Costs and correlates of evolution of increased dispersal in *Drosophila melanogaster*

Thesis submitted in partial fulfilment of the requirements of Five Year BS-MS Dual Degree Program at

Indian Institute of Science Education and Research, Pune

Mohammed Aamir Sadiq

20121102

Biology Division, IISER Pune

Under the supervision of

Dr. Sutirth Dey

Biology Division, IISER Pune

CERTIFICATE

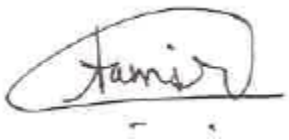
This is to certify that this dissertation entitled "*Costs and correlates of evolution of increased dispersal in Drosophila melanogaster*" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Mohammed Aamir Sadiq at IISER Pune under the supervision of Dr. Sutirth Dey, Associate Professor, Biology Division, IISER Pune during the academic year 2016-2017.

Dr. Sutirth Dey Date: 20/03/2017

Biology Division, IISER Pune

DECLARATION

I hereby declare that the matter embodied in the report entitled "*Costs and correlates of evolution of increased dispersal in Drosophila melanogaster*" are the results of the work carried out by me at the Department of Biology, IISER Pune, under the supervision of Dr. Sutirth Dey and the same has not been submitted elsewhere for any other degree.



Mohammed Aamir Sadiq Date: 20/03/2017

20121102

Abstract

Dispersal is a vital process that influences species distribution patterns and allows organisms to track favourable environmental conditions. Dispersal evolution is intimately linked with biological invasions and range shifts due to climate change. Hence it is important to understand the ecological and evolutionary consequences of dispersal. Studies on dispersal often focus on association of traits with dispersal, thereby neglecting how the traits themselves can change with evolution of dispersal. Here, I investigate the effects of dispersal evolution using populations of *Drosophila melanogaster* selected for increased dispersal. I find that evolution of increased dispersal leads to an increase in locomotion without affecting critical components of fitness such as fecundity and lifespan. Moreover, dispersal evolution does not lead to a change in body glucose and glycogen content. Finally, I show that, traits associated with increased dispersal do not deteriorate when selection pressures are relaxed. These findings imply that dispersal evolution entails no major costs and that higher dispersive phenotype can persist in populations even when dispersal is not advantageous. These findings coupled with the fact that dispersal is heritable, imply that populations can rapidly evolve higher dispersal under favorable conditions. Thus, the results of this study can improve our insights on processes like range shifts, invasions and response of populations to various environmental stresses including the effects of climate change.

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Acknowledgement

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Chapter 1: Introduction

Dispersal is a process which involves movement of individuals or propagules resulting in gene flow across geographic space (Ronce, 2007). It is a major factor governing species distribution patterns and therefore has huge ecological implications. Studies have shown that dispersal can rescue small populations from local extinction (Brown and Kodric-Brown, 1977). However, dispersal can also exacerbate the risk of global extinction in metapopulations by increasing synchrony between constituent subpopulations (Molofsky and Ferdy, 2005). Since dispersal facilitates gene flow, it can influence genetic diversity of populations. In fact, it has been reported that dispersal can increase genetic diversity within populations, rather than between populations (reviewed by Ronce, 2007). At the same time, by facilitating gene flow, dispersal can potentially bring about uniformity in genetic diversity among populations. Furthermore, dispersal may affect the process of speciation as well, as it can impede (Lenormand, 2002) or facilitate local adaptation (Gandon et al., 1996). Dispersal may also lessen the probability of interaction with kin. On one hand, this could counter inbreeding depression and kin-competition. On the other hand, it could lead to a loss of cooperation with kin (Clobert et al., 2012). Dispersal also allows populations to track favorable conditions. As a result of such wide-ranging effects, dispersal plays a huge role in range shifts of populations due to climate change and habitat fragmentation. Also, dispersal is a crucial phenomenon for range expansions and species invasions. Previous theoretical studies have shown that dispersal can evolve in the context of spatiotemporally varying environments (McPeek and Holt, 1992; Travis and Dytham, 1999). Moreover some of the recent empirical studies have shown that with suitable selection pressure, dispersal can evolve (Li and Margolies, 1994; Tung et al., 2016). Thus, particularly in the context of contemporary spatio-temporal changes in environments due to habitat fragmentations and climate change, it is timely and important to understand the ecological and evolutionary aspects of the consequences of dispersal evolution. This understanding will help us to predict the response of natural populations to changing environmental conditions.

Dispersal consists of mainly three stages, namely, emigration, transfer and immigration (Clobert et al., 2012). Emigration refers to departure from a current patch, transfer denotes the movement between patches and immigration alludes to the arrival and settlement into a new patch. This act of moving from one region to another entails major risks and challenges for the dispersing individuals. For example, the area between the habitats may be hostile/unsuitable for living, and the distance between two suitable habitats might be large, thus requiring a lot of energy for travel. Settling into new environments can also pose a challenge due to lack of familiarity with resource locations, predators and conspecifics in the new habitat. Thus it is unlikely that all the individuals in a population will be equally adept at dealing with the challenges of dispersal. More specifically, the individuals who have certain physiological, morphological or behavioural features which help them to overcome or minimize the costs of dispersal will have an advantage to disperse over longer distance successfully. For example, it has been observed in birds and fishes that bold individuals disperse over large distances (Dingemanse et al., 2003; Duckworth and Badyaev, 2007; Fraser et al., 2001). Boldness may increase the likelihood of emigration and exploration of new habitats. Similarly, studies have shown that, in cane toads, dispersal ability is associated with an increase in the length of hind limbs (Phillips et al., 2006) which is believed to increase mobility, a critical component of dispersal. Furthermore, aggressiveness may facilitate dispersal by increasing an individual's chances of integrating into a new environment. In fact, it has been observed that higher aggressiveness allows western bluebirds to displace an interspecific competitor while settling into a new habitat (Duckworth and Badyaev, 2007).

Since certain morphological, physiological or behavioural traits are often associated with greater dispersive phenotypes (Bonte et al., 2012; Corcobado et al., 2012; van Overveld et al., 2010), it is interesting to investigate how these traits themselves evolve with the evolution of dispersal. Additionally, detailed study of behavioral and life-history traits in course of dispersal evolution can potentially reveal the underlying genetic relationship dispersal has with other traits. Although some studies have previously shown association of certain traits with dispersal (Altwegg et al., 2000; Stevens et al., 2012), they fail to predict how these traits change with evolution of dispersal. Also, one has to

be careful in interpreting the results of such one generational studies because, it could so happen that the observed association between dispersal and other traits could arise due to environmental effects rather than any underlying genetic mechanisms.

In this study, I have studied how dispersal is related to certain traits in populations that have been selected for increased dispersal. I have investigated how locomotion and other energy demanding life-history traits like lifespan and reproductive output have evolved in the course of dispersal evolution in laboratory maintained, large, outbred populations *Drosophila melanogaster*. In the process, I have also addressed whether there is a change in the amount of energy reserves in the individuals of the selected populations. Finally, I also explore the costs of maintaining dispersal related traits when selection pressures are relaxed.

Chapter 2: Materials and Methods

2.1 Description of experimental populations

All the experimental populations were originally derived from four outbred laboratory populations of *Drosophila melanogaster*, namely DB_{1-4}. The DB populations trace their history back to four large outbred populations called JB_{1-4}. The detailed ancestry of JB populations and maintenance regime of DB populations can be found elsewhere (Sheeba et al., 1998; Tung et al., 2016).

From each of the DB populations, two populations, namely VB and VBC were derived. Thus four pairs of VB (VB_{1-4}) and VBC populations (VBC_{1-4}) were generated. VB and VBC populations that share a common subscript, are related by ancestry (e.g. VB_1 and VBC_1 are descendants of DB_1 and so on). The VB populations were subjected to selection for increased dispersal and the VBC populations were maintained as their corresponding controls. Each pair of VB-VBC sharing common ancestry were assayed together and were treated as blocks in subsequent statistical analyses.

To check for the effects of relaxation of selection pressure, four ReVB lines were derived from the four VB populations after 50 generations of selection. Again, each set of VB, VBC and ReVB populations, sharing common ancestry, were assayed together and were treated as blocks in statistical analyses. The selection regime of ReVB lines was similar to that of VBC lines.

2.2 Maintenance regime of experimental populations

All the experimental populations, namely, VB, VBC and ReVB are maintained on a 15 day discrete generation cycle in a constantly lit environment maintained at 25˚C.The first 11 days of the flies' lifecycle is spent in 37 ml transparent plastic vials containing approximately 6ml banana-jaggery food (for composition see Appendix).During this time, the egg, larval and pupal stages are completed and the flies emerge as adults. On the 12^{th} day post egg-collection, selection is imposed on the flies (see below) after which, the populations are transferred to plexi-glass population cages (25 cm × 20 cm ×15 cm) (Fig.1). The cages contain food laced with excessive live yeast paste to boost

fecundity. The population densities in these population cages are maintained at approximately 2500 individuals to avoid adverse effects of very small population size such as inbreeding and stochastic extinctions. Approximately 40 hours later, the flies are provided with petri-plates containing fresh banana-jaggery food. The food surface acts as an oviposition site. On the 15th day of the lifecycle, eggs are collected from the food plate and transferred to individual plastic vials containing food. The eggs so collected form the next generation and the parents are discarded, thus maintaining a 15 day discrete generation cycle. Around 60-80 eggs are transferred to each vial. This ensures that the larvae develop in low to moderate levels of crowding. Thus no density dependent selection is enforced during larval development. Every generation, each VB population is generated from 80 vials containing 60-80 eggs each, thus giving rise to ~4800 individuals. Each VBC and ReVB populations are generated from 40 such vials. This ensures that after selection (see next section), the population sizes of VB, VBC and ReVB populations are similar.

Figure 1| Maintenance enclosures of flies. Adult flies are maintained in population cages containing food plates. The population cages contain ~2500 flies which minimizes the chances of inbreeding-like effects.

2.3 Selection regime

Complete details of the selection protocol can be found elsewhere (Tung et al., 2016). Briefly, the selection apparatus consists of a source connected to a sink via a small diameter pipe (Fig. 2). The sink contains a water source. On the 12th day post egg collection, ~2500 flies are introduced into the source. The whole apparatus is kept in a well-lit environment maintained at 25˚C. The flies are allowed to migrate for 6 hours or till approximately 50 percent of the flies in the source reach the sink, whichever occurs first. Six hours is chosen as the cut-off for dispersal duration as there is no mortality due to desiccation stress in the first six hours (personal observation). Only the flies that reach the sink are allowed to lay eggs for the next generation. During selection, two independent selection apparatus are setup for each VB population with each source having ~2500 individuals each. Post selection, the flies in the two sinks for a given VB population are mixed and transferred to a population cage. Since only 50% of the flies in the source are selected, this mixing ensures that the population sizes of each VB population are similar to that of their controls. The length of the pipe was initially kept constant at 2 m. The length was increased intermittently at later rounds of selection. At the time of the assays (~gen 50), the length of the path was approximately 16m.

Parallel to the selection process, VBC populations are also introduced into source containers but are not allowed to migrate. About 2500 individuals are introduced into the source containers. They are provided with water after 3 hours in order to mimic the desiccation conditions faced by the VB populations as part of the selection protocol. All the flies that survived in the sources containing VBC population are allowed to form the next generation, thus ensuring that there is no selection for dispersal.

The ReVB lines were derived from the VB lines after 50 generations of selection. These are maintained along with VB and VBC lines and are exposed to the same treatment as the VBC populations.

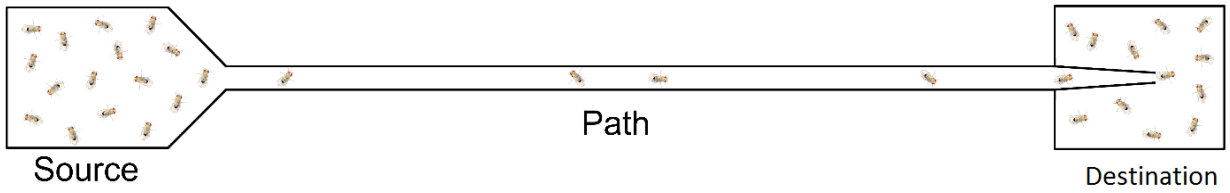

Figure 2| Selection apparatus. The source and the sink are transparent containers. The source is connected to the destination by a narrow transparent pipe. The protrusion of the sink in the pipe reduces backflow of flies. The tiny objects inside the apparatus denote flies. Only flies that reach the sink are allowed to form the next generation.

2.4 Assays

All assays were performed after relaxing the selection on the experimental populations for one generation. For this, the experimental populations were transferred directly into population cages on the 12th day. They were allowed to grow under similar environmental conditions and their progeny were used in performing the assays. This ensured minimization of non-genetic parental effects on the assay populations (Singh et al., 2016).

2.4.1 VB-VBC locomotor activity assay:

VB and VBC populations were simultaneously assayed for locomotor activity after 49 generations of selection following standard protocols (Chiu et al., 2010). The populations were assayed independently under two conditions, namely, in the presence and absence of food. For the assay in the presence of food, 11 day old male flies were used. On the 11th day post egg collection, single male flies were aspirated into separate glass vial tubes of 5mm diameter containing banana-jaggery food at one end. Thus, each vial with a single male fly in it formed a replicate. Aspiration was preferred over CO_2 anesthesia to avoid any effects of anesthesia on the activity of flies (Van Dijken et al. 1977)**.** The protocol for cleaning and preparation of glass vials can be found elsewhere (Chiu et al., 2010). The vials containing individual flies were then inserted

into the Drosophila Activity Monitors (Fig. 3) data collection system (Trikinetics Inc, Waltham, MA)**.** The apparatus was left undisturbed and data was collected for the next 6 hours. The whole assay was run in a well-lit environment maintained at 25˚C. VB and VBC flies belonging to a particular block were assayed together and there were approximately 32 replicates for each of the populations. From the data obtained over the first 6 hours, average activity per hour and percentage of time spent resting in were calculated. Average activity was calculated as total number of activity counts divided by total time, that is, 6 hours. Percentage of time spent resting was calculated using the definition that continuous inactivity for 5 minutes is equivalent to rest (Chiu et al., 2010; Hendricks et al., 2000). While performing the assay in the absence of food, the same procedure was employed with the exception that 12 day old male flies were used and the glass vials contained no food.

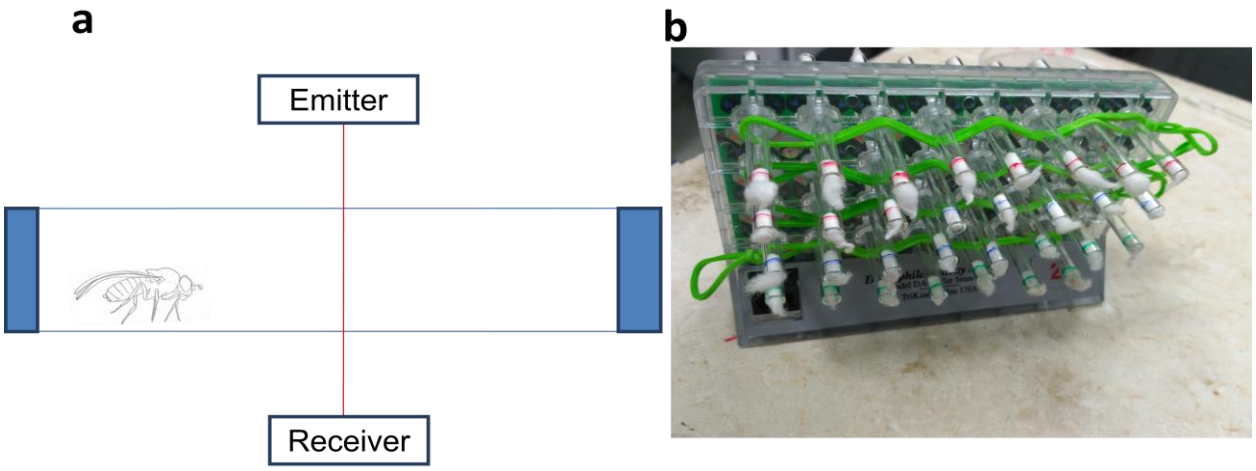

Figure 3| Drosophila Activity monitor (DAM 2) data collection system. (a) Schematic to show working of a DAM2 monitor; each vial containing a fly is bisected by an infrared beam (red line) sent from the emitter. Each time the fly intersects the beam, the receiver registers a signal. Thus, the counts are an indicator of locomotor activity. **(b)** DAM 2 monitor with vials containing individual flies.

2.4.2 Longevity assay

After 51 generations of selection, VB and VBC adults were assayed for their longevity. To avoid the potentially confounding effects of mating on longevity (Kidwell, 1966), we used virgin flies for this assay. 10 replicates per sex per VB / VBC population were setup at 1500 hrs IST on the 10^{th} day post egg collection. Each replicate consisted of 10 virgin flies of the same sex kept in 40 ml transparent plastic vials containing 6ml of banana-jaggery food. Flies were anesthetized before introduction into the food vials. This was done to separate flies according to sex. In order to ensure that the flies received fresh food, they were transferred into fresh food vials every alternate day. Also, the vials were kept in a constantly lit environment maintained at 25˚C for the entre duration of the experiment. The flies were counted every day at 1500 hrs IST in order to determine mortality. This was done until all the flies died. Flies that escaped or died due to human induced causes were not included in the analysis. Average lifespan for a replicate was calculated as the sum of lifespans of all individual flies in that replicate divided by the total number of flies.

2.4.3 Fecundity assay

Female fecundity of selected (i.e., VB) and control (i.e. VBC) populations were assayed after 53 generations of selection for all blocks. Female fecundity was assayed both during early and late life. The assay to determine early life fecundity was set up on the 14^{th} day post egg collection, at approximately 2200hrs IST. There were approximately 40 replicates for each VB and VBC population. Each replicate consisted of a 14-day old male-female pair. On the 14^{th} day post egg collection, flies were anaesthetized and single male-female pairs were introduced into 50 ml falcon tubes containing food cups(Fig. 4). The replicates were then left undisturbed for 12 hours in a well-lit environment maintained at 25˚C. At the end of 12 hours, the flies were discarded and the eggs laid on the food cup were counted under a microscope. Thus, early life fecundity was measured on the same day on which the flies laid eggs during the process of selection.

Late life fecundity was measured in a similar manner. On the 33rd day post egg collection, single anesthetized male-female pairs were introduced into 50 ml falcon tubes containing food cups. The flies were introduced again at approximately 2200hrs IST. The setup was left undisturbed for 12 hrs in constant light and at 25˚C. At the end of 12 hours, the flies were discarded and the eggs on the food cup were counted. Again, 40 replicates were setup for each VB/VBC population.

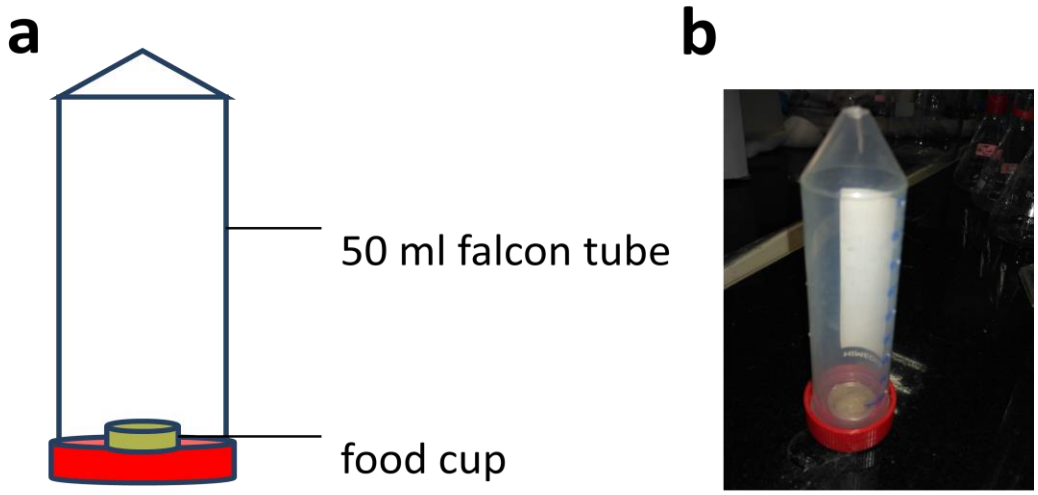

Figure 4| Apparatus for fecundity assay. (a) Schematic representation of a 50 ml falcon tube containing a food cup. **(b)** A real image of falcon tube. Small holes are pierced in the falcon tube that allowed exchange of gases with the environment.

2.4.4 Glucose and glycogen assay

After 49 generations of selection, the VB and VBC flies were assayed to check for differences in their glucose and glycogen content. Six replicates were set up for each VB/VBC populations with each replicate containing five flies. Only virgin males were assayed for their glycogen content. On the twelfth day post egg collection, male flies were collected in groups of five in Eppendorf tubes. The flies were then killed by

completely immersing the tubes in liquid nitrogen, and stored at -80˚C until the assay was performed.

At the time of the assay, each set of five flies was homogenized in 50µl of ice cold water. This was followed by immediately adding 200µl of water to the homogenate and heating the homogenate to 95˚C for 5 minutes in order to inactivate the enzymes. The homogenates were then centrifuged at 13,000g for 5 minutes to remove insoluble materials. Glucose and glycogen was estimated on the supernatant using the glycogen assay kit and protocol (catalogue number MAK016) provided by Sigma Aldrich. For each replicate, the obtained glucose and glycogen content was divided by 5 to get the mass of glycogen/glucose per fly.

2.4.5 Effects of relaxation of Selection Pressure

To check for the effects of relaxation of selection for increased dispersal, ReVB flies were assayed along with the VB and VBC flies. The flies were assayed for their desiccation resistance and locomotor activity. At the time of the assays, ReVB flies had undergone 9-10 generations of selection and VB and VBC flies had undergone 59-60 generations of selection. Blocks 1 and 2 were assayed simultaneously with ReVB having undergone 9 rounds of selection and VB and VBC consequently having undergone 59 generations of selection. Blocks 3 and 4 were assayed after one further generation of selection.

a. Locomotor Activity Assay

VB, VBC and ReVB lines were assayed for their locomotor activity in the absence of food. The protocol for this assay is exactly as mentioned earlier for the VB-VBC Locomotor activity assay. Briefly, the assay involved aspiration of single male flies into glass vials of 5mm diameter. The aspiration was done on the 12^{th} day post egg-collection at approximately 1200hrs IST. The activity of the flies for the next 6 hours was recorded using Drosophila Activity Monitor (DAM 2) data collection (Trikinetics Inc, Waltham, MA) system following standard protocol(Chiu et al., 2010).There were 21

replicates for each VB,VBC and ReVB populations of each block. Average activity was calculated as total number of activity counts divided by total time, that is, 6 hours. Percentage of time spent resting was calculated using the definition that continuous inactivity for 5 minutes is equivalent to rest (Chiu et al., 2010; Hendricks et al., 2000).

b. Desiccation Resistance

All VB, VBC and ReVB lines of a particular block were assayed simultaneously for desiccation resistance. The assay was setup on the 12^{th} day post egg collection at approximately 1000hrs IST. Each replicate consisted of 10 flies of the same sex in a 40ml transparent plastic vial. Flies were anesthetized using CO_2 before being introduced in to the vials. This was done to aid sex-based separation and counting of flies. Ten replicates were set up for each sex of each VB and VBC population. After set up, the flies were counted every 2 hours to check for mortality. This was done until all flies died. Desiccation resistance of each fly was calculated as the duration of time for which the fly was alive post the setting up of the assay. Mean desiccation resistance for a replicate was calculated as the average of the desiccation resistances of all flies in that replicate.

2.5 Statistical analyses

As mentioned earlier, each pair of VB/VBC populations sharing common ancestry was treated as block for statistical analyses. Thus, there were 4 blocks in all. For assays involving ReVB populations, each triad of VB, VBC and ReVB populations sharing common ancestry was treated as a block. Data for fecundity, glucose and glycogen content, locomotor activity and percentage sleep (arcsine-square root transformed) were subjected to independent two factor mixed-model ANOVA with treatment (VB/VBC/ReVB) considered as fixed factor crossed with block (1-4) which was considered as a random factor. Data for longevity and desiccation resistance assay was

subjected to independent three factor mixed-model ANOVA with treatment (VB/VBC/ReVB) and sex (Male/Female) of flies considered as fixed factors and block (1-4) considered as random factor crossed with each other. Post hoc-Tukey tests were performed on all data generated from assays involving ReVB populations when the main effect was significant. All statistical analyses were performed using STATISTICA® v5 (StatSoft. Inc., Tulsa, Oklahoma).

Chapter 3: Results

3.1 Evolution of increased dispersal is correlated with enhanced locomotor activity

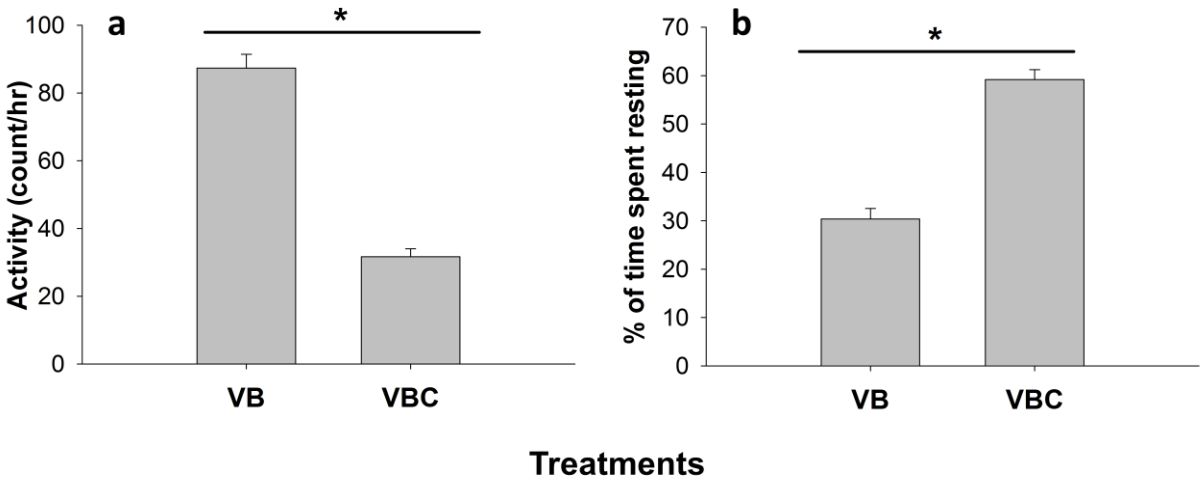

Figure 5| Locomotor activity and sleep profiles of VB and VBC males in the presence of food. (**a**) Average activity per hour (±SEM); VB showed significantly greater activity than VBC. (**b**) Average percentage of time spent resting (±SEM); VB spent lesser percentage of time resting than VBC. ***** indicate that the differences are significant(i.e., $p<0.05$).

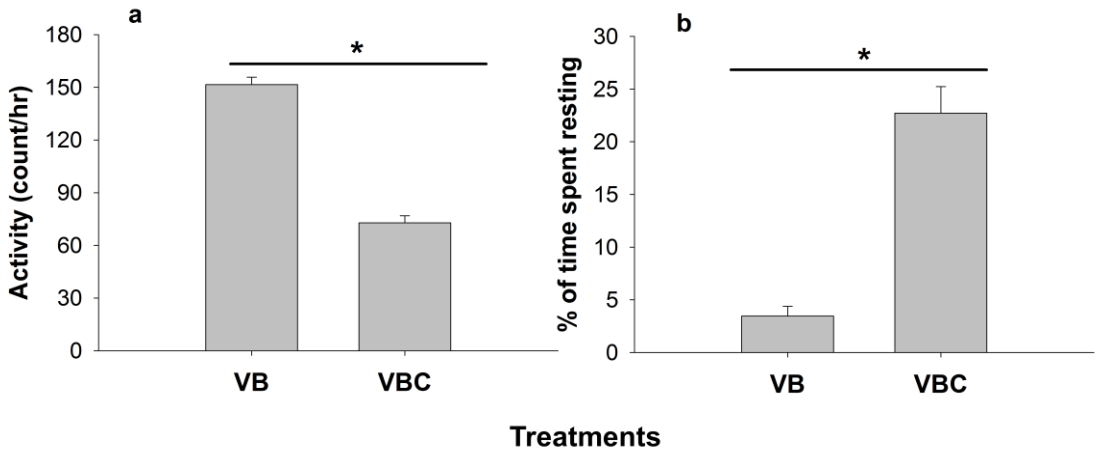

Figure 6| Locomotor activity and sleep profiles of VB and VBC males in the absence of food. (**a**) Average activity per hour (±SEM); VB showed significantly greater activity than VBC. (**b**) Average percentage of time spent resting (±SEM); VB showed lesser percentage of time resting than VBCs. ***** indicate that the differences are significant (i.e., $p<0.05$).

VB and VBC males were tested for their activity levels in the presence and absence of food. Locomotor activity and rest time were measured over the first 6 hours in both the cases. In the presence of food, VB males showed significantly greater locomotor activity than VBC males (Fig. 5a, $F_{1,3}=423.211$, $p=0.0002$, Cohen's $d=1.482$). Also, VB males spent significantly lesser time resting as compared to VBC males(Fig. 5b, $F_{1,3}=386.730$, $p=0.0002$, Cohen's $d=1.208$). This pattern persisted even in the absence of food with VB males showing greater activity (Fig. 6a, $F_{1,3}=60.343$, $p=0.004$, Cohen's $d=1.649$) and lesser resting time (Fig. 6b, $F_{1,3}=50.441$, $p=0.005$, Cohen's $d=0.898$). Thus, VB males show higher activity levels than VBC males, both in the presence and absence of food. This suggests that evolution of increased dispersal is correlated with an increase in physical activity.

3.2 Evolution of increased dispersal does not trade-off with lifespan

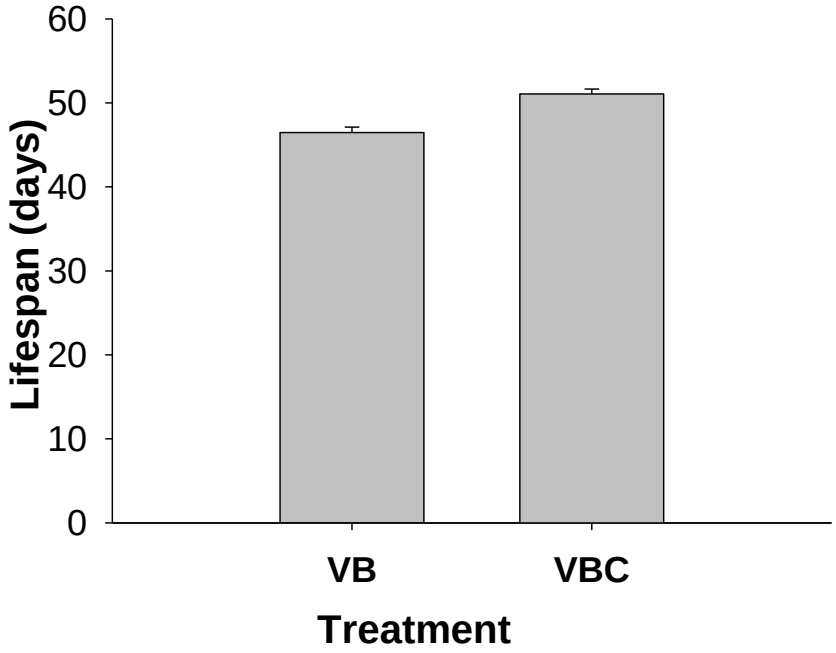

Figure 7|Lifespanof VB and VBC. Average lifespan (±SEM) of VB and VBC. No significant difference in survivorship was observed between VB and VBC.

Lifespan of virgin VB and VBC flies was measured to check whether evolution of increased dispersal traded-off with lifespan. Results showed that VB and VBC flies had similar lifespans suggesting that selection for increased dispersal does not lead to the evolution of reduced lifespan (Fig. 7, $F_{1,3}$=4.914, p=0.113, Cohen's d=0.83). Thus, dispersal evolution does not entail lifespan costs.

3.3 Female fecundity does not correlate with evolution of increased dispersal

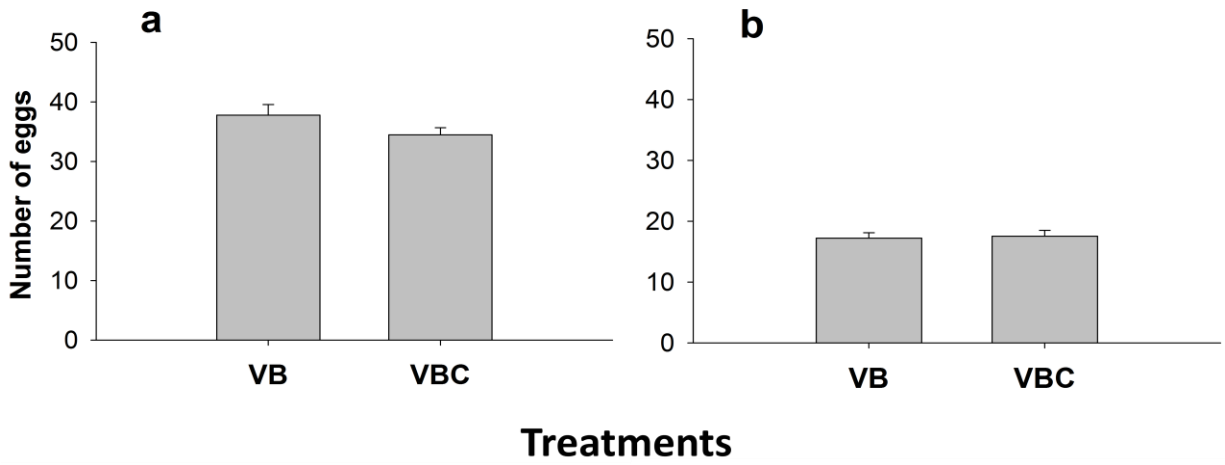

Figure 8| Female fecundity comparisons of VB and VBC. (**a**) Average early life female fecundity (±SEM); no significant difference in early female fecundity was observed between VB and VBC. (**b**) Average late-life female fecundity (±SEM); no significant difference in early female fecundity was observed between VB and VBC.

Female fecundity was measured both during early and late life. Early life fecundity was measured on the 14th day post egg collection and late life fecundity was measured on the 33rdday post egg collection. VB and VBC female flies showed similar early life fecundity levels (Fig. 8a, $F_{1,3}$=0.252, p=0.65, Cohen's d=0.17). Since fecundity is age dependent, effect of dispersal evolution on early and late-life fecundity may be different. However, late life fecundity also remained similar for both the treatments (Fig. 8b, $F_{1,3}$=0.205, p=0.682, Cohen's d=0.03). This indicates that evolution of increased dispersal does not trade-off with female fecundity, both during early and late life.

3.4 Selected flies do not differ from control flies in their body glucose and glycogen content

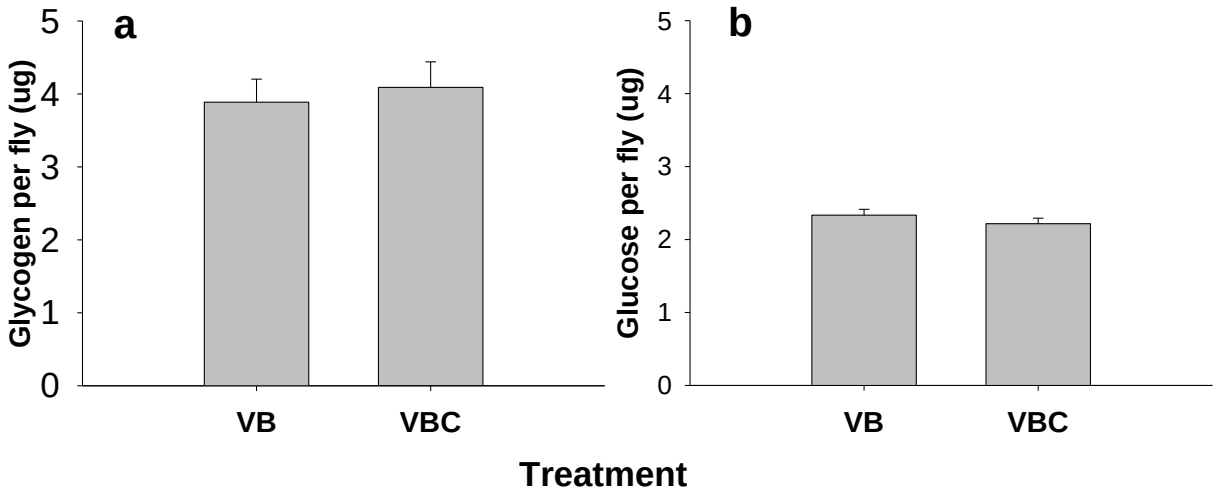

Figure 9| Glycogen and glucose levels in VB and VBC males. (**a**) Average body glycogen content in 5 males each of VB and VBC males (±SEM); no significant difference in body glycogen levels was observed between VB and VBC males. (**b**) Average body glucose content in 5 males each of VB and VBC males (±SEM); no significant difference in body glucose levels was observed between VB and VBC males.

In order to check whether dispersal evolution led to a change in storage capacities of energy reserves, glucose and glycogen content in the bodies of virgin VB and VBC males was measured. Both VB and VBC males were observed to have similar body glycogen (Fig. 9a, $F_{1,3}$=4.267, p=0.131, Cohen's d=0.12) and glucose content (Fig. 9b, $F_{1,3}$=2.546, p=0.209, Cohen's d=0.31) . This suggests that the evolution of increased dispersal does not lead to an alteration in resource storage levels in males.

3.5 Effects of relaxation of selection pressure:

a. VB-VBC-ReVB locomotor activity and sleep profiles.

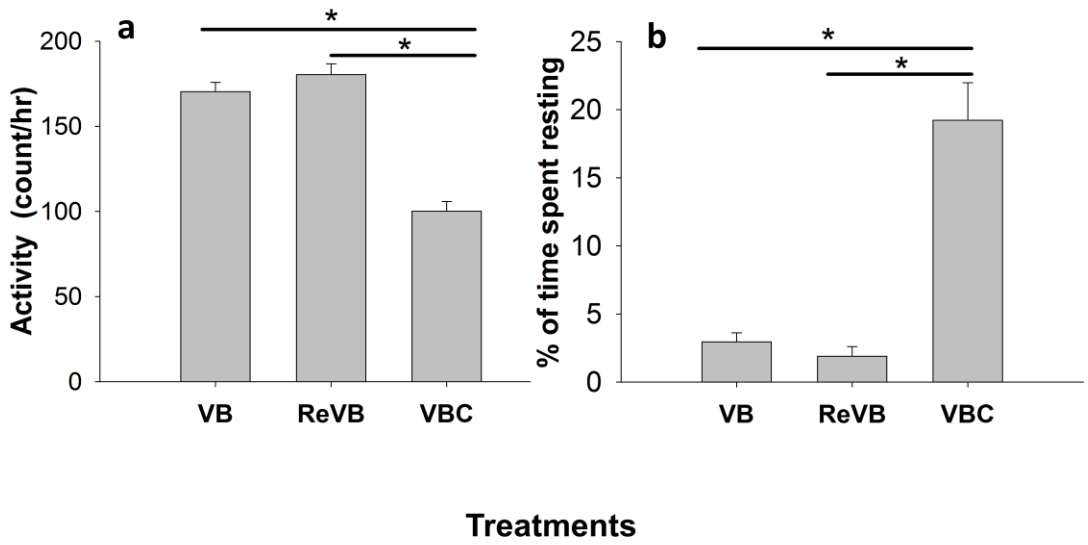

Figure 10| Locomotor activity and sleep profiles of VB, ReVB and VBC males in the absence of food. (**a**) Average activity (±SEM); VB and ReVB showed significantly greater activity than VBC. (**b**) Average percentage of time spent resting (±SEM); VB and ReVB spent lesser percentage of time resting than VBCs. No significant difference was observed between VB and ReVB with respect to locomotor activity and rest. ***** indicate that the differences are significant ($p<0.05$).

To check for the effects of relaxation of selection pressure on locomotor activity, male ReVB flies were assayed along with VB and VBC males. ANOVA yielded a significant effect of treatment on activity/hour (Fig. 10a, $F=28.948$, $p=0.001$).Post-hoc Tukey test showed that locomotor activity levels of VB and ReVB are similar ($p=0.418$). However, VB and ReVB had significantly higher activity/hour than ReVB ($p=0.000$ for both the pairwise comparisons).

A separate ANOVA yielded a significant effect of treatment on percentage of time spent resting (Fig. 10b, $F=20.562$, $p=0.002$).Post-hoc Tukey test showed VB and ReVB spent similar proportions of time resting ($p=0.580$). However, VB and ReVB spent significantly lesser time resting than VBC ($p=0.000$ for both the pairwise comparisons). Thus the activity levels of VB and ReVB are similar and greater than that of VBC.

b. Desiccation assay:

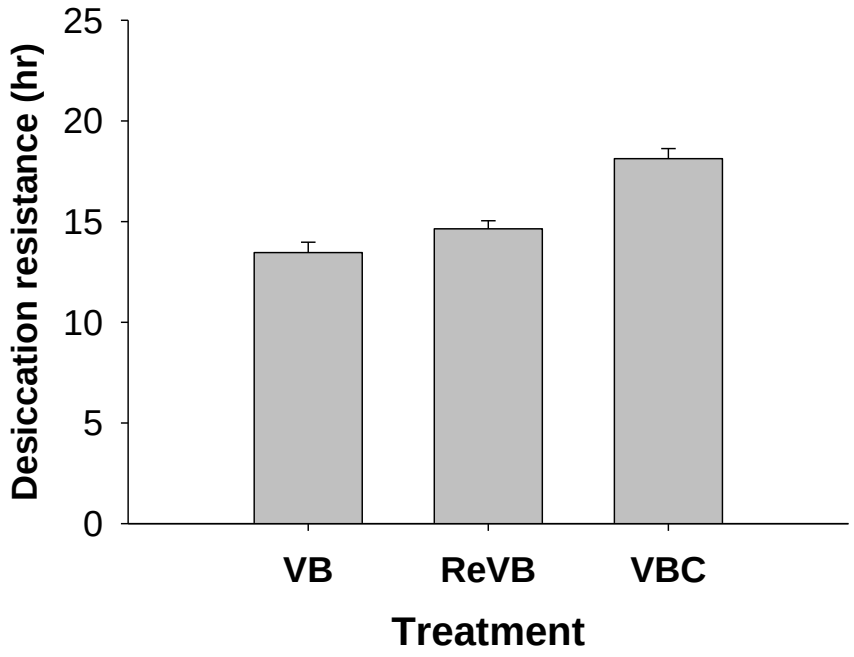

Figure 11| Desiccation resistance profiles of VB, ReVB and VBC. Average desiccation resistance of VB, ReVB and VBC (±SEM); VB and ReVB populations showed similar levels of desiccation resistance. VBC populations had the highest desiccation resistance among the three populations.

ANOVA yielded no significant effect of treatment (Fig.11, F=3.864, p=0.084). When effect sizes were considered, VB-ReVB comparison yielded low effect size (Cohen's d=0.288). ReVB-VBC and VB-VBC comparisons yielded large effect sizes (Cohen's d is 0.86 and 1.02 respectively). Thus, VB and ReVB populations seem to have similar desiccation resistances. The desiccation resistances of VB and ReVB seem to be lower than that of VBC populations.

Chapter 4: Discussion

Dispersal is a complex life history trait whose evolution may necessitate morphological, behavioural and physiological adaptations. While many studies have reported traits that co-vary with dispersal, very few have been aimed at studying traits that evolve with dispersal. This is partly because, in the field, organisms face a multitude of selective pressures simultaneously acting upon multiple traits. Thus discerning out whether a trait is evolving as a byproduct of evolution of some other trait (here greater dispersal) or as a result of independent selection pressure on the trait itself is often very difficult. Additionally, these kind of studies warrant long-term selection for the focal trait over several generations. Due to logistic reasons, this is often not possible even under laboratory conditions. One field study involving cane toads, failed to establish the genetic basis behind the observed co-variation of morphological changes due to dispersal evolution (Phillips et al., 2010). In another study involving spider mite, researchers had sought to study the correlated response to selection for dispersal on a suite of life-history traits but failed to detect any (Fronhofer et al., 2014).This study explores how evolution of dispersal affects locomotor activity, lifespan, fecundity and storage of energy reserves in populations selected for increased dispersal. It also explores the cost of maintaining dispersal related traits when highly dispersive populations are not subjected to selection for increased dispersal.

4.1 Evolution of locomotor activity.

Dispersal consists of three successive stages: departure from a current habitat, movement, and settlement in a new habitat. Since movement has a crucial role in dispersal, increase in mobility with evolution of dispersal is expected. Not surprisingly, I found that selection for increased dispersal results in a correlated increase in locomotor activity. The selected populations showed greater average activity per hour and spent lesser time resting than the control populations (Fig. 5, Fig. 6). This finding is consistent with a study on butterflies which shows a positive relationship between dispersal and mobility (Hanski et al., 2006). Also, higher locomotor activity may facilitate exploration of

new settlement patches. In fact, the VB populations have been shown to have higher exploratory activity compared to VBC populations (Shree Sruti, 2017, Master's Thesis, IISER-Pune.). Interestingly, the fact that locomotor activity and resting time are inversely related in the VB flies, may not be intuitive as higher activity could result in more exhaustion. This may lead to an increase in resting period. It is important to note here that the VB populations are subjected to intense selection pressure to reach a new destination within 6 hours after being introduced into the source. Thus, having greater locomotor activity and minimizing rest may be advantageous to the VB populations. Moreover, this pattern was observed both in the presence and absence of food. This indicates that the difference in locomotor activity between the control and the selected flies is independent of starvation and desiccation stress. Interestingly, both the selected and the control populations showed greater physical activity in the absence of food (Fig. 5, Fig. 6). A possible explanation for this could be that starvation induces the flies to show higher foraging activity, which requires higher locomotor activity. It is important to note that here the locomotor activity assay was performed only on male flies. However, following the same logic, the same pattern can be expected for females as well. This is because, in the selection protocol, it is absolutely essential for VB females to reach the sink in order to foster the next generation. Thus, having higher locomotor activity is advantageous to females also.

4.2 Why does lifespan not change with evolution of increased dispersal?

Although having higher locomotor activity could facilitate dispersal, it may trade-off with lifespan. This is because, more active organisms are expected to have higher metabolic rates, resulting in greater oxidative stress (Adelman et al., 1988). This stress is shown to cause organisms to age faster, leading to a reduction in lifespan (Cui et al., 2012). Furthermore, studies have shown that metabolic rate correlates positively with the distance travelled (Niitepõald et al., 2009). In our study as well, the selected flies (VBs) were seen to exhibit higher dispersal ability (Tung et al., 2016) and locomotor activity (Fig.5, Fig.6). Consequently, VB populations are expected to have lower lifespan. Also, dispersal is an energy intensive process (Roff, 1977). Thus, evolution of dispersal may

require organisms to allocate more resources towards dispersal. This may in turn reduce the amount of resources required for other body maintenance functions, thus reducing lifespan. Therefore, evolution of increased dispersal should result in a reduction in lifespan.

However, contrary to our expectations, it was observed that no trade-off exists between lifespan and dispersal. The VB populations did not show a significant reduction in lifespan as compared to that of VBC populations (Fig. 7). This implies that the more active VB populations may not have higher metabolic rates. An alternative explanation is that dispersal evolution has not led to a reduction in resource allocation towards body maintenance.

Although reduction in lifespan with evolution of increased dispersal is expected, there exist contradictory studies. For example, it has been observed that dispersal varies positively with lifespan (Saastamoinen et al., 2009). The ability to find new food resources and reallocation of existing resources may be possible mechanisms by which dispersing individuals increase their lifespan. However, our results do not concur with these findings as the selected populations do not show an increase in lifespan when provided with *ad libitum* food. In fact, although not significant, the VB populations show a decrease in lifespan when compared with VBC populations. This is apparent when effect size is considered, which is large (Cohen's d=0.83). It would be interesting to observe how lifespan evolves after further generations of selection.

4.3 Dispersal evolution and fecundity

The Y-model postulates that, when resources are limited, increased allocation of resources towards reproduction compromises the resource investment towards body maintenance (de Jong and van Noordwijk, 1992) and it is this irreversible resource allocation which gives rise to trade-offs. An organism's life history is characterized by a variety of trade-offs, as resources cannot simultaneously be invested in dispersal, body maintenance and reproduction. It has already been seen that evolution of increased dispersal has led to an increase in locomotor activity without any significant reduction in

lifespan. One consequence of this could be that resource allocation towards reproduction has been compromised, which implies that a decrease in fecundity with evolution of increased dispersal is expected. However, no such patterns were observed as early and late life fecundities were similar for both control and selected populations(Fig.8) suggesting that fecundity does not reduce with evolution of increased dispersal. A possible reason for this could be that resources are not limiting as the flies had access to *ad libitum* food during the course of the assay. Another possibility is that currently the flies are not facing resource limitation in terms of reproduction and body maintenance which in turn implies that there is potential for further increase in dispersal ability and rate due to selection. In other words, possible trade-offs with fecundity are likely to be observed once the limits of increasing dispersal ability are reached.

4.4 Effect of dispersal evolution on energy reserves

The fact that evolution of increased dispersal has led to an increase in locomotor activity without affecting fecundity and lifespan suggests that the selected populations may have higher amounts of energy reserves. To check this, we measured glucose and glycogen content in the flies as these are the primary sources of energy apart from lipids. No significant difference in glucose and glycogen content was found between the selected and control populations (Fig. 9). A possible explanation for this could be that the selected populations have evolved a greater efficiency in utilizing their energy reserves. Furthermore, studies have shown that desiccation resistance and glycogen content are positively correlated (Graves et al., 1992). Since the selected populations have lower desiccation resistance (Tung et al., 2016), they are expected to have reduced glycogen content as well. The fact that this correlation has not been observed here may imply that the selected populations are preferentially investing their glycogen resources into locomotor activity instead of desiccation resistance. This could explain the increase in locomotor activity with a corresponding decrease in desiccation resistance in the selected populations.

Although, no significant difference in glucose and glycogen content was found, there may exist differences in lipid content. An assay in this direction could possibly reveal hitherto unknown differences between VB and VBC populations with respect to storage of energy reserves. Also, this assay was performed only on males and assays on females could give us entirely different patterns.

4.5 Effects of relaxation of selection pressure for increased dispersal

Selection for a particular trait may entail costs and potential trade-offs with other traits. In this study, no such trade-offs or costs are found when populations are selected for increased dispersal. Thus, does evolution of increased dispersal entail any costs at all? To investigate this further, I decided to investigate the effects of relaxation of selection for increased dispersal. For this, I derived the ReVB populations VB populations and subjected them to the same regime as that of VBC populations. Thus, the ReVB populations are relieved from the selection pressure for dispersal. If dispersal is a costly phenotype to maintain, then the ReVB populations should show reverse evolution of dispersal related traits. To check this, we assayed for locomotor activity and desiccation resistance which, as shown above, had increased and decreased respectively in the selected VB populations.

The locomotor activity levels of relaxed populations (i.e, ReVB) and selected populations were found to be similar, and significantly greater than those of control populations (Fig.10). Additionally, the desiccation resistances of VB and ReVB populations are similar and seem to be lower than those of VBC populations (Fig. 11). This similarity in trait values between VB and ReVB populations indicates that relaxation of selection pressure does not lead to a reverse evolution of dispersal related traits. This implies that maintenance of dispersal phenotype in the VB populations does not entail major phenotypic costs. The fact that fecundity and lifespan, critical components of fitness, are not affected by evolution of increased dispersal, could explain why dispersal is an inexpensive trait to maintain. An alternative explanation commonly cited for the failure of reversal evolution is the lack of genetic variation (Teotónio and Rose, 2001).

Due to intense directional selection, the genetic variation in the population declines (Keightley and Hill, 1987), thus making it impossible for the relaxed strains to achieve the phenotype of the control populations. This is unlikely to be the case in our populations since the VB populations are continuing to respond to selection for dispersal, which would have not been the case if all variation had been depleted. It should however be noted that, although no deterioration of locomotor activity is observed in the relaxed populations, the dispersal kernel (Clobert et al., 2012; Nathan et al., 2003) of these populations needs quantification. This will truly ascertain whether dispersal is an expensive trait to maintain. Also, this locomotor activity assay was performed after only 10 generations of relaxation. It has been shown that reversal of traits due to relaxation of selection pressure can sometimes require up to 50 generations to show sufficient divergence in trait values (Teotónio and Rose, 2000).Thus, an assay performed after further generations of selection could yield different results.

In summary, this study shows that selection for increased dispersal can lead to enhanced locomotor activity without any reduction in lifespan and fecundity. The amount of energy reserves like glucose and glycogen need not change in course of dispersal evolution. Lastly, the study demonstrates that dispersal related traits, such as reduced desiccation resistance and increased locomotor activity, might persist even in the absence of selection pressure for dispersal.

4.6 Conclusion

In recent times, range shifts due to climate change (Parmesan and Yohe, 2003) and the invasion of habitats by exotic species have become major areas of investigation in ecology(Travis and Dytham, 1999). Dispersal is intimately linked with such range expansions. In fact, it has been reported that range expansions are associated with evolution of higher dispersal propensity (Travis and Dytham, 1999). This, coupled with the fact that dispersal is a rapidly evolvable trait (Fronhofer et al., 2014), can lead to larger range expansions than previously predicted. Thus understanding the ecological

and evolutionary aspects of dispersal is imperative. One way to do this is by understanding traits that change with dispersal. This study aims at identifying traits that evolve as a result of selection for increased dispersal. In particular, it demonstrates that evolution of dispersal is associated with increased physical activity without affecting lifespan and fecundity. Additionally, it shows that dispersal is an inexpensive trait to maintain. These findings, coupled with the fact that dispersal is a heritable trait (Hansson et al., 2003; Saastamoinen, 2008), suggest that the dispersal phenotype can persist in populations for a long time and can evolve rapidly when selected for. Thus, the understanding of traits that co-evolve with dispersal can help us predict range shifts due to climate change and assess the possible invasion dynamics of exotic species. Furthermore, studies have shown that dispersal is linked with a myriad of behavioral traits such as aggression and boldness (van Overveld et al., 2010). It would be interesting to see how these behavioural traits change with evolution of dispersal. This is of particular interest because evolution of behavioural and life-history traits could affect interactions between organisms of a community. Thus the effect of dispersal evolution on community dynamics could be an interesting avenue for further research.

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Appendix

Dispersal apparatus:

The dispersal apparatus employed during selection consists of a source container connected to a sink container via transparent tube (i.e, path) of small diameter (~1cm). The source and sink containers are also transparent. The source is a cylindrical plastic container whose height and diameter are 16 cm and 11 cm respectively. A conical flask is attached to one end of the source container. The sink is again a transparent cylindrical container (diameter=11cm, height=16cm). A strip of wet cotton is paced inside the sink. The end of the path protrudes (~10 cm) into the sink. This minimizes backflow of flies that have reached the sink. The path is coiled to make the setup compact.

Banana jaggery medium:

Following is the composition of standard fly food (bulk volume=1 litre) used in the lab:

Water (1litre), Barley (25g), Jaggery (35g), Yeast (36 g), Agar (12.4g), Water+Ethanol (120ml+22ml), Methyl paraben + Ethanol (2.4g+23 ml). Weight (or volume, wherever applicable) of each ingredient is proportionally altered in order to make fly food with different bulk volume.