evolution and the loss of sex differences in VB populations? The VB populations have undergone continuous selection for higher dispersal via spatial sorting every generation. The selection setup is such that females need to complete the *source*-to-*destination* dispersal in order to contribute to the next generation, whereas males could theoretically mate with several females before the dispersal run and still be able to sire progeny in the next generation (Fig. 1; Tung *et al.* 2018b)). The high selection pressure on dispersal speed (only the first 50% dispersers are selected each generation) means that, over generations, the rate at which the eventually successful female dispersers would leave the *source* container would tend to increase. Additionally, leaving the *source* container quickly can also allow the females to escape excessive harassment by males (Byrne *et al.* 2008; Malek & Long 2019). However, the high female dispersal thus evolved could create a shortage of mates for males, likely resulting in increasingly higher mate-finding dispersal by them (Shaw & Kokko 2014; Mishra *et al.* 2020b). This way, spatial sorting would then ensure that both males and females evolve increasingly similar dispersal kernels (Meier *et al.* 2011; Shaw & Kokko 2014) with high dispersal propensity, ability and speed. As a result, the dispersal-selected females can emigrate away to escape the excessive mate harassment experienced at high densities, while the males track their movement via mate-finding dispersal (Fig. 3B, Figs. 4C and 4D). Over generations, the continuous evolution of higher dispersal could thus lead to a similar magnitude of DDD loss in both sexes.

4.3 Implications

Our results showed that, contrary to the previous expectations from literature, dispersal evolution completely reversed the negative DDD seen in control and ancestral populations. This was accompanied by a loss of sex differences in dispersal, leading to fewer instances of sex-biased dispersal. Below, we discuss some ecological and evolutionary implications of these results.

First, our results demonstrate a strong effect of dispersal evolution, resulting in uniformly high dispersal of males and females irrespective of the environmental context. In other words, this is yet another piece of strong evidence that dispersal evolution leads to context-independent dispersal (Tung *et al.* 2018b). As a result, we predict that populations undergoing strong spatial selection would exhibit increasingly more phenotype-dependent movement than context-dependent movement (*sensu* Clobert *et al.* 2009; Clobert *et al.* 2012).

Second, the narrowing of sex differences confirms that males and females can evolve strikingly similar dispersal patterns, even if their initial dispersal patterns are very different. In species where dispersal costs differ significantly between males and females (Gros *et al.* 2008; Trochet *et al.* 2016; Mishra *et al.* 2018a), this could alter the physiology and life history of either or both sexes.

Finally, the loss of negative DDD is incongruent with the classical prediction for DDD evolution in dispersing populations. This is because these previous studies typically started with an initial positive DDD, which was then lost or dampened by dispersal evolution (Travis *et al.* 2009; Fronhofer *et al.* 2017). By starting with a negative DDD, we show that the type of initial DDD pattern and its underlying causes could affect the evolutionary outcome. Therefore, future empirical studies, especially on taxa with non-positive DDD, would help understand the potential evolutionary outcomes of spatial selection on DDD patterns.

CHAPTER 5

Sex differences in dispersal syndrome are modulated by environment and evolution

Highlights

- Dispersal syndrome of males and females studied using three traits: body size, desiccation resistance and exploratory activity
- Sexes differed markedly in their dispersal syndrome, modulated by nutrition availability
- Dispersal evolution significantly altered the sex differences in dispersal syndrome

Published as and adapted from:
Mishra, A.*, Tung, S.*, Shreenidhi, P. M., Aamir Sadiq, M., Shree Sruti, V. R., Chakraborty, P. P., & Dey, S. (2018). Sex differences in dispersal syndrome are modulated by environment and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1757), 20170428. *Equal contribution

1 INTRODUCTION

Many natural populations are spatially structured with some amount of dispersal across local habitats (Hanski 2001). Environmental heterogeneity across these habitats can then lead to local adaptation (Kawecki & Ebert 2004), which allows the subpopulations to increase their average fitness in the context of their local environments. Dispersal plays a crucial role in this process and can either favour local adaptation by increasing the amount of available genetic variation or hinder it by introducing less adapted individuals to the population (Lenormand 2002). To complicate matters further, theory suggests that dispersal can interact in complex ways with other factors like genetic drift (Blanquart *et al.* 2012), genetic architecture (Billiard & Lenormand 2005) and environmental stochasticity (Kisdi 2002) in shaping the local adaptation. Some of these insights have been empirically verified. For example, it has been shown that increasing the rate of migration enhances the rate of evolution for antibiotic resistance and reduces the associated costs in the bacterium *Pseudomonas aeruginosa* (Perron *et al.* 2007). Similarly, the extent of local adaptation in parasite populations is determined by their migration rates relative to their hosts (Gandon *et al.* 1996).

Another important way by which dispersal can potentially affect local adaptation is through its association with other phenotypic traits. Being a key life-history trait in individuals (Bonte & Dahirel 2017), dispersal typically has strong associations with other morphological, life-history or behavioural traits (Clobert *et al.* 2009). These trait associations with dispersal collectively comprise a 'dispersal syndrome' (Ronce & Clobert 2012) and affect the local adaptation in new environments (Clobert *et al.* 2009; Cote *et al.* 2010) through the process of habitat matching (Edelaar *et al.* 2008; Jacob *et al.* 2015). Moreover, the capacity of subsequent movement by such dispersers would likely be modulated via the changes in their existing dispersal syndrome under the new environment. Consequently, dispersal syndromes have been extensively studied over the last two decades (reviewed in Ronce & Clobert 2012)). However, several important questions remain unanswered. For example, relatively little is understood about the patterns and consequences of sex differences in dispersal syndromes (Legrand *et al.* 2016). It is well known that several life-history and behavioural traits are significantly different across sexes in many taxa (Kelley 1988; Prasad & Joshi 2003; Zajitschek *et al.* 2009) and therefore it is reasonable to assume that dispersal syndromes would also vary across sexes. Both demographic and genetic effects of sex-biased dispersal (e.g. Kawecki 2003; Lopez *et al.* 2008; Aguilée *et al.* 2013)) could amplify or weaken, if the two sexes have dissimilar dispersal syndromes due to differences in their physiology. Therefore, in species

where both males and females disperse, sex differences in dispersal syndromes could play a major role in determining their local adaptation.

A general overview of the literature on dispersal syndromes indicates that a majority of the evidence for their existence comes from same-generation trait-association data, i.e. by comparing the traits of dispersive individuals with non-dispersive individuals (e.g. Stevens *et al.* 2014; Comte & Olden 2018)). We call the dispersal syndromes inferred in this manner as "ecological dispersal syndromes". However, it is not known whether these ecological dispersal syndromes allow us to make any predictions regarding the trait associations that take shape during evolution of dispersal (henceforth "evolutionary dispersal syndromes"). If an ecological dispersal syndrome could reliably predict the direction or extent of association between dispersal and other traits in the evolutionary dispersal syndrome (within the same environment), it would be a rich source of information on the heritable trade-offs involved with dispersal, besides being a good proxy of the dispersal status of individuals in the long-term. In contrast, an ecological dispersal syndrome that is incongruent with the evolutionary dispersal syndrome, even under the same environment, would be of limited utility. This becomes even more interesting in the context of the evolution of sex differences in dispersal syndrome. Depending on the exact nature of the selection pressure, males and females could also be differentially affected during dispersal evolution. For instance, if mating precedes dispersal but oviposition or giving birth follows dispersal, males and females might evolve very different resource allocation patterns. For passing their genes to the next generation, the females the females in this scenario have to undertake dispersal and pay its associated costs (Bonte *et al.* 2012). However, the males can potentially avoid dispersal, and hence the cost, by impregnating a large number of females prior to the latter's dispersal. This sex-based divergence in strategies could result in a physiological adaptation for dispersal in females but not in males, leading to evolutionary differences in their dispersal syndromes. Thus, in principle, it is possible for dispersal syndromes to evolve differentially across sexes.

In this study, we investigated the dispersal syndrome in adult fruit flies, *Drosophila melanogaster*, as a composite of three traits: body size, desiccation resistance and exploratory tendency. Dispersers typically have larger body size across several taxonomic groups, *inter alia*, milkweed bugs (Dingle *et al.* 1980), butterflies (Sekar 2012; Stevens *et al.* 2012), birds (Böhning-Gaese *et al.* 1998; Paradis *et al.* 1998; Sutherland *et al.* 2000; Dawideit *et al.* 2009) and mammals (Sutherland *et al.* 2000; Santini *et al.* 2013; Whitmee & Orme 2013). Stress resistance is also likely to be

positively associated with dispersal, as enduring hostile environments during the transition phase can be a beneficial trait for dispersers (Bowler & Benton 2005). Similarly, exploratory tendency is expected to have a strong positive association with dispersal (Cote *et al.* 2010; Korsten *et al.* 2013) as exploring the surrounding areas before initiating the journey is thought to be an integral part of the dispersal process (Debeffe *et al.* 2013). Consequently, it is not surprising to find more exploratory individuals at the range margins (Liebl & Martin 2012) and in invasive species (Rehage *et al.* 2005; Cote *et al.* 2010; Russell *et al.* 2010). Unfortunately, the nature of sex differences in the context of dispersal is not well known for these traits (although see van Overveld *et al.* 2014)). Therefore, we assessed the sex differences in dispersal syndrome of *D. melanogaster* by examining how body size, exploratory tendency and desiccation stress resistance varies in males and females between dispersers and non-dispersers. Specifically, we asked three questions: (a) Are dispersal syndromes in *D. melanogaster* sex-specific? (b) How do sex differences in dispersal syndrome respond to a change in the environment (here, nutrition level)? (c) How well are the evolutionary dispersal syndrome and its corresponding sex differences predicted by the ecological dispersal syndrome under the same environment?

To address these questions, we conducted three experiments. Experiment 1 involved the study of ecological dispersal syndrome under low nutrition, whereas Experiment 2 examined the ecological dispersal syndrome under standard nutrition. Experiment 3 assessed the evolutionary dispersal syndrome under standard nutrition. Thus, a comparison of data from Experiments 1 and 2 shows how an environmental factor (i.e. nutrient availability) modulates the sex differences in ecological dispersal syndrome, while a comparison of data from Experiments 2 and 3 shows how the sex differences differ between the ecological and evolutionary dispersal syndromes under a uniform environment. We found that dispersal syndromes are not only sex-specific, but these sex differences also vary across environments. We also show that the ecological and evolutionary dispersal syndromes differ substantially, with respect to both sex-independent and sex-specific effects.

2 MATERIALS AND METHODS

2.1 Fly populations

We used individuals from a large (breeding size of ~2400 individuals) laboratory population of *Drosophila melanogaster*, named DB_4, for investigating the ecological dispersal syndrome in Experiments 1 and 2. The DB populations in turn trace their ancestry back to the IV lines, which were wild-caught at South Amherst, MA, USA in 1970 (Ives 1970). Ever since, these flies have been maintained in the laboratory at large population sizes to ameliorate inbreeding-like effects. For examining the evolutionary dispersal syndrome, we used eight *D. melanogaster* populations derived from DB populations, four of which (VB_{1-4}) have been subjected to selection for higher dispersal over 70 generations, with the other four populations (VBC_{1-4}) serving as the corresponding control populations (Tung *et al.* 2018a; Tung *et al.* 2018b). The ancestral relationship among the populations is outlined in Fig. 1.

The larvae and adults of all the populations (i.e. DB_4, VB_{1-4} and VBC_{1-4}) were maintained at 25 °C and constant light conditions. During regular maintenance, the flies are made to oviposit on petri-plates containing standard banana-jaggery medium for 12-16 h. After oviposition, we cut 40 small strips of the medium, each containing 60–80 eggs that are sampled randomly, and introduce them individually into 35-mL plastic vials that had ~6 mL of the same banana-jaggery medium. This ensures that the larvae are raised under low-to-moderate level of crowding, to avoid any confounding effect of density-dependent selection (Joshi 1997). In these vials, the adults start emerging by the 8^{th}–9^{th} day after egg collection. For the DB_4 population, on the 12^{th} day, the adults are transferred to plexi-glass cages (25 cm × 20 cm ×15 cm) and are provided with fresh banana-jaggery medium every alternate day. This process continues until the 18^{th} day, when the adult flies are supplied with excess live yeast paste along with standard banana-jaggery food. Whereas, for the VB and VBC populations, on the 12^{th} day from the day of egg collection, the adults are subjected to the dispersal selection protocol (detailed in Tung *et al.* 2018a; Tung *et al.* 2018b). Immediately after this, the adults of a given population are transferred to a plexi-glass cage and provided with yeast supplement along with standard banana-jaggery food. For all the populations, after ~40 h of yeast provisioning, eggs are collected for the next generation. The adults are discarded after oviposition, thus ensuring that individuals of two successive generations never co-exist. Thus, the length of egg-to-egg cycle is 21 days for DB_4, whereas it is 15 days for VB and VBC populations.

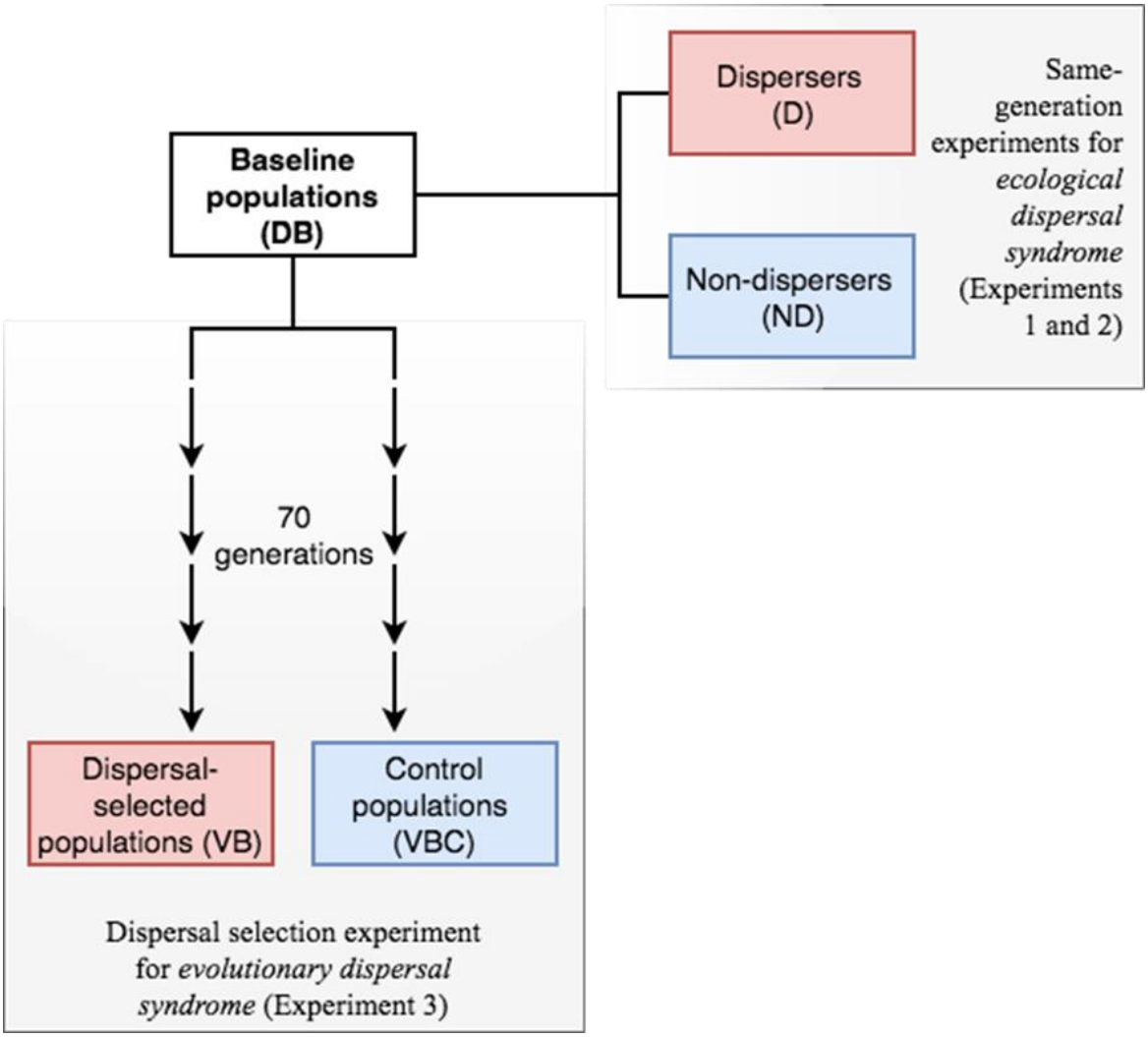

Fig. 1 *Drosophila melanogaster* populations used in the study. Starting from a baseline population (DB_4), flies were taken for the same-generation experiments (Experiments 1 and 2). The same DB_4 population also served as the ancestral population for a pair of the dispersal-selected (VB_4) and control (VBC_4) populations in the dispersal evolution experiment (Experiment 3).

2.2 Experiments

To identify flies as dispersive or non-dispersive individuals, we used two-patch dispersal setups, each comprising a *source*, a *path* and a *destination* (similar to Mishra *et al.* 2018b; Tung *et al.* 2018b)). In this setup, age-matched adult flies are introduced into a plastic container (*source*) that is connected to another empty plastic container (*destination*) via a long transparent plastic tube (*path*). The flies can thus disperse from the *source* to the *destination*, via the *path*. The *path* length and the time period allowed for dispersal can be varied as per the experimental requirements. This two-patch setup has been used in the long-term dispersal selection experiment that gave

rise to the aforementioned VB and VBC populations (Tung *et al.* 2018b), as well as for investigation of density-dependent and sex-biased dispersal in *D. melanogaster* (Mishra *et al.* 2018b).

Experiment 1: Ecological dispersal syndrome under low nutrition

For this experiment, ~38,000 eggs of the same age were randomly sampled from the DB_4 population and reared under low nutrition conditions (33%-diluted banana-jaggery medium) in 640 vials, at a density of ~60 eggs/vial. Upon completion of their development into adults, we segregated these flies into dispersers and non-dispersers by subjecting them to three rounds of successive filtering, starting with 16 independent two-patch dispersal setups (Fig. 2). Through each of these dispersal rounds, flies that were consistently dispersive/non-dispersive were collected, while the rest were discarded. This ensured that we chose only those flies which showed a high repeatability (Bell *et al.* 2009; Dingemanse *et al.* 2010) in their dispersive/non-dispersive behaviour. Dispersers and non-dispersers were then compared in terms of their life history and behaviour, to assess the ecological dispersal syndrome of flies under low nutrition.

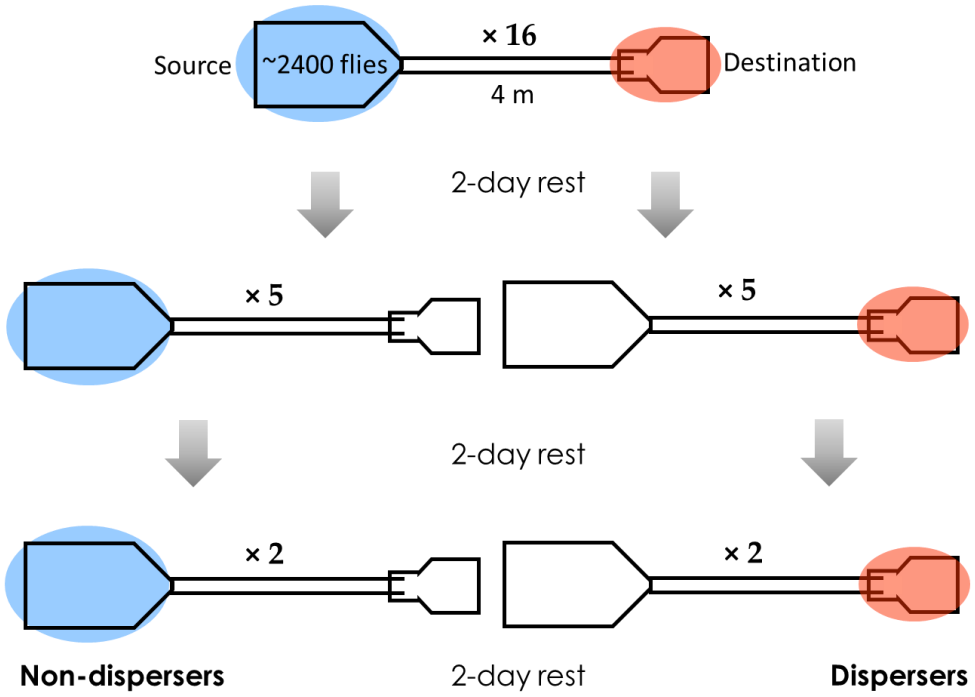

Fig. 2 Schematic diagram for segregation of dispersers and non-dispersers in the same-generation experiments (Experiments 1 and 2). The three successive steps of filtering (at 2-day intervals) ensured that at the end, we retained only those flies that had consistently shown either dispersive or non-dispersive behaviour.

Experiment 2: Ecological dispersal syndrome under standard nutrition

This experiment was identical to Experiment 1, with the sole exception that here, flies were reared under standard nutrition conditions (standard banana-jaggery medium). Dispersers and non-dispersers were then segregated using three rounds of successive filtering (Fig. 2) and compared against each other to evaluate the ecological dispersal syndrome (see section 2.3).

Experiment 3: Evolutionary dispersal syndrome under standard nutrition

For this experiment, we used VB_{1-4} (dispersal-selected) populations and their corresponding control populations, VBC_{1-4} (Tung *et al.* 2018a; Tung *et al.* 2018b; Fig. 3). At the time of this study, these flies had undergone selection for 70 generations.

A potential way to investigate the evolutionary dispersal syndrome would be to compare the dispersers and non-dispersers within a given dispersal-selected population. That is, one could subject each VB population to similar kind of dispersal segregation as in Experiments 1 and 2, and then compare the dispersers and non-dispersers to assess the dispersal syndrome. However, there are two major problems with this approach. First, since VBs have undergone dispersal selection for 70 generations, it would be nearly impossible to obtain enough flies in these populations that do not disperse (i.e. non-dispersers). Second, during the course of dispersal evolution, there may have been some inadvertent selection for uncontrolled environmental variation acting on these populations, the effects of which would then get confounded with the effects of dispersal evolution. In order to circumvent these issues, we used a different approach to assess the evolutionary dispersal syndrome. Like many other *Drosophila* life-history evolution experiments (for example, see Prasad *et al.* 2001; Tung *et al.* 2018a)), we compared the VBs with their corresponding controls (VBCs), which have been maintained and evolved in parallel to these populations. Except for the selection for dispersal, the VBCs have undergone identical maintenance as the VBs (including the duration of desiccation stress faced by VBs during dispersal). Since Experiment 2 involved the ancestral population (DB_4) of VB_4 and VBC_4, the dispersal syndrome estimated therein would serve as an approximation of the dispersal syndrome at the beginning of the dispersal-selection experiment (where the flies were first sorted into dispersers and non-dispersers), whereas the dispersal syndrome assessed by comparing VBs and VBCs would serve as the evolutionary dispersal syndrome after 70 generations of

selection. Prior to the assays, the VB and VBC populations were reared under common conditions for one generation to minimize the influence of non-genetic parental effects (Watson & Hoffmann 1996). From these flies, eggs were collected for the assays (section 2.3) while maintaining a low egg density (~50 eggs on ~6 mL food in each vial) to avoid any confounding effect of larval crowding on the traits assayed. On the 12th day from egg collection, assays were performed on the adult flies. For all the assays, environmental (constant light, 25°C temperature, abundant nutritional availability, low rearing density) and physiological (similar age) conditions of the flies were strictly controlled and maintained identically across all VB and VBC populations.

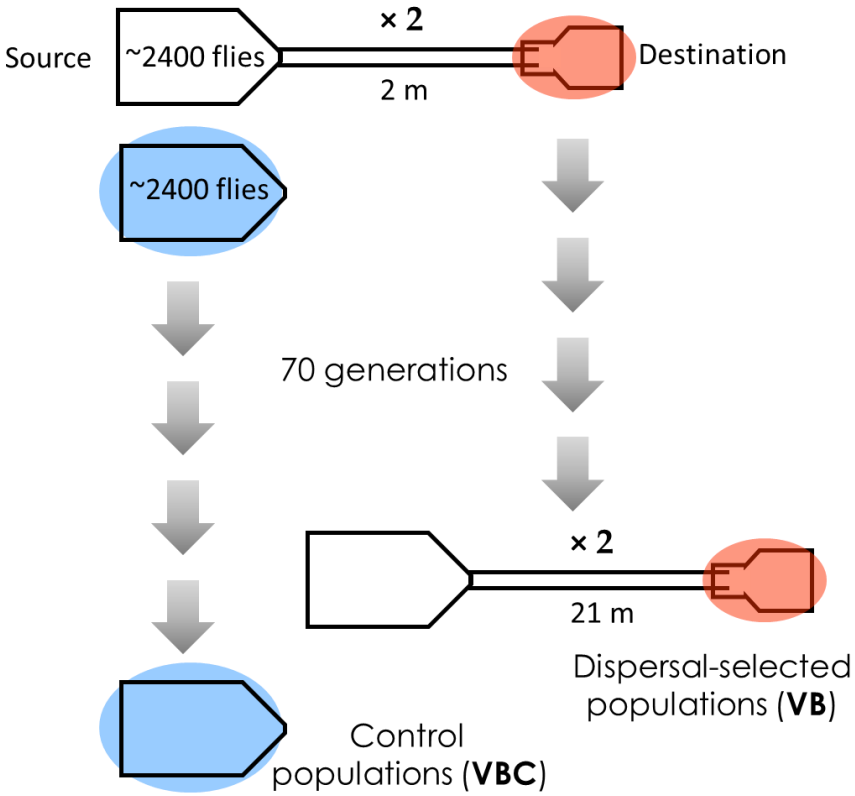

Fig. 3 Schematic diagram for selection of dispersal in the dispersal evolution experiment (Experiment 3).

2.3 Dispersal syndrome traits

A total of three traits were used to assess the dispersal syndrome of flies: dry body weight, desiccation resistance and exploratory activity. The details of assay for each trait are provided below.

Dry body weight of the adult flies was measured as a proxy for body size. For this, the flies were first sorted by sex, then killed by flash freezing and dried at 60 °C for 72 h in a hot-air oven. After thawing to the room temperature, the flies were weighed to the nearest 0.1 mg using Shimadzu (model AUY220) weighing balance. In Experiments 1 and 2, 10 batches of 20 males or 20 females were weighed for both non-dispersers and dispersers. In Experiment 3, 10 batches of 20 males or 20 females were weighed for each pair of VB and VBC populations.

Desiccation resistance for a fly was measured as the duration that it can survive without food and moisture. To quantify this trait in Experiments 1 and 2, 10 flies of either sex from non-dispersers and dispersers were introduced into empty transparent vials and monitored until the death of the last fly in each vial. The survivorship checks were conducted every 2 h and 10 such replicates were used per sex. Similarly, in Experiment 3, for each of the VB and VBC populations, the duration of survival in the absence of food and moisture was recorded for 10 sets of 10 flies of either sex.

For exploratory activity assay, flies of either sex were aspirated from the egg-collection vials and introduced individually into the experimental arena (modified from Soibam *et al.* 2012 and identical to Tung *et al.* 2018a), which comprised a clear polycarbonate petri dish lid of 10-cm inner diameter. The flies typically prefer to walk along the boundary of the arena (i.e. the side-wall of the lid) and avoid the inner zone. Thus, movements away from the boundary indicate the exploratory tendency of an individual (Soibam *et al.* 2012). Upon introduction into the arena, we allowed 1 minute for each fly to acclimatize to the new environment, after which it was observed for 10 subsequent minutes. Following an established paradigm (Liu *et al.* 2007), the number of times it entered the inner two-third area of the experimental arena (marked *a priori*) was recorded as the number of exploratory trips. In Experiments 1 and 2, the number of exploratory trips was measured for 32 individuals per sex for both non-dispersers and dispersers. For Experiment 3, we used a part of the dataset presented in (Tung *et al.* 2018a), comprising the exploratory tendency data for 32 individuals per sex of the VB and VBC populations.

2.4 Statistical analyses

This study involved three experiments (1, 2 and 3) that were designed separately and conducted one after the other. In each experiment, we separately compared

three traits (body size, desiccation resistance and exploratory tendency) across dispersers/non-dispersers (Experiments 1 and 2) or VB/VBC (Experiment 3). Among these, since Experiment 3 involved four blocks of dispersal-selected and control populations (VB_{1-4} and VBC_{1-4}, respectively), the data for only the direct descendants of the DB_4 population (used in Experiments 1 and 2), i.e. VB_4 and VBC_4, were used whenever a comparison was made between Experiments 2 and 3.

One potential way to analyse the data for each trait would be to use three-way ANOVAs with *experiment* (1/2/3), *dispersal* (disperser/non-disperser) and *sex* (male/female) as fixed factors. In order to do this, we would have to use the data for only the direct descendants of the DB_4 population used in Experiments 1 and 2, i.e. VB_4 and VBC_4. This would allow us to directly test for the *experiment* × *dispersal* × *sex* interaction, thereby elucidating the effects of *experiment* (i.e. nutritional status or evolution) on how *dispersal* status interacts with *sex*. However, if analysed this way, day-to-day environmental variations (since the three experiments were conducted on separate days) would be confounded with *experiment* identity, thereby increasing the noise in the data. This is the reason why, in the *Drosophila* life-history literature, such experiments are typically performed with a blocked design, such that the effects of day-to-day variations can be explicitly accounted for using a mixed-model ANOVA (for example, see Prasad *et al.* 2001; Tung *et al.* 2018a)). In the context of our study, this would mean that one replicate for each experiment (1/2/3) should have been performed on the same day, and this entire thing should have been repeated on multiple days to get the desired number of replicates. Unfortunately, this study was neither designed nor performed that way, thus rendering such a statistical analysis inappropriate. A better way to analyse this data would be to conduct separate ANOVAs for each trait in each of the three experiments with *dispersal* and *sex* as fixed factors (Experiment 3 would have an additional random factor of *population block*). This would allow one to compare the dispersers with the non-dispersers under each of the three experimental conditions. One can then *qualitatively* compare, say, the responses of the dispersers vs. the non-dispersers in Experiment 1 with that of the dispersers vs. the non-dispersers in Experiment 2 to assess the effects of nutrition on dispersal syndrome. The assumption here would be that if there are any systemic differences in the assay conditions (say, changed atmospheric pressure Dagaeff *et al.* 2016)) across days, then they similarly affect the measured traits (i.e. body weight/desiccation resistance/exploratory tendency) of the dispersers and the non-dispersers. Unfortunately, although this statistical analysis better takes into account how the data were collected, it will not allow one to conduct the above-

mentioned explicit statistical test for the *experiment* × *dispersal* × *sex* interaction for each of the three traits.

In this study, we analyse the same data in both the ways mentioned above. We believe that the analysis consisting of the separate experiment-wise ANOVAs is the more appropriate one, and present its results first. We then briefly present the analysis consisting of the pooled three-way ANOVAs and discuss its implications in Section 3.3.

All the ANOVAs were carried out using STATISTICA v5 (StatSoft Inc. Tulsa, Oklahoma). Tukey's HSD test was used to adjudge the pairwise differences between means, whenever a significant *dispersal* × *sex* (or *experiment* × *dispersal* × *sex*) interaction was observed. Cohen's *d* was used as a measure of effect size for such significant differences, and the value of *d* was interpreted as large, medium and small for $d \geq 0.8$, $0.8 > d \geq 0.5$ and $d < 0.5$, respectively (Cohen 1988).

3 RESULTS

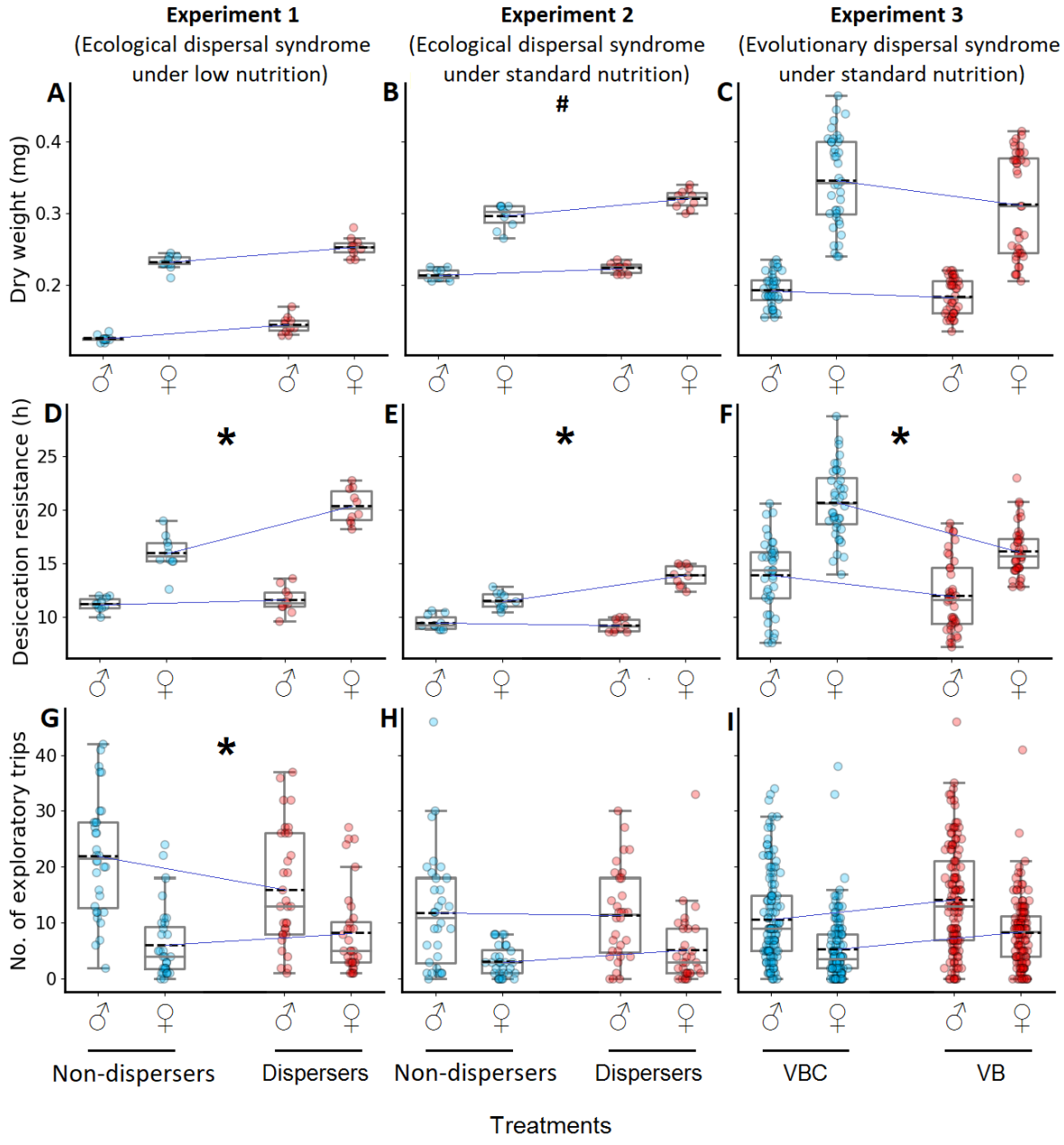

Fig. 4 *Dispersal* × *sex* interaction in the three experiments. Cleveland-box plots show male and female data for the three traits investigated (arranged row-wise in panels), across Experiments 1, 2 and 3 (arranged column-wise in panels). The circles (blue for non-dispersers/VBCs; red for dispersers/VBs) represent individual replicates, with a small random jitter along the X-axis. Box edges denote 25th and 75th percentiles, while the black solid and broken lines represent the median and mean, respectively. * denotes $p < 0.05$ for the *dispersal* × *sex* interaction, whereas # denotes $0.05 < p < 0.1$ for the *dispersal* × *sex* interaction. For the exact *p* values of ANOVA, refer to Tables 1, 3 and 5. For the *p* values of pairwise differences (Tukey's HSD test), refer to Tables 2, 4 and 6.

As stated in Section 2.4, the data were analysed in two different ways. Sections 3.1 and 3.2 present the results when, for a given trait, each experiment was analysed separately. Section 3.3 presents the results when data from all the experiments for a given trait were analysed using a pooled, three-way ANOVA.

3.1 Sex differences in ecological dispersal syndrome varied under different nutrition levels

The results from two-way ANOVAs for Experiment 1 (low nutrition) and Experiment 2 (standard nutrition) were compared to qualitatively assess the differences in the ecological dispersal syndrome under the two nutrition regimes. The complete ANOVA tables are provided as Tables 1–6.

Dispersers had a greater body weight than non-dispersers in both Experiment 1 ($p = 6.3 \times 10^{-7}$, $F_{1,36} = 36.38$) and Experiment 2 ($p = 4.9 \times 10^{-5}$, $F_{1,36} = 21.21$). The *dispersal* × *sex* interaction was not significant in Experiment 1 ($p = 0.71$, $F_{1,36} = 0.13$) (Fig. 4A), whereas in Experiment 2, there was a marginally significant *dispersal* × *sex* interaction ($p = 0.06$, $F_{1,36} = 3.60$) (Fig. 4B). Post-hoc tests for Experiment 2 revealed that the dispersive females were significantly heavier (Tukey's HSD $p = 4.3 \times 10^{-4}$, $d = 1.7$) than non-dispersive females, whereas no such difference was observed for males (Tukey's HSD $p = 0.24$).

For desiccation resistance, both experiments yielded a significant *dispersal* × *sex* interaction (Experiment 1: $p = 3.9 \times 10^{-5}$, $F_{1,36} = 21.91$; Experiment 2: $p = 3.5 \times 10^{-6}$, $F_{1,36} = 29.99$) (Fig. 4D and 4E). While dispersive females were consistently more resistant to desiccation than non-dispersive females (Experiment 1: Tukey's HSD $p = 1.6 \times 10^{-4}$, $d = 2.8$; Experiment 2: Tukey's HSD $p = 1.6 \times 10^{-4}$, $d = 2.9$), no such trend was observed for males (Experiment 1: Tukey's HSD $p = 0.93$; Experiment 2: Tukey's HSD $p = 0.87$, also see Fig. 5).

The most discernible difference between low and standard nutrition conditions was observed for exploratory tendency. Experiment 1 revealed a significant *dispersal* × *sex* interaction ($p = 0.01$, $F_{1,124} = 6.67$) (Fig. 4G). While dispersive males had significantly lower exploratory tendency than non-dispersive ones (Tukey's HSD $p = 0.04$, $d = 0.6$), no such difference was observed in the female flies (Tukey's HSD $p = 0.75$). In contrast, Experiment 2 showed neither a significant *dispersal* × *sex* interaction ($p = 0.32$, $F_{1,124} = 0.98$) (Fig. 4H), nor a significant main effect for *dispersal* ($p = 0.56$, $F_{1,124} = 0.35$).

In summary, when the dispersers were compared with the non-dispersers in the context of a given experiment: (a) sex difference in body size was apparent under standard nutrition but not under low nutrition, (b) sex difference in desiccation resistance was observed under both nutrition regimes, and (c) sex difference in exploratory behaviour was apparent under low nutrition but not under standard nutrition.

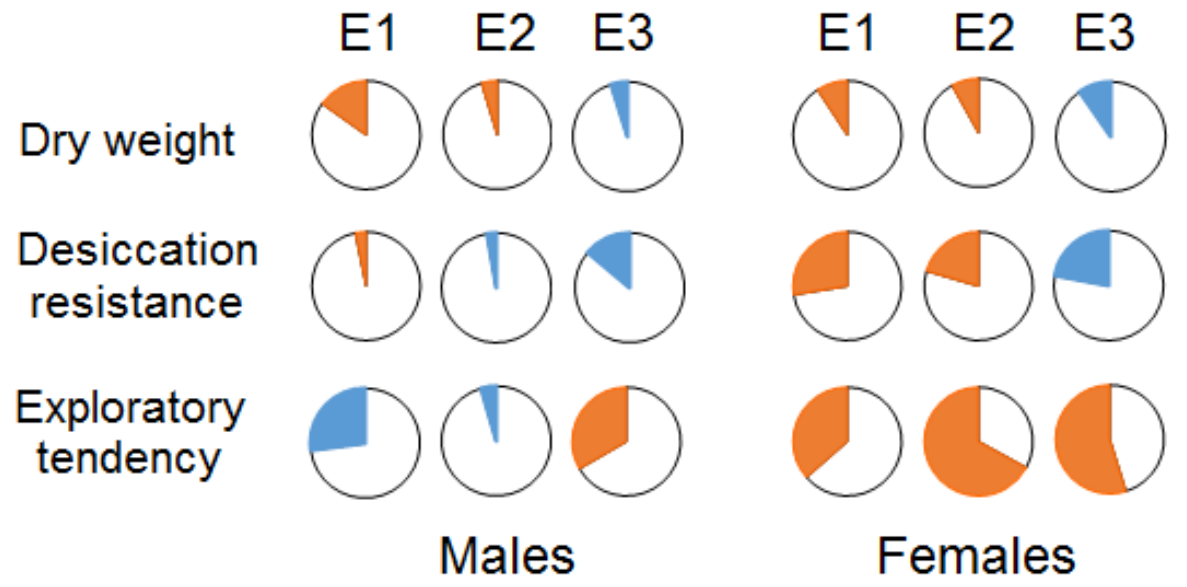

Fig. 5 Dispersal syndromes of males and females in the three experiments. E1, E2 and E3 represent Experiments 1, 2 and 3, respectively. Area of the coloured sector in the circles represents the percentage difference in the corresponding trait value for dispersers (for E1 and E2) or VBs (for E3), with respect to the non-dispersers and VBCs, respectively. Orange and blue colours denote positive and negative changes, respectively, in the dispersers (or VBs). It can be noted that the overall dispersal syndrome differs between males and females. Furthermore, this sex difference in dispersal syndromes varies across the three experiments. For exact values of the effect size (Cohen's *d*) associated with these differences, refer to Tables 2, 4 and 6.

3.2 Evolutionary dispersal syndrome and the associated sex differences differed markedly from the ecological dispersal syndrome

To assess the differences in dispersal syndrome across different temporal scales (same-generation measurements vs. post-dispersal evolution), results from individual ANOVAs for Experiment 2 and Experiment 3 were compared. The complete ANOVA tables are provided as Tables 1–6.

While Experiment 2 revealed a marginally significant *dispersal* × *sex* interaction as well as a main effect of *dispersal* on the body size (Section 3.1), neither of these effects were found to be significant for Experiment 3. Dispersal-selected flies (VBs) had similar dry body weight as their controls (VBCs) ($p = 0.13$, $F_{1,3} = 4.30$), and *dispersal* did not show a significant interaction with *sex* ($p = 0.1$, $F_{1,3} = 5.56$) (Fig. 4C).

Interestingly, the pattern for desiccation resistance was completely reversed between the ecological and evolutionary dispersal syndromes. Dispersive females had a significantly greater desiccation resistance than their non-dispersive counterparts in Experiment 2 (Section 3.1). In contrast, dispersal-selected flies in Experiment 3 had a lower desiccation resistance compared with the controls. Moreover, a significant *dispersal* × *sex* response was observed ($p = 0.04$, $F_{1,3} = 10.99$) (Fig. 4F) in Experiment 3, although dispersal-selected flies of both sexes had a significantly lower desiccation than the corresponding control flies (Males: Tukey's HSD $p = 1.2 \times 10^{-5}$, $d = 0.6$; Females: Tukey's HSD $p = 7.7 \times 10^{-6}$, $d = 2.0$). This was likely due to the larger magnitude of difference in females than in males (Fig. 4F, Fig. 5).

For exploratory tendency, while Experiment 2 did not show a significant effect of *dispersal* (Section 3.1), Experiment 3 revealed a significant main effect of *dispersal* ($p = 0.04$, $F_{1,3} = 11.96$). The dispersal-selected flies had a higher exploratory tendency in both sexes, and the *dispersal* × *sex* interaction was not significant ($p = 0.93$, $F_{1,3} = 0.01$) (Fig. 4I).

Thus, the evolutionary dispersal syndrome differed substantially from the ecological dispersal syndrome, in terms of both sex-independent and sex-specific effects. It should be noted here that while assessing the ecological dispersal syndrome in Experiment 2, the dispersers and non-dispersers belonged to the same population (i.e. DB_4). On the other hand, while investigating the evolutionary syndrome, a given pair of dispersers (VBs) and non-dispersers (VBCs) belonged to two different populations, both of which were descendants from a common DB population (Section 2.1).

3.3 Analysing the three experiments together

In this pooled analysis, data were considered from only VB_4 and VBC_4 populations (i.e. the direct ancestors of DB_4 population), to lend symmetry to the ANOVA design. The complete ANOVA tables are provided as Tables 7–11.

Three-way ANOVA for the body weight data pooled over the three experiments yielded a significant *experiment* × *dispersal* interaction ($p = 3.5 \times 10^{-6}$, $F_{2,108} = 14.13$), whereas the *dispersal* × *sex* and *experiment* × *dispersal* × *sex* interactions were not significant ($p = 0.50$, $F_{1,108} = 0.46$, and $p = 0.15$, $F_{2,108} = 1.96$, respectively).

In contrast, for desiccation resistance, all the tested effects, including *experiment* × *dispersal* ($p<10^{-6}$, $F_{2,108} = 94.24$), *dispersal* × *sex* ($p = 0.007$, $F_{1,108} = 7.64$) and *experiment* × *dispersal* × *sex* ($p = 7.1 \times 10^{-7}$, $F_{2,108} = 16.18$), were found to be significant.

For exploratory tendency, the effect of *experiment* × *dispersal* ($p = 0.18$, $F_{2,372} = 1.71$) was not significant, but the *dispersal* × *sex* interaction was significant ($p = 0.04$, $F_{1,372} = 4.40$). Further, The *experiment* × *dispersal* × *sex* interaction was marginally significant ($p = 0.06$, $F_{2,372} = 2.75$).

The presence of an *experiment* × *dispersal* × *sex* interaction in two of the three traits studied indicates that sex differences in dispersal syndrome were being affected differently across the three experiments. The post-hoc results for these traits are provided in Tables 9 and 11. In summary, this analysis (along with the post-hoc results) shows that while significant sex-specific differences were observed in desiccation resistance, these differences were not significant for exploration and body size. This outcome is not surprising as this analysis confounds the effects of experimental treatments with day-to-day variations, thereby increasing the amount of noise in the data. Therefore, we refrain from interpreting the results of these pooled, three-way ANOVAs. We report them here solely for the sake of comprehensiveness.

Table 1. ANOVA results for dry body weight data from Experiments 1, 2 and 3.

	Factor	df Effect	MS Effect	df Error	MS Error	F value	p-value
Experiment 1	***dispersal***	1	0.004	36	0.00011	36.38	6.3×10^{-7}
	sex	1	0.12	36	0.00011	1020.56	5.1×10^{-28}
	dispersal × sex	1	1.6×10^{-5}	36	0.00011	0.13	0.71
Experiment 2	***dispersal***	1	0.003	36	0.00014	21.21	4.9×10^{-5}
	sex	1	0.08	36	0.00014	587.91	6.8×10^{-24}
	dispersal × sex	1	0.0005	36	0.00014	3.60	0.06
Experiment 3	***dispersal***	1	0.018	3	0.004	4.30	0.13
	sex	1	0.79	3	0.022	35.32	0.01
	dispersal × sex	1	0.006	3	0.001	5.56	0.1

Table 2. Tukey's HSD *p*-values for the pairwise differences in dry body weight data from Experiments 1, 2 and 3. Cohen's *d* is computed as a measure of effect size for the significant pairwise differences. M: male, F: female.

	***p*-value for *dispersal × sex* interaction**	**Pairwise difference**	**Tukey's HSD *p***	**Cohen's *d***	**Effect size interpretation**
Experiment 1	0.71	M Dispersers – M Non-dispersers	Not applicable		
		F Dispersers – F Non-dispersers			
		M Dispersers – F Dispersers			
		M Non-dispersers – F Non-dispersers			
Experiment 2	0.06	M Dispersers – M Non-dispersers	0.24	-	-
		F Dispersers – F Non-dispersers	0.00043	1.7	Large
		M Dispersers – F Dispersers	0.00016	9.7	Large
		M Non-dispersers – F Non-dispersers	0.00016	6.8	Large
Experiment 3	0.1	M Dispersers – M Non-dispersers	Not applicable		
		F Dispersers – F Non-dispersers			
		M Dispersers – F Dispersers			
		M Non-dispersers – F Non-dispersers			

Table 3. ANOVA results for desiccation resistance data from Experiments 1, 2 and 3.

	Factor	df Effect	MS Effect	df Error	MS Error	F value	p-value
Experiment 1	***dispersal***	1	56.64	36	1.86	30.42	3.1×10^{-6}
	sex	1	462.4	36	1.86	248.34	9.8×10^{-18}
	dispersal × sex	1	40.80	36	1.86	21.91	3.9×10^{-5}
Experiment 2	***dispersal***	1	11.02	36	0.57	19.27	9.5×10^{-5}
	sex	1	113.57	36	0.57	198.51	3.2×10^{-16}
	dispersal × sex	1	17.16	36	0.57	29.99	3.5×10^{-6}
Experiment 3	***dispersal***	1	421.04	3	55.86	7.54	0.07
	sex	1	1223.51	3	11.94	102.54	0.002
	dispersal × sex	1	69.63	3	6.33	10.99	0.04

Table 4. Tukey's *p*-values for the pairwise differences in desiccation resistance data from Experiments 1, 2 and 3. Cohen's *d* is computed as a measure of effect size for the significant pairwise differences. M: male, F: female.

	***p*-value for *dispersal × sex* interaction**	**Pairwise difference**	**Tukey's HSD *p***	**Cohen's *d***	**Effect size interpretation**
Experiment 1	4.0×10^{-5}	M Dispersers – M Non-dispersers	0.93	-	-
		F Dispersers – F Non-dispersers	0.00016	2.8	Large
		M Dispersers – F Dispersers	0.00016	6.5	Large
		M Non-dispersers – F Non-dispersers	0.00016	3.9	Large
Experiment 2	3.5×10^{-6}	M Dispersers – M Non-dispersers	0.87	-	-
		F Dispersers – F Non-dispersers	0.00016	2.9	Large
		M Dispersers – F Dispersers	0.00016	6.2	Large
		M Non-dispersers – F Non-dispersers	0.00016	3.0	Large
Experiment 3	0.04	M Dispersers – M Non-dispersers	1.2×10^{-5}	0.6	Small
		F Dispersers – F Non-dispersers	7.7×10^{-6}	2.0	Large
		M Dispersers – F Dispersers	7.7×10^{-6}	1.8	Large
		M Non-dispersers – F Non-dispersers	7.7×10^{-6}	2.4	Large

Table 5. ANOVA results for exploratory tendency data from Experiments 1, 2 and 3.

	Factor	df Effect	MS Effect	df Error	MS Error	F value	p-value
Experiment 1	***dispersal***	1	110.63	124	79.74	1.39	0.24
	sex	1	4429.76	124	79.74	55.55	1.4×10^{-11}
	dispersal × sex	1	532.20	124	79.74	6.67	0.01
Experiment 2	***dispersal***	1	19.53	124	56.41	0.35	0.56
	sex	1	1785.03	124	56.41	31.64	1.2×10^{-7}
	dispersal × sex	1	55.12	124	56.41	0.98	0.32
Experiment 3	***dispersal***	1	1485.12	3	124.14	11.96	0.04
	sex	1	3949.38	3	145.64	27.12	0.01
	dispersal × sex	1	1.53	3	167.93	0.01	0.93

Table 6. Tukey's *p*-values for the pairwise differences in exploratory tendency data from Experiments 1, 2 and 3. Cohen's *d* is computed as a measure of effect size for the significant pairwise differences. M: male, F: female.

	***p*-value for *dispersal × sex* interaction**	**Pairwise difference**	**Tukey's HSD *p***	**Cohen's *d***	**Effect size interpretation**
Experiment 1	0.01	M Dispersers – M Non-dispersers	0.04	0.6	Medium
		F Dispersers – F Non-dispersers	0.75	-	-
		M Dispersers – F Dispersers	0.003	0.8	Large
		M Non-dispersers – F Non-dispersers	0.000007	1.9	Large
Experiment 2	0.32	M Dispersers – M Non-dispersers	Not applicable		
		F Dispersers – F Non-dispersers			
		M Dispersers – F Dispersers			
		M Non-dispersers – F Non-dispersers			
Experiment 3	0.93	M Dispersers – M Non-dispersers	Not applicable		
		F Dispersers – F Non-dispersers			
		M Dispersers – F Dispersers			
		M Non-dispersers – F Non-dispersers			

Table 7. Pooled three-way ANOVA for dry body weight data over Experiments 1, 2 and 3.

Factor	df Effect	MS Effect	df Error	MS Error	F value	p-value
experiment	2	0.13	108	0.00015	864.49	$< 1 \times 10^{-28}$
dispersal	1	0.003	108	0.00015	21.66	9.3×10^{-6}
sex	1	0.50	108	0.00015	3410.52	$< 1 \times 10^{-28}$
experiment × dispersal	2	0.002	108	0.00015	14.12	3.5×10^{-6}
experiment × sex	2	0.03	108	0.00015	203.95	$< 1 \times 10^{-28}$
dispersal × sex	1	6.8×10^{-5}	108	0.00015	0.46	0.50
experiment × dispersal × sex	2	0.0003	108	0.00015	1.96	0.15

Table 8. Pooled three-way ANOVA for desiccation resistance data over Experiments 1, 2 and 3.

Factor	df Effect	MS Effect	df Error	MS Error	F value	p-value
experiment	2	301.73	108	1.85	162.86	$< 1 \times 10^{-28}$
dispersal	1	13.33	108	1.85	7.2	0.008
sex	1	726.19	108	1.85	391.97	$< 1 \times 10^{-28}$
experiment × dispersal	2	174.59	108	1.85	94.24	$< 1 \times 10^{-6}$
experiment × sex	2	30.23	108	1.85	16.32	6.4×10^{-7}
dispersal × sex	1	14.14	108	1.85	7.64	0.007
experiment × dispersal × sex	2	29.97	108	1.85	16.18	7.1×10^{-6}

Table 9. Post-hoc (Tukey's HSD) results for *experiment × dispersal × sex* interaction for desiccation resistance data.

	Tukey's HSD p-value for difference between dispersers and non-dispersers	
	Males	**Females**
Experiment 1	0.99	1.2×10^{-4}
Experiment 2	0.99	0.009
Experiment 3	1.2×10^{-5}	7.7×10^{-6}

Table 10. Pooled three-way ANOVA for exploratory tendency data over Experiments 1, 2 and 3.

Factor	df Effect	MS Effect	df Error	MS Error	F value	p-value
experiment	2	1021.94	372	59.85	17.07	8.0×10^{-8}
dispersal	1	2.34	372	59.85	0.039	0.84
sex	1	5642.67	372	59.85	94.28	$< 1 \times 10^{-28}$
experiment × dispersal	2	102.20	372	59.85	1.71	0.18
experiment × sex	2	512.94	372	59.85	8.57	2.3×10^{-4}
dispersal × sex	1	263.34	372	59.85	4.40	0.04
experiment × dispersal × sex	2	164.84	372	59.85	2.75	0.06

Table 11. Post-hoc (Tukey's HSD) results for *experiment × dispersal × sex* interaction for exploratory tendency data.

	Tukey's HSD p-value for difference between dispersers and non-dispersers	
	Males	**Females**
Experiment 1	0.09	0.99
Experiment 2	0.99	0.99
Experiment 3	0.99	0.99

4 DISCUSSION

4.1 Dispersal syndromes in *D. melanogaster* are sex-specific

In sexually dimorphic species, the associations among life-history traits are often sex-specific (Prasad & Joshi 2003). However, despite the increasing realization that dispersal is a key life-history component (Bonte & Dahirel 2017), sex differences in dispersal syndromes remain poorly understood. This is even more surprising in light of the fact that sex differences in dispersal traits (i.e. sex-biased dispersal) are quite ubiquitous and well-studied, both empirically and theoretically (reviewed in Trochet *et al.* 2016; Li & Kokko 2019)). Comparing the traits of dispersers and non-dispersers, we show that the dispersal syndrome in *Drosophila melanogaster* differs substantially between males and females (Fig. 5). Across the three experiments, these sex differences were apparent in the life-history traits (body size and desiccation resistance) as well as the behavioural trait (exploratory tendency) (Fig. 4). As the environment is a crucial determinant of ecological trait-associations (Jessup & Bohannan 2008), we next investigated whether and how the sex differences in dispersal syndrome vary across environments.

4.2 Nutrition level can modulate the sex differences in dispersal syndrome

Any ecological trait association is likely to change in the face of environmental shifts. This is also expected in the case of dispersal syndromes (Ronce & Clobert 2012; Cote *et al.* 2017), and recent studies have devoted much effort into delineating the contributions of genetic and environmental factors in shaping the dispersal syndromes, most notably in butterflies (Saastamoinen *et al.* 2012; Legrand *et al.* 2016). However, it is not known how sex differences in dispersal syndromes respond to environmental changes.

Comparing the results from Experiment 1 and 2, we show that the pattern of sex differences in *Drosophila* dispersal syndrome varied across the two nutritional regimes (Fig. 5), quite interestingly, in different directions for different traits. Low nutrition obscured the existing sex difference in body size (*cf.* Fig. 4A and 4B), maintained the sex difference in desiccation resistance (*cf.* Fig. 4D and 4E), and uncovered a sex difference in exploratory tendency (*cf.* Fig. 4G and 4H). While sex differences in life-history traits have already been shown to change with nutrition availability (Kolss *et al.* 2009), the observation that diet changes sex differences in a behavioural trait (exploratory tendency) is novel to the best of our knowledge. In addition to supporting the already established notion that nutrition levels influence

overall behaviour (Han & Dingemanse 2017; Strang *et al.* 2017), it demonstrates the effect of nutrition on the sex differences in behaviour.

In terms of body size, we found that dispersers of both sexes were larger than non-dispersers in Experiment 1 (Fig. 4A), which is consistent with the results of trait-association studies across taxa (Dingle *et al.* 1980; Sutherland *et al.* 2000), including *Drosophila melanogaster* (Roff 1977). In terms of desiccation resistance, the female dispersers fared better than their non-dispersive counterparts in both nutritional regimes, whereas males showed similar desiccation resistance irrespective of their dispersal status (Fig. 4D and 4E). The exploratory tendency of dispersers was not higher than that of the non-dispersers in any of the nutritional regimes or sexes, indicating that exploratory tendency was not an essential prerequisite for successful dispersal in our experimental setup. This is unexpected because even in our setup, the flies had to locate the single aperture in the source through exploration (i.e. there were no cues that guided them towards the exit). The lack of greater exploratory tendencies in the dispersers is also in contrast with most of the literature on personality-dependent dispersal, where behaviours such as exploration are expected to confer an advantage during dispersal (Korsten *et al.* 2013; Cote *et al.* 2017). More surprisingly, dispersive males even showed a lower exploratory tendency than non-dispersive males under low nutrition (Fig. 4G). In short, although environment is known to play a crucial role in the shaping of dispersal syndrome (Saastamoinen *et al.* 2012), we show that the pattern of sex differences in dispersal syndrome can vary across different environmental contexts (here, nutrition level), with possibilities ranging from obscuring of existing sex differences to appearance of new ones (Fig. 5). Hence, care must be taken while extrapolating observed dispersal syndromes as well as any underlying sex differences to other environmental conditions.

Over long time scales, same-generation trait associations are liable to change in three ways: first, due to changes in the environment alone, second, via evolutionary changes in the populations, and finally, through an interaction of the two mechanisms. Understandably, it is quite difficult to disentangle these three causes of changes in dispersal syndrome in natural populations. To address the issue of changed environments confounding the possible effects of phenotypic evolution, we next investigated the evolutionary dispersal syndrome using dispersal-selected populations that have been maintained under identical laboratory conditions of light and temperature as Experiments 1 and 2, and selected using the same dispersal setup that was used for segregation of dispersers/non-dispersers in Experiments 1 and 2. Furthermore, the flies in the evolved populations were reared under standard

nutrition (as in Experiment 2), and were assayed under conditions identical to those in Experiments 1 and 2. Thus, using highly controlled and reproducible laboratory conditions, we sought to remove the effects of environment as a confounding factor as much as practicable.

4.3 Ecological and evolutionary dispersal syndromes can differ substantially, even under similar environmental conditions

The notion that ecological dispersal syndromes, obtained from same-generation association studies, can potentially predict the trait associations after dispersal evolution (Ronce & Clobert 2012) has never been investigated empirically. Here, we investigated the sex-specific changes in dispersal syndrome due to dispersal evolution by comparing the dispersal syndromes among males and females of dispersers and non-dispersers in Experiments 2 with the corresponding syndromes of males and females of selected and control populations in Experiment 3. In Experiment 2, we found a sex-specific difference of dispersal syndrome in terms of body size, with dispersers being significantly larger only in females but not in males. However, Experiment 3 revealed that the body size of the males and females of the dispersal selected populations were not different from the males and females of the VBCs (Fig. 4C, Table 1), thus suggesting a change in the pattern of sex-specific dispersal syndrome. More critically, contrary to the expectations from literature (Roff 1977; Dingle *et al.* 1980; Sutherland *et al.* 2000), neither males nor females in dispersal-selected populations had a greater body size than the corresponding controls. One potential reason for this might be the fact that under our standard nutrition, the flies have access to *ad libitum* resources at any given point, and therefore do not need to acquire and store greater amount of resources in the body. This automatically leads to the prediction that if selection for dispersal were to happen under nutrient-limited condition, the dispersal syndrome with respect to body size might be very different.

For desiccation resistance, a number of interesting and contrasting observations were obtained in Experiment 3. First, contrary to the ecological dispersal syndrome (Experiment 2, Fig. 4E), desiccation resistance of the dispersal-selected flies (VBs) was significantly lower than corresponding controls (VBCs) for both sexes. This suggests that, at an evolutionary time scale, dispersal traded-off with desiccation resistance. Second, although the *dispersal* × *sex* interaction was significant in Experiment 3, the direction had reversed completely, i.e. there was a reduction in

desiccation resistance of VBs, which was more apparent in females than in males (*cf.* Fig. 4E and 4F, Table 4). Third, despite having a comparable body size, the desiccation resistance of VBs was significantly lower compared to the VBCs. This contradicts a well-documented positive correlation between body size and desiccation resistance (Parsons 1970; Clark & Doane 1983). These observations make sense when we note that VBs have evolved significantly greater levels of locomotor activity (Tung *et al.* 2018b). The constitutively higher activity of the VB flies is likely to exhaust them faster in the absence of food and moisture, thus making them more susceptible to desiccation stress, particularly given that their body size is comparable to that of VBC flies (Fig. 4C).

Differences between the ecological and evolutionary dispersal syndromes were also observed for exploratory tendency, a key component of behavioural syndromes and personalities in animals (Korsten *et al.* 2013; Cote *et al.* 2017). There was no difference between dispersers and non-dispersers in Experiment 2, whereas dispersal-selected flies of both sexes in Experiment 3 evolved a significantly higher exploratory tendency (Fig. 5, Table 5). This is in accordance with the results of studies on natural populations reporting the presence of more exploratory individuals at range-expansion fronts (Liebl & Martin 2012) and in invasive species (Rehage *et al.* 2005; Russell *et al.* 2010).

Together, these results highlight that the short-term association of traits observed in same-generation studies (i.e. ecological dispersal syndrome) may not be a good predictor of the long-term evolutionary changes (i.e. evolutionary dispersal syndrome), even if the environment remains relatively unchanged.

There are two potential reasons for this observation. First, in addition to the obvious role played by additive genetic variance, the non-additive components of genetic variance and environmental effects could play a role in shaping the same-generation phenotypic correlations. However, during the course of evolution, only the heritable components of the phenotypic variation get transmitted to the future generations. The evolution of correlated traits is also shaped by phenomena such as pleiotropy and linkage disequilibrium. Second, there is a theoretical possibility of some uncontrolled environmental variation acting on the dispersal-selected and control populations which could contribute to the divergence between ecological and evolutionary dispersal syndromes. However, given that environments under laboratory conditions are tightly controlled, such a situation is extremely unlikely.

The idea that same-generation phenotypic associations may not always be a good predictor of the direction of evolutionary responses has been empirically examined before (Leroi *et al.* 1994). However, it had never been tested in the context of dispersal syndromes or their sex differences.

While studies on dispersal syndromes have discussed the labile nature of dispersal syndromes in the face of evolution (Ronce & Clobert 2012), the focus has mostly been on the effect of changing environments during the course of evolution (Legrand *et al.* 2016). By demonstrating the differences in ecological and evolutionary dispersal syndromes under a uniform environment, we show that such evolutionary predictions are risky even when environments do not change during evolution.

4.4 Implications for local adaptation

We demonstrate that not only can there be sex differences in dispersal syndromes, but they can also reorient due to environmental changes (here, low nutrition) as well as dispersal evolution by spatial sorting. These findings have several implications in the context of ecological and evolutionary role of dispersal in determining the degree of local adaptation. First, sex differences in dispersal syndromes can lead to indirect demographic consequences. For instance, various studies have reported positive, negative or no correlation of dispersal with body size (Sutherland *et al.* 2000; Stevens *et al.* 2013), mating success (Madsen *et al.* 1993; Gerloff *et al.* 1999; Jack & Fedigan 2004) and fecundity (Guerra 2011; Bonte *et al.* 2012), traits that have major effects on the temporal dynamics of populations. Sex-specific variations in these traits would further affect the dynamics of populations that are connected by migration (i.e. metapopulations) (Hanski & Gaggiotti 2004). While this kind of non-random (i.e. phenotype-dependent) gene flow across populations has been recently recognized in the context of dispersal syndromes (Cote *et al.* 2017), this observation has not yet been made in the context of syndromic sex differences, probably due to the lack of empirical evidences for the latter. Second, like any other phenotype-dependent dispersal event (Edelaar & Bolnick 2012), sex-specific dispersal syndromes can also have evolutionary consequences. In fact, behavioural syndromes have already been suggested to have sexually dimorphic fitness consequences, including the possibility of generating evolutionary conflict between males and females (Pruitt & Riechert 2009). In addition to such individual-level effects, sex differences in dispersal syndromes would also modulate the degree of habitat-matching in the newly colonized areas, with potential evolutionary consequences for source-sink dynamics

(Holt & Barfield 2015) as well as functioning of metasystems (Jacob *et al.* 2015). Finally, this study reinforces the lability of dispersal syndromes across both environments (Experiments 1 and 2) and evolutionary time scales (Experiments 2 and 3), thus calling into question their utility as predictors of dispersal.

CHAPTER 6

Desiccation stress as a cause and a cost of dispersal in *Drosophila melanogaster*

Highlights

- Desiccation stress acts as a significant cause of dispersal in both sexes
- Significant desiccation cost of dispersal seen in males, but not in females
- Dispersal evolution did not change this sex-bias in the desiccation costs of dispersal
- Females instead paid a higher cost of dispersal in terms of their fecundity

This chapter is being written up as the following research article:

Mishra, A, *Tung, S, Sruti, VRS, Shreenidhi, PM, & Dey, S. Desiccation stress as a cause and cost of dispersal in Drosophila melanogaster. (in prep)*

1 INTRODUCTION

Biological dispersal is often driven by numerous biotic and abiotic causes that promote movement across space (Matthysen 2012). However, the very process of movement can be costly to the dispersing organisms in several ways (Bonte & Dahirel 2017). Investigating the causes and costs of dispersal can therefore help understand the constraints faced by individual organisms (Ronce & Clobert 2012), as well as their potential effects on the population- and community-level consequences of dispersal (Bowler & Benton 2005).

Among the many possible factors that can influence dispersal as a cause or a cost, stress is a common one. On the one hand, abiotic and biotic stresses can influence dispersal as a cause, by driving movement of individuals away from an area (Matthysen 2012). On the other hand, the process of movement can make the dispersing organisms stressed or increase their susceptibility to stress, as dispersal is often an energy-intensive endeavour (Bonte *et al.* 2012). Thus, stress is likely to play a role as both a cause and a cost of dispersal. However, despite ample evidence across taxa that dispersal is correlated with enhanced body levels of stress hormones such as corticosterone (Silverin 1997; Belthoff & Dufty 1998; Meylan *et al.* 2002), it is typically not easy to tease apart the two distinct roles of stress as a cause and a cost of dispersal. Therefore, while stress can influence dispersal in multiple ways, its exact relationship with dispersal remains largely unclear in most instances.

Desiccation is one of the most commonly faced environmental stresses for numerous taxa (Black & Pritchard 2002; Holmstrup *et al.* 2002; Kranner *et al.* 2008; Holzinger & Karsten 2013). Understandably, desiccation not only affects the physiology of individual organisms (e.g. Gibbs *et al.* 1997; Folk & Bradley 2004; Bazinet *et al.* 2010), but is also an important determinant of species distributions (e.g. Kellermann *et al.* 2009; Rajpurohit *et al.* 2013). Furthermore, organisms' responses to desiccation stress are also important in the context of climate change and its biological implications (Parsons 1991; Hoffmann *et al.* 2003; Tuba *et al.* 2011; Van Heerwaarden & Sgrò 2014). Given that dispersal often serves as the first line of defence against unfavourable environments for many taxa (Gerber & Kokko 2018; Riotte-Lambert & Matthiopoulos 2020), it is crucial to investigate the exact relationship between biological dispersal and desiccation stress.

Furthermore, an often overlooked topic in this context is sex differences. While the relationship among environmental stress, dispersal and sex have been recently discussed (Gerber & Kokko 2018), sex differences in the role of dispersal as a stress-

escape mechanism have typically not been studied. This is not surprising given that, until recently, sex differences in dispersal syndromes had not been highlighted (Legrand *et al.* 2016; Mishra *et al.* 2018a). The presence of pervasive sex differences in life-history and behaviour literature leads us to anticipate some sex differences in the relationship between dispersal and stress as well.

Here, we investigate the relationship between desiccation stress and dispersal of *Drosophila melanogaster* under controlled environmental conditions. Specifically, we asked the following questions: (1) Does desiccation stress act as a cause of dispersal? (2) Does desiccation stress act as a cost of dispersal? (3) Are the answers to the above two questions different for males and females? Specifically, we use an experimental setup that allows us to categorize dispersal-desiccation correlations into cause and cost scenarios. Our results showed that desiccation stress acts as a significant cause for dispersal for both sexes. However, the role of desiccation stress as a cost of dispersal was limited to males, at least under our experimental conditions. Instead, the females showed a significant cost of dispersal in terms of their fecundity. We discuss these results in the context of *Drosophila* physiology, along with their implications for dispersal patterns.

2 METHODS

2.1 Fly populations

We used large, outbred laboratory populations (breeding size ~2400 individuals) of *D. melanogaster* for all the experiments in this study. The ancestry of these populations can be traced back to the IV lines, which were wild-caught in South Amherst, MA, USA (Ives 1970). The single-generation experiments in this study were conducted using a baseline population named DB_4 (Sah *et al.* 2013; Mishra *et al.* 2020b). In addition, we used four dispersal-selected populations (namely, VB_{1-4}) and their corresponding controls, the non-selected populations (VBC_{1-4}), for one experiment. As a result of the ongoing selection for higher dispersal every generation, the VB populations have evolved a higher dispersal propensity and ability (Tung *et al.* 2018b), as well as lower desiccation resistance (Mishra *et al.* 2018a), compared with the VBC populations. All the populations were maintained in 15-day discrete-generation cycles under uniform environmental conditions of 25 °C temperature and 24-h light.

2.2 Dispersal setup

Following previous studies (Mishra *et al.* 2018a; Mishra *et al.* 2018b; Tung *et al.* 2018b; Mishra *et al.* 2020a; Mishra *et al.* 2020b), we used a two-patch dispersal setup for observing fly dispersal. Each dispersal setup comprised a *source* container, a *path* tube and a *destination* container (Fig. 1). In this setup, all the flies for a given treatment/group are first introduced into the *source* container, which opens into a transparent plastic tube (internal diameter ~1 cm) that serves as the *path*. The other end of the path tube leads into the *destination* container, thereby allowing the dispersal of flies from the *source* to the *destination* container, for a fixed duration. Depending on the experiment, the size of the *source* and *destination* containers, as well as the length of the *path* tube, can be customized. A single experiment typically involves multiple such dispersal setups, maintained under uniform environmental conditions. At the end of a dispersal run, these dispersal setups are dismantled, and the flies found in each part (*source*/*path*/*destination*) are used as per the experimental requirements.

2.3 Experiments

We carried out a series of experiments to address various questions related to causes and costs of dispersal. The protocols, type of data obtained and the statistical analyses are presented separately for each experiment below.

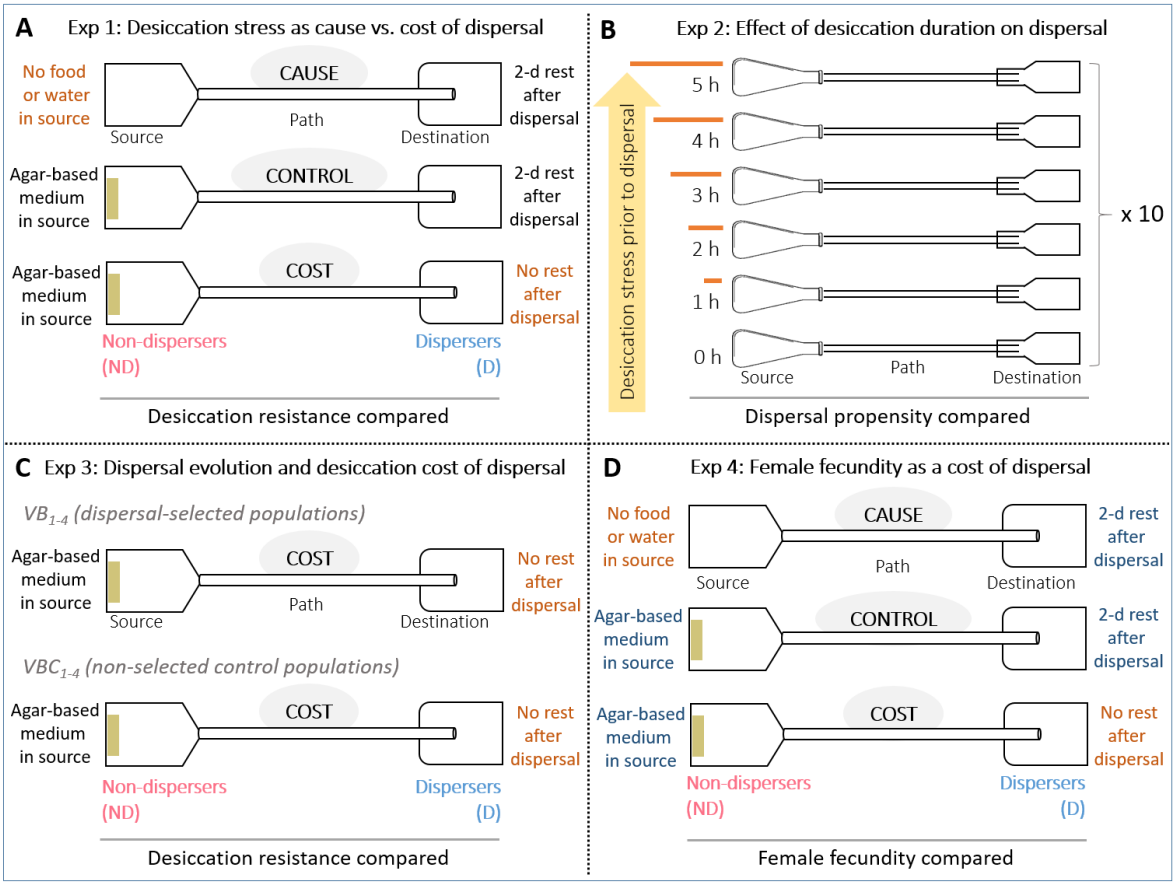

Fig. 1: Schematics of the experimental design. (A) Experiment 1 investigated the role of desiccation stress as a cause vs. cost of dispersal. Using a *source-path-destination* setup, age-matched flies from an outbred baseline population (DB_4) were segregated into non-dispersers (ND) and dispersers (D) under three scenarios: Cause (no food or water in *source*; rest provided after dispersal run), Control (agar-based banana-jaggery medium in *source*; rest provided after dispersal run), and Cost (agar-based banana-jaggery medium in *source*; no rest provided after dispersal run). ND and D flies within each scenario were then assayed for their desiccation resistance. **(B)** Experiment 2 further examined the role of desiccation stress as a cause of dispersal. Groups of age-matched flies from DB_4 population were subjected to different durations of desiccation stress (0–5 h) before being subjected to dispersal assay. Dispersal propensity and temporal dispersal profile were then compared across these treatments. **(C)** Experiment 3 investigated whether the desiccation cost of dispersal differs between populations selected for higher dispersal ($VB_{1–4}$) and their non-selected controls ($VBC_{1–4}$). Desiccation resistance of all eight population blocks was compared under the Cost scenario similar to Experiment 1. **(D)** Experiment 4 examined the role of female fecundity as a cause vs. cost of dispersal. Here, female ND and D flies for the three scenarios (Cause, Control and Cost) were assayed for their fecundity.

2.3.1 Experiment 1: Desiccation stress as cause vs. cost of dispersal

We first examined whether desiccation stress plays a role as a cause and a cost of dispersal in *D. melanogaster*. For this, we started with ~19,200 age-matched (12-day-old from egg collection) adult flies from the DB_4 population that were reared under identical conditions of *ad libitum* food and water. Cylindrical, translucent plastic containers (~1.5 L volume) were used as *source* and *destination*, along with a *path* length of 6 m, to assemble two-patch dispersal setups (described in section 2.2). Batches of the aforementioned DB_4 individuals were then introduced into eight such dispersal setups (~2400 individuals per setup) and allowed to disperse for 5 h. By modulating two factors, i.e. presence of agar-based food (banana-jaggery medium) in the *source* container, and the provision of rest to flies after the dispersal run, we devised three scenarios (Fig. 1A, see explanation in next paragraph): (a) *Cause* scenario, where we could identify whether desiccation stress was a cause of dispersal, (b) *Control* scenario, where desiccation stress was expected to be neither a cause nor a cost of dispersal, and (c) *Cost* scenario, where we could identify whether desiccation stress was a cost of dispersal (Fig. 1A). In each of the three scenarios, the flies that completed dispersal from the *source* to the *destination* were termed as dispersers (D), whereas the flies that were found inside the *source* container were termed as non-dispersers (ND). The flies found in the path at the end of the dispersal run were not used in this experiment.

In the *Cause* scenario, there was no food or water in the *source*, making desiccation stress a likely driver of dispersal away from the source. After the dispersal event, we collected the ND and D flies separately and provided them a 2-day rest with *ad libitum* food and water, so that the D flies could recuperate any energy costs of dispersal run. Thereafter, we assayed 200 ND and 200 D flies (100 males+100 females each) for their desiccation resistance (following Mishra *et al.* 2018a), to assess whether they differed in terms of their inherent desiccation sensitivity (Fig. 1A: Cause scenario). Here the assumption was that the rest of two-days is sufficient to ameliorate any negative effects on desiccation sensitivity (Mishra *et al.* 2018a).

In the *Control* scenario, we provided agar-based banana-jaggery medium in the *source* container during the dispersal run, thereby removing desiccation stress as a possible driver of dispersal. Similar to the *Cause* scenario, the dispersal event was followed by a 2-day rest to both ND and D flies, to offset any energy costs of dispersal (Fig. 1A: Control scenario). Subsequently, we compared the desiccation resistance of 200 ND and 200 D flies, to ascertain if there were any unaccounted-for differences between them, i.e. other than those detected in *Cause* and *Cost* scenarios.

The *Cost* scenario was complementary to the *Cause* scenario. Here, we provided banana-jaggery medium in the *source* container, thereby removing desiccation stress as a cause of dispersal, but did not allow any rest after dispersal. Same as above, we then compared the desiccation resistance of 200 ND and 200 D flies, with any difference attributed to the energy costs of dispersal (Fig. 1A: Cost scenario).

The desiccation data thus collected were analysed together in a single mixed-model ANOVA, with *scenario* (Cause/Control/Cost), *dispersal* (ND/D) and *sex* (male/female) as the fixed factors. As the flies were assayed in single-sex groups of 10 individuals within a vial (following Mishra *et al.* 2018a), we included *vial identity* (1–10) as a random factor that was nested within the *scenario* × *dispersal* × *sex* interaction. Following the ANOVA, we used Tukey's post-hoc test for pairwise comparisons. These analyses, along with the ones described in subsequent sections, were carried out using STATISTICA® v8 (StatSoft. Inc., Tulsa, Oklahoma).

2.3.2 Experiment 2: Effect of desiccation duration on dispersal

Here, we investigated how dispersal changes with the duration of desiccation stress. For this, we segregated age-matched (12-day-old from egg collection) DB_4 flies into multiple groups of 120 individuals (60 males + 60 females) that were subjected to varying durations of desiccation stress (0, 1, 2, 3, 4, and 5 h) before being subjected to dispersal assay in separate dispersal setups. The *source* here was a 100-mL glass flask without any food or water, the *path* length was 2 m, and the destination was a 250-mL plastic bottle. The dispersal assay lasted for 2 h. Following a previous protocol (Mishra *et al.* 2018b; Mishra *et al.* 2020a; Mishra *et al.* 2020b), the experiment was carried out over 10 consecutive days with a fresh set of age-matched flies every day. This allowed us to assay one replicate of every desiccation treatment each day, yielding 10 replicates blocked by *day*. In total, 6000 flies (5 desiccation treatments × 2 sexes × 10 days × 60 flies $treatment^{-1}$ sex^{-1} day^{-1}) were assayed for this experiment. From the dispersal assay, we collected data on dispersal propensity (Friedenberg 2003) and the temporal dispersal profile (similar to Mishra *et al.* 2018b; Mishra *et al.* 2020a; Mishra *et al.* 2020b). To account for any day-to-day microenvironmental variation, we analysed the dispersal propensity data in a randomized complete block design (RCBD), with *day* as the random blocking factor. Therefore, the three-way mixed model ANOVA for dispersal propensity included *desiccation duration* (0, 1, 2, 3, 4, and 5 h) and *sex* (male and female) as fixed factors, and *day* (1–10) as the random factor. The temporal dispersal profile data were similarly analysed, where separate

two-way mixed-model ANOVAs were performed at each time point for males and females, with *desiccation duration* (fixed factor) and *day* (random factor). The family-wise error rates were then controlled using the sequential Holm-Šidák correction (Abdi 2010). As both dispersal propensity and temporal profile data were in the form of proportions, they were arcsine-square root transformed prior to ANOVA (Zar 1999).

2.3.3 Experiment 3: Dispersal evolution and desiccation cost of dispersal

Here, we used dispersal-selected populations (VB_{1-4}), which have a higher dispersal propensity and ability, as well as a lower desiccation resistance, than their non-selected controls (VBC_{1-4}) (Mishra *et al.* 2018a; Tung *et al.* 2018b). In this experiment, we investigated whether the VB and VBC populations differ in their desiccation cost of dispersal. This would help determine if selection for dispersal under desiccated conditions has altered the magnitude of proximate cost paid by dispersers. We subjected ~2400 age-matched individuals per population block (1–4) of each population type (VB/VBC) to segregation into ND and D individuals under the *Cost* scenario as described in section 2.3.1. Thereafter, we assayed 100 males and 100 females (in groups of 10 individuals/vial) from each of the eight populations (VB_{1-4} and VBC_{1-4}) for their desiccation resistance (following Mishra *et al.* 2018a). The entire desiccation resistance data were analysed using a mixed-model ANOVA, with *dispersal selection* (VB/VBC), *dispersal* (ND/D) and *sex* (male/female) as fixed factors, and *population block* (1–4) and *vial identity* (1–10) as random factors. Here, *vial identity* was nested inside the *dispersal selection* × *dispersal* × *sex* × *population block* term. Following the ANOVA, we used Tukey's post-hoc test for pairwise comparisons.

2.3.4 Experiment 4: Female fecundity as cause vs. cost of dispersal

This experiment aimed to examine whether the lack of an oviposition surface served as a cause of dispersal in female flies and whether females paid a dispersal cost in terms of their fecundity. The female flies in this experiment were from the same ND and D groups of flies that were segregated in Experiment 1, giving rise to: (a) *cause* scenario, defined by the lack of suitable oviposition site in *source* container, (b) *control* scenario, with suitable oviposition surface (i.e. banana-jaggery medium) in the *source* and provision of rest after dispersal run, and (c) *cost* scenario, where no rest is provided and flies were assayed for their fecundity immediately after dispersal (Fig. 1D). We measured the female fecundity as the number of eggs laid

over a 12-h period (following Tung *et al.* 2018a), with the ND and D flies for each scenario assayed together. The entire fecundity data were analysed together using a two-way ANOVA, with *scenario* (*cause*, *control*, and *cost*) and *dispersal* (ND and D) as the fixed factors.

3 RESULTS

3.1 Desiccation stress as a cause vs. cost of dispersal

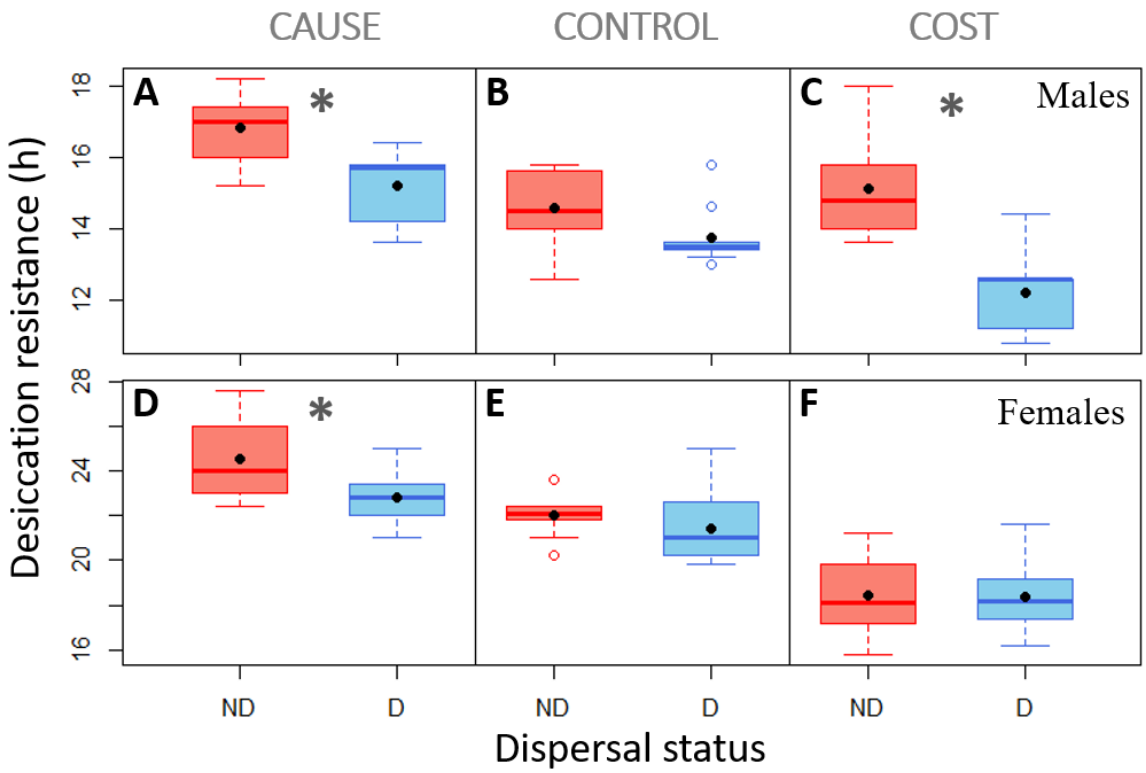

Fig. 2: Desiccation stress as cause vs. cost of dispersal (Experiment 1). Desiccation resistance for non-disperser (ND) and disperser (D) flies from an outbred, baseline population (DB_4), under three scenarios: Cause, Control and Cost. Data for males and females are presented in the top and bottom rows, respectively. Edges of the boxplots represent the 25th and 75th percentiles of the data. The black dots represent means and the horizontal lines inside the boxes represent medians. Asterisks (*) indicate a significant difference (Tukey's $p < 0.05$) between ND and D flies within a given panel. Note that the scale of the Y-axis differs between the males (top row) and the females (bottom row).

Desiccation resistance data from Experiment 1 showed a significant *scenario* × *dispersal* × *sex* interaction ($F_{2,1070} = 3.59$, $p = 0.031$). Tukey's post-hoc analysis for this interaction revealed a number of results. First, there was no difference in the desiccation resistance of dispersers vs. non-dispersers for the control case (Tukey's p for males = 0.87; Tukey's p for females = 0.99) (Fig. 2B, 2E). This was expected, as all these flies had access to ad libitum food and water in the source container, as well as a 2-day rest after the dispersal event. Second, dispersers in the cause scenario had a lower desiccation resistance than non-dispersers (Tukey's p for males = 0.039, d = 1.63 (large); Tukey's p for females = 0.032, d = 1.10 (large)) (Fig. 2A, 2D). This

suggests that desiccation stress served as a cause of dispersal in both sexes. Third, while males experienced a cost of dispersal in terms of their desiccation resistance (Tukey's $p = 1.8 \times 10^{-5}$, $d = 1.21$ (large)), no such cost was seen in females (Tukey's $p > 0.99$) (Fig. 2C, 2F).

3.2 Desiccation stress as a cause of dispersal in both sexes

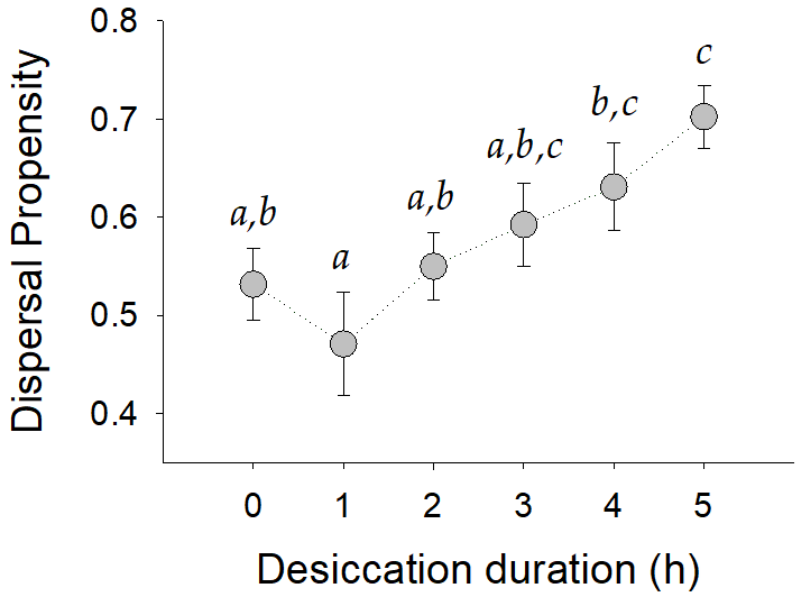

Fig. 3: Effect of desiccation duration on dispersal propensity (Experiment 2). Dispersal propensity (± SE) for age-matched flies from an outbred baseline population (DB_4) subjected to desiccation stress for different durations (0–5 h). Each point represents the average of 10 replicates (each with 120 individuals). Means with the same lower-case alphabets are not significantly different from each other. See Table 1 for the exact Tukey's p values and the associated effect sizes.

The role of desiccation stress as a cause of dispersal was further investigated in Experiment 2. Analysis of data from this experiment revealed a significant effect of *desiccation duration* on the dispersal propensity ($F_{5,99} = 5.71$, $p = 1.1 \times 10^{-4}$). Tukey's post-hoc analysis revealed an increasing trend of dispersal propensity with increasing duration of desiccation stress (Fig. 3; Table 1). Moreover, the *desiccation duration* × *sex* interaction was not significant ($F_{5,99} = 0.59$, $p = 0.70$), indicating that this trend was symmetric across males and females. Moreover, males and females had similar temporal dispersal profiles, and no significant differences were detected across any treatments for any of the time points (Fig. 4). Therefore, the results from both Experiments 1 and 2 suggested that desiccation stress served as a cause of

dispersal in both sexes, with longer durations of desiccation leading to greater dispersal.

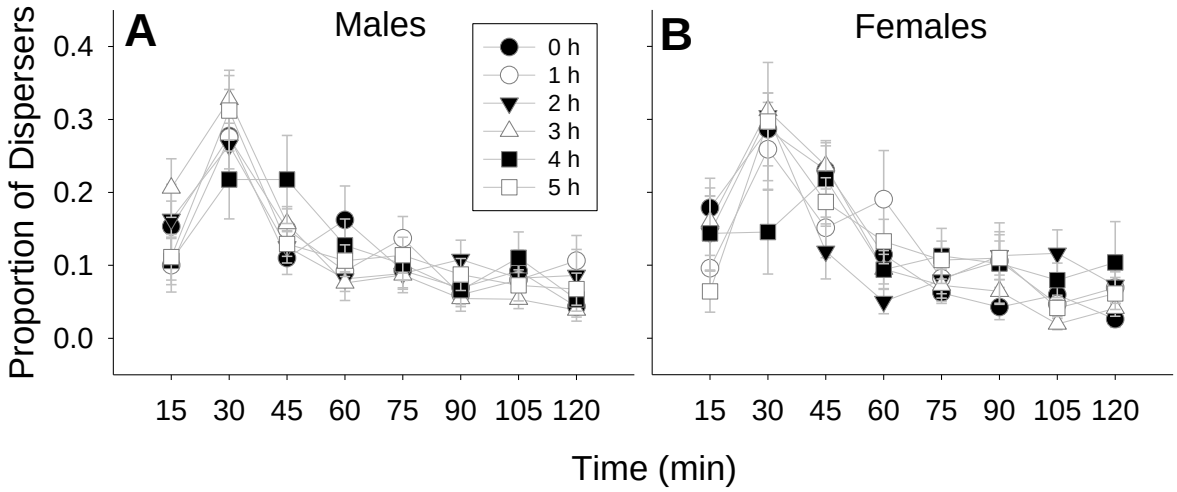

Fig. 4: Temporal dispersal profile of males and females (Experiment 2). Proportion of dispersers (± SE) obtained during each of the 15-min windows, for males and females subjected to desiccation stress for different durations (0–5 h).

Table 1. Pairwise comparisons of dispersal propensity for the desiccation-duration treatments in Experiment 2. For significant pairwise differences (Tukey's $p < 0.05$), effect sizes (Cohen's *d*) are computed.

* denotes a significant pairwise difference (Tukey's $p < 0.05$).

Pairwise comparison	Tukey's p	Cohen's *d*	Effect size interpretation
0 h – 1 h	0.736639	-	-
0 h – 2 h	0.999614	-	-
0 h – 3 h	0.805291	-	-
0 h – 4 h	0.329780	-	-
0 h – 5 h	0.010559*	1.108280	Large
1 h – 2 h	0.535305	-	-
1 h – 3 h	0.096330	-	-
1 h – 4 h	0.011524*	0.731080	Medium
1 h – 5 h	0.000190*	1.186380	Large
2 h – 3 h	0.933709	-	-
2 h – 4 h	0.521928	-	-
2 h – 5 h	0.026958*	1.036640	Large
3 h – 4 h	0.971132	-	-
3 h – 5 h	0.252996	-	-
4 h – 5 h	0.719592	-	-

3.3 Desiccation stress as a sex-biased cost of dispersal

Next, we examined the role of desiccation stress as a cost of dispersal using four dispersal-selected populations (VB_{1-4}) and their corresponding non-selected controls (VBC_{1-4}) (Experiment 3). Desiccation resistance data from this experiment revealed a significant *dispersal* × *sex* interaction ($F_{1,2838} = 23.38$, $p = 0.017$), with males experiencing a relatively larger desiccation cost of dispersal (Tukey's $p = 7.7 \times 10^{-6}$, $d = 1.86$ (large)) (Fig. 5A, 4B) than females (Tukey's $p = 1.4 \times 10^{-5}$, $d = 0.42$ (small)) (Fig. 5C, 4D). Moreover, the *dispersal selection* × *dispersal* × *sex* interaction was not significant ($F_{1,2838} = 0.15$, $p = 0.73$), indicating that this result was consistent for both control (VBC) and dispersal-selected (VB) populations.

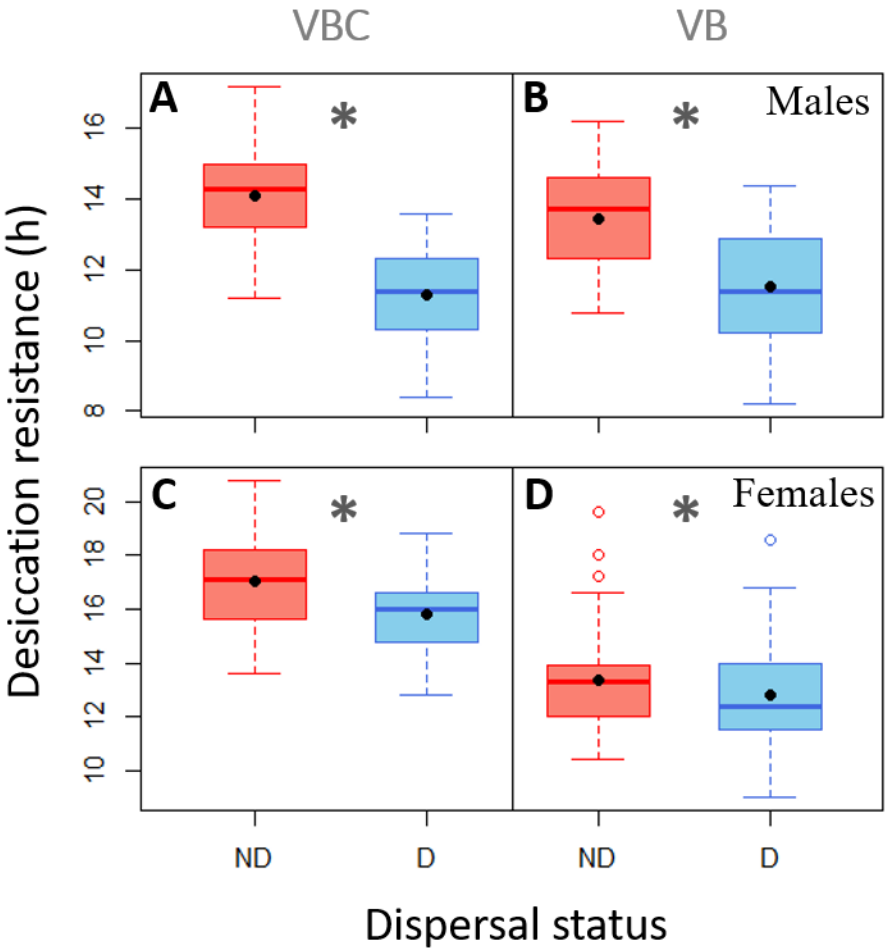

Fig. 5: Dispersal evolution and desiccation cost of dispersal (Experiment 3). Desiccation resistance of non-dispersers (ND) and dispersers (D) from VB_{1-4} (dispersal-selected) and VBC_{1-4} (control) populations. Data for males and females are presented in the top and bottom rows, respectively. Edges of the boxplots represent 25th and 75th percentile of the data. Asterisks (*) indicate a significant difference (Tukey's $p < 0.05$) between ND and D flies within a given panel.

3.4 Significant cost of dispersal for females in terms of fecundity

As minimal or no desiccation cost of dispersal was observed for females (Sections 3.1 and 3.3), we investigated if there was a reproductive cost of dispersal for the females (Experiment 4). Analysis of the female fecundity data from this experiment revealed a significant *scenario* × *dispersal* interaction ($F_{2,233} = 6.49$, $p = 0.0018$). Tukey's post-hoc analysis for this interaction revealed no significant difference between dispersers and non-dispersers under the control (Tukey's $p > 0.99$) (Fig. 6B) and cause (Tukey's $p = 0.48$) scenarios (Fig. 6A), but a significant difference in the cost scenario: disperser females had a lower fecundity than non-disperser females (Tukey's $p = 0.02$, $d = 0.68$ (medium)) (Fig. 6C). Therefore, we concluded that female flies pay a cost of dispersal in terms of their fecundity.

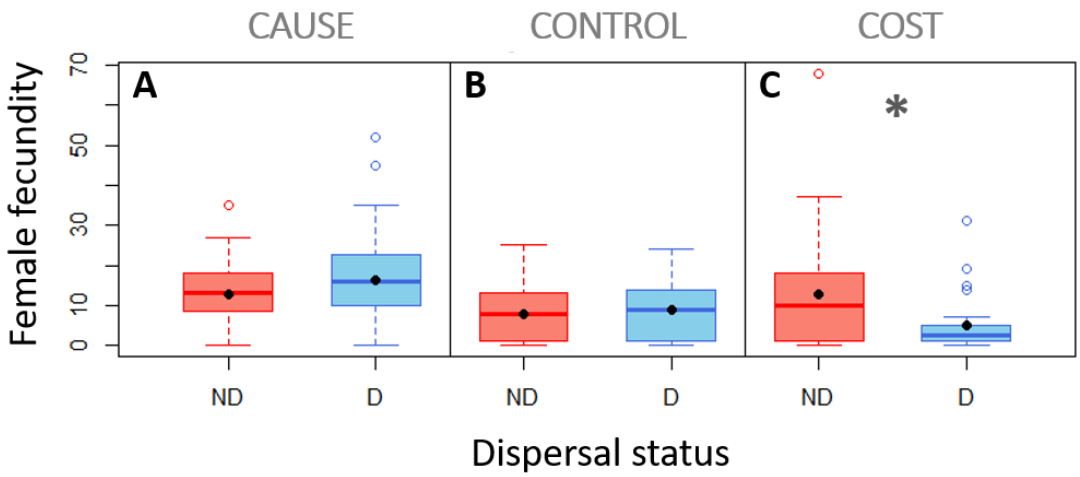

Fig. 6: Female fecundity as cause vs. cost of dispersal (Experiment 4). Female fecundity for non-disperser (ND) and disperser (D) flies from an outbred, baseline population (DB_4), under three scenarios: Cause, Control and Cost. Edges of the boxplots represent the 25th and 75th percentiles of the data. Asterisks (*) indicate a significant difference (Tukey's $p < 0.05$) between ND and D flies within a given panel.

4 DISCUSSION

4.1 Desiccation stress as a cause of dispersal in both sexes

Environmental stress, among other things, can serve as a major cause of biological dispersal. At the same time, the very process of dispersal can be stressful to the individuals. When monitored after a dispersal event, the stress-resistance ability of organisms is often found to be lower (Graves *et al.* 2004). This decrease can come about in three different ways. First, the dispersers might be the ones that were more susceptible to the stress, and therefore they dispersed. Second, even if the stress resistance of the dispersers is inherently similar to that of the non-dispersers, the energy spent in the act of dispersal reduces the stress-resistance ability of the former. Third, it might be an interaction of the two. Unfortunately, these questions are very difficult to answer, particularly when there is no way of distinguishing *a priori* between a disperser and a non-disperser. Here, we investigated this complex relationship using desiccation as the type of stress and fruit flies as a model system. Our experimental design allowed us to explicitly control for other confounds when a particular aspect of the desiccation-dispersal relationship was being examined.

To begin with, Experiment 1 revealed that the disperser (D) flies had a lower desiccation resistance than the non-disperser (ND) flies under the *Cause* scenario (Figs. 2A and 2D). Comparing the results with the *Control* scenario, which showed no difference between ND and D flies (Figs. 2B and 2E), we could conclude that desiccation stress indeed served as a significant driver of dispersal for both male and female flies. This is in line with the expectation from literature that dispersal is one of the foremost ways for escaping unfavourable conditions (Gerber & Kokko 2018), not only in animal taxa (Cremer & Heinze 2003; Riotte-Lambert & Matthiopoulos 2020) but also in plants (Martorell & Martínez-López 2014). While this is not a surprising result, our study demonstrates it explicitly using a unique setup, where we were able to control for the possible confound of desiccation as a cost of dispersal (Fig. 1A).

Going a step further, we demonstrate in Experiment 2 how *Drosophila* dispersal changes with increasing desiccation stress (Fig. 3). Given that desiccation resistance is highly correlated with glycogen content in fruit flies (Gibbs *et al.* 1997), one might have expected a decrease in dispersal at high desiccation durations, where the flies likely faced a severe depletion of their glycogen reserves (Folk & Bradley 2004; Bazinet *et al.* 2010). Surprisingly however, this was not the case in Experiment 2, where flies of both sexes showed a nearly monotonic increase in their dispersal

propensity with increasing desiccation stress (Fig 3). This means that, at least for the durations of desiccation stress (up to 5 h) imposed in Experiment 2, the flies were in a state to successfully initiate dispersal. However, as a corollary, it also means that organisms often do not disperse until the stress turns acute, which may make them more susceptible to dispersal-related risks and costs (see Section 4.2). It is possible that this delay in emigration could be a function of how long it takes to initiate a physiological response to the stress. Overall, we speculate that the ability to perceive stress would play a role in shaping the dispersal-mediated escape response from stressful habitats.

Since dispersal is also known to incur various costs (reviewed in Bonte *et al.* 2012), the process of dispersal itself can induce stress or increase the susceptibility of dispersing individuals to stress. We explored the potential desiccation cost of dispersal using the *Cost* scenario, in Experiments 1 and 3.

4.2 Sex-biased cost of dispersal in terms of desiccation stress

Given that active dispersal involves expenditure of energy, it is likely that flies spend a part of their glycogen reserves during dispersal (Graves *et al.* 2004), which can reduce their desiccation resistance following a dispersal event. Experiment 1 confirmed a cost of dispersal in terms of their desiccation resistance, although it was not symmetric between the two sexes. A significant desiccation cost of dispersal was observed for males (Fig. 2C) but not for females (Fig. 2F) in the DB_4 population. Similarly, Experiment 3 revealed that the desiccation cost of dispersal was much higher in males (Fig. 5A, 4B) than in females (Fig. 5C, 4D) (see section 3.3 for the exact effect sizes). As both dispersal-selected (VB) and non-selected control (VBC) flies showed a male-biased desiccation cost, we concluded that the evolution of dispersal did not alter the immediate desiccation cost of dispersal between these populations..

A potential explanation for the sex bias in desiccation cost is the sexual dimorphism in body size and desiccation resistance of *D. melanogaster* adults. A positive association between desiccation resistance and body size is well documented in adult fruit flies (Parsons 1970; Clark & Doane 1983). Given that female fruit flies are typically larger than their male counterparts, they typically tend to have a higher desiccation resistance as well (Gibbs *et al.* 1997; Matzkin *et al.* 2007; Mishra *et al.* 2018a). As a result, the females likely had greater resources to begin with, which allowed them to successfully undertake dispersal without paying a high desiccation

cost. This is also congruent with the fact that dispersal evolution has not led to a change in the body size of VB females relative to their VBC controls (Mishra *et al.* 2018a; Tung *et al.* 2018a).

It is possible that the dispersal cost for females manifests not in terms of their somatic maintenance (here, desiccation resistance), but instead their reproductive potential. This is in line with the results of several life-history studies on trade-offs that show a reproductive cost instead of somatic costs in females (Miyatake 1997; Ghalambor & Martin 2001; Djawdan *et al.* 2004; Muller-Landau 2010). In such cases, female fecundity is often one of the first traits to exhibit this cost. Given the energy-intensive nature of active dispersal (as evidenced by the dispersal cost borne by males in this study), female fecundity could show a cost of dispersal. Therefore, we next investigated the association between female fecundity and dispersal.

4.3 Fecundity cost of dispersal for female flies

The relationship between dispersal and fecundity varies across taxa. A negative association between dispersal and fecundity has been reported in several wing-dimorphic insects (reviewed in Guerra 2011), wing-monomorphic insects (reviewed in Tigreros & Davidowitz 2019), as well as other taxa such as *C. elegans* (Friedenberg 2003). These results are typically explained as a developmental or energetic cost of dispersal in terms of fecundity. In contrast, a positive association between dispersal and fecundity has been observed in many mammalian taxa (reviewed in Stevens *et al.* 2014). Here, the typical explanation is twofold. First, individuals with better body condition, including higher fecundity, could be better able to complete dispersal. Second, high fecundity could lead to high dispersal via increased kin competition in a given habitat. Of course, it is also possible that the dispersal-fecundity relationship, like other dispersal-trait associations, is modulated by the environmental context (e.g. Legrand *et al.* 2016; Mishra *et al.* 2018a). For instance, the fecundity cost of dispersal may be particularly strong under limiting resources. Similarly, the positive association between dispersal and fecundity might be altered by the population density and level of resources in the originating patch (e.g. Einum *et al.* 2006). Therefore, experiments under controlled conditions, which can take the ecological context into account, can provide important insights into the relationship between fecundity and dispersal.

Experiment 4 revealed that, while there was no difference under the *Cause* and *Control* scenarios, D females had a significantly lower fecundity than ND flies in the

Cost scenario (Fig. 6). What makes our result interesting is that females showed a fecundity cost before the somatic cost of dispersal, at least in terms of desiccation resistance (*cf.* Figs. 2F and FC). A plausible explanation for this is that, under stressful conditions, individuals may prioritize survival over potential reproduction. This has been observed in other life-history traits as well, where allocation of resources into somatic maintenance can, at times, take priority over reproductive investment (e.g. Miyatake 1997; Ghalambor & Martin 2001; Djawdan *et al.* 2004; Muller-Landau 2010). In particular, given that dispersal is a key life-history trait (Bonte & Dahirel 2017) with several potential costs (Bonte *et al.* 2012), the fecundity trade-off observed here is in line with the observations for other wing-monomorphic insects (Tigreros & Davidowitz 2019).

4.4. Implications

Our results revealed desiccation as a cause of dispersal for both sexes in *Drosophila melanogaster*, and dispersal propensity of both male and female flies increased with increasing desiccation duration. In addition, we observed a male-biased cost of dispersal in terms of desiccation resistance, while the female flies paid a fecundity cost of dispersal. We discuss some implications of our results below.

First, these results demonstrate that the relationship between stress and dispersal is likely complicated. On one hand, stress is likely to drive dispersal of individuals away from an area. On the other hand, dispersing individuals incur a further cost of dispersal in terms of increased stress. Therefore, early dispersers from a population may be the least stress-tolerant individuals. In contrast, highly stress-tolerant individuals could delay emigration in response to a stress. As a result, if dispersal occurs across habitats with high connectivity, stress-intolerant individuals may have the highest dispersal propensity (e.g. Fig. 3). However, if the inter-habitat connectivity is poor, only the relatively stress-resistant individuals in a population would be able to undertake dispersal successfully by surviving the large dispersal costs.

Second, sex differences in the somatic costs of dispersal may effectively lead to instances of sex-biased dispersal, even if a similar number of male and female individuals emigrate from a given area. This is because the stress-sensitive sex (e.g. males in the current study) may not be able to complete dispersal as successfully as the stress-resistant sex (here, females). As a result, in the species where mating occurs after a dispersal event, such differences can lead to a skew in the local sex

ratio of the dispersed population and consequently mate limitation. Moreover, the sex-biased nature of dispersal costs can result in demographic consequences through dispersal syndromes (Mishra *et al.* 2018a; Shaw *et al.* 2018). For instance, if the fecundity of immigrant females in a new area is reduced as a consequence of dispersal, then they may not be able to compete with the resident females in that area. As a result, the apparent prioritization of fitness cost over somatic cost in females, as observed here, can hamper their settlement ability in a new habitat.

Finally, while dispersal is often considered an effective escape route against environmental stress (Boeye *et al.* 2013; Travis *et al.* 2013), it might not be enough to offset the fitness reduction caused by changing climatic conditions (Buckley *et al.* 2013). The situation might worsen further with dispersal-associated costs that hamper the stress tolerance of individuals and their biological fitness (Cheptou *et al.* 2008). Consequently, there is a need to incorporate information on the physiological condition of dispersers in models that consider dispersal as a mode of escape from stressful habitats.

CHAPTER 7

Conclusions

In this thesis, I examined some facets of biological dispersal using microcosm studies with *Drosophila melanogaster*. Together, these chapters addressed both population- and individual-level phenomena associated with dispersal, thereby encompassing three major themes.

The first overarching theme of this thesis was sex differences in dispersal. This encompassed sex-biased dispersal (SBD), sex differences in density-dependent dispersal (DDD), mate-finding dispersal, and sex-specific dispersal syndromes. While SBD remains the most commonly studied aspect of the dispersal-sex relationship, I demonstrate its sensitivity to both ecological (Chapters 2 and 3) and evolutionary contexts (Chapter 4), even under uniform abiotic conditions. To my knowledge, these findings constitute the first empirical evidence of a switch in the direction of SBD for the same population. These changes were partly a result of the interaction between DDD and SBD, something that had been theoretically proposed (Gilroy & Lockwood 2012; Trochet *et al.* 2016) but never empirically shown. Finally, by highlighting the extent of sex differences in dispersal syndromes (Chapters 5 and 6), I attempted to expand the focus of sex differences in movement beyond the most commonly studied topic, i.e. SBD. I speculate that sex-specific adaptations to movement might be the norm rather than the exception in sexually dimorphic species, and understanding them could substantially improve our understanding of movement patterns in mixed-sex populations.

The second running theme through the chapters was the effect of environment on dispersal. Unsurprisingly, the external environment is a prominent factor that influences dispersal patterns, as evidenced by the multiple studies that showcase 'context-dependent dispersal' (*sensu* Clobert *et al.* 2012). As the studies in this thesis involved microcosm experiments, I could use a bottom-up approach to study the role of environmental factors in various aspects of dispersal. In other words, these experiments involved a strictly defined abiotic and biotic environment, where I was able to tinker with a single environmental factor at a time to study its effect on dispersal or its correlates. This included the population density (Chapters 2 and 4), mate availability (Chapters 2 and 3), nutrition level (Chapter 5), and water availability (Chapter 6). Although these examples represent just a subset of the environmental factors that potentially shape dispersal (Matthysen 2012), my rationale for using them was to examine specific hypotheses in each context. While some of the results thus obtained were intuitive or expected (based on the extant literature), a few others were surprising or seemingly counterintuitive. For instance, stronger DDD with a longer exposure to the density treatments was intuitive (Chapter 2), but a negative DDD in the absence of resources was unexpected (Lambin 1994; Matthysen 2005; Kokko & Rankin 2006). Similarly, significant mate-finding dispersal in response to skewed sex ratios was expected in males, but based

on the mate-finding literature (Shaw & Kokko 2014; Fromhage *et al.* 2016), it was a counterintuitive observation for the female flies (Chapter 3). Finally, male flies under low nutrition showed a negative association between dispersal and exploratory tendency (Chapter 5), even though the latter is considered a crucial behavioural trait for dispersal (Cote *et al.* 2010; Korsten *et al.* 2013; Tung *et al.* 2018a). Overall, these results highlight how: a) our knowledge about the role of environment in shaping dispersal is far from complete, and b) even seemingly simple differences in environment can produce drastic changes in dispersal patterns.

Finally, the third major theme of this thesis was evolutionary changes in dispersal. The empirical work on dispersal evolution has lagged behind the modelling studies on the topic (e.g. Gadgil 1971; Ronce 2007; Duputié & Massol 2013), likely due to the difficulties in tracking evolution in natural habitats. The past decade, however, has seen a wealth of studies on the evolution of higher dispersal, mostly using laboratory populations (e.g. Fronhofer *et al.* 2014; Williams *et al.* 2016; Weiss-Lehman *et al.* 2017; Tung *et al.* 2018b). It stands to reason that if evolutionary changes in dispersal traits are commonplace, they must affect other dispersal-associated patterns as well. In this vein, I demonstrate that mate-finding dispersal (Chapter 3) can close the gap between male and female movement, making it a crucial factor for dispersal evolution in mixed-sex populations. I also investigated the evolutionary changes in DDD and SBD, which showed a complete erasure and reversal in direction, respectively, in dispersal-selected populations (Chapter 4). Finally, I compared the ecological dispersal syndrome with the evolutionary one (Chapter 5), and further investigated if evolution changed the desiccation cost of dispersal (Chapter 6). Surprisingly, the relationship between dispersal and desiccation resistance switched from positive to negative in the evolutionary syndrome (Chapter 5), highlighting that evolution can shape trait-associations in counterintuitive ways, even under a constant environment. Overall, these results exemplify that evolutionary changes in dispersal can be quite commonplace, not only in dispersal traits (e.g. dispersal distance and propensity), but also in other phenomena (here, DDD, SBD and syndromes). Therefore, we need to consider these potential evolutionary changes, for both forecasting species' responses to habitat change and making long-term decisions regarding biological conservation and invasive species management.

In addition to the three broad themes discussed above, the findings in each chapter had a number of specific implications. Moreover, these proof-of-concept results could simulate new directions of empirical work and synthesis. Below, I outline the implications of my findings and a few potential directions for future research.

The most surprising observation in **Chapter 2** was negative DDD in the absence of resources such as food, water and oviposition site. By comparing data across three experiments, we propose male mate harassment as a plausible hypothesis for this observation. This hypothesis is thus complementary to the suggestion that dispersal in females serves as an escape mechanism against male harassment (e.g. Byrne *et al.* 2008; Malek & Long 2019). Moreover, the marked differences between the results of same-sex and mixed-sex experiments exemplify the susceptibility of DDD patterns to mate availability. As a result, it would be interesting to see how the mating status of individuals (i.e. virgin vs. mated) affects these observations and dispersal patterns in general. The chronological order of mating and dispersal is already a crucial topic in the context of SBD (reviewed in Li & Kokko 2019), and I speculate that it plays an important role for other phenomena such as DDD as well. Finally, in addition to the effect of individual environmental components on dispersal, this study highlights the need to study the effect of multiple environmental factors (here, population density, duration of exposure to density, and presence of mates), which can interact to produce interesting and unexpected patterns of dispersal.

Chapter 3 provides empirical evidence for mate-finding dispersal, in the aftermath of SBD. To begin with, the strong mate-finding dispersal observed in males helps explain the DDD results above, as males showed a similar but slightly weaker DDD response than females in all cases. Moreover, mate-finding dispersal by female flies, which are already well adapted to mate limitation (Fuerst *et al.* 1973; Pyle & Gromko 1978; Pitnick *et al.* 1999), suggests that mate-finding dispersal might be an especially prevalent form of movement in many other taxa. If true, this would a key plastic trait for dealing with mate limitation that arises out of demographic stochasticity or SBD (Miller *et al.* 2011). In addition, it can directly modulate the evolution of dispersal via spatial sorting (*sensu* Shine *et al.* 2011), where it can hinder the assortative mating of phenotype-dependent dispersers at population range fronts. As mate-finding dispersal is one of the two predicted outcomes of SBD-induced mate limitation (Shaw & Kokko 2014; Fromhage *et al.* 2016), an obvious future direction is to find empirical evidence for the reciprocal response, i.e. a decrease in the movement of less-dispersive sex following an SBD event. Finally, to establish its effect on spatial sorting, a comparative study between the taxa with and without mate-finding dispersal should be carried out, where the rate of dispersal evolution in both cases can be assessed.

To my knowledge, **Chapter 4** represents only the second empirical study on DDD evolution. However, in contrast to the previous empirical and theoretical studies, I started with a population that had a strong negative DDD, which is instead expected to evolve after spatial selection (Travis *et al.* 2009; Fronhofer *et al.* 2017; Weiss-Lehman *et al.* 2017). Interestingly, dispersal evolution completely erased the DDD

signature in both males and females, resulting in density-independent dispersal, thereby contradicting the previous predictions. This highlights the importance of studying and modelling DDD patterns other than positive DDD, which although the most common form of DDD, accounts for less than 50% of empirical DDD observations (Harman *et al.* 2020). Furthermore, I observed an evolutionary reversal in the direction of SBD, indicating that not only DDD, but also SBD could be subject to drastic evolutionary changes, particularly in polygamous species. Together with the mate-finding dispersal discussed above, this means that DDD and SBD may not be as characteristic a feature of a taxon as currently believed.

Chapter 5 dealt with two primary topics, i.e. sex differences and robustness of dispersal syndromes. Here, I chose three traits that are either commonly associated with dispersal across taxa (i.e. body size and exploratory tendency) (e.g. Sutherland *et al.* 2000; Cote *et al.* 2010; Stevens *et al.* 2012; Korsten *et al.* 2013) or were likely to have a strong association with dispersal under the experimental conditions used in this study (i.e. desiccation resistance). Not only were pervasive sex differences found for all three traits, the syndromes in the two ecological and the one evolutionary scenario were substantially different from each other. This is the case when all the flies had a common recent ancestry and were reared under identical conditions prior to the study. Therefore, a straightforward implication of these findings is that dispersal syndromes can be highly labile and sex-specific, which directly limits their efficacy as possible proxies of dispersal (Ronce & Clobert 2012; Stevens *et al.* 2013). This is especially true for populations in their natural habitats, which not only vary in terms of resource availability, but likely also have distinct evolutionary histories. Finally, it would be interesting to examine how sex-specific trait associations of dispersal interact with sex differences in movement to affect ecological phenomena such as novel habitat colonization and community assembly, a topic featured in a recent modelling study (Shaw *et al.* 2018) but with no empirical data to date.

Chapter 6 aimed to delineate the exact role of a dispersal-correlated trait (here, desiccation resistance) in modulating dispersal. On a conceptual level, the clearly demarcated cause and cost role of desiccation stress reported here provides a phenotypic understanding of how dispersal syndromes might take shape. Moreover, as desiccation is an extremely common environmental stress, especially in arid regions (Black & Pritchard 2002; Holmstrup *et al.* 2002; Kranner *et al.* 2008; Holzinger & Karsten 2013), I hypothesize that dispersal in many other taxa could also exhibit a similar cause-cost relationship with desiccation stress. While the exact shape of this relationship would likely vary with the species and population history, my experimental setup, with provisions of pre-dispersal stress and post-dispersal rest, can easily be modified and extended for other studies. Finally, the dual nature of desiccation stress as a cause and cost of dispersal reveals an interesting trade-off at

the population level: stress-resistant individuals might serve as effective dispersers but may show a delayed emigration from a deteriorating habitat, whereas stress-intolerant individuals may be early emigrants but in a poorer state to complete dispersal. Therefore, the habitat resource availability and body condition of individuals would likely determine the efficacy of dispersal-mediated conservation and population management strategies.

To summarize, this thesis examined several aspects of biological dispersal from the viewpoint of sex differences, context dependence and evolutionary changes. While the results from these microcosm experiments should not be directly extrapolated to *Drosophila* dispersal in natural habitats, they provide a proof-of-concept evidence for several hypotheses in the dispersal literature. As a result, these studies can serve as a useful bridge between the modelling studies on dispersal and empirical studies carried out in the wild. Furthermore, many of the novel results obtained here should open up avenues for future research, some of which I discussed in this chapter. I hope that these findings are of relevance to not only ecology and evolutionary biology, but also conservation sciences and invasive species management.

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