Effect of dispersal evolution on pre-adult traits in laboratory populations of *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

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CERTIFICATE

This is to certify that this dissertation entitled *“Effect of dispersal evolution on pre-adult traits in laboratory populations of 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 P.M. Shreenidhi 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 *"Effect of dispersal evolution on pre-adult traits in laboratory populations of 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.

P. M. Shreenidhi Date: 20-03-2017

Abstract:

Dispersal is a complex spatial process that allows individuals to track favourable environmental conditions. In recent times, with increased incidents of habitat degradation and fragmentation, studies have predicted the evolution of increased dispersal. Given the various causes and costs of dispersal, several traits of an individual are expected to evolve in response to dispersal evolution. Using laboratory populations of *Drosophila melanogaster* selected for higher dispersal, this study aimed at understanding: (1) how dispersal traits across different life-stages were correlated, (2) the effect of dispersal evolution on pre-adult traits, and (3) how these changes affect the subsequent settlement success in new environments. Additionally, the transmission pattern of dispersal phenotype from parents to offspring was investigated. Results suggest that there was no correlation between adult and larval locomotor activity. The dispersal-selected flies had a lower larval competitive ability than the control flies, and this result was substantiated by a trend of longer development time in dispersal-selected flies and no overall change in body-size. This would imply that dispersers are relatively inefficient at resource-utilization during development, explaining their poor performance in competitive environments. However, despite being competitively inferior in a resource-limited condition, dispersal-selected flies were as successful as control flies with respect to the settlement success in new environments. Lastly, it was found that mother and father did not contribute differentially to the dispersal phenotype of the offspring. The increased dispersal ability of selected populations, combined with no trade-off in proliferative success, makes them potent invaders and colonizers across varied environments.

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Acknowledgements

First and foremost, I would like to thank my mentor Dr. Sutirth Dey. It was his beginner's course 'Population Biology, Ecology and Evolution' that stimulated my interest in Evolutionary Biology. Since then, I have been in his lab for three years and I sincerely thank him for this wonderful opportunity. I think he has created a lab with just the perfect amount of freedom, fun and guidance. I hope that I can be at least half as passionate as him towards all things science and life.

I owe all my fly skills to two of my closest friends and lab-mates; Saint Sudipta Tung and Abhishek Mishra. Sudipta Tung is really no less than a saint; his patience, enthusiasm, and intelligence is inspiring. His one fatal flaw is his jokes. There is definitely a chance that it might prove to be deadly. Abhishek Mishra is not a saint, but he is definitely amongst the most important influences in my life. I thank him sincerely, for all his insights on every silly/serious question I have ever had, and for spending so much time and effort proof-reading this thesis.

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Finally, I would like to thank my parents and my brother for being there for everything. Without their unwavering support, guidance and freedom none of this would have been possible.

Introduction:

Dispersal is defined as the movement of individuals, which has implications for gene flow across space (Ronce, 2007). It is of particular relevance in current times, which are plagued by increased incidences of habitat fragmentation and destruction, and rapid changes in climatological conditions. In such a scenario, dispersal is likely to play a vital role in the persistence of a wide range of species though means such as climatological niche tracking among other things. (Maggini et al., 2011; Comte and Grenouillet, 2013). The dispersal of individuals into a new population or community can have profound effects on population stability, extinction probabilities (Brown and Kodric-Brown, 1977; Heino et al., 1997), and community dynamics (Mooney and Cleland, 2001), thus substantially affecting a wide variety of ecological processes.

Just as dispersal affects many ecological processes, a diverse range of factors affect dispersal (Bowler and Benton, 2005). The multi-causality of dispersal stems from it being a product of interactions between the phenotype of an individual and its environmental conditions (Clobert et al., 2009). Organisms, depending on their internal physiological, behavioural or morphological states, could differ in their sensitivity to these factors (Clobert et al., 2012) which in turn could influence their individual dispersal decisions. Additionally, the causal factors vary in their importance across the multiple-stages of dispersal, namely ‘departure’, ‘transient’, and ‘settlement’ (Clobert et al.,2012), and thus, each stage could place different selection pressures on the individual. For example, kin-competition may play a role in ‘departure’ of individuals from natal site, but it might not play such a major role in the ‘transient’ stage (Clobert et al.,2012). Moreover, dispersal is known to be an energetically expensive process (Ronce, 2007) that can place considerable demands on the energy reserves of an individual. This multifaceted causation of dispersal links it to several physiological, morphological and behavioural attributes of the organism (Clobert et al., 2009). Therefore, the evolution of such a complex-trait can lead to several interesting questions concerning the life history of organisms.

First, the extent of dispersal shown by an individual depends on its life-stage (Clobert et al., 2012). An individual may be motile only in a single life stage, in which case, its dispersal capacity would be determined by that life stage. But in species such as insects, with multiple motile life-stages, there could be considerable variation in

dispersal ability across different life-stages (Bowler and Benton, 2009; Clobert et al., 2012). The total dispersal could, in principle, then be a result of movement realized across the life stages in the respective environments. In addition, the environmental experiences of an individual during the pre-adult stage could affect its life-history as an adult, consequently altering the dispersal ability at a later age (Clobert et al., 2012). Given the intricate relationship between life-history traits and dispersal, the evolution of dispersal in one life-stage can potentially modulate the dispersal capability in other life-stages. Therefore, it is of interest to examine the correlation of dispersal traits across different life-stages.

Second, the costs and causes of dispersal could lead to trait correlations and trade-offs (Bonte et al., 2012). The realisation of these costs and causes of dispersal varies from one individual to another and is determined by their physiological constitution. This constitution is in turn determined by developmental processes (Clobert et al., 2012). In this context, the association of several life-history and developmental traits with dispersal has been well-studied. Body size of an individual, an important life-history trait, is known to correlate both positively and negatively with increased dispersal (Hanski et al., 1991; Lawrence, 1987; Anholt, 1990; Barbraud et al., 2003). Additionally, there are correlational studies which show that increased flight capacity (related to dispersal ability), is correlated with slower development time (Gu and Danthanarayana, 1992). Similarly, the competitive ability of adults is also known to influence dispersal decisions (Duckworth and Badyaev, 2007; Eden, 1987; Clarke et al., 2008). However, all these existing studies only establish the relation between dispersal and these traits, and do not provide an understanding into how evolution of dispersal could affect evolutionary trajectories of other traits. Such an understanding is essential, considering how widespread habitat degradation is expected to lead to evolution of dispersal traits (Fronhofer et al., 2014), which could lead to subsequent changes in various other life-history traits.

Lastly, following a dispersal event, the reproductive success of an organism would depend on producing viable offspring in the final habitat. This would, in turn, depend on its fecundity as well as the development of offspring from egg/embryo to viable adults. Since dispersal evolution is expected to affect several developmental and life-history traits in an individual, it could also affect the success of dispersers in a new environment. Although there is evidence from multiple studies that dispersal

phenotypes are exhibited by superior individuals (related to fecundity, aggression, boldness) (Lavie and Ritte, 1978; Duckworth and Badyaev, 2007; Clobert et al., 1988), there is little understanding of how the evolution of dispersal traits and its consequences on other life-history traits could affect the success of dispersers in new habitats. In addition, the transmission patterns of dispersal phenotype from one generation to the next could provide insights into the speed of dispersal evolution. For example, spatial sorting and assortative mating of dispersers at range expansion fronts is known to increase the speed of dispersal evolution (Shine et al., 2011). But the speed of evolution of dispersal traits could be reduced if the expression of dispersal ability in the offspring is governed by phenomenon such as genomic imprinting. Such transmission patterns have been reported (Saastamoinen, 2008). Hence, depending on the demography of disperses that settle into a new population, subsequent dispersal profile of the next generation could be altered.

To improve the current understanding of dispersal evolution and its effect on individuals, a selection experiment for higher dispersal on laboratory maintained populations of *Drosophila melanogaster* in a controlled environment is underway. It has already been shown that the selected populations have higher dispersal ability and propensity than the control populations, demonstrating that the dispersal traits have evolved (Tung et al., 2016). Currently, with over 50 generations of selection, these populations can be used to address several questions related to the effects of long-term dispersal evolution on various individual-level traits. This study is aimed at investigating the above-mentioned questions using these dispersal-selected lines and the respective control populations.

D. melanogaster are holometabolous insects, and go through four distinct developmental stages, namely egg, larva, pupa and adult. *D. melanogaster* in their larval stages are mobile, and actively move around in search of food. Previous studies from our lab have shown that dispersal ability of adult flies is correlated with increased locomotor activity (Tung et al., 2016). Thus, the larval locomotor activity was assayed to understand its correlation with adult locomotor activity, and the relation between dispersal ability across the two motile stages of *D. melanogaster*.

Next, the changes in developmental attributes of *Drosophila melanogaster* were investigated in response to the evolution of dispersal. Pre-adult traits in *Drosophila*

melanogaster are an important determinant of the adult life-history (Prasad and Joshi, 2003), and thus, dispersal evolution in adults could lead to changes in pre-adult traits. The post-eclosion body size of adults is determined by the size of the third-instar larvae at pupation (Prasad and Joshi, 2003). Body size of the fly is therefore, dependent on resource acquisition in larval stages. An increase in resource requirement owing to the energy costs of dispersal (Ronce, 2007) could influence changes in larval traits such as larval competitive ability and development time. Larval competitive ability can be a determinant of the quality and quantity of resources acquired by an individual, and prolonged resource acquisition to meet the increased requirements might lead to a change in development time. Any changes in these traits might also be reflected in the body size of the fly. Thus, the larval competitive ability, development time and body size of the flies was assayed to understand the effect of dispersal evolution on these traits.

In addition, the changes in development and life-history traits due to dispersal evolution were investigated in different environments. Assuming successful immigration into a habitat, I define the reproductive success of an organism in that habitat as its settlement success. The settlement success of dispersal-selected and control flies, measured as the number of viable offspring produced, was tested across three different environments.

Finally, given that dispersal-selected flies have higher activity compared with control flies (Tung et al., 2016), I used locomotor activity as a proxy for dispersal ability, and tested the activity of purebred dispersal and control flies along with their hybrids. This was done to see if dispersal traits are transmitted to subsequent generations in a parent-specific manner.

Materials and Methods

Ancestral populations:

All flies used in this study were derived from four laboratory populations of *Drosophila melanogaster*, namely DB_{1-4} (**D**ey **B**aselines). They are housed in plexi-glass cages at an adult population size of approximately 2500 flies. They are maintained in incubators at a temperature of 25˚C and 90% humidity, on standard banana-jaggery food under constant light conditions on a 21-day discrete generation cycle. The eggs are reared in food vials containing ~6 mL of food at a density of 60-80 eggs, in incubators at 25˚C. Apart from stock populations, two eye-colour mutants are also maintained in the lab, namely DBW (**D**ey **B**aseline **W**hite-eyed) and SE (**S**carlet **E**ye). Their maintenance regime is similar to the DB populations.

Dispersal selected populations:

There are four dispersal selected populations VB_{1-4} (**V**aga**B**ond) and their corresponding controls VBC_{1-4} (**V**aga**B**ond **C**ontrol). Populations that share a subscript also share ancestry, and have been derived from the corresponding DB population. Thus, for example, VB_1 and VBC_1 share ancestry and have been derived from DB_1. They are held in cages at a population size of approximately 2500 flies that are housed in incubators at a temperature of 25˚C under constant light conditions. The VB and VBC populations are maintained on standard banana-jaggery food on a 15-day discrete generation cycle. They face dispersal selection on 12^{th} day from egg collection, are supplied with live-yeast paste for a single day on the 13^{th}, and the eggs to constitute the next generation are collected on the 15^{th} day. The eggs are reared in food vials containing ~6 mL of food at a density of 60-80 eggs, in incubators at 25˚C.

Dispersal Selection:

On the 12^{th} day from egg collection, all the offspring flies of VB_{1-4} populations undergo dispersal selection to subsequently form the current generation of VB_{1-4}. The corresponding VBC_{1-4} are kept in similar conditions as VB_{1-4} the except that they do not undergo dispersal selection. Though the details of the selection regime are not directly pertinent to understanding methodological details of the assays performed, it is of relevance to understand the environmental context in which the

dispersal evolution happens, for different environmental scenarios could lead to different trajectories of dispersal evolution. Therefore I present a detailed account of the dispersal selection protocol here.

The dispersal selection set-up consists of a source bottle, a path and a destination/sink bottle, as shown in Illustration 1. The source bottle is connected to a coiled path, which opens into the destination bottle. The path is coiled to make the set-up compact.

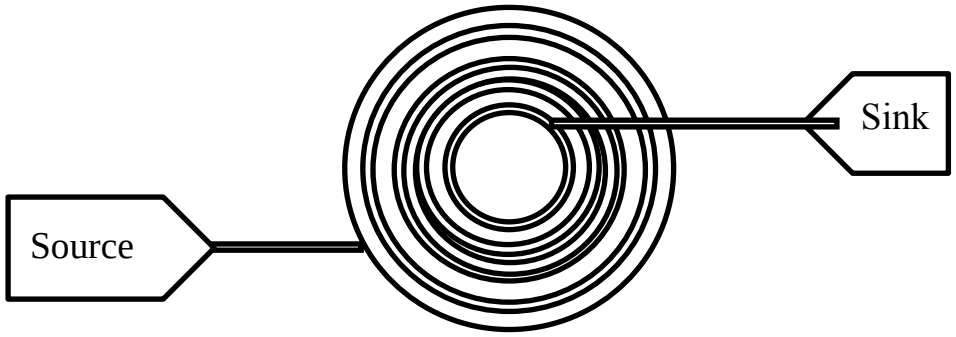

Illustration 1: Dispersal selection set-up

The source bottle is a plastic see-through container of volume 1.5 L. It does not contain any supply of food or moisture. The path consists of a transparent plastic pipe of approximately 1 cm inner diameter. The sink bottle is also a container similar to the source, except that the path protrudes about 10 cm into the container. This protrusion prevents the backflow of the flies that reach the sink into the path. The sink also contains a thin, wet cotton strip which acts as a supply of water and moisture. The entire set-up is placed in a uniformly lit region at 25˚C.

Approximately 2500 of 12-day old flies are introduced into the source bottle. The flies are then allowed to disperse in the set-up till approximately 50% of the flies reach the sink or for 6 hours, whichever comes first. Each of the four populations of VB undergo dispersal selection similarly. The dispersal selection is terminated at 6 hours to avoid desiccation stress. To maintain the final population size of ~2500, there are

two such dispersal selection set-ups for each VB population. The flies from the sink of both of these dispersal set-ups of a given VB population are mixed together to form the breeding population of the VBs. While the VB populations undergo dispersal selection, the corresponding control populations (VBC) are introduced into a plugged source bottle and are maintained at similar conditions to the dispersal set-up. This source bottle also does not contain food or water, but a wet cotton is plugged into the control source bottle when about 25% of the flies reach the destination in the corresponding VB setup. At the end of the selection, the flies are introduced into cages containing standard banana-jaggery medium. When selection was imposed for the first time in these populations, the path length was kept at 2 metres. As the populations quickly responded to selection, the path length has been increased regularly and is currently at 20 meters after 70 generations of selection.

Assays:

All assays were performed on populations of VB_{1-4} and VBC_{1-4} after one generation of relaxation, *i.e*, rearing under common conditions, without selection for dispersal, to ameliorate the effects of short-term non-genetic inheritance (Rose 1984). Populations sharing a subscript, say VB_1 and VBC_1 are assayed together and hence, act as statistical blocks.

Larval Competitive Ability

The larval competitive ability of the selected lines (VBs) and control lines (VBCs) was tested against a white-eyed common competitor (DBW). The assay was performed after 51 generations of selection. Exactly 150 eggs each of a given VB population and DBW were introduced in a vial containing exactly 2mL of standard banana-jaggery medium, and maintained at 25˚C in the incubator. There were 5 such replicate vials per VB or VBC population. The vials were checked for flies every 12 hours from the 8^{th} day since set-up, until no fly eclosed for 3 consecutive days. The number of red-eyed flies (VB) and the number of white-eyed flies (DBW) that eclosed were noted down as data for the particular time point. The fraction of the given population of VB flies in the total number of eclosed flies (i.e. VB+DBW) was calculated. The same procedure was followed for the corresponding VBC. All the four dispersal selected populations and their controls were assayed similarly. The fractions were then arcsine square root transformed to obtain a nearly normal

distribution of data (Zar, 1999), on which a two way mixed model ANOVA was performed, with selection as fixed factor (VB, VBC), and blocks (1-4) as random factor crossed with each other.

Larval Locomotor Activity

In this experiment, the locomotor activity of 76-hour old VB and VBC larvae were measured using Drosophila Activity Monitor (DAM) (Trikinetics Inc.). The assay was performed after 53 generations of selection. Drosophila females typically retain eggs at different stages of development inside their body, and therefore the larvae that hatch from a given clutch can be of different age. Since larvae of different ages can potentially have different motility, this can, in principle, affect the measurement of the trait. To ensure that all larvae assayed were of the same age, synchronized egg collection was conducted as per the following procedure. An egg collection plate with yeast paste was introduced into the cage. After one hour, the plate was discarded and another similar plate was introduced into the cage. After another hour, the plate was yet again discarded, and a final collection plate was introduced. This collection plate was then removed from the cage after 30 minutes. Approximately 50 eggs from this plate were introduced into a food vial containing ~6 mL of standard banana-jaggery medium. These vials were then incubated at 25˚C. There were 5 such vials for a given population. After precisely 76 hours from egg collection, the larvae were extracted from the vials and their motility was measured. Following established protocol (McParland et al., 2015), a larva was introduced into a moist DAM tube, and the ends of the tube were plugged with 4% agar plugs of 1cm length. The moisture and the agar help in preventing the desiccation of the larva. There were 32 such replicate tubes for each of the VBs and VBCs. The instrument comprised of an Infra-Red beam emitter and receiver to measure the activity of the individual. The DAM tube was inserted into the instrument such that the tube was bisected by the infra-red beam. The instrument counted the number of times the beam got obstructed by the movement of the individual in a time interval of 5 minutes. This count was reported as the activity of the individual for that time interval. The total number of counts over a period 20 minutes was recorded as data. All the four dispersal selected populations and their controls were assayed similarly. The data was analyzed using a two-way mixed model ANOVA with selection as fixed factor, and blocks (1-4) as random factor crossed with each other.

Egg to Adult Development Time

The egg to adult development time of VB and VBC populations was assayed after 51 generations of selection. Exactly 30 synchronised eggs of a given population were introduced into a vial containing ~6mL of standard banana-jaggery medium, and maintained at 25˚C in the incubator. The vials were checked for flies every 2 hours from 8th day since set-up, until there were 3 consecutive days without any eclosion. The number of male and female flies eclosing at each time point was recorded and the flies were discarded. There were 20 such replicate vials for each of the four selected and control populations. The data was analyzed using a three-way mixed model ANOVA with selection (VB, VBC) and sex (Male, Female) as fixed factors, and blocks (1- 4) as random factor, all factors crossed with each other.

Dry body weight assay

To estimate the body size of VB and VBC populations, the dry body weight of the flies was measured after 49 generations of selection. For a given population, approximately 50 eggs were introduced into food vials containing ~6 mL of standard banana-jaggery medium, ensuring *ad libitum* food conditions. After 12 days from egg collection, the adult flies were collected from these food vials. These flies were then killed by flash freezing using liquid N_2 and stored at -80˚C till assay date. During the assay, batches of 20 flies of a given sex were made. There were 5 such replicate batches for a given sex. These batches were then dried at 60˚C for 72 hours and their dry-body weight was measured to the nearest 0.1 mg. The average value for a single fly of a given batch was calculated by dividing the total measured weight by 20. The data was analyzed using a three-way mixed model ANOVA with selection (VB, VBC) and sex (Male, Female) as fixed factors, and blocks (1- 4) as random factor, all crossed with each other.

Settlement Success Assay

In this experiment, the success of VB and VBC flies, measured as the number of viable offspring produced, is tested across different environments. Two mating pairs of 12 day old flies (from egg collection), were introduced in a vial containing a given food environment (see below). They were left overnight in the vial and thus allowed to mate and oviposit for 12 hours. There were 20 such replicates for a given

population and food environment. At the end of 12 hours, the flies were discarded and the vials were maintained in the incubator at 25˚C. The vials were checked for eclosing offspring flies every 12 hours from 8th day since the setup, until there were 3 consecutive days with no eclosion. The total number of flies eclosing in this time period were termed as the total number of viable offspring and entered as data. All the four dispersal selected populations and their controls were assayed similarly. There were 3 different food environments across which the performance of the flies was assayed:

1. **Normal food environment:**

This environment comprised of the standard banana jaggery medium (Appendix: Table 1). The vial corresponding to this environment contained ~6 mL of this standard medium.

2. **33% Diluted food environment:**

This environment was composed of the same ingredients as the standard banana jaggery medium, but at a lower concentration (Appendix: Table 2). All the comprising ingredients (except agar agar), i.e. banana, jaggery, yeast and barley were exactly one-third of their usual concentration in the standard medium. The amount of dilution was based on previous standardizations, and was sufficient to cause noticeable delay in fly development, and hence the environment was interpreted to be stressful. The vial corresponding to this environment contained ~6 mL of this diluted medium.

3. **Urea rich food environment:**

This medium was exactly similar to the normal food environment, except that it contained urea at a concentration of 12 g/L. Urea was dissolved into water and then added to the medium post preparation and before cooling. The vial corresponding to this environment contained ~6 mL of this urea enriched medium. The concentration of urea was based on previous standardizations in the laboratory, and was sufficient to cause noticeable delay in fly development, and hence the environment was interpreted to be stressful.

This assay was performed after 59 generations of selection. The data was analyzed using a three-way mixed model ANOVA with selection (VB, VBC) and environment

(Normal, Urea, 33%) as fixed factors, and blocks (1- 4) as random factor, all factors crossed with each other.

Inheritance of activity assay

Inheritance of activity assay was conducted to understand the transmission pattern of the heritable variation for dispersal traits from parents to the offspring. This assay was performed on VB_4 and VBC_4 populations. For this assay, the following crosses were set up:

1. VB_4 females X VB_4 males
2. VB_4 females X VBC_4 males
3. VBC_4 females X VB_4 males
4. VBC_4 females X VBC_4 males

For the crosses, on the 9th day from egg collection, virgin flies were collected from food vials containing approximately 50 eggs in ~6mL of standard banana-jaggery medium. The populations had undergone 53 generations of selection at the time of the assay. For a given cross, 5 mating pairs i.e. 5 virgin males and 5 virgin females were introduced into a fly bottle with ~30 mL of standard banana-jaggery medium. There were two such bottles for each cross. These mating pairs were then left to mate and oviposit in the bottles for 12 hours. The adult flies were then discarded after 12 hours, and the bottles, containing eggs from the crosses, were maintained in the incubator at 25˚C.

The activity of 12 day old male offspring of the crosses was measured using Drosophila Activity Monitor (DAM) and standard protocol (Tung et al., 2016). A single male was introduced into a DAM tube. The ends of the tube were secured with cotton. The activity of the male was measured for a total duration of 6 hours. There were 32 such replicates for each cross. Due to logistic constraints, only 64 flies could be assayed at a given time. Hence, the assay was staggered over two days, with 16 replicates of a given cross assayed on each day. The activity per hour and percentage of time spent resting (arcsine square root transformed) was calculated. The instrument reports activity in intervals of 5 minutes. 5 minutes of total inactivity is termed as resting period. The percentage of time spent resting was in the total assay

duration was calculated. Two types of ANOVA were done on the data. The data was first analyzed using a two-way mixed model ANOVA, with identity of type of progeny as fixed factor (4 types) and day as random factor. The data was then analyzed using a three-way mixed model ANOVA, with the identity of Mother (VB, VBC) and Father (VB, VBC) as fixed factors, and day as a random factor, all factors crossed with each other.

Results:

1. Evolution of adult dispersal traits do not affect larval locomotor activity

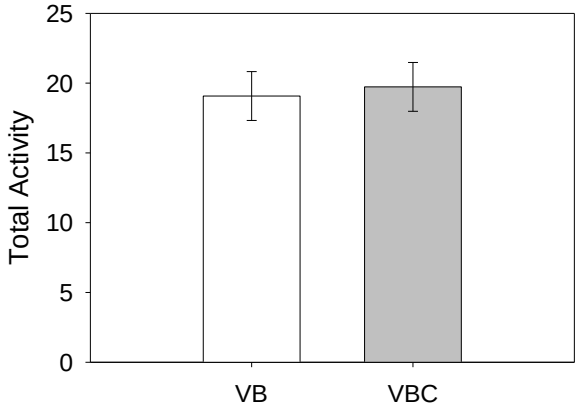

Figure 1: Larval locomotor activity of VB and VBC populations. Average total activity in an interval of 20 minutes (±SEM); no significant difference in the observed larval activity levels of VB and VBC

The total locomotor activity of 76-hour old larvae (for a time period of 20 minutes) was assayed. Both VB and VBC larvae were observed to have similar locomotor activity profiles (Fig. 1, $F_{1,\ 3}$= 0.24, p=0.66). This suggests that evolution of dispersal did not lead to changes in locomotor activity levels of the larvae.

2. Dispersal evolution does not affect egg-to-adult development time

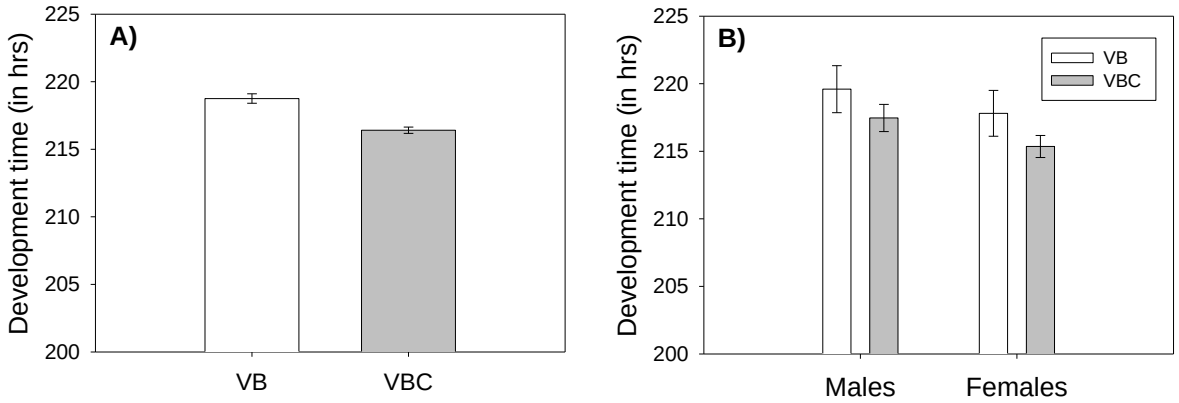

Figure 2: Egg to adult development time of VB and VBC populations. **A)** Average development time (in hours) ± S.E.M of VB and VBC; No significant difference was observed in the development time of VB and VBC. **B)** Selection × Sex interaction; no sex-specific responses were observed.

The development time of VB and VBC was measured to check whether it evolves in response to evolution of dispersal. Results show that VB and VBC have similar development time (Fig. 2A, $F_{1, 3}$= 4.89, p=0.11). However, there does seem to be a trend of VB taking longer to develop as compared to the VBC, and the effect size is medium (Cohen's d = 0.63). Additionally, both males and females respond similarly to selection with respect to their development time (Fig. 2B, $F_{1, 3}$= 0.43, p=0.56). Thus, there is no sex specific response in development time to selection for increased dispersal.

3. Dispersal evolution does not change dry body weight

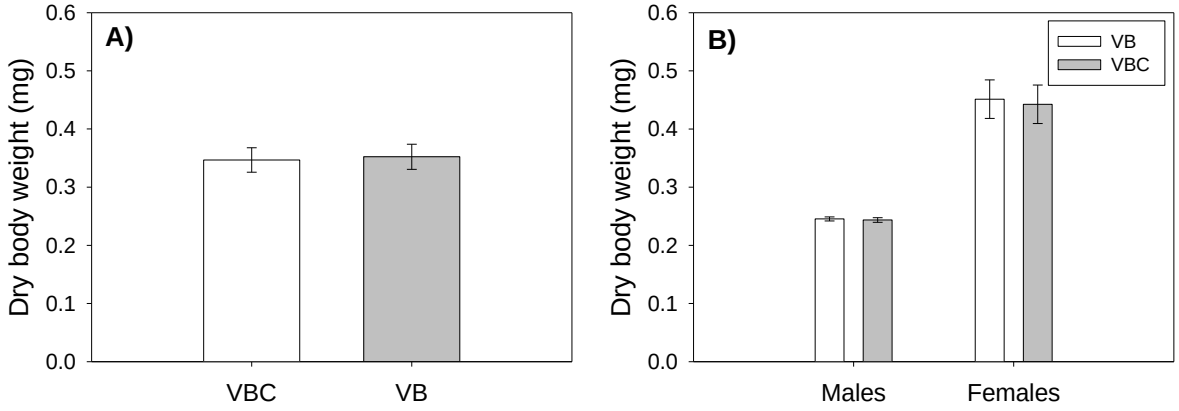

Figure 3: Dry body weight comparison of VB and VBC populations. **A)** Average dry body weight (in mg) ± S.E.M of VB and VBC; No significant difference was observed in the dry body weight of VB and VBC. **B)** Selection × Sex interaction; no sex-specific responses were observed.

The dry body weight of VB and VBC was assayed. Results show that VB and VBC have similar dry body weight (Fig 3A. $F_{1, 3}$= 0.76, p=0.45), suggesting that dry body weight does not respond to selection for increased dispersal. Furthermore, both males and females respond similarly to selection for increased dispersal with respect to their body size, and there was no selection × sex interaction (Fig 3B. $F_{1, 3}$= 2.12, p= 0.24). Thus, there is no sex-specific response to selection for increased dispersal in dry body-weight.

4. Dispersal evolution reduces larval competitive ability

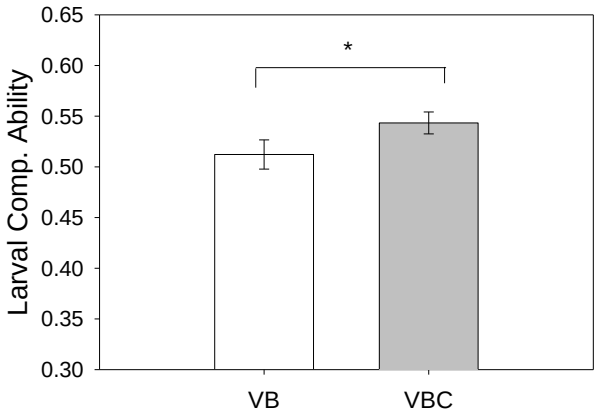

Figure 4: Larval Competitive Ability of VB and VBC. Average Larval Competitively Ability ± S.E.M of VB were found to be lower as compared to VBC. * indicates that differences are significant at 95% confidence interval.

The larval competitive ability of VB and VBC larvae was tested against common white-eyed competitor, DBW. Results show that VB were found to lower larval competitive ability as compared to VBC (Fig. 4, $F_{1,\ 3}$= 9.63, p=0.053), suggesting that larval competitive ability decreases in response to selection for increased dispersal.

5. Selection for dispersal ability and rate have no effects on settlement success

The settlement success, measured as the average number of viable offspring produced by a single mating pair of VB and VBC was tested across several environments. Analysis revealed that there was no significant difference between the number of viable offspring produced by VB and VBC across all environments flies (Fig. 5A, $F_{1,\ 3}$= 0.028, p= 0.88). There was no significant interaction of Selection × Environment ($F_{2,\ 6}$= 0.99, p= 0.42), implying that both VB and VBC behaved similarly across all environments (Fig. 5B, 5C, 5D).

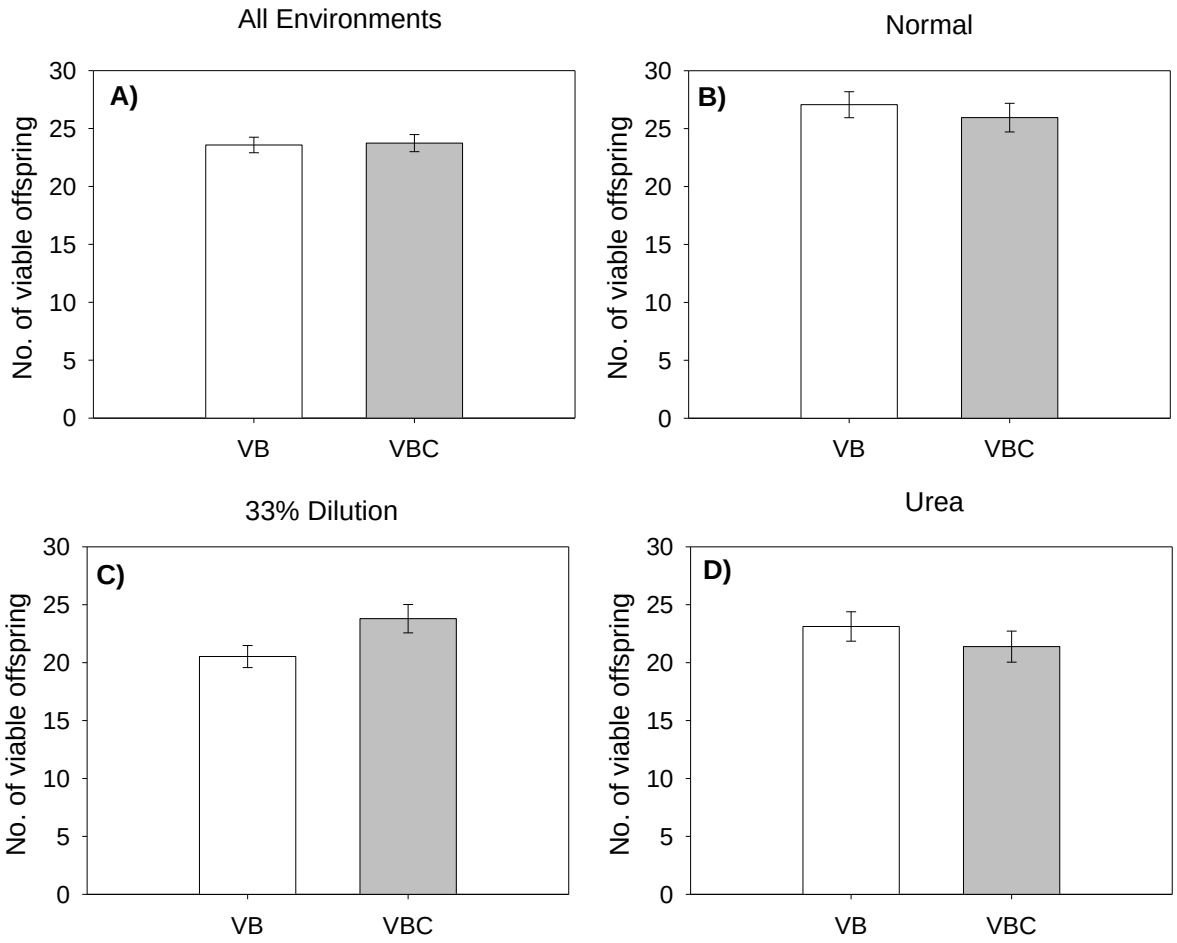

Figure 5: Settlement success of VB and VBC across several environments. The average number of viable offspring ± S.E.M produced by a single mating pair of VB and VBC is plotted, **A)** pooled across all environments, **B)** in normal food environment, **C)** in 33% dilution environment, and **D)** in Urea rich environment. No significant difference was found in the settlement success of VB and VBC pooled over all environments. No significant effect of Selection × Environment was found.

6. Effect of sex of parent on inheritance of activity

The following notation is used to represent the offspring of the two homotypic and heterotypic crosses. The notation is a two letter code with the letters B and C. The letter B corresponds to the dispersal selected population and C corresponds to control population. The first letter in the notation corresponds to the identity of the mother and second letter represents identity of the father. Hence, the four types of offspring are:

5. V**B** Mother X V**B** Father ⇒ **BB**
6. V**B** Mother X VB**C** Father ⇒ **BC**
7. VB**C** Mother X V**B** Father ⇒ **CB**
8. VB**C** Mother X VB**C** Father ⇒ **CC**

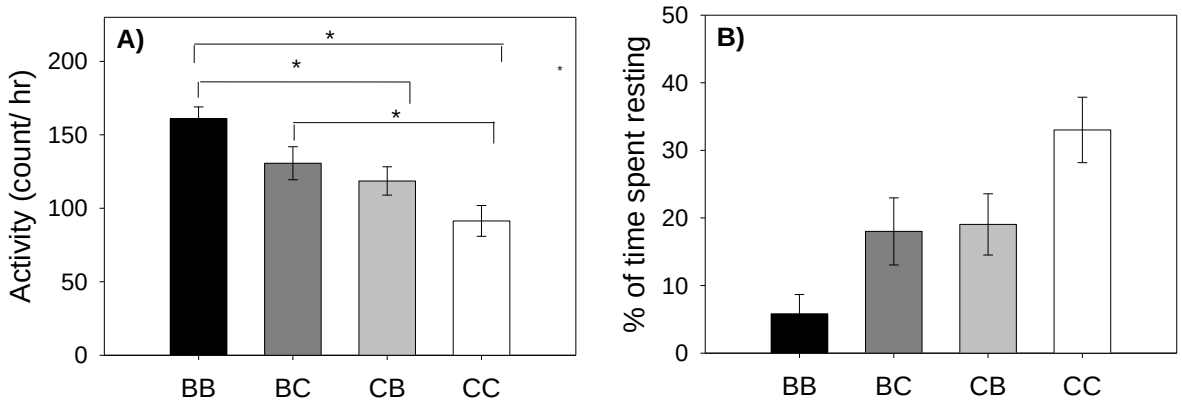

Figure 6: The locomotor activity and resting profiles of the four offspring types. A) Average activity per hour ± S.E.M. There was significant difference between the activity profiles among the four offspring types. **B)** Average percentage of time spent resting ± S.E.M. Marginally significant difference in the resting profile of the four offspring types.

The locomotor activity and resting profiles of the four offspring types were assayed. On performing ANOVA with day as random factor, crossed with offspring type as fixed factor, it was found that there were significant differences between the locomotor activity levels of the four offspring types (Fig. 6A, $F_{3,3}$= 19.10, p=0.018). The difference between the resting patterns of the four offspring types were found to be of borderline significance (Fig. 6B, $F_{3,3}$= 7.99, p=0.06).

Tukey HSD test on the locomotor activity pattern of the four offspring types revealed the following. When the activity of the offspring of VB mothers and VBC mothers with a background of VB father was compared, there was a significant difference in activity (BB- CB comparison, p= 0.031). But when activity of the offspring of VB fathers and VBC fathers with a background of VB mother was compared, there was no significant difference in activity (BB- BC comparison, p = 0.14). Similarly, when the activity of offspring of VB mothers and VBC mothers with a background of VBC fathers was compared,

there was a significant difference in activity (BC-CC comparison, p= 0.016). But when the activity of offspring of VB fathers and VBC fathers with a background of VBC mothers was compared, there was no significant difference in activity (CB-CC comparison, p=0.22).

To understand the implication of the results in the activity profile of the offspring flies, a three-way mixed model ANOVA was performed with day as random factor, the identity of mother (VB/VBC) and father (VB/VBC) as fixed factors, all factors crossed with each other.

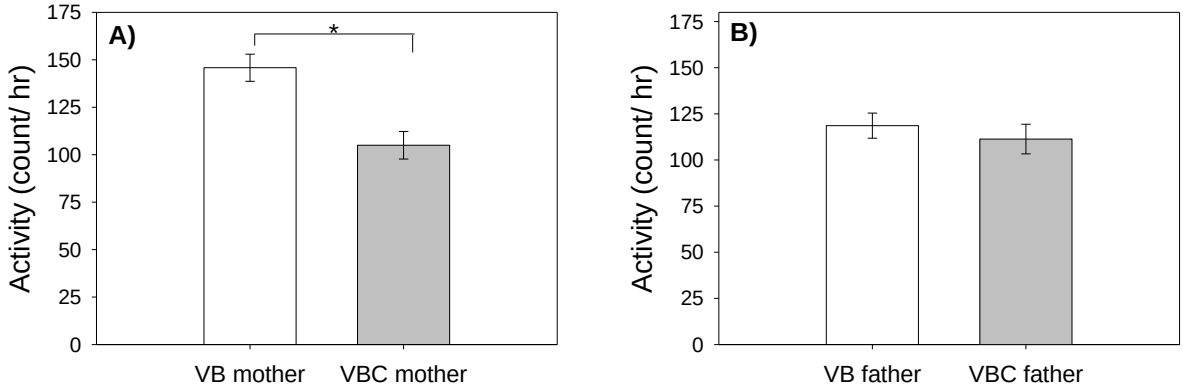

Figure 7: Average locomotor activity ± S.E.M of offspring pooled over A) maternal identity, and **B)** paternal identity. There was significant difference in the offspring of VB and VBC mothers, while no significant difference was found in the offspring of VB and VBC fathers.

When pooled over maternal identity, it was found that the offspring of VB mothers had significantly higher activity as compared to the offspring of VBC mothers (Fig. 7A, $F_{1,1}$ =342.19, p=0.034). The effect size calculated as Cohen's d is large (Cohen's d = 5.67). When the average activity per hour of offspring was pooled over paternal identity, no significant difference was found (Fig. 7B, $F_{1,1}$ = 64.38, p= 0.079), even though the effect size calculated as Cohen's d was large (Cohen's d = 0.98).

Discussion:

Dispersal is a complex process of great ecological significance, and it depends on interactions between multiple factors. These factors can be broadly classified into three categories: the motives of the individual for dispersal, the energy costs of dispersal and the condition of the individual (Clobert et al., 2012). Hence, evolution of such a complex trait could lead to several behavioural, morphological and behavioural trade-offs in the organism. In the lab, *Drosophila melanogaster* have been selected for higher dispersal ability in adults, and it has been shown that the dispersal traits have evolved in the adults of these selected populations (Tung et al., 2016). The dispersal evolved flies had greater dispersal propensity, greater dispersal ability, and increased locomotor activity. Since *Drosophila melanogaster* are motile in the larval life-stage as well, this system provides an opportunity to understand how locomotor activity in pre-adult stages is affected by increased activity in adults. Given that dispersal is energy/resource intensive and that the amount of resource at eclosion entirely depends on the resource gathered in the pre-adult period, it is possible that pre-adult traits are also affected in response to selection for increased dispersal on the adults. To check this hypothesis, I assayed some key pre-adult traits such as development time, body size and larval competitive ability.

Evolution of increased dispersal does not correlate with larval locomotor activity

Drosophila melanogaster larvae actively move around in search of food. This movement of the larva for the purpose of foraging is influenced by a gene called the '*for*' gene (de Belle et al., 1989), of which there are two variants: 'for^R' and 'for^S'. In the presence of food, it has been shown that larvae with 'for^R' gene move a lot more to procure food than the ones with 'for^S' gene (de Belle et al., 1989). It has also been seen that adult flies with 'for^R' allele traverses a lot more distance while feeding as compared to the flies with the other allele (Pereira and Sokolowski, 1993).

Our lab has earlier shown that evolution of dispersal is correlated with adult activity, as quantified using the Drosophila activity Monitor (DAM) (Tung et al., 2016) . Since there is evidence that larval and adult activities are correlated (Pereira and

Sokolowski, 1993), I tried to see if evolution of increased adult activity, as a correlated response to selection for dispersal, also leads to a simultaneous increase in larval activity. Interestingly though, evolution of dispersal did not lead to any changes in larval locomotor activity between VB and VBC populations (Fig. 1) suggesting that adult and larval locomotor activity are not correlated. One reason for this apparent discrepancy between the previous results (Pereira and Sokolowski, 1993) can be that the previous study reported that larval activity maps on to adult activity, while I was looking at whether enhanced adult activity implies increased larval activity and variation in adult activity is not directly affect the variation in larval activity.

Dispersal evolution is not correlated with egg to adult development time

Dispersal is an energetically intensive process (Ronce, 2007), and hence, may require increased resource acquisition to match the increased energy requirement. The body size of the fly, an indicator of energy reserves, remains fairly constant post eclosion, and is largely determined by the size of the third instar larva at pupation (Prasad and Joshi, 2003). It is possible that increased resource acquisition requires prolonged larval foraging and hence, longer development time. Thus, it is likely that dispersal selected lines might take a longer time to develop. However, my assays show that there is no significant difference between the development time of VB and VBC (Fig. 2A) although the VBs do seem to take a longer time to develop than the VBCs. One reason for this discrepancy between the expectations and our empirical observations might be the fact that at the time of this assay, the VBs had undergone only 51 generations of selection which may not be sufficient for this trade-off to be exhibited. Another possible reason might be that although the VBs need more energy, they get it through increased efficiency of resource utilization and not by acquiring greater amount of resources during larval development. To investigate this possibility, I estimated the body weight at eclosion of these flies.

Dry body weight of flies is not affected by dispersal evolution

There are two predominant hypotheses that could explain the relation between body-size and dispersal (Bowler and Benton, 2005). It is known that dispersal is correlated with smaller individuals that cannot access resources in their natal site because of their inferior status or lower competitive ability. In other words, this line of reasoning suggests that dispersers are expected to have low body sizes (Hanski et al., 1991; Lawrence, 1987). However, on the other hand, if the energy expenditure of dispersal is too high, then individuals with less amount of resources would not be able to reach their destinations, which would result in a positive correlation between dispersal success and body size (Anholt, 1990; Barbraud et al., 2003). Note here that the first prediction is applicable for dispersal initiators, while the second one pertains to those individuals who are able to reach a destination patch. This once again highlights the point that different selection pressures are at play for different stages of dispersal, which can lead to contrasting predictions about how a given life-history trait (here body-size of the dispersing individuals) is expected to respond to selection for dispersal .

In *Drosophila melanogaster*, the adult body size is largely fixed by the size at which third instar larvae undergo pupation (Prasad and Joshi, 2003), and body size of an individual is known to influence dispersal decision (Bowler and Benton, 2005). The dry body weight of VB and VBC flies was assayed to understand the effect of dispersal evolution on body size. No significant difference in the dry-body weight of VB and VBC flies was observed (Fig. 3A). On one hand, this could suggest that dispersal in our system is neither energy expensive nor means of escaping competition. On the other hand, it is also possible that there are methods by which VB flies compensate for the energy cost: they could be more energy efficient. For example, it is known that insects minimize the high energy cost of flight by using highly efficient muscles (Dickinson and Lighton, 1995), and it is possible that the VB flies have evolved something similar for ambulatory dispersal. But, it is also possible that a difference in body-weight might manifest further down in the selection. Unfortunately, with the present data, it is not possible to distinguish between these various possibilities.

Larval competitive ability decreases in response to evolution of dispersal

The capability of an individual to acquire resources in a resource limited environment hinges on the competitive ability of the individual. As discussed earlier, there have been several studies that investigate the relationship between dispersing individuals and their competitive ability. Using a common competitor assay, I show that VBC larvae were competitively superior as compared to the VB larvae (Fig. 4).

There are two possible explanations for how this difference could manifest. Severely crowded conditions such as in the assay (300 eggs in 2 ml of food), could lead to accumulation of high levels of urea, a naturally occurring nitrogenous waste product in the environment. Urea is known to be toxic to *Drosophila* larvae, and affects both development time and larval survival (Botella et al., 1985). It is possible that VBC larvae could have higher tolerance levels for urea toxicity as compared to VB larvae and hence, perform and survive better in crowded environments. However, the equal settlement success of VB and VBC in urea-rich environment challenges this possibility (Fig 5D.)

An intuitive understanding of the observed result comes from integrating information about the development time and body size of VBs. Although there is no significant difference, VBs take a longer time to develop than VBCs. If the trend in development time arises due to a prolonged larval growth period coupled with no change in body-size of the flies, it implies that VBs are slow feeders or have reduced efficiency in converting larval food to biomass (note that this different from the above-mentioned efficiency of resource utilization in the adults). As a result, they take a longer time to reach the same body-size as the VBCs, and hence, they cannot compete well in a resource-limited scenario, and thus have lower larval competitive ability than the VBCs. This line of reasoning assumes that the pupal development time of VBs and VBCs are similar and therefore, the observed changes in egg-to-adult development are primarily due to differences in the duration of the larval phase. One of the ways to check this prediction is to separately measure the larval and pupal development time, an assay that I hope to undertake in near future.

Dispersal evolution does not affect settlement success of VBs and VBCs

Dispersal is a three-stage process, consisting of 'departure', 'transient', and 'settlement' stages (Clobert et al., 2009). A successful dispersal event involves completing all three stages of dispersal, and producing viable offspring in the final patch. I defined the success of an organism in the final patch as settlement success. The total number of viable offspring produced by an individual is dependent on adult fecundity, the hatchability of the egg, and the entire developmental pathway from the egg, larva, pupa to a viable adult. Here, I tried to make predictions about the settlement success of VBs and VBCs across various types of environment by measuring the number of viable offspring produced. Three types of environments were used: i) normal food, favourable environment, ii) 33% diluted food, nutrient poor environment, iii) urea-enriched, toxic environment. Analysis showed that VBs and VBCs produce similar number of viable offspring across all assayed environmental conditions (Fig. 5A, 5B, 5C, 5D)

Hence, if it is assumed that both VB and VBC complete the first two stages of dispersal with the same level of success, they do not seem to differ in their ability to effectively settle in the assayed set of environments. But here in lies the key point. The ability to successfully navigate through the 'departure' and 'transient' of dispersal depends on two very important dispersal traits, dispersal propensity (keenness to move out of a habitat) and dispersal ability (ability to travel distances), respectively. VBs have both superior dispersal propensity and ability as compared to the VBCs (Tung et al., 2016). Therefore, if the assay was modified to contain all the three stages of dispersal, it can be expected that the sheer number of individuals reaching the settlement environment would be different, which would then contribute to population level differences in the colonizing ability of VB and VBC.

Both males and females contribute additively to the dispersal phenotype of the offspring

In addition to the effects of dispersal evolution on developmental traits and settlement success, I also looked at the transmission pattern of dispersal ability from parents to offspring. There are some studies that report that heritability estimates of

dispersal vary between the sexes (Saastamoinen, 2008) and that two sexes could contribute differentially to the dispersal phenotype of the offspring. In our system, evolution of dispersal ability is known to be strongly correlated with increased adult activity (Tung et al., 2016). Hence, I used adult activity as a proxy for dispersal ability. In this experiment, homotypic and heterotypic crosses between VB and VBC population were set up, and the activity levels and resting patterns of the offspring were profiled. Analysis revealed that offspring of VB mothers had significantly higher activity as compared to offspring of VBC mothers (Fig. 7A), and there was no significant difference between the offspring of VB fathers and VBC fathers (Fig. 7B), although the effect size of this factor was large. The offspring types differed marginally in their resting pattern (Fig. 6B). Though ANOVA indicates that dispersal ability of the mother influences the dispersal ability of the offspring more profoundly than the dispersal ability of the father, the effect sizes of both maternal and paternal identity are large. Hence, I conclude that both paternal and maternal identity contribute to the dispersal phenotype of the offspring.

Conclusion & Future directions:

The most prominent result of this study is that dispersers, despite being competitively inferior in larval stages, show no trade-off in reproductive success across three different environment types (nutrient-poor, nutrient-rich and toxic), suggesting that dispersers could successfully settle in varied habitat types. The settlement success of dispersers should be further tested in other habitat types to verify this result. Relevant environmental stresses could include temperature, water availability, and intra/interspecific interactions.

Another significant conclusion from this study is the efficiency of energy usage in dispersers. Though it is likely that dispersers have reduced efficiency at the larval stage in converting food to biomass, the results suggest that dispersers might be energy efficient as adults. The observation that there was no change in the body-size of flies upon selection for dispersal, combined with the results from other assays in the lab (Sadiq, 2017, Master's Thesis, IISER-Pune), suggests that dispersers do not seem to be afflicted by the energy costs of dispersal. One possible explanation is that there is no significant energy cost of dispersal in the current setup, or that enough generations have not passed for a significant difference to appear. A more intriguing possibility is that dispersers have become more energy efficient to accommodate the energy costs of dispersal, and as a result, this improved efficiency has precluded the occurrence of trade-offs. If such a change is possible, then it is interesting to reflect on the fact that there is scope to improve efficiency in normal populations, which is realised through dispersal evolution. If this were the case, quantifying the energy costs of dispersal in our experimental system, and measuring the energy efficiency of dispersers should be the logical next step.

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Appendix

Table 1: Composition of 1 L of standard banana-jaggery medium

Water (L)	1
Banana(g)	205
Barley (g)	25
Jaggery (g)	35
Yeast (g)	36
Agar(g)	12.4
Water (mL)	120
Ethanol (mL)	22
Methyl-4-hydroxy benzoate(g)	2.4

Table 2: Composition of 1 L of 33% diluted food environment

Water (L)	1
Banana(g)	68.33
Barley (g)	8.33
Jaggery (g)	11.66
Yeast (g)	12
Agar(g)	12.4
Water (mL)	120
Ethanol (mL)	22
Methyl-4-hydroxy benzoate(g)	2.4