Study of dispersal and associated life history traits in laboratory populations of *Drosophila melanogaster*

BS-MS Thesis

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Under the guidance of

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CERTIFICATE

This is to certify that this dissertation entitled "*Study of dispersal and associated life history 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 original research carried out by Abhishek Mishra at IISER Pune under the supervision of Dr. Sutirth Dey, Associate Professor, Biology Department during the academic year 2014-2015.

Signature of the Supervisor Date: 25th March 2015

(Dr. Sutirth Dey)

DECLARATION

I hereby declare that the matter embodied in the report entitled "*Study of dispersal and associated life history traits in laboratory populations of Drosophila melanogaster*" are the results of the investigations 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.

Signature of the Student Date: 25th March 2015

(Abhishek Mishra)

Abstract

Dispersal has long occupied a pivotal position in many ecological and evolutionary studies, but the evolution of dispersal as a trait still remains under-studied. The study reported here empirically demonstrates for the first time, the evolution of dispersal kernel (distribution of dispersed organisms across space). Within 20 generations of selection on laboratory populations of *Drosophila melanogaster*, a 100% increase in dispersal propensity and an increment of 35% in dispersal ability has been observed. Life-history assays done on desiccation resistance and female fecundity have not shown a significant trade-off in the selected populations, which may hint at the ease and speed with which dispersal can evolve. Additionally, in contrast to some past studies, we could not find any significant association of life-history traits between dispersers and non-dispersers within a given population. The findings can have major implications on a variety of theoretical models and experimental studies involving assumptions about the constancy of kernel in the case of active dispersal. The results of this work also stress the importance of longer selection experiments in order to delineate the relationship between life-history and dispersal traits.

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Acknowledgements

I would like to start by thanking my mentor Dr. Sutirth Dey, who has been kind enough to allow me an opportunity to work in his lab. Over the past three years, I have learned a lot from him, not just in science, but also about generally looking at things in life. I am grateful to him for the freedom and guidance I was provided with during all my projects, something that has really helped me grow as a researcher. Words aren't enough to express my gratitude towards him. Thank you, sir.

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There are my batch mates and friends whom I can't thank enough. They made the 5-year stay at IISER interesting, joyful and worth remembering. My thanks to the 2010 batch and to all my classmates in biology courses.

Finally, and most importantly, any words of gratitude towards my parents are insufficient. Mummy and Papa, you are the best. Thank you for being with me and for all the love and guidance over the years.

Introduction

Dispersal may be defined as “any movement of individuals or propagules with potential consequences of gene flow across space” (Ronce, 2007). It is an important phenomenon in the living world with diverse effects ranging from the spatio-temporal dynamics of populations to ecology, evolution and conservation of numerous species of animals and plants (Clobert et al., 2012). Earlier studies have shown that dispersal can rescue a population of small size from local extinction (Brown & Kodric-Brown, 1977) and relax the adverse effect of genetic drift (Tallmon et al., 2004), among other things. In the short-term however, and at an individual level, dispersal can be an expensive affair. As active dispersal is an energy-intensive process, it requires a reallocation of body resources on the part of the organism. For instance, flight activity has been shown to significantly decrease egg production in *Drosophila* females (Roff, 1977). Additionally there is an element of risk, for example, predation, or starvation due to non-availability of resources. This has in part, led to the study of behavioural correlates of dispersal, such as boldness, sociability or aggressiveness (Cote et al., 2010).

The costs of dispersal are thus, expected to modulate the life-history and behaviour of organisms. In such a scenario, directed selection experiments can play a crucial role in enhancing our understanding of the evolution of dispersal as a trait. These experiments can help us gain insights about the various dispersal related trade-offs and their underlying mechanisms. Additionally, they can improve our comprehension about the heritability of dispersal attributes such as ability, propensity, efficiency, etc. A few such studies already exist in the literature but some of them failed to get any response to selection (Tien et al., 2011; Bitume et al. 2011). Other studies which do report a response and possible life history trade-off(s), suffer from some limitations; for example, a small initial size of populations used for selection (Yano and Takafuji 2002). Some other studies have used indirect methods for selection, such as wing size (Gu and Danthanarayana 1992) and wing polymorphism (Zera and Zhao 2006) instead of direct selection on dispersal. Additionally, all these studies involve really short selection time (≤ 10 generations) and hence, it is not clear whether the observed response is a transient phenomenon or a stable evolutionary outcome.

The aim of this project was to run a laboratory selection on fruit flies to understand dispersal evolution. In contrast to the past studies, our experimental setup involves (a) selection directly on the dispersal *kernel* (actual distribution of dispersed individuals), (b) large population sizes (≥ 2400 individuals), and (c) longer selection period (results till 20 generations of selection reported here). The idea is to allow for a better understanding of dispersal evolution. Further, the objective has been to integrate the various axes of investigation, such as dispersal attributes (propensity, ability, etc.), life history traits, behavioural traits and biochemical correlates (lipid content, glycogen content, etc.). This report deals with the direct response to selection and association with the life-history traits.

In preparation for the selection, a few other experiments were also done. Previous work on the ciliate *Tetrahymena* has shown correlations between different dispersal strategies and some life-history traits (Fjerdingstad et al., 2007; Pennekamp et al., 2014), pointing to a phenotypic association of dispersal with life-history. Similar results have been obtained from experiments on Glanville fritillary butterfly, where higher dispersal was associated with a trade-off in longevity (Hanski et al., 2006).
In an effort to see if such a correlation holds for *Drosophila*, a Life History Association experiment at generation zero (LHA 0) was performed, in which two core life-history traits, namely (a) Female fecundity, and (b) Dry weight were checked for dispersers and non-dispersers in the population. While difference in fecundity would help see any shift in reproductive fitness, dry weight is a measure of body size which has been suggested to positively correlate with dispersal ability (Roff 1977).
Apart from life history correlations, some recent work has investigated density dependence of dispersal as well. Evidence ranges from negative density dependence (Pennekamp et al., 2014) to a U-shaped density dependence (Fronhofer et al., 2014) in *Tetrahymena*. An experiment was designed and conducted to check the density dependence, if any, in *Drosophila* dispersal. Three densities were chosen and dispersal kernel recorded for all the three densities. This experiment is hitherto referred to as Density Dependence of Kernel at generation zero (DDK 0).
After the start of selection, groups of assays were conducted to check the response to selection as well as any life history trade-off. Two rounds were conducted, once after 9^{th} and 10^{th} generations of selection, and again at the end of 19^{th} and 20^{th}

generations. Both rounds of assays involved (a) dispersal kernel assay, to check the direct response to selection, (b) female fecundity assay, to check any changes in reproductive strategy due to selection, and (c) desiccation resistance assay, to see any trade-offs in body maintenance.

Materials and methods

Fly stocks

All experiments were performed using four laboratory populations of *Drosophila melanogaster* (**DB**/**D**ey **B**aseline $_{1-4}$), which are maintained under constant light at a temperature of 25℃, and are provided with *ad libitum* banana-jaggery medium as food. A 21-day discrete generation cycle is followed where the adult flies are kept enclosed in plexi-glass cages. The eggs for the next generation are collected at a density of ~60 eggs/ ~6ml medium, in cotton-plugged plastic vials. In order to minimise inbreeding, a large population size of about 2400 adults is maintained every generation for each of the populations. These populations are direct descendants of laboratory populations JB $_{1-4}$ at Evolutionary Biology Laboratory, JNCASR, whose detailed maintenance regime has been described elsewhere (Sheeba et al., 1998).

From each of these populations (DB_{1-4}), two populations were derived, one of which was subjected to selection for dispersal (henceforth VB_{1-4}) and the other served as the matching control (henceforth, VBC_{1-4}). Thus, populations bearing the same numerical subscripts (e.g. VB_1 and VBC_1) are related by ancestry. Such pairs were always assayed together and were treated as blocks during statistical analysis.

Dispersal setup

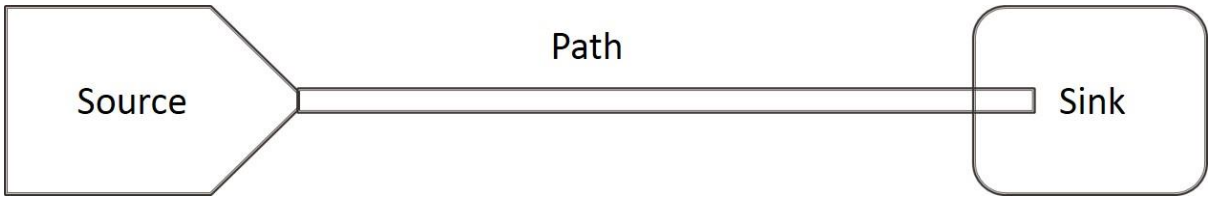

Figure 1. Dispersal setup

The setup for dispersal in all the experiments and assays consisted of the following three parts (see Fig. 1):

1. *Source*:

   A cylindrical plastic container of about 1.5 L volume with its bottom face cut out and mounted on by a plastic funnel was used as the *source*. The lid on the

other end was kept closed. The flies were transferred into the *source* through the funnel-end, as anaesthetisation is not a feasible option just before a dispersal run. The plastic funnel thus, served a twofold purpose; (a) it is difficult for the flies to escape (while being transferred) through the narrow opening of the funnel, and (b) the tapering opening of the *source* has been found effective to promote dispersal levels in earlier standardisation experiments carried out in the lab (Mishra *sem. proj.* 2012).

2. *Path*:
   Connected to the funnel-end of *source* was a plastic pipe with inner diameter of about 1 cm and a fixed length (length varied according to the experiment). This plastic pipe provided the flies with a channel to leave the *source* and disperse away from it. In some of the experiments, the path was made compact by coiling the pipe into a spiral shape in order to save space.

3. *Sink*:
   At the other end of the path, another plastic container (size depends on the experiment) was attached which served to collect the dispersed flies. Following earlier standardisation experiments (Mishra *sem. proj.* 2012), a length of about 3 cm of the plastic pipe at this end was kept protruded into the bottle, as it helps prevent backflow of flies from the *sink* into the path.

Following previous standardisation experiments, the *source* and the *sink* were placed on their lateral surface, as it helps in greater dispersal (Mishra *sem. proj.* 2012). The pipe was also placed in such a way that the entire *path* rests horizontally with minimum differences in height across the sections. The 3-component setup as described above was fixed to a surface using scotch tape and left undisturbed once the flies have been transferred into the *source*. To minimise any local differences, temperature was kept constant at 25℃ and sufficient lighting provided with the help of fluorescent tubes. Following earlier data on desiccation resistance of the lab fly stocks, any dispersal run was not allowed for more than 6 hours, as this time period is comfortably short of the time it takes for first recorded deaths due to desiccation.

Life-history association at generation zero (LHA 0)

For this experiment, 60-80 eggs were collected from each of the four DBs into 40 food vials each. The resulting number (~2400) thus, was close to the size at which these stocks are maintained. Upon eclosion as adults, the flies of each population were transferred to a dispersal setup with *path* length 2 metres. Earlier standardisation had demonstrated that it took close to 6 hours for the dispersal of about 50% of the flies at this *path* length and density (Mishra *sem. proj.* 2012). Plastic containers identical to the ones used as *sources* were used as *sinks*, but without the funnel attachment.

Following the dispersal part of the experiment (6 hours or ~50% dispersal, whichever happened earlier), the flies from the *source* and the *sink* were collected into separate plexi-glass cages for each population, and provided immediately with food. Owing to the energy expenditure incurred during dispersal, the individuals were given a rest of three days before any of the assays were done. Routine food change (every alternate day) was done for the cages during this resting period.

The following two assays were performed once the resting period was over:

1. *Female fecundity assay*:
   Female fecundity for this experiment was estimated as the number of eggs laid in a period of 24 hours on the 15th day of age since egg collection. The setup included a plastic cup filled with food (which also served as the egg laying surface) attached on to the open end of a standard plastic vial containing a pair of male and female flies, using medical grade tissue tape to allow for air exchange. This setup was kept vertically upright with the food cup part at the bottom, allowing the females a horizontal surface to deposit their eggs. 35 such replicates were used for each of the two groups of '*source*' and '*sink*' flies, and this was done for all the four populations. After 24 hours from setup, the number of eggs in each of the food cups was counted and recorded.

2. *Dry weight assay*:
   For this assay, freshly eclosed flies were first collected and kept at -80℃. This was followed by sorting them into males and females. For each sex, 10

replicates, containing 10 flies each were exposed to a 72-hour long drying period in a hot-air oven at 50℃ to eliminate moisture from the individuals' bodies. The weight of each group of 10 flies was then measured using a sensitive balance.

Density dependence of dispersal kernel at generation zero (DDK 0)

Distribution of flies after a dispersal run was used as a measure of estimating the dispersal kernel. An arbitrarily long *path* length of 10 metres (5 times the standardised length of 2 metres) was chosen for this experiment. However, this time the *path* didn't involve a single, long plastic pipe and was instead partitioned into 20 bins of length 0.5 metre each. This has been done to get an estimate of dispersal distance. As calculating the distance for each of the thousands of flies is not feasible, the flies in a bin are grouped together and given the value of distance for that particular bin. As the diameter of the pipe used for making these bins was same, these bins had to be joined using a plastic adapter (obtained by cutting 5 mL micropipette tips and using their wider end) and tissue tape. The usual *source* containers were used and standard plastic fly culture bottles were used as *sinks*. The assembled *path* took a linear shape and was fixed onto the floor using scotch tape. The replicates were arranged parallel to each other.

To see if density has a discernible effect on the kernel, three densities were chosen. For the *lowest density*, 50 eggs were collected in 20 vials each, giving a final adult population size of about 1000. For the *medium density*, 40 such vials were used, resulting in about 2000 adult flies, and for the *highest density* the number of vials was 60, giving about 3000 adult individuals. All the four populations had two replicates for each density.

Dispersal was allowed to happen for exactly 6 hours, at the end of which, the setup was dismantled quickly by disconnecting all the bins and plugging them with cotton. Labels with block, replicate and bin number helped with the identification of bins after the dismantling. The bins and containers were then kept in either a hot-air oven or in a refrigerator at -20˚C till the flies were dead. They were then separated into males and females, and counted.

Three quantities were calculated using the data obtained from each replicate of kernel assay:

a) $Dispersal\ propensity =$
$\text{Number of flies who left the source}/\text{Total number of flies}$

b) $Dispersal\ ability = \text{Average distance travelled by the flies who left the source}$

c) $Proportion\ reaching\ the\ sink =$
$\text{Number of flies who reached the sink}/\text{Total number of flies}$

Selection regime

The four baseline populations **DB** $_{1\text{-}4}$ were used for generating the selection lines (**VB** $_{1\text{-}4}$). As mentioned earlier, the DBs are reared at a 21-day generation cycle. However, most of the adults are eclosed by the 12th day of egg collection. It takes another 3 days (2 days of yeasting and 12-16 hours of cut-plate) before eggs can be collected from these adults in sufficient amounts, to constitute the next generation. Therefore in the interest of faster selection, VBs are maintained at a 15-day generation cycle, with the selection step (dispersal run) being carried out on the 12th day. Hence, DBs cannot serve as controls to the selected populations and instead, new control populations (**VBC** $_{1\text{-}4}$) reared at 15-day generation cycle were started. The rest of the general maintenance for the VBs/VBCs remains similar to the stock populations (DBs).

The selection regime for VBs involves the usual *source*, *path* and *sink*. The path length at generation 0 was chosen to be 2 metres (following standardization for ~50% dispersal in 6 hours). *Sink* containers used are the same as ones in the LHA 0 experiment, except that they are provided with a wet cotton strip. This serves two purposes; (a) it provides moisture for flies which otherwise face a desiccative pressure, and (b) it reduces the backflow of flies from *sink* to the *path*. The setup is arranged in a well-lit area (currently a multi-level steel rack with extended fluorescent lights attached to the edges) and is left undisturbed until almost half of the flies have migrated to the *sink* (takes ≤ 6 hours). Only the flies reaching the *sink* are then allowed contribute to the next generation.

To preserve the density at which the dispersal runs were standardised, two replicate dispersal runs for each of the VBs are done. The selected flies (~50% of total) from the two replicate runs are then pooled together to form the stock for that particular generation, thus maintaining the adult density.

To ensure correspondence in the controls (VBCs), they are transferred into a *source* container which is not connected to a path but is instead plugged with cotton. This container with VBC population is then placed next to the replicate setups of corresponding VB for the entire period of dispersal run. In order to alleviate the difference in desiccation stress faced by VBs and VBCs, cotton plug of the VBC container is substituted by a wet cotton plug when half the desired level of dispersal has been achieved (~25% of flies reaching the *sink*) in corresponding VB setups. Hence, all the flies in a VBC setup face desiccation stress until roughly half of the VB flies have gained access to moisture by reaching the *sink* (containing wet cotton strip).

Over the course of selection, as a shift towards quicker dispersal was observed, the path length has been increased in intermittent generations.

<u>Assays after 9-10 generations of selection</u>

Owing to logistic and time constraints, assays for only 2 blocks out of 4 could be done in a given generation. The assays for VB/VBC 1 and 2 were done at 9th generation of selection, while the assays for the remaining blocks (VB/VBC 3-4) were done at 10th generation of selection. Following previous *Drosophila* selection experiments, a relaxation period of one generation was allowed before the assays, where there was no selection imposed and the VB/VBC populations were reared under identical conditions. This is done to minimise any non-genetic effects the selection regime could have on the flies (Rose 1984).

The following assays were conducted:

1. *Dispersal kernel assay*:
   The experimental setup was the same as DDK 0 experiment. But instead of density, the effect of selection was investigated. Each combination of treatment (VB/VBC) and block (1/2/3/4) was replicated thrice. The density of

adult flies used was chosen as *medium* (~2000 flies per replicate).

2. *Female fecundity assay*:
   The fecundity of females was estimated over three days (14^{th}-16^{th} days of age since egg collection). Instead of counting the eggs as done in LHA 0, the number of adult offspring was used. Each replicate consisted of two pairs of flies (2 males and 2 females) transferred into a food vial on the 14^{th} day. They were then transferred to a fresh food vial the next (15^{th}) day, and the old vial was incubated at 25℃. This was repeated after 24 hours (16^{th} day). After the 16^{th} day, the parent flies were discarded and the progeny from all three incubated vials was counted daily, as and when they eclosed as adults. The number of such replicates for each block of VBs and VBCs was 30.

3. *Desiccation resistance assay*:
   Three replicates with 100 males and 100 females each were used to measure the desiccation resistance of each population. The setup was a 1.5 L container with a cloth tied across the open face to ensure ventilation. Checks were done every 2 hours from setup (9^{th} day of age from egg collection) and dead flies were sucked out using a lab-made aspirator. The number and sex of these flies was then recorded.

<u>Assays after 19-20 generations of selection</u>

As in the previous round (9-10^{th} generations), the following three assays were conducted, albeit with slightly different protocols:

1. *Dispersal kernel assay*:
   The *path* length was kept at 20 metres, with 0.5 m long bins for the first 10 metres and 1 m long bins for the last 10 metres. Also, to accommodate the increased *path* length, the pipe was coiled into a spiral shape. Everything else remained similar to the assay done in 9-10^{th} generation.

2. *Female fecundity assay*:
   The fecundity was estimated over the first week of adulthood. Virgin flies

(collected within 6 hours of eclosion) were used for the setup, which had an inverted 50 mL macro-centrifuge tube with a food cup attached on the inside of the lid. Five small holes were poked in the tube body for ventilation. Each replicate had a pair (1 male and 1 female) transferred into a food vial on the 9th day of age. The lid was replaced with another lid containing a fresh food cup every day for the next 7 days, and the eggs in old food cups counted. This was replicated 35 times for each population.

3. *Desiccation resistance assay*:
   For all the populations, males and females were assayed separately in 10 replicates with 10 flies each. Setup was done with flies of age 9 days (from egg collection) and data was collected every 2 hours hence. Readings involved number and sex of dead flies at every time point.

Statistical analysis

The data from all assays (except fecundity) were subjected to separate three-way mixed model ANOVAs, with treatment (fixed factor; two levels: VBC and VB, or '*source*' and '*sink*') crossed with population (random factor; four levels: 1/2/3/4) and sex (fixed factor; two levels: male and female). In fecundity assays where only females were used, sex was omitted as a factor. All the ANOVAs were performed on STATISTICA^{TM} version 5.

Results

Life-history association at generation zero (LHA 0)

1. *Female fecundity assay*

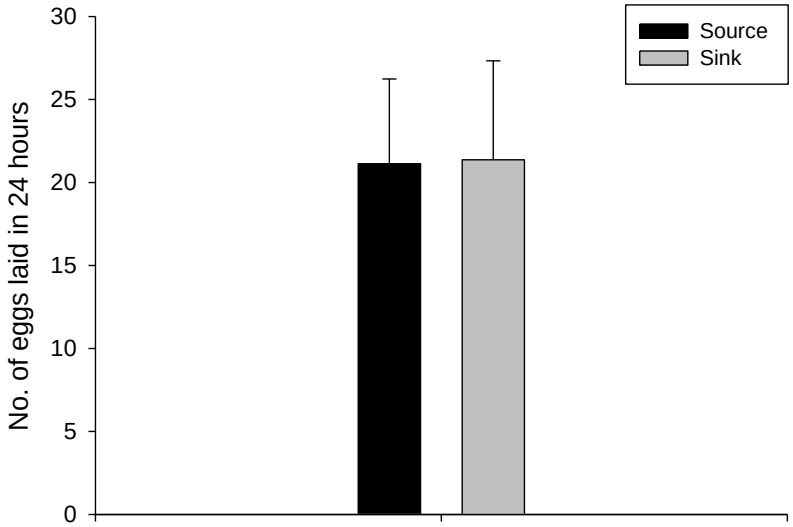

Fig. 2. Average Female Fecundity (LHA 0) ± SE

No significant difference was observed in female fecundity over 24 hours for the two groups '*source*' and '*sink*' (p=.846, F=.045).

2. *Dry weight assay*

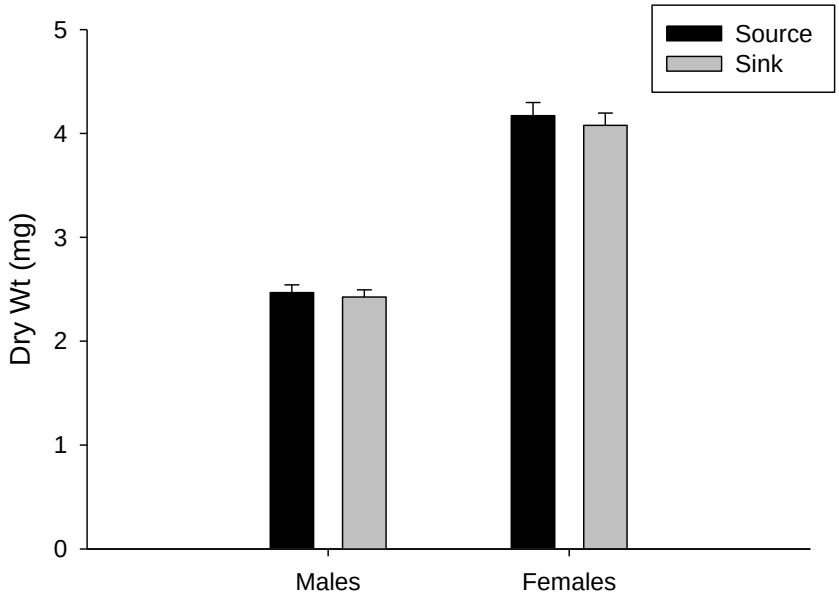

Fig. 3. Average Dry Weight of 10 individuals (LHA 0) ± SE

No significant difference was observed in the dry weight of flies in the two groups '*source*' and '*sink*' (p=.138, F=4.027). The females however, had significantly high dry weight as compared to males (p=.000, F=456.105).

Density dependence of dispersal kernel at generation zero (DDK 0)

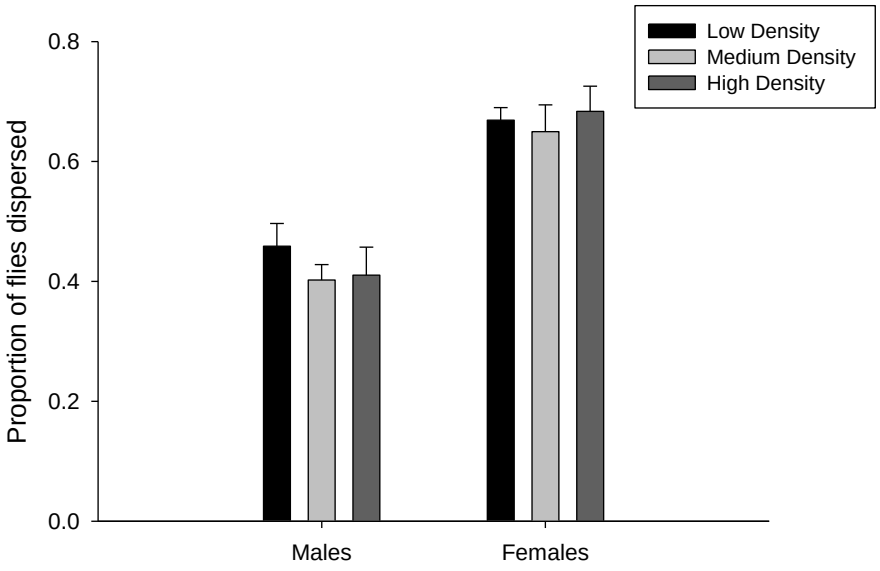

Fig. 4. Average Dispersal Propensity (DDK 0) ± SE

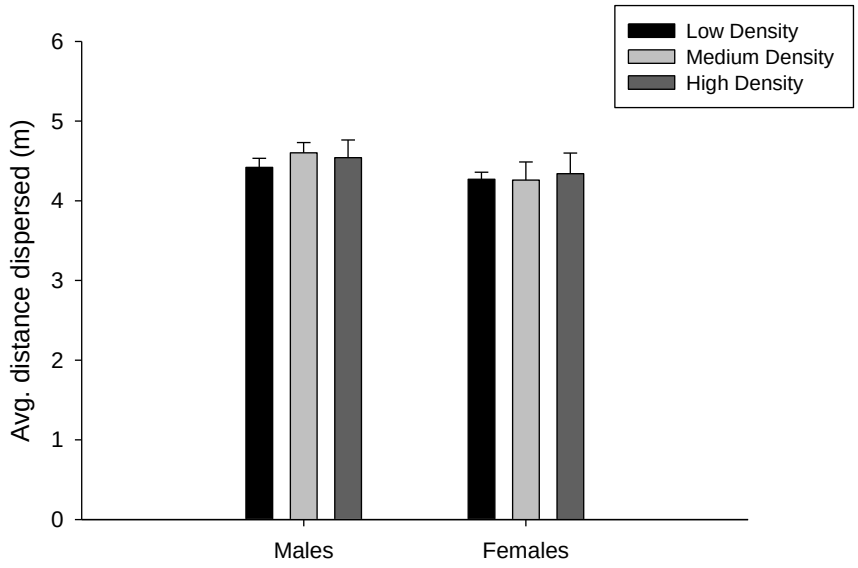

Fig. 5. Average Dispersal Ability (DDK 0) ± SE

Density didn't have a significant effect on any of the three quantities measured: dispersal propensity (p=.793, F=.242), dispersal ability (p=.86, F=.155) and the proportion of flies reaching *sink* (p=.242, F=1.815). On the other hand, sex showed a

significant effect on the dispersal propensity (p=.006, F=48.19) with females showing higher propensity than males. However, males and females didn't have a significant difference in dispersal ability (p=.301, F=1.555) or the proportion of flies reaching the *sink* (p=.224, F=2.333).

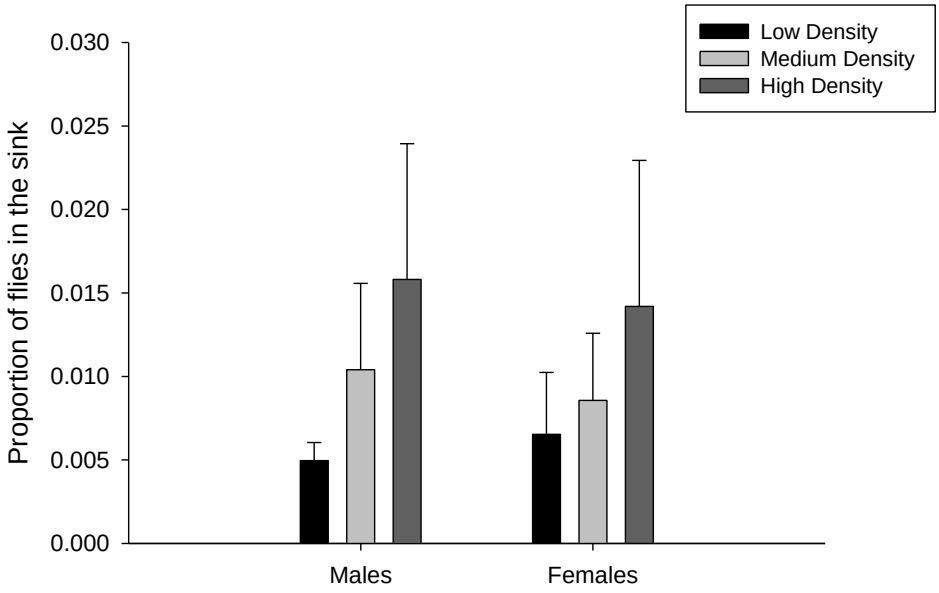

Fig. 6. Proportion reaching Destination (DDK 0) ± SE

<u>Assays after 9-10 generations of selection</u>

1. *Dispersal kernel assay*

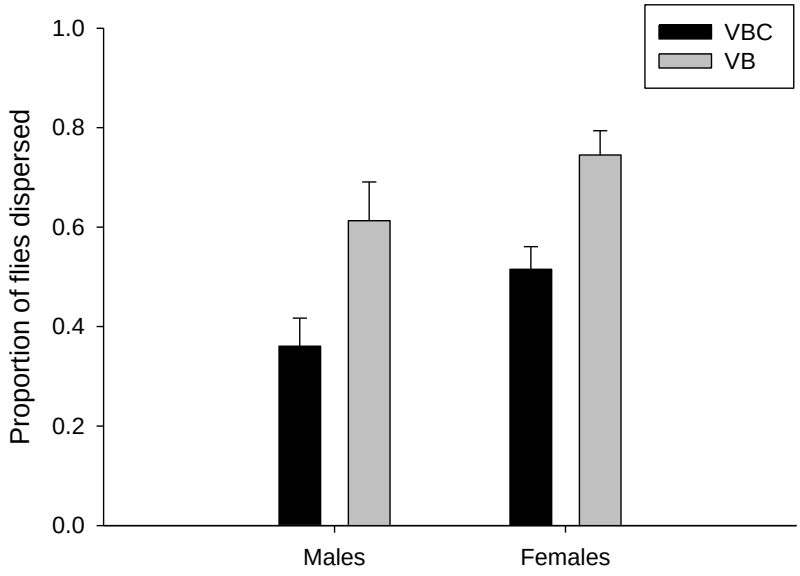

Fig. 7. Average Dispersal Propensity (Gen: 9, 10) ± SE

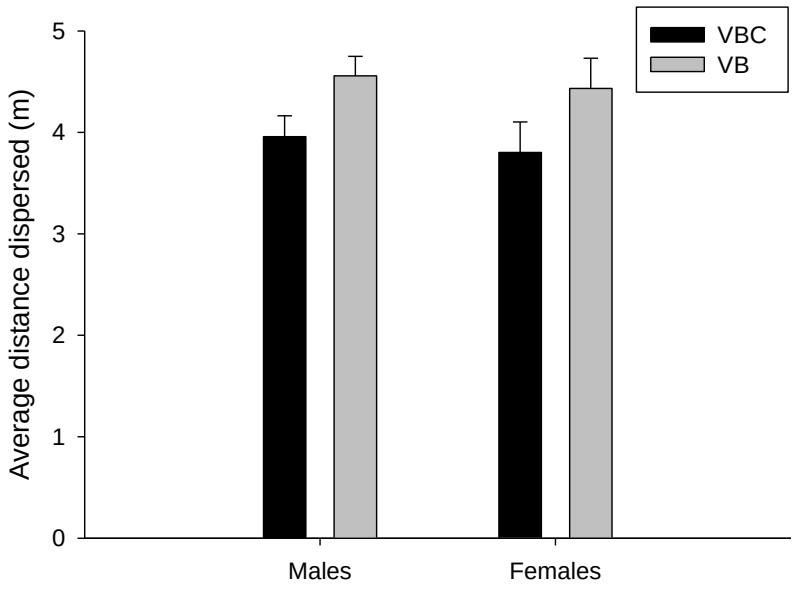

Fig. 8. Average Dispersal Ability (Gen: 9, 10) ± SE

Selection had a significant effect on dispersal propensity (p=.001, F=145.205), dispersal ability (p=.019, F=21.018) and the proportion of flies reaching *sink* (p=.000, F=403.841). VBs had higher values than VBCs for all the three quantities. Also, females had significantly higher dispersal propensity than males (p=.007, F=41.421), but the effect of sex was not significant on dispersal ability (p=.419, F=0.87) and the proportion reaching *sink* (p=.312, F=1.468).

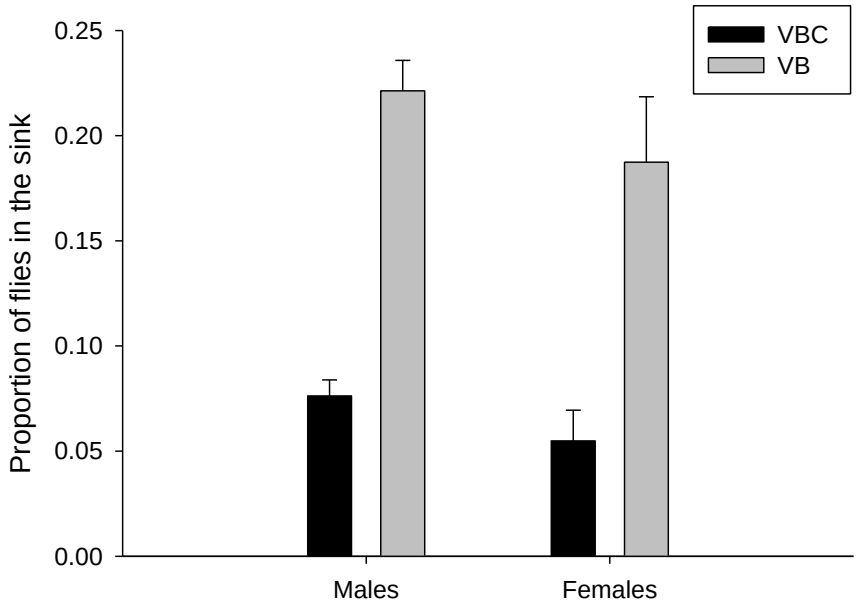

Fig. 9. Proportion reaching Destination (Gen: 9, 10) ± SE

2. *Female fecundity assay*

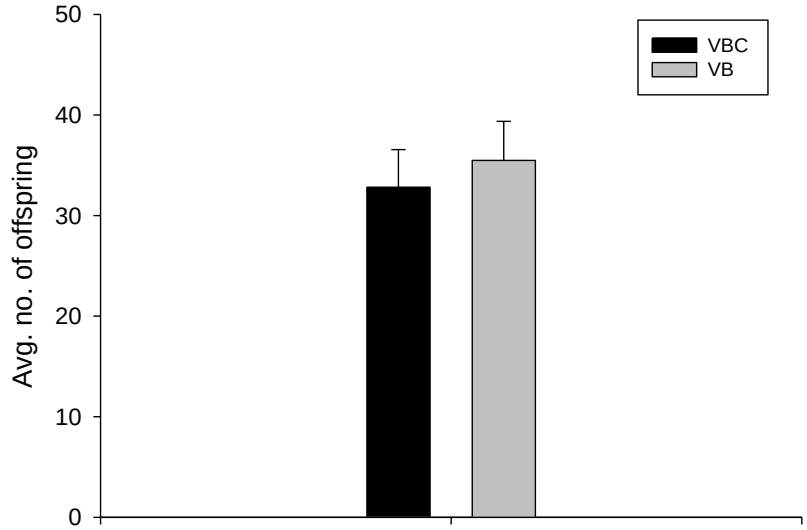

Fig. 10. Average Female Fecundity of 2 females (Gen: 9, 10) ± SE

No significant effect of selection was observed on female fecundity over 3 days (p=.141, F=3.948).

3. *Desiccation assay*

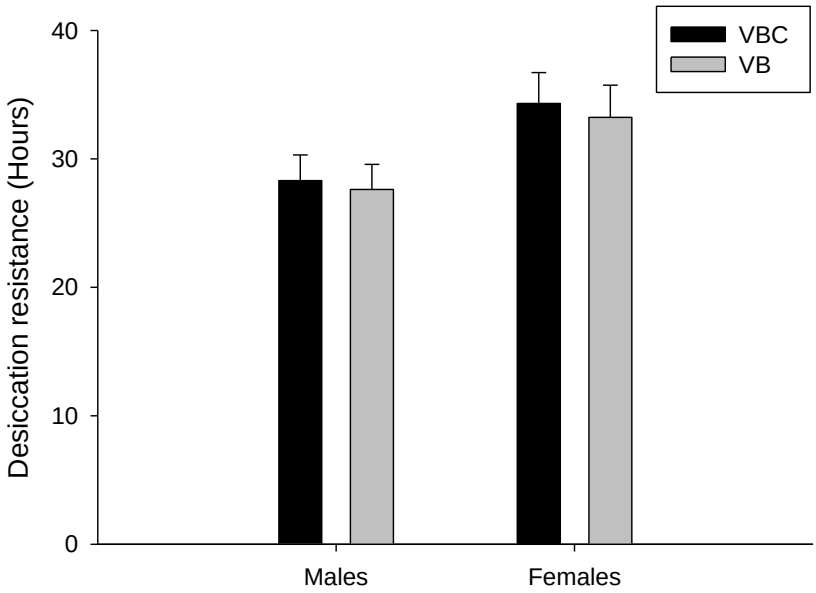

Fig. 11. Average Desiccation resistance (Gen: 9, 10) ± SE

No significant effect of selection was observed on desiccation resistance (p=.125, F=4.383). Females had a significantly higher desiccation resistance than males (p=.002, F=107.917).

Assays after 19-20 generations of selection

1. *Dispersal kernel assay*

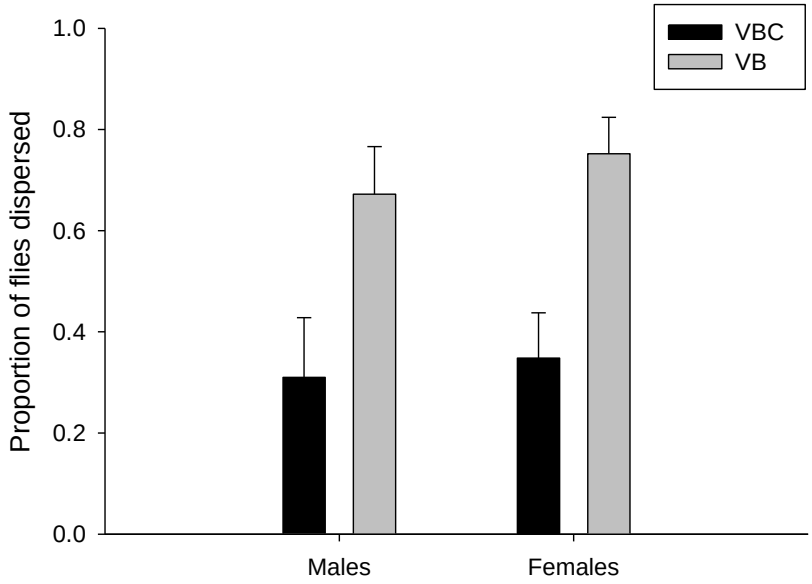

Fig. 12. Average Dispersal Propensity (Gen: 19, 20) ± SE

Selection had a significant effect on dispersal propensity (p=.018, F=22.684), dispersal ability (p=.002, F=94.2) but not on the proportion of flies reaching *sink* (p=.095, F=5.828). VBs had higher mean values than VBCs for all the three quantities. Sex did not have a significant effect on propensity (p=.159, F=3.472), but males had a significantly higher dispersal ability (p=.031, F=14.77) and proportion reaching *sink* (p=.019, F=21.279).

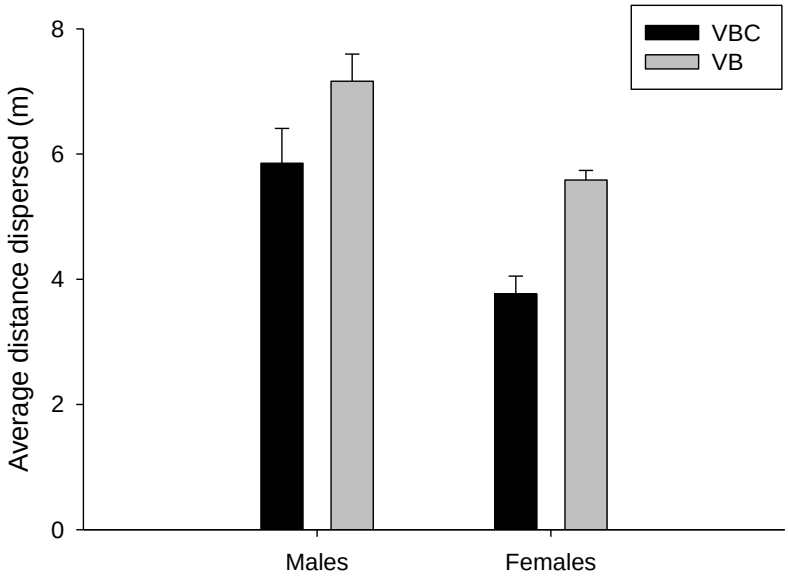

Fig. 13. Average Dispersal Ability (Gen: 19, 20) ± SE

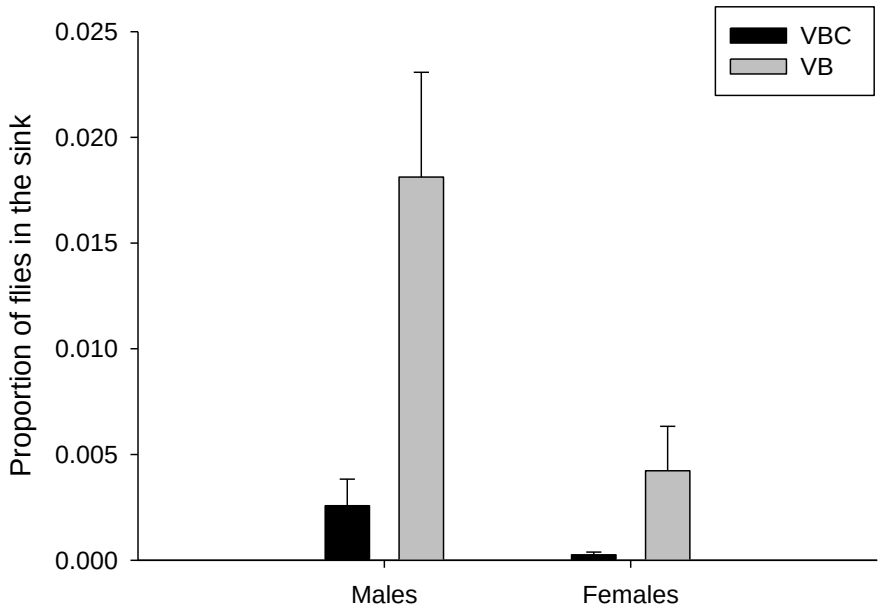

Fig. 14. Proportion reaching Destination (Gen: 19, 20) ± SE

2. *Female fecundity assay*

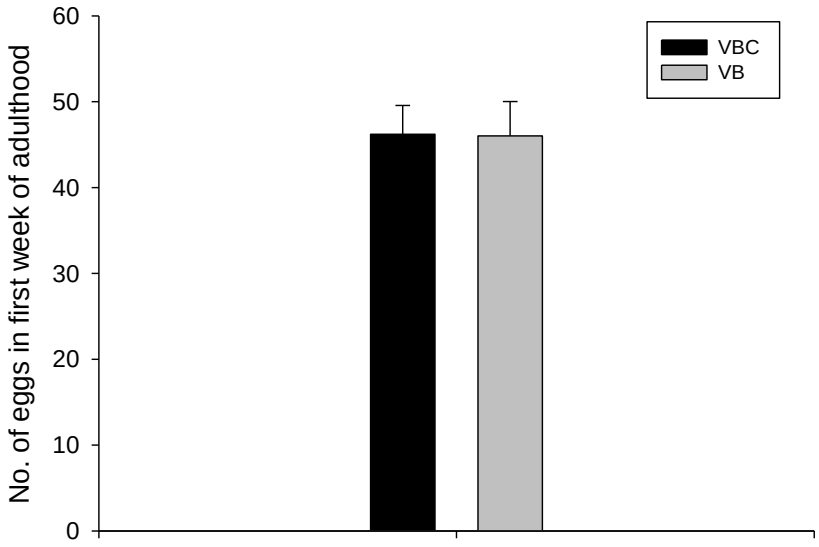

Fig. 15. Early Life Female Fecundity (Gen: 19, 20) ± SE

Early life fecundity was not significantly different for VB and VBC females (p=.903, F=.018).

3. *Desiccation assay*

No significant effect of selection was observed on desiccation resistance (p=.175, F=3.139). Females and males did not have a statistically significant difference in desiccation resistance (p=.348, F=1.232).

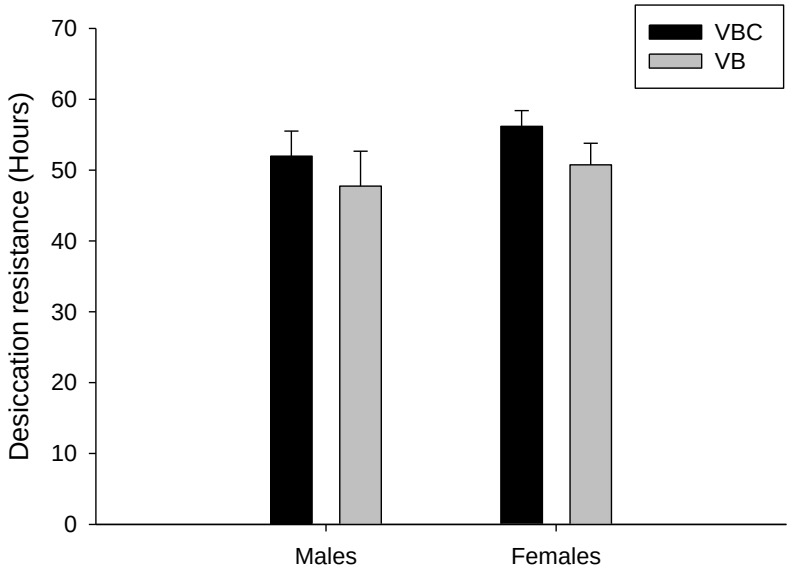

Fig. 16. Average Desiccation resistance (Gen: 19, 20) ± SE

Discussion

Contrary to the past studies that showed different patterns of association of dispersal ability/propensity with life history (Hanski et al., 2006; Fjerdingstad et al., 2007; Pennekamp et al., 2014), results of the life history assays performed at the zeroth generation (LHA 0) failed to detect any correlation of fecundity or dry weight with dispersers and non-dispersers in *Drosophila*. There is a possibility that the correlation could instead exist with one of the traits we did not measure. Alternatively, it might have resulted due to differences in the genetic architecture of the model organism used and its populations. If this were to be true, it would imply that dispersal modulates life history quite differently in different species.

The effect of density was also not apparent in the experiment measuring density dependence of dispersal kernel at generation zero (DDK 0). All three quantities calculated (dispersal propensity, dispersal ability and proportion reaching sink) could not be shown to be significantly affected by the density of flies. Many past studies have pointed out population density as an important aspect of dispersal, with some of them reporting a clear density dependence (Pennekamp et al., 2014; Fronhofer et al., 2015). Intra-specific competition has long been speculated as one of the major causes for dispersal (Lambin et al., 2012). The three densities used in the DDK 0 experiment were an attempt at artificial crowding, but the absence of density dependence in results could indicate that either (a) the density levels were not enough to induce sufficient crowding, or (b) that over-crowding is not as important a cause for dispersal in *Drosophila* as it is in some other species. Again, in the case of the latter being true, it would be an evidence of different causes of dispersal working for different organisms.

The results of kernel assays at both 9-10th generations and 19-20th generations indicate that the dispersal kernel has evolved in the selected populations (VBs). The effect of selection is quite clearly visible on all three quantities measured from the kernel: dispersal propensity, dispersal ability and the proportion of flies reaching the *sink*. Although the last quantity doesn't show a significant difference between VBs and VBCs in the 19-20th gen assay, it may be due to the fact that the *path* length was increased to 20 metres, and hence the flies that reached the sink from both groups

were probably just the outliers. In any case, the proportion is really low for both the groups (< 2.5%), and so drawing any major conclusions with such small numbers is difficult. As for the dispersal propensity, VBs registered more than a 50% increase at the 9-10th generation as compared to VBCs for both the sexes. By the 20th generation of selection, the propensity of VBs was twice as that of VBCs. Although the assay protocols have been modified over generations to accommodate logistic challenges (e.g. 20 metres path length per replicate), and therefore the results aren't directly comparable across generations, it is safe to say that dispersal propensity has continued to show a positive response to the selection. The same is true for dispersal ability. At the 9-10th generation, there is easily a difference of 0.5 metres between VBs and VBCs for both the sexes. For 19-20th generations, the difference has increased to more than a metre.

The fact that the dispersal kernel evolved leads to a very crucial point. In ecological studies, dispersal kernel is typically treated as a constant or as a 'neutral', stochastic quantity (Lowe and McPeek 2014). Our study is the first empirical demonstration that dispersal kernel can and does evolve. Thus any theoretical models on dispersal or any studies that make the assumption about the kernel being constant should be re-evaluated with respect to the findings.

Another key result is that both dispersal ability and dispersal propensity evolved in response to selection. While propensity is crucial for an individual to leave a population, ability is important in deciding how far and how fast it can disperse. Selection studies in the past have focussed either on ability (Gu and Danthanarayana 1992; Yano and Takafuji 2002; Zera and Zhao 2006) or on propensity (Tien et al., 2011). By showing that propensity and ability can evolve together, our results strengthen the idea that dispersal is a complex process and one should be careful while extrapolating the results about one of the dispersal traits (propensity, ability, etc.) to the actual dispersal in nature.

Previous work in wild populations of cactophilic *Drosophila pachea* has shown that males have higher dispersal propensity than females (Markow and Castrenza, 2000). Even in *Drosophila melanogaster*, females have been shown to have higher emigration activity (Iliadi et al., 2002), while another study has suggested that mating experience modulates dispersal in males and females differently (Simon et al., 2011). The kernel assays reported here also offer some interesting insights on sex

bias in dispersal. Starting with the DDK 0 experiment, where the only significant difference was in higher dispersal propensity of females, the trends seem to change as selection progressed through generations. Propensity stays significantly higher for females at 9-10th gen assay, but the significance disappears at the 19-20th generation of selection. Added to this is the fact that dispersal ability and proportion reaching *sink* went from being not different significantly (at DDK 0 and 9-10th generation) to being significantly higher for the males (at 19-20th generation). Again, due to changes in protocol, any direct comparison across generations is not feasible. However, if the trends continue in further rounds of assays, there is a possibility of selection for sexual dimorphism w.r.t dispersal traits operating here. Further work needs to be done to confirm or deny any hypothesis in this regard.

Although selection has been clearly demonstrated to be working, we are yet to find a trade-off in terms of life-history. Attempts to link increased dispersal ability and propensity with a life-history trait have not yielded any significant result till the 20th generation. Possible reasons for this observation include (a) the number of generations of selection has not been sufficient to produce a discernible trade-off, (b) the traits assayed were not the ones with trade-off(s) or (c) both. Further rounds of assays will be required to say anything conclusive about the effect of dispersal selection on life history, as a significant difference might be visible at later generations. Since the selection regime involves desiccation stress for a few hours, a positive result later on might provide insights into the causes for dispersal, as well as a possible trade-off in terms of body maintenance.

Conclusions

The chief result of this study is the empirical demonstration that dispersal kernel can evolve rapidly. This is contrary to the often-quoted assumption that dispersal kernel is an immutable property of a species (Lowe and McPeek 2014). It should be noted here that our results are applicable only to the case where dispersal is an active process. For all those organisms where dispersal happens passively, due to environmental factors like wind or water currents, the assumption of a constant dispersal kernel (given a particular environmental condition), might still hold. The other important result of this study is that at least in the first twenty generations, there seems to be no trade-off associated with the evolution of dispersal. This indicates that it might be easy for an organism to evolve w.r.t dispersal abilities, at least in the short run. Selection over a much longer span of time, coupled with assays on a larger set of life-history traits, would be needed to dissect out how the genetic architecture of traits related to dispersal evolve in the long run. Finally, the fact that the dispersal kernel evolves suggests that a significant amount of theoretical and empirical work needs to be done to understand the effects of an evolving kernel on the spread of vector-borne diseases and invasion front for introduced species in natural ecosystems. This in turn might have an impact on the management strategies adopted for these threats.

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