Title: Selection for dispersal leads to evolution of kernel and increased locomotor activity in *Drosophila melanogaster*

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Abstract

Dispersal enables organisms to respond to climate change and habitat degradation and hence it is important to understand dispersal evolution and its consequences. Studies on dispersal evolution often investigate isolated components of dispersal like propensity or ability, thus neglecting how these components and their related costs interact with each other to shape the dispersal kernel. We investigated these issues by evolving replicate populations of *Drosophila melanogaster* using a setup analogous to increasing habitat-fragmentation over generations. The selected populations showed significantly higher dispersal propensity, ability and rate, which translated into an evolved kernel with a lower skew and kurtosis and a 67% increase in spatial extent. The enhanced dispersal was attributable to significantly greater locomotor activity. The flies paid a cost in terms of reduced desiccation resistance, but showed no reduction in fecundity. Thus, under the right conditions, multiple aspects of dispersal can evolve simultaneously, with potentially serious ecological consequences.

INTRODUCTION

Climate change and human activity related habitat degradation / destruction has affected many natural populations all over the world (reviewed in Root *et al.* 2003). One of the ways by which organisms cope with such stresses is dispersal, which allows them to increase their survival probability by tracking favorable environmental conditions (Travis *et al.* 2013). As a result, evolution of dispersal and its consequences have been a major focus of research in both theoretical and empirical evolutionary ecology for the last few decades (reviewed in Bowler & Benton 2005; Ronce 2007).

In the classical theoretical literature, dispersal is supposed to evolve due to three major reasons, namely, inbreeding avoidance (Charlesworth & Charlesworth 1987), reduction of kin-competition (Gandon 1999) and spatio-temporal environmental heterogeneity (McPeek & Holt 1992). However, substantial bodies of empirical and theoretical work over the last few decades suggest that the picture may not be so simple after all (reviewed in Bonte *et al.* 2012)). Dispersal is typically studied in terms of its component processes like dispersal propensity (i.e. the fraction of dispersers leaving the current habitat) (Friedenberg 2003), dispersal ability (i.e. the mean distance travelled) (Bitume *et al.* 2011) and dispersal rate (i.e. the speed with which they travel) (Phillips *et al.* 2008). Evidently, which component(s) of dispersal evolve(s) is contingent upon the nature of the selection pressure faced by each component, the costs associated with them, how these costs interact with each other and how they are countered by the organisms (Bonte *et al.* 2012). For example, in laboratory populations of *C. elegans*, dispersal propensity evolves when patch fitness is varied by externally imposed extinctions but does not evolve when patch fitness is altered by varying resource density (Friedenberg 2003). Similarly, although the various components of dispersal are related to each other, evolution of a given component does not

necessarily make an organism better in terms of another component (Fronhofer *et al.* 2014). Thus, for any organism under a given ecological scenario, a complete picture of dispersal evolution is possible only when the costs faced by each dispersal component and the response of the organism to those costs are simultaneously investigated. Unfortunately, most theoretical and empirical studies on dispersal evolution typically focus on the evolution of any one of the several components of dispersal, and there is little empirical understanding of how these various components of dispersal interact with each other to affect the evolution of the dispersal kernel (although see Fronhofer *et al.* 2014)).

To address this issue, we subjected four replicate populations of *Drosophila melanogaster* to directional selection for increased dispersal, and compared them with matched controls. Firstly, we found the simultaneous evolution of dispersal propensity, ability and rate in the selected populations, both in the presence and absence of proximal stresses for dispersal. Secondly, we show that due to the increase in the frequency of the long-distance dispersers (LDDs), dispersal kernels of the selected populations have evolved significantly greater standard deviation and reduced values of skew and kurtosis, which ultimately leads to a 67% higher spatial extent (Kot *et al.* 1996). Thirdly, we show that the changes in the dispersal properties of the populations are primarily related to increased locomotor activity in the selected flies and present a detailed picture of how different aspects of the locomotor activity have evolved. Fourthly, we demonstrate that the selected flies pay a cost in terms of reduced desiccation resistance but none in terms of fecundity. Finally, in the discussion section, we elucidate the nature of the costs that operated on the various components of dispersal and how that could have had an impact on the evolution of these components. We also discuss why some of our results do not match with previous observations in the literature and what that implies. These results can have important

ecological, conservation- related and economic implications related to, among other things, the evolution of invasive species and destabilization of metapopulation dynamics.

MATERIALS AND METHODS

Details of the methods are presented as Appendix S1 in Supporting Information (SI). However, we present sufficient information here so that the reader can follow the study without referring to the SI.

Description of the populations

The experimental populations used in this study were derived from four independent large laboratory populations of *Drosophila melanogaster* (DB_{1-4}). From each DB_i population (where $i \in [1, 4]$), we derived two populations: VB_i (short for 'vagabond', subjected to selection for dispersal) and VBC_i (corresponding no-dispersal control). Thus, VB and VBC populations that share a numerical subscript (e.g. say VB_1 and VBC_1) were related by ancestry (DB_1 in this case). The adults of both VBs and VBCs were maintained under 15-day discrete generation cycles on banana-jaggery medium.

Selection for dispersal

The apparatus for selection for dispersal consisted of three components whose detailed dimensions are presented in the SI (Appendix S1). Briefly, the flies were placed in a large plastic container called the source, which was connected to another plastic container (the destination) through a plastic tube (the path). In order to impose selection, we introduced ~2400 flies 12 days post egg-collection to an empty source. Only the flies that travelled through the path to the destination within six hours, were allowed to breed for the next generation. The arbitrary cut-off of six hours was chosen because under desiccating conditions, there is almost no mortality in these flies during the first six hours (S. Tung personal observations). Since the imposed selection allowed ~50% of the flies to breed, there were two independent "source-path-

destination" setups, with ~2400 flies in the source, for each VB_i population. Post-selection, the dispersed flies in the two destination containers for a given VB_i population were mixed and transferred to a population cage. The VBCs were also introduced into the source bottles under desiccating conditions. However, they were not allowed to migrate (no selection for dispersal) and were supplied with a source of moisture after 3 hrs.

Assays

All assays were performed after growing both VB and VBC populations for one generation under common conditions, to rule out the contributions of non-genetic parental effects.

Dispersal kernel assay in presence and absence of food

This assay was used to assess the difference in dispersal propensity and ability between the VBs and the VBCs. The assay-setup was similar to the selection setup described above except for the path, which was 20 m long and divided into several detachable sections. On the 12^{th} day after egg collection, ~2000 adult flies were introduced into the source container and were allowed to disperse for 6 hours. After the end of dispersal run, each section of the path was independently sealed with all the flies in it. The flies were then heat killed and the number of flies in each section was recorded. For each VB_i and VBC_i population, there were three such replicate kernel setups. We performed two kinds of kernel assays: a) with an empty source and b) in the presence of ~20 ml banana-jaggery medium in the source. In total, this set of assays involved scoring ~96,000 flies.

Dispersal rate assay

For this assay, we again used a source-path-destination set up with a path length of 2 meters and there were six replicate setups for every VB_i and VBC_i population. The assay was initiated by introducing 120 12-day old flies (60 males + 60 females) into each source. The total duration for this assay was 2 hours, with the destination being replaced with a fresh bottle after every 15 minutes. The flies in the destination at each time point were then heat-killed, segregated according to sex, and counted.

Measures of dispersal components, kernel and spatial extent

The proportion of total flies in the source that initiated dispersal was taken as the dispersal propensity (Friedenberg 2003). Dispersal ability was the distance (in meters) travelled by the flies that left the source. Since dispersal ability is measured only on the flies that came out of the source, the measure of propensity and ability were independent of each other. Dispersal rate was computed as the average speed of the flies for covering the 2 m path in the dispersal rate assay. The mathematical expressions for each of these quantities are given in the SI (Appendix S1).

Dispersal kernels of the VB/VBCs were characterized using the various percentiles of the distribution. Change in the mean distance travelled, in principle, can shift the kernel without changing its shape. We eliminated this effect by computing the percentiles after subtracting the mean distance travelled in a given kernel replicate from the distance travelled by each individual in the replicate. We also measured the higher moments of the dispersal kernel like standard deviation, skew and kurtosis. To further characterize the kernel, we fitted the data with the negative exponential distribution $y=ae^{-bx}$ (Schtickzelle *et al.* 2012), where x is the distance from the source, y is the frequency of individuals found at x, and a, b are the intercept and slope

parameters respectively. The value of spatial extent was estimated as b^{-1}. ln (a/ 0.01), i.e. the distance from the source beyond which 1% of the population is expected to disperse.

Locomotor activity assay

After 49 generations of selection, locomotor activity of the selected and control lines were checked both in presence and absence of food. For this, we used the *Drosophila* Activity Monitoring (DAM2) data collection system (Trikinetics Inc, Waltham, MA) and followed standard protocol (Chiu *et al.* 2010). The obtained data were divided into two parts- i) first 6 hours and ii) next 24 hours. The first set captured the activity-rest pattern immediately after introduction of the flies in the tubes. The next set measured the steady state activity-rest pattern for a complete 24 hour cycle including the time when selection was imposed during the routine maintenance protocol (i.e. six hours on the 12th day after egg collection). For each of these two sets, average activity per hour and the fraction of time flies spent in sleeping/resting were computed. Following standard definitions (Hendricks *et al.* 2000; Chiu *et al.* 2010), continuous inactivity of five minutes or more was considered as sleep/rest. For each VB/VBC population, we measured the activity of 30-32 12-day old male flies.

Fecundity and Desiccation resistance assay

The number of eggs laid by 14 day old (i.e. the age of oviposition in the selection lines) females was estimated as a measure of fecundity. Desiccation resistance was measured as the time to death for 12-day old (i.e. the age at which dispersal occurs during selection) flies in the absence of food or water. For each VB/VBC population, fecundity was measured on 40 pairs while desiccation resistance was measured on 10 sets of 10 flies for each sex.

Statistical analyses

Since VB_i and VBC_j that shared a subscript (i.e.$i = j$) were related to each other by ancestry, they were analyzed together as a block. Data for dispersal propensity, dispersal distance and dispersal rate were subjected to separate three factor mixed-model ANOVA with selection (VB and VBC) and sex (male and female) as fixed factors and block (1-4) as a random factor. The propensity data, being fractions, were arcsine-square root transformed (Zar 1999) before analysis. The standard deviation, skew, kurtosis, *a*, *b* and spatial extent data for each population were computed after pooling the data for the three replicate kernels. For these six quantities, we used separate Mann-Whitney U tests to compare the VBs and the VBCs. For locomotor activity, % sleep (arcsine-square root transformed) and female fecundity data, we used two factor mixed-model ANOVA with selection (VB and VBC) as fixed factor and block (1-4) as a random factor. Three factor mixed-model ANOVA was performed for desiccation resistance assay with selection (VB and VBC) and sex (male and female) as fixed factors and block (1-4) as a random factor. All statistical analyses were performed using STATISTICA® v5 (StatSoft. Inc., Tulsa, Oklahoma).

RESULTS

Evolution of various dispersal components and their persistence in the absence of stress

In the presence of food in the source, the VB populations were found to have significantly greater dispersal propensity VBCs (Fig. 1a, $F_{1,3}$=60.78, *P*=0.004), ability (Fig. 1b, $F_{1,3}$=15.23, *P*=0.03) and rate (Fig. 1c, $F_{1,3}$=31.5, *P*=0.01) compared to the VBCs. This suggested that in the selected populations, a larger fraction of flies initiated dispersal, travelled farther and at a faster speed. We also measured these components of dispersal under conditions similar to the selection protocol (i.e. in the absence of food in the source).The VB populations again had a significantly higher propensity (Fig. S1a, $F_{1,3}$=22.68, *P*=0.02), ability (Fig. S1b, $F_{1,3}$=68.8, *P*=0.004) and rate (Fig. S1c, $F_{1,3}$=48.21, *P*=0.006,) than the corresponding VBCs, thus highlighting the robustness of the results.

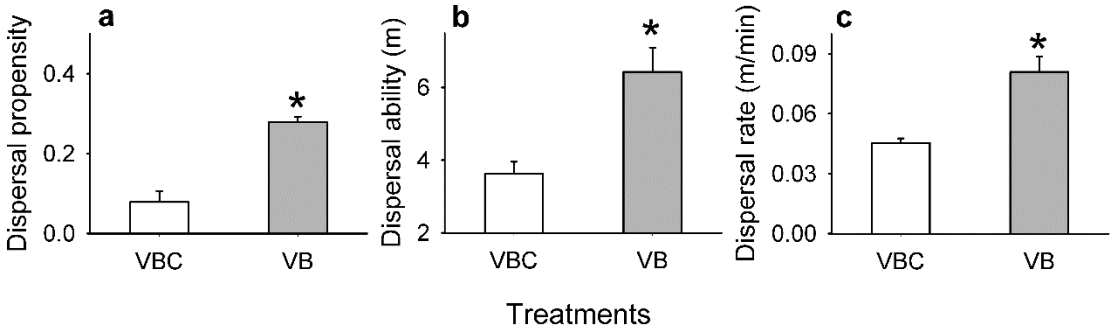

Figure 1 | Evolution of dispersal components of VB and VBC populations. (**a**) Propensity refers to the fraction of the total population that disperses from the source. (**b**) Ability refers to the mean distance travelled by those flies that come out of the source. (**c**) Rate measured as the speed of the flies while covering the path. The selected populations (VBs) had significantly greater propensity, ability and rate compared to the controls VBCs). Each bar is a mean over four replicate populations and the error bars represent standard errors around the mean (SEM). * denotes *P*< 0.05 for the main effect of selection in the ANOVA.

Taken together, these results imply that multiple components of dispersal had simultaneously evolved in the selected populations, and this difference was observable irrespective of the presence or absence of a proximate reason for them to disperse. We next investigated the implications of these changes in dispersal components, on the spatial distribution of the organisms, i.e. the dispersal kernel.

Evolution of dispersal kernel due to increased frequency of LDDs

All the higher percentiles (75 onwards) of VB-kernels were higher than the corresponding percentiles of VBCs (Fig. 2a). This indicates the presence of greater number of Long-Distance-Dispersers (LDDs) (Nathan *et al.* 2012) in the selected populations and suggests that the overall kernel shape has changed. This conclusion was further strengthened by the observation that the VB populations had greater standard deviation (Fig. 2b, Mann-Whitney U=0.0, P= 0.02), lesser positive skew (Fig. 2c, Mann-Whitney U=0.0, P= 0.02) and more negative kurtosis (Fig. 2d, Mann-Whitney U=0.0, P= 0.02) compared to the VBCs. When we fit a negative exponential distribution ($y=ae^{-bx}$) to the data (Schtickzelle *et al.* 2012), we found that the values of the intercept parameter a (Fig. 3a, Mann-Whitney U=0.0, P= 0.02) and the slope parameter b (Fig. 3b, Mann-Whitney U=0.0, P= 0.02) for the VB kernels were significantly lower than the VBCs (see Table S1 for R^2 values). This indicates a general flattening of the shape and fattening of the tail of the kernel in the selected populations.

We used the estimated average values of a and b from the equation $y=ae^{-bx}$ to calculate the spatial extent (Kot *et al.* 1996) as the distance from the source up to which 1% of the population was expected to reach. The mean spatial extent of the VBs and VBCs were found to be 28 m and 16.8 m respectively which implies an increase of 67% (Fig. 3c, Mann-Whitney U=0.0, P=

0.02).This implies an increase in the proportion of LDDs in the population (i.e. the fatness of the tail of the distribution).

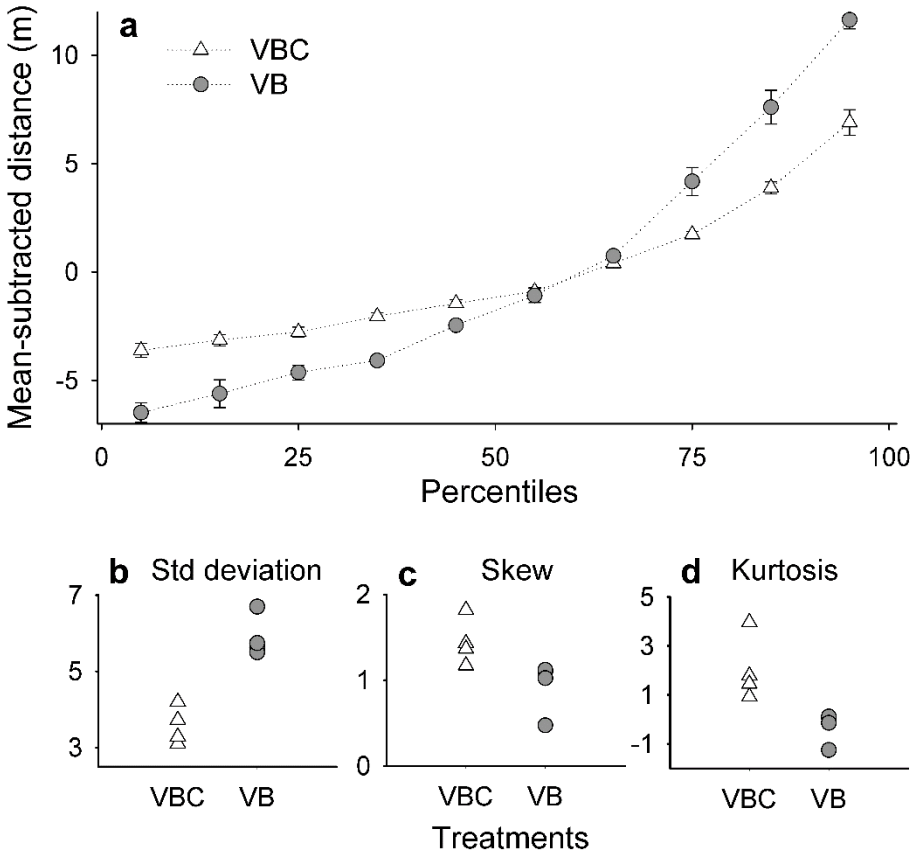

Figure 2 | Location and shape parameters of dispersal kernel for VB and VBC populations. (**a**) 5th to 95th percentile for the mean-subtracted kernels of VB and VBC populations. The error bars represent standard errors around the mean. In few cases the error bars are too small to be visible. In terms of the upper (> 65) percentiles VB > VBC; while for the lower (<50 percentiles), VB < VBC. Each percentile point represents data pooled over four VB or VBC populations. (**b**) Standard deviation, (**c**) Skew and (**d**) Kurtosis of the dispersal kernels. Each point (triangle for VBC and circle for VB) represents data from one replicate population, pooled over three independent kernel measurements. Together these panels indicate that the dispersal kernels of VBs have become flatter and their tails have become fatter.

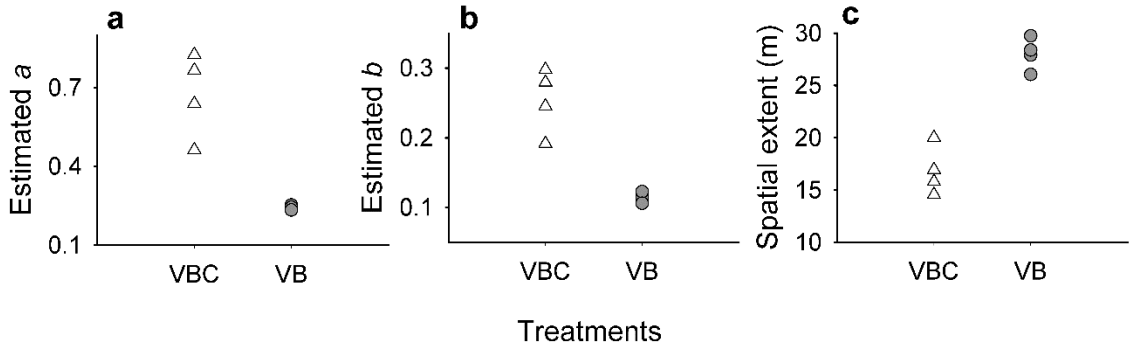

Figure 3 | Parameters of dispersal kernel and estimated spatial extent. Dispersal kernel parameters were estimated by fitting the negative exponential $y=ae^{-bx}$, where x is the distance from the source and y is the frequency of individuals found at x. Estimated values of (**a**) a and (**b**) b are significantly lower for VBs than VBCs (Mann-Whitney U tests, P = 0.02 for both). (**c**) Using the fitted curve, spatial extent of each of VB and VBC populations was computed by finding the distance from the source, beyond which 1% of the population is expected to reach. Population extents of VBs were greater than VBCs, indicating an increase in LDDs in the population (Mann-Whitney U tests, P = 0.02).

To summarize, our results indicate that even if we account for the increased mean, the shape of the dispersal kernel of the VBs had evolved to be substantially different from that of the VBCs.

Selected flies have increased locomotor activity

In the presence of food, after six hours of acclimatization, the VB populations were found to have significantly greater locomotor activity than the VBCs over a 24-hour duration (Fig. 4a, $F_{1,3}$= 59.9, P= 0.004). Interestingly, this difference persisted even during the first six hours after setup irrespective of the presence (Fig. 4b, $F_{1,3}$=423.3, P=0.0003) or absence (Fig. 4c, $F_{1,3}$=60.3, P=0.004) of food. Furthermore, after an acclimatization of six hours, over a 24 hour period, the VBs spent similar amount of time in rest/sleep as the VBCs (Fig. 4d, $F_{1,3}$= 5.47, P=0.1). However, for the first six hours after set up, the VBs spent significantly less time in rest/sleep in the presence (Fig. 4e, $F_{1,3}$=386.9, P=0.0003) or absence (Fig. 4f, $F_{1,3}$=50.4, P=0.006) of food.

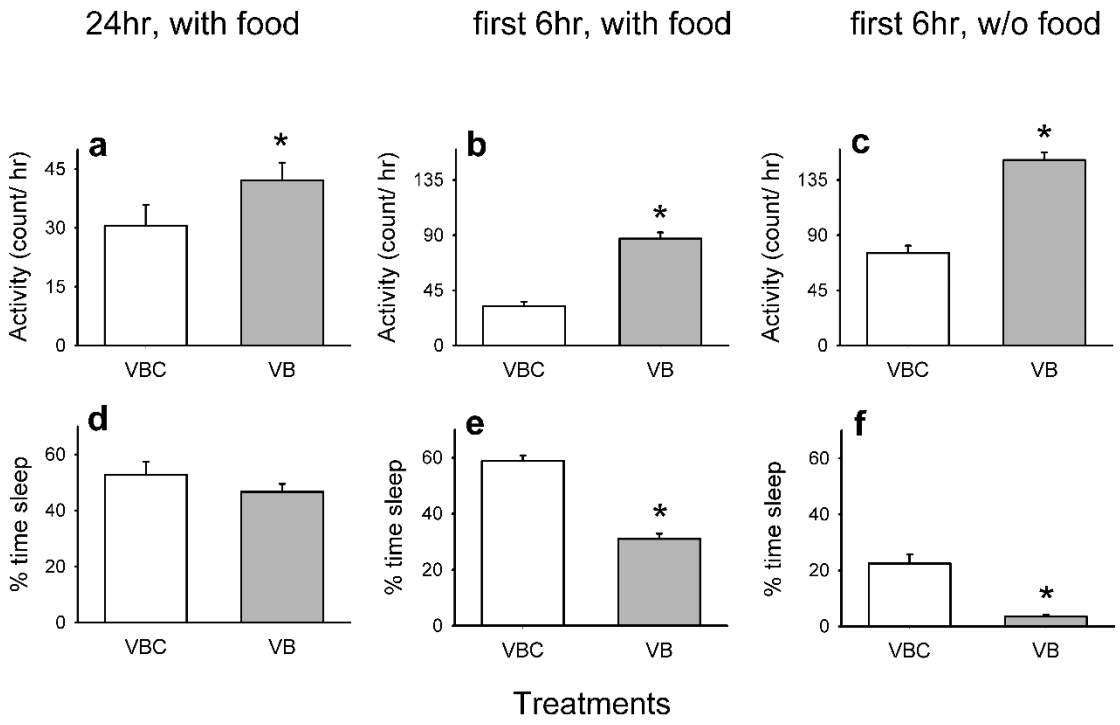

Figure 4 | Locomotor activity-sleep profiles in the presence and absence of food. Locomotor activity of VBs was significantly greater than VBCs, (**a**) over 24 hours post acclimatization and (**b**) during the first 6 hours of acclimatization period in presence of food, as well as (**c**) during the first 6 hours of acclimatization period in the absence of food. (**d**) The percentage of time spent in sleep/rest in 24 hours post acclimatization in presence of food is similar for both VBs and VBCs. However, during the first six hours VBs spent significantly lesser percentage of their time in sleep/rest than VBCs, both in the presence (**e**) and absence of food (**f**). The error bars represent standard errors around the mean (SEM) and * denotes $P<0.05$.

Trade-offs: selected flies have similar fecundity but reduced desiccation resistance

There was no significant difference between the fecundity of the VB and VBC flies (Fig. 5a, $F_{1, 3}=2.54$, $P=0.2$), indicating an absence of a trade-off between increased dispersal ability and reproductive output. However, the VBs were found to be significantly more susceptible to desiccation than the VBCs (Fig. 5b, $F_{1, 3}=15.8$, $P=0.03$).

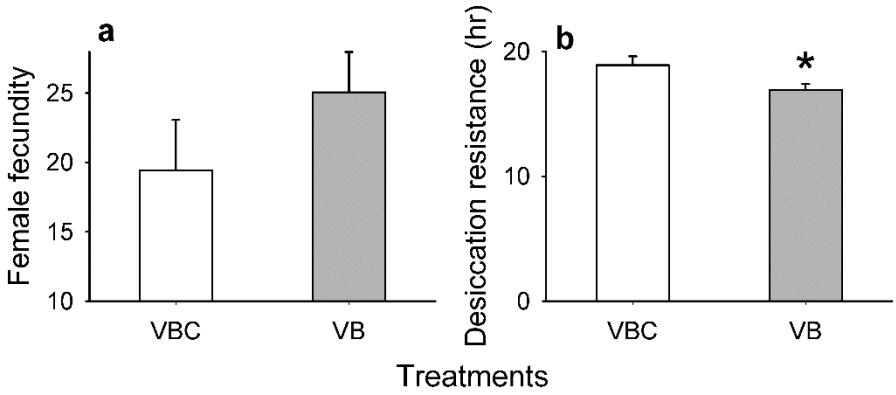

Figure 5 | Life-history traits of VBs and VBCs. (**a**) Average (± SEM) female fecundity of VB and VBC populations were not statistically different from each other. But, in terms of (**b**) average desiccation resistance (± SEM), VBs performed poorly compared to the VBC populations. * denotes $P<0.05$.

DISCUSSION

Evolution of dispersal is a highly complex phenomenon which depends on the interaction of a large number of factors. For dispersal evolution, physiological / behavioral attributes pertaining to the different phases of dispersal (i.e. emigration, travel and arrival/settlement) (Bowler & Benton 2005; Cote *et al.* 2010) interact with each other and the corresponding environmental pressures / stresses to impose a variety of costs on the population (Bonte *et al.* 2012). Consequently, the population evolves by optimizing over the various components of dispersal (propensity, ability, rate etc.), subject to the availability of the required genetic variation. The composite outcome of this process is reflected in the dispersal kernel and spatial extent of the population. Unfortunately, most theoretical and empirical studies in the dispersal literature typically focus on only a few of these issues at a time, which might be a reason behind the wide-spread variation in the results across such studies. For example, it has been shown that in spider-mites (*Tetranychus urticae*), dispersal propensity evolves when there is direct selection on dispersal rate (i.e. those who disperse early are selected) (Yano & Takafuji 2002). Interestingly, in the same model system, propensity fails to evolve when the selection is imposed directly on propensity (Tien *et al.* 2011) or on dispersal ability (Bitume *et al.* 2011). Similarly, dispersal propensity evolves in *C. elegans* populations in response to externally imposed extinctions but not due to variations in resource-densities (Friedenberg 2003). However, in the absence of information on how these selection pressures interact with the biology of the organism, it is not possible to understand why some components of dispersal evolved under certain circumstances while others did not.

In our study, only the first 50% of the adults that reached the destination were allowed to breed for the next generation. Thus, there was a direct selection for both dispersal propensity (i.e.

tendency to leave the source patch) and dispersal rate (i.e. speed of dispersal). Moreover, as the length of the path increased over generations, there was also a direct selection on dispersal ability (i.e. the ability to travel the required distance). Most importantly, by virtue of the way they were imposed, the selection pressures on these three components of dispersal were not antagonistic to each other. In other words, increase in any one of the components of dispersal (propensity, ability, rate) did not reduce the fitness in context of another component and consequently, all three could increase simultaneously. This is in contrast to an earlier empirical study that documented the evolution of LDDs but not dispersal propensity or mean dispersal distance in spider-mites (Fronhofer *et al.* 2014). The selective agent in the previous study was spatially-correlated extinctions, i.e. nearby patches were more likely to go extinct, which favored the LDDs. However, propensity did not evolve under this scenario as positive spatial-correlation of extinction, in the absence of a significant increase in dispersal ability, substantially increases the cost of leaving the current habitat. Another theoretical study on the evolution of passive dispersal of seeds on a fragmented landscape has also suggested that spatial autocorrelation among nearby habitats can lead to the evolution of long-distance dispersal, but not propensity (Hovestadt *et al.* 2001). Our results also differ from theoretical (North *et al.* 2011) and field (Baguette *et al.* 2003; Schtickzelle *et al.* 2006; Cheptou *et al.* 2008) studies predicting that increased habitat fragmentation should have a negative effect on dispersal propensity. Again, this apparent discrepancy is resolved when we observe that in some of these studies, the mortality during the travelling phase is so high that individuals with lower dispersal propensity have greater fitness even in the presence of habitat destruction (Baguette *et al.* 2003; Cheptou *et al.* 2008) (although see Schtickzelle *et al.* 2006). In our study, since ~50% of the flies were able to reach the destination, the cost of dispersal was not prohibitively high, which allowed dispersal propensity

to evolve, as predicted in some of the earlier theoretical studies (Heino & Hanski 2001; Zheng *et al.* 2009). It should be noted here that we estimated dispersal ability only on the individuals that emigrated from the source, thus avoiding a potentially confounding effect of increased propensity on ability. In the absence of this, the estimated values of dispersal ability for the VBs would have been even higher. Thus, our interpretation of increase in the dispersal ability of VBs is conservative.

To summarize, our results show that under the proper set of ecological and evolutionary conditions, simultaneous evolution of dispersal propensity, ability and rate is possible. Thus, when investigating the eco-evolutionary dynamics of dispersal (Travis *et al.* 2012), it is imperative to consider the interplay of the costs experienced during the various phases of dispersal and their impact on the various dispersal components. Evidently, the sign and magnitude of the evolutionary response in each of these dispersal components would interact to shape the spatial distribution of the organisms (i.e. the dispersal kernel). That was our next object of investigation.

The progenitors of the VB and VBC flies trace their ancestry to the IV populations, which were collected from the wild in 1970 (Ives 1970). Over the last 46 years (~few thousand generations) of their existence in vials and cages, these flies have not been subjected to any conscious selection for any movement-related traits. In spite of this, the unselected VBC populations harbored an appreciable number of long-distance dispersers (LDDs). This is evidenced by the long tail of the dispersal kernel (Fig. 2a) and a spatial extent of 16.8 m (Fig. 3c) even though the mean dispersal ability was only 3.6 m (Fig. 1b). This corroborates the general notion that in the absence of any external constraints, the presence of LDDs in a population is perhaps the norm and not an exception (Nathan *et al.* 2012). This is of considerable ecological and evolutionary

importance and can affect a range of processes including rate of range expansion (Hill *et al.* 1999), gene transfer across fragmented populations (Nichols & Hewitt 1994), the ability of a population to adjust to climate change (Travis *et al.* 2013) etc. This has prompted a number of studies on factors that affect long distance dispersal and its evolution in nature (Muller-Landau *et al.* 2003; Nathan *et al.* 2008).

Although the dispersal kernel is often considered to be a static entity in the literature (Chapman *et al.* 2007; Krkošek *et al.* 2007), recent theoretical (Phillips *et al.* 2008; Starrfelt & Kokko 2010) and empirical studies (Fronhofer *et al.* 2014) have suggested that the kernel should be evolutionarily dynamic. Our results (Fig. 2 and 3) support this notion. We also confirm a previous theoretical prediction (Phillips *et al.* 2008) that the kernel of the evolved population would have reduced skew and kurtosis (Fig. 2c and 2d). The important question now was the mechanism that led to an increase in the fraction of LDDs in the VB populations.

Increase in dispersal propensity or rate, by themselves; do not necessarily entail evolution of LDDs. However, selection for greater dispersal ability can potentially lead to an increase in the frequency of LDDs. Studies on natural populations of cane toads have suggested that increased dispersal ability can evolve due to a change in locomotor activity associated with an increase in the length of the hind limb (Phillips *et al.* 2006). Although the genetic basis for this change was equivocal (Phillips *et al.* 2010), enhanced locomotion would be a strong candidate mechanism for any observed increase in dispersal ability or rate.

Locomotion is a critical component of several behaviors including foraging, mate-finding, predator / stress avoidance etc. (Martin 2003), and has been shown to respond to artificial selection in *Drosophila melanogaster* (Connolly 1966; Jordan *et al.* 2007). Intuitively, evolution

of increased locomotor activity could be favored in our flies which were under strong selection for dispersal propensity, ability and rate, and this was indeed found to be the case (Fig. 4a). Furthermore, the selected populations had greater locomotor activity than the controls during the first six hours after introduction to a new environment (same duration as the selection protocol) (Fig. 4b), and spent lesser time in rest (Fig. 4e). This observation is consistent with the fact that the VBs were under intense selection to reach a new environment within the first six hours of introduction to the source. Consequently, maximizing the amount of activity and minimizing the resting period during that time would be of obvious advantage to the VB flies. More interestingly, we found similar activity/rest patterns in the absence of food (Fig. 4c and 4f), which suggests that the increased activity is independent of starvation or desiccation cues. This is again consistent with the observation that the dispersal propensity, ability and rate differences between VBs and VBCs were observed irrespective of the presence or absence of food (Fig. 1 and Fig. S1). In *Drosophila*, increased locomotor activity is associated with changes in a large number of genes (Jordan *et al.* 2007) as well as neuro-chemicals like noradrenaline (Tunnicliff *et al.* 1969) or tachykinin-related peptides (Winther *et al.* 2006). However, intuitively, it is not clear which subset of these genetic mechanisms might have evolved in our VB flies, thus creating a potentially fruitful avenue for future research. At the physiological level though, evolution of dispersal due to an energetically expensive enhancement of locomotor activity is expected to lead to various trade-offs related to both reproduction and body-maintenance.

The relationship between dispersal and fecundity has been somewhat controversial in the literature. On one hand, flight ability/ dispersal has been shown to be negatively correlated with fecundity in several insects including *Drosophila* (Roff 1977), long-winged crickets (Roff & Fairbairn 2007) and aphids (Dixon *et al.* 1993). This is thought to be due to energy limitation as

allocation of resources to the muscles reduces the availability of the same for reproductive functions. On the other hand, several investigators have reported a positive correlation between dispersal and fecundity (Hanski *et al.* 2006), (reviewed in Rankin & Burchsted 1992). One way by which such positive correlations can be observed is if the dispersers are also the physically superior organisms of the population who abound in resources and therefore escape the dispersal-fecundity trade-off (Bonte & de la Peña 2009). However, our results varied from both these observations in the literature since there was no significant difference between the fecundity of the VB and the VBC populations (Fig. 5a), in spite of a significant increase in all dispersal-related traits in the former (Fig. 1). This observation is consistent with an earlier study on spotted-mites that reported dispersal evolution accompanied by no change in fecundity (Fronhofer *et al.* 2014). However, in spite of the lack of statistical significance, the VBs had ~25% greater fecundity than the VBCs which is consistent with the dispersers-are-superior line of argument. It was therefore unintuitive to find that the desiccation resistance of the VBs had been significantly reduced (Fig. 5b) even though these flies did face desiccation in the dispersal path during the process of selection. However, it should be noted that the VBs have a significantly greater locomotor activity (Fig. 4a), which requires a lot of metabolic energy, and hence could potentially lead to reduced desiccation resistance.

In our study, the VBs were only experiencing active dispersal, whereas in nature flies undergo a mixture of active and passive dispersal processes (Dobzhansky 1973). Moreover, in our study, the dispersal landscape (i.e. the path) was linear and homogeneous whereas, in nature, the landscape is likely to be heterogeneous. Thus, under natural conditions, both these factors could conceivably lead to other kinds of interactions between the various dispersal components,

ultimately resulting in a somewhat different set of trade-offs. In spite of this, our results have some general implications.

There is a growing realization in the literature that multiple components of dispersal must be investigated simultaneously to attain a complete picture of dispersal evolution (Bonte *et al.* 2012). In such a framework, our result about concurrent evolution of multiple dispersal components can be taken as a null model. In other words, whenever a particular component of dispersal is seen not to evolve, elucidating the reasons for that can become a focus of investigation. Furthermore, our study shows that under gradual directional selection of moderate intensity and in the absence of conflicting selection pressures, dispersal can evolve rapidly, substantially and with non-significant reproductive trade-offs. Such conditions are expected to be fairly common in nature, particularly in regions where climate changes or habitat degradations are gradual but steady. More critically, our results indicate that once evolved, these traits can express themselves even in the absence of any proximal stresses. This could lead to organisms with intrinsically high rates of dispersal. On the positive side, this could reduce the chances of local extinction (Brown & Kodric-Brown 1977; Forney & Gilpin 1989) and ensure greater gene flow between populations (Vilà *et al.* 2003). On the negative side, this could increase the invasiveness of species (Kot *et al.* 1996; Neubert & Caswell 2000), increase the rate of spread of diseases (Keeling *et al.* 2001) and induce instability in metapopulation dynamics through enhanced synchrony between neighboring subpopulations (Dey & Joshi 2006). Either way, evolution of enhanced dispersal is almost guaranteed to have wide-spread economic and /or conservation implications.

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ONLINE SUPPORTING INFORMATION FOR

Selection for dispersal leads to evolution of kernel and increased locomotor activity in *Drosophila melanogaster*

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Appendix S1: Detailed materials and methods

Ancestral populations:

The experimental populations used in this study were derived from four independent large (breeding size of ~2400) laboratory populations of *Drosophila melanogaster* (DB_{1-4}) which in turn trace their ancestry to four outbred populations called JB_{1-4}. The detailed maintenance regime and ancestry of the JB_{1-4} populations has been described elsewhere (Sheeba *et al.* 1998). The maintenance regime of the DB_{1-4} populations are similar to the JB_{1-4}, except that the former set of flies are introduced into population cages on the 12th day after egg collection

From each DB_i population (where $i \in [1, 4]$), we derived two populations: VB_i (short for 'vagabond', subjected to selection for dispersal) and VBC_i (corresponding no-dispersal control). Thus VB and VBC populations that share a numerical subscript (e.g. say VB_1 and VBC_1) were related by ancestry (DB_1 in this case), and hence were always assayed together and treated as blocks in statistical analyses.

Maintenance regime of experimental populations:

The adults of both VBs and VBCs were maintained in plexi-glass population cages (25 cm × 20 cm ×15 cm) at a high adult number (~2400 individuals) to avoid inbreeding. Following earlier protocols, both the larvae and the adults were maintained at 25°C and constant light conditions (Sheeba *et al.* 1998). The flies were made to oviposit on petri-plates containing banana-jaggery medium for 12-16 hours. After oviposition, we cut small strips of the medium, each containing ~60-70 eggs, and introduced them individually into 35 ml plastic vials that had ~6 ml of the same banana-jaggery medium. This ensured that the larvae were raised under low to moderate level of crowding, and there was no confounding effect of density-dependent selection (Joshi

1997). The adults started emerging by the 8^{th}-9^{th} day after egg collection and on the 12^{th} day, the VB populations underwent selection for dispersal (see below). Since at 25°C temperature, all normally developing adults eclose by 10^{th} -11^{th} day, our selection protocol ensured that there was no inadvertent selection for faster larval development (Prasad *et al.* 2001). After the imposition of selection, the flies were transferred to the population cages and immediately supplied with excess live yeast- paste to boost their fecundity. Around 40 hours after this, the flies were supplied with a fresh petri-plate containing banana-jaggery medium for oviposition. The eggs so collected formed the next generation and the egg-laying adults were discarded, ensuring that adults from two different generations never co-exist. Thus, both VBs and VBCs were maintained under 15-day discrete generation cycles. For each VB population, we collected eggs in 80 vials (thus leading to approximately 4800 adults) while for VBCs, the corresponding number of vials was 40. This ensured that after selection (see next section), the breeding population of the VB populations was similar to that of the VBCs.

Selection protocol

The apparatus for selection for dispersal consisted of three components: a *source*, a *path* and a *destination*. The source was an empty transparent cylindrical plastic container of diameter 11 cm and height 16 cm with a funnel attached to one end (S2 Fig). The diameter of the broad end of the funnel matched that of the source, while the diameter of the exit to the stem was 1.8 cm. The path connecting the source with the destination consisted of a transparent plastic tube of inner diameter ~1 cm. The destination was again a cylindrical plastic container (diameter 11 cm and height 16 cm) and contained a supply of moisture in the form of a strip of wet cotton. The end of the path protruded ~10 cm inside the destination (S2 Fig). This protrusion helped in reducing the rate of backflow as, after getting out of the path, the flies typically spent most of their time on the

walls or floors of the container, and hence mostly failed to locate this aperture. To make the overall setup compact, the path was coiled (in the horizontal plane). The length of the path was 2 m at the beginning of the selection, but was increased intermittently. By generation 33 (when most of the assays were done), the path length had reached 10 m.

In order to impose the selection, on the 12th day after egg-collection, ~2400 adults (coming out of 40 vials) of a given VB_i population were placed in a source, which was then connected to the destination via the path. The entire setup was placed in a well-lit room maintained at 25 °C. Since the source had no moisture, the flies experienced desiccation. Pilot runs with the ancestral DB populations had shown that under these environmental conditions, a subset of the flies tended to move through the opening towards the destination. Pilot studies also showed that very few flies dispersed in the presence of food in the source and therefore we decided to impose selection in the absence of food. The flies were allowed to disperse for six hours or till roughly 50% of the population reached the destination (whichever happened earlier). The arbitrary cut-off of six hours was chosen because assays in the lab had demonstrated that under desiccating conditions, there was almost no mortality during the first six hours (S. Tung personal observations). Only the flies that reached the destination were allowed to breed for the next generation. Since the imposed selection allowed ~50% of the flies to breed, there were two independent "source-path-destination" setups, with ~2400 flies in the source, for each VB_i population. Post-selection, the dispersed flies in the two destination containers for a given VB_i population were mixed and transferred to a population cage. They were then supplied with live-yeast paste and after ~40 hours, eggs were collected (as mentioned above). The VBCs were maintained similarly as the VBs except two major differences. Firstly, after transferring the flies into the source, the exit was blocked by a cotton plug and the flies were allowed to desiccate for

3 hours (which was half the total time allowed for the VB flies to migrate). Following the protocol for the VB flies, the VBC flies were then supplied with a moist cotton plug for the next three hours. This controlled for the inadvertent desiccation experienced by the VB flies in the source and the path, as part of the selection protocol. It should be noted here that there was almost zero mortality in the VBC flies during this time, thus ensuring that the selection pressure for desiccation resistance was at best, mild. Secondly, all the flies in the VBC populations were allowed to breed, thus ensuring no selection for dispersal.

Assays:

All assays were performed after relaxing the selection on both VB and VBC populations for one generation. For this, the VB and VBC flies were transferred directly into the corresponding cages on the 12^{th} day after egg collection. The progeny of these flies, henceforth referred to as the relaxed populations, were used for the assays. This common-rearing ensured that influence of phenotypic plasticity or non-genetic parental effects were ameliorated. Additionally, to remove any extraneous influence due to larval crowding, egg density was kept to ~50 eggs on ~6mL food in each vial.

Dispersal kernel assay in presence and absence of food

This assay was used to assess the difference in dispersal propensity and ability between the VBs and the VBCs. The assay-setup was similar to the selection setup (see section 'Selection protocol' and S2 Fig) except for the length of the path, which was 20 m. Furthermore, to obtain the location kernel (i.e. the distribution of the location of the files after dispersal) the path was divided into multiple detachable sections: 20 sections of length 0.5 m each, followed by 10 sections of length 1 m each. The destination container (a 250 ml plastic bottle) did not contain

food or water but had a long protrusion to reduce backflow. On the 12th day after egg collection, ~2000 adult flies were introduced into the source container and were allowed to disperse for 6 hours. During this interval, the entire setup was kept undisturbed under constant light and at a temperature of 25˚C. After the end of dispersal run, the setup was dismantled; the openings of the source, the destination, and each section of the path were secured carefully with cotton plugs, and labeled appropriately. The flies were then heat killed and the location (in terms of the distance from the source) and sex of each fly was recorded. For each VB_i and VBC_i population, there were three such replicate kernel setups.

We performed two kinds of kernel assays: a) with an empty source and b) in the presence of ~20 ml banana-jaggery medium in the source. The former set of assays was performed after 19-20 generations of selection while the latter set of assays happened after 32-33 generations of selection. In total, this set of assays involved scoring ~96,000 flies.

Dispersal rate assay

Dispersal rate assay was performed after 39 generations of selection. To measure the rate of dispersal, the path length was kept constant at 2 meters. A 100-mL glass flask (actual volume ~135 ml), with ~35 ml of banana-jaggery medium covering the bottom was used as the source, while the destination was a 250 ml plastic bottle. For every VB_i and VBC_i population, there were six replicate setups. 12-day old (from egg-collection) adults were used for this assay. One day prior to the assay, we anaesthetized the flies under carbon-dioxide, separated them by sex and maintained them overnight at a density of 60 flies (30 males + 30 females) in vials containing ~6 ml of banana-jaggery food. This ensured that the effects of adult crowding were controlled for and the flies had enough time to recover from the stress due to anesthesia. The assay was

initiated by introducing 120 flies (60 males + 60 females) into each source. The total duration for this assay was 2 hours, with the destination being replaced with a fresh bottle after every 15 minutes. The flies in the destination at each time point were then heat-killed, segregated according to sex, and counted.

Locomotor activity assay

After 49 generations of selection, locomotor activity of the selected and control lines were checked both in presence and absence of food using *Drosophila* Activity Monitoring (DAM2) data collection system (Trikinetics Inc, Waltham, MA). For the activity assay in presence of food, flies were generated from the relaxed populations. On the 11^{th} day from the day of egg collection, between 1830 hr -1930 hr, single adult male flies were aspirated into freshly prepared glass activity tubes of 5 mm diameter, containing standard banana-jaggery food at one end. We preferred aspiration over CO_2 anesthesia to avoid any lingering effects of anaesthetization on the activity of the flies (Van Dijken *et al.* 1977). Details of the preparation of the tubes and the cleaning of the same can be found elsewhere (Chiu *et al.* 2010). The selected populations were always assayed along with their matched control populations (i.e. VB_i was always assayed with the corresponding VBC_i) and there were 30-32 replicates for each population. Activity data were collected for 30 hours and during this entire time the monitors containing the activity tubes were kept undisturbed, inside an incubator maintaining 25 °C and constant light.

The obtained data were divided into two parts- i) first 6 hours and ii) next 24 hours. The first set captured the activity-rest pattern immediately after introduction of the flies in the tubes. The next set measured the steady state activity-rest pattern for a complete 24 hour cycle including the time when selection was imposed during the routine maintenance protocol (i.e. six hours on the 12^{th}

day after egg collection). For each of these two sets, average activity per hour and the fraction of time flies spent in sleeping/resting were computed. Following standard definitions (Hendricks *et al.* 2000; Chiu *et al.* 2010), continuous inactivity of five minutes or more was considered as sleep/rest.

The locomotor activity assay in the absence of food was similar to the one mentioned above except that no food was provided and both ends of the activity tubes were secured with clean, dry cotton plugs. The setup for this assay was done on the 12^{th} day from the day of egg collection between 1200 hr-1300 hr which roughly corresponds to the time at which selection was imposed during regular maintenance of VBs. Moreover, in the no food case, locomotor activity was recorded only for 6 hours from the time of setup, as after this period, the flies become stressed and slowly start dying due to desiccation.

Fecundity assay

After 40 generations of selection, fecundity of 14 day (post egg collection) old flies was assayed. This is the day on which eggs were collected from the VB and the VBCs during their routine maintenance regime. The flies were segregated into pairs of 1 male + 1 female under mild CO_2 anesthesia and each pair was transferred into individual 50 ml falcon tubes. The falcon tubes had a small (~1.5 ml) food cup attached at the centre of the inner surface of the lid and had small pores on the tube wall to allow for exchange of gases. 12 hours after introduction, the flies were discarded, and the number of eggs in each food cup was counted under stereo microscopes. 40 such replicates were used for each of the eight selection × block combinations. The time window allowed for oviposition in this assay (12 hours) was the same as that used for the VBs and VBCs under their routine maintenance regime.

Desiccation resistance assay

For this assay, adult flies were collected from relaxed populations (after 44 generations of selection) on the 12th day after egg collection. The flies were sexed under light CO_2 anesthesia and 10 individuals of a given sex were introduced into a clean, transparent, plastic vial. For each VB/VBC population there were 10 replicate vials per sex (i.e. 100 males and 100 females were assayed per population). The vials were kept in an incubator maintained at 25 °C with constant light and monitored every two hours for mortality, till all the flies died. From this data, desiccation resistance of each fly was computed as the difference between the time of setup and the time when it died.

Dispersal components

Dispersal propensity

The proportion of total flies in the source that initiated dispersal was taken as the dispersal propensity (Friedenberg 2003). Thus propensity = (Number of flies found outside the source/ Total number of flies).

Dispersal ability

The dispersal ability was computed only on the flies that left the source, based on the section of the path in which they were found after 6 hours. All flies found in a given section of the path were deemed to have travelled the distance between the source and the midpoint of the section. The destination container was considered as a part of the last path-section. Thus mathematically,

$$Dispersal\ distance = \sum_{i=1}^{y} \frac{x_i n_i}{Total\ number\ of\ flies\ outside\ source}$$

where, n_i is the number of flies found in the i^{th} path-section, x_i is the distance of the mid-point of this section from source and y is the total number of path-sections (here $y = 30$, see Section 'Dispersal kernel assay in presence and absence of food' for details). Since dispersal ability is measured only on the flies that came out of the source, the measure of propensity and ability were independent of each other.

Dispersal rate

Dispersal rate was computed as the average speed of the flies for covering the 2 m path in the dispersal rate assay. Thus mathematically,

$$Average\ speed = \frac{\sum_i (\frac{d}{T_i} \times n_i)}{\sum_i n_i}$$

where, d is the distance of the path (here, $d = 2$m), n_i is the number of flies that crossed a distance of 2 m during the t^{th} time interval and T_i is the time in minutes after the i^{th} interval since the setup-time. Here, data were collected for 2 hours, in 15-minutes intervals. Thus $T_i \in \{15, 30, 45, 60, 75, 90, 105, 120\}$.

Curve-fitting for estimating population extent

The data obtained from the dispersal kernel assay in presence of food, were fitted with the negative exponential distribution $y=ae^{-bx}$, where x is the distance from the source, y is the frequency of individuals found at x, and a, b are the intercept and slope parameters respectively. For this we pooled the data of the three replicates for each of the four populations of VB and VBC, estimated the frequency for each distance, natural log-transformed all values and fitted the equation $\ln(y) = \ln(a) - bx$ using linear regression. The estimated R^2 values (Supplementary

Table S1) ranged between 0.67 and 0.99 and the residuals showed no major trends. The value of population extent was estimated as b^{-1}. ln (a/ 0.01), i.e. the distance from the source beyond which 1% of the population is expected to disperse.

During the linear regression, we observed that one data point in the kernel of the VB_3 population seemed to be an outlier. Excluding this point from the kernel considerably improved the fit (R^2 = 0.26 became R^2 = 0.91) and the distribution of the residuals improved considerably. However, removing this outlier reduced the mean value of the spatial extent of VBs from 32.6 m to 28.01m. Incidentally, there were no changes in terms of the statistical significance in the Mann-Whitney U-tests for *a*, *b* or the spatial extent irrespective of whether the outlier is included or excluded. Therefore, in this study, we chose to report the value of population extent omitting the outlier. Note that this removal only makes our estimate of the spatial extent of VBs more conservative.

S1 Table. R^2 values of the fitted kernels

Populations	R^2
VB1	0.67
VB2	0.73
VB3	0.91
VB4	0.65
VBC1	0.97
VBC2	0.97
VBC3	0.99
VBC4	0.98

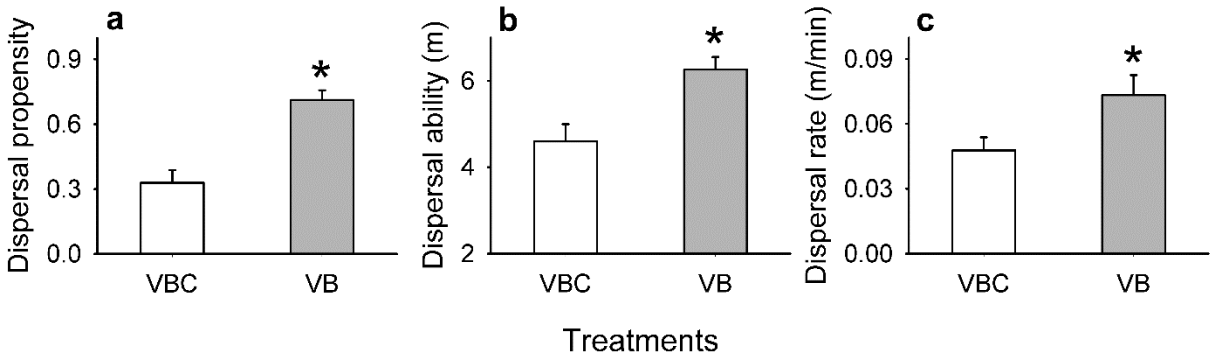

Figure S1. Average (± SEM) values of the dispersal components in the absence of food in the source. VBs had greater (**a**) dispersal propensity, (**b**) dispersal ability and (**c**) dispersal rate compared to VBCs even when food was not present in the source container during the corresponding assay. * denotes $P<0.05$.

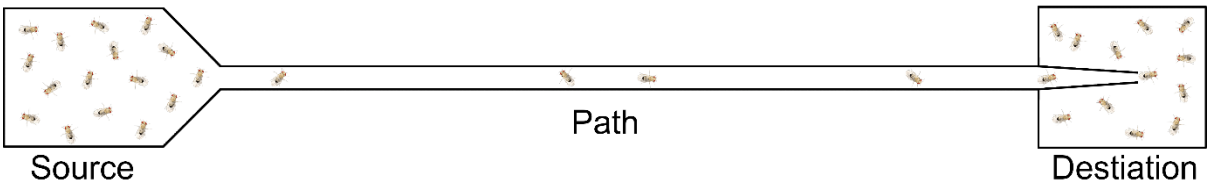

Figure S2. Schematic diagram of source-path-destination setup. The source and the destination are transparent plastic containers. The path is a transparent plastic tube. The path protrudes inside the destination by ~10 cm; this protrusion considerably reduces backflow of the flies. Here, all the three parts-- the source, path and the destination are detachable. The tiny objects oriented randomly inside the setup denote the flies. The length of the path increased from 2m to 10m during the selection. During the kernel and the rate assays, the lengths of the paths were 20 m and 2m respectively.

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