Title: Selection for greater dispersal in early life leads to faster age-dependent decline in locomotor activity and shorter lifespan

Authors: Ruchitha BG[1,2], Nishant Kumar[1], Chand Sura[1], Sudipta Tung[1]*

Affiliations:

Integrated Genetics and Evolution Laboratory (IGEL), Department of Biology, Ashoka University, Sonipat, Haryana, India, 131029.

Indian Institute of Science Education and Research (IISER) Pune, Pune, Maharashtra, India, 411008.

1. ruchitha.bg@students.iiserpune.ac.in
2. nishant.kumar@ashoka.edu.in
3. chand@ashoka.edu.in
4. sudipta.tung@ashoka.edu.in [ORCID 0000-0002-3201-3303]

* **Name and address of the corresponding author:**

Sudipta Tung
DBT/Wellcome Trust India Alliance Early Career Fellow
Department of Biology, Ashoka University
Plot No. 2, Rajiv Gandhi Education City,
Post Office Rai, Near Rai Police Station,
Sonipat, Rai, Haryana, India 131029

Tel: +91-130 2300669
Email: sudipta.tung@ashoka.edu.in

Abstract

Locomotor activity is one of the major traits that is affected by age. Greater locomotor activity is also known to evolve in the course of dispersal evolution. However, the fate of evolved locomotor activity in absence of selection pressure for dispersal over several generations, and the impact of dispersal evolution on the functional senescence of locomotor activity are unknown. We addressed these knowledge gaps using large outbred populations of *Drosophila melanogaster*, which evolved greater locomotor activity in their early life as a correlated response to early life selection for increased dispersal. For this, we maintained the selected and ancestry-matched control populations under identical conditions for nine generations in absence of dispersal selection. Subsequently, we tracked locomotor activity of individual flies of both sexes at regular intervals until a late age. Longevity of these flies was also recorded. Interestingly, we found that locomotor activity declines with age in general, but activity level of dispersal selected populations never drops below the controls. More importantly, the rate of age-dependent decline in activity of the dispersal selected population was found to be much larger; while the two sexes perform similarly with respect to the ageing profile of activity. Dispersal selected populations were also found to have a shorter lifespan as compared to the control, a potential cost of elevated level of activity throughout their life. These results are crucial in the context of invasion biology as contemporary climate change, habitat degradation, and destruction provide congenial conditions for dispersal evolution. Such controlled and tractable studies investigating the ageing pattern of important functional traits are important in the field of biogerontology as well, which looks into improving the biological quality of life during late-life by postponing the onset of senescence.

Introduction

Dispersal is a central life-history trait (Bonte & Dahirel 2017). At an individual level, it can confer survival advantage against proximate environmental stresses (Studds *et al.* 2008), as well as reproductive advantage through a greater prospect of finding mates and suitable breeding grounds in case of sexually reproducing organisms (Greenwood & Harvey 1982; Studds *et al.* 2008). At the population level, it modulates the tempo and extent of adaptive dynamics by affecting the degree of gene flow between populations (Lenormand 2002; Garant *et al.* 2007). Dispersal also shapes local ecology by influencing processes such as, *inter alia*, territoriality, range expansion, species invasion, etc. (Hargreaves & Eckert 2014; Andrade-Restrepo *et al.* 2019).

It has been shown through both theoretical and empirical evidence that the dispersal abilities of individuals can evolve rather quickly in the presence of favourable environmental conditions (Alex Perkins *et al.* 2013; Huang *et al.* 2015; Ochocki & Miller 2017; Tung *et al.* 2018b). These conditions are prevalent in the contemporary times due to, *inter alia*, climate change, human-induced landscape changes, and habitat destruction. Notably, dispersal is known to have strong associations with a number of traits of an individual, such as locomotor activity, aggression, body size, boldness, etc. (Wahlström 1994; Michelangeli *et al.* 2017; Searcy *et al.* 2018; McGhee *et al.* 2021; Wu & Seebacher 2022), which are collectively known as dispersal syndrome. Consequently, these traits also often evolve as a result of dispersal evolution (Fjerdingstad *et al.* 2007; Tung *et al.* 2018a). Such changes in organismal trait distributions can lead to the evolution of organisms with combinations of dispersal and dispersal syndromic traits with significantly higher potential for range expansion and biological invasion, and thereby threaten local ecological stability (Lambrinos 2004; Alex Perkins *et al.* 2013; Renault *et al.* 2018). Thus, not surprisingly, investigating different

aspects of dispersal evolution is a prime focus among contemporary ecologists, evolutionary biologists, and conservation biologists alike.

Despite this growing interest in studying dispersal evolution, its impacts on functional senescence (Grotewiel *et al.* 2005), or the gradual decline in an organism's ability to function as it ages, has largely been ignored. This is important, as senescence can have a significant impact on an organism's ability to disperse and colonize new areas. For example, individuals with accelerated functional senescence may have a decreased ability to travel long distances or may be more vulnerable to predation. Additionally, senescence can also affect an organism's ability to reproduce and pass on their genes, which can impact the evolution of dispersal traits in a population. The lack of understanding and consideration of functional senescence in dispersal evolution studies can lead to incomplete or inaccurate conclusions about the evolution of these traits.

As humans age, one of the major challenges they face is the impairment of locomotor activity (McGibbon & Krebs 2004), which is a key trait that evolves as a correlated response to dispersal evolution. While the performance of physical activity is known to deteriorate with age in humans, the relationship between age and locomotion activity is not consistent among other organisms. For example, some animals, such as senescence-accelerated mice (Miyamoto *et al.* 1986) and rabbits in open-field tests (Deyo *et al.* 1989) show an increase in activity with age; mice, rhesus monkeys and some rats show a decrease in activity with age (Goodrick 1971; Dean *et al.* 1981; Emborg *et al.* 1998). In contrast, no significant change in activity with age has been seen in cats and some rats (Levine *et al.* 1987; Nagahara & Handa 1997). In the case of the insect model *Drosophila melanogaster*, Le Bourg (1987) has shown an age-dependent decrease in activity in females, and an increase in activity in males till 5 weeks of age, after which activity declines. However, some studies have shown that such

sexual dimorphism in the age-dependent activity of *Drosophila* is strongly influenced by the genotype of the flies used (van Dijken *et al.* 1979; Fernández *et al.* 1999). Therefore, it is not yet clear how mobility changes with age in sexually dimorphic organisms and how those changes are manifested in populations that have evolved greater locomotor activity as a by-product of selection for greater dispersal during early life.

In this study, we aimed to fill this knowledge gap by investigating age-related patterns of dispersal in large, outbred populations of *Drosophila melanogaster* that have been selected for greater dispersal during their early life for over seven years (153 generations). *D. melanogaster* was chosen as the model organism due to its short lifespan of around three months, which allowed for the study of age-dependent traits across the entire lifespan of the organism. Furthermore, the short lifespan of *D. melanogaster* makes evolutionary studies feasible, and its low maintenance cost and small size allows for experimentation with large population sizes (here, each population has n = ~2400) to reduce issues of individual variations in aging and aging-related traits.

We examined the locomotor activity of the evolved populations and their ancestry-matched controls after maintaining both types of populations under identical conditions for nine generations, or approximately five months, without any further selection for dispersal. The aging profiles of the evolved and control populations were compared for males and females at multiple time points across the lifespan to understand the impact of dispersal evolution on the functional senescence of locomotor activity in these flies. We began by analysing the aging profile in a cross-sectional study to understand how the trait has changed in these populations. Next, considering variation in lifespan among individuals within a population and to eliminate potential survivorship bias because of it, we conducted a longitudinal study by following flies individually and measuring their locomotor activity across age.

Additionally, in this longitudinal study, we compared the longevity of the evolved and control populations to understand the impact of functional aging of locomotor activity on their lifespan.

Methods

Experimental Populations

We used four large (n = ~2400), outbred, laboratory-maintained populations of *Drosophila melanogaster* (VB_{1-4}) that were selected for greater dispersal propensity and ability for 153 generations (>6 years), and their respective ancestry-matched control (VBC_{1-4}) populations. Details of the selection protocol and maintenance regime of these populations were reported previously (Tung *et al.* 2018b). Briefly, the VB_{1-4} and VBC_{1-4} populations were maintained in 15-day discrete generation cycles on a standard banana-jaggery medium (following the recipe of Sheeba & Joshi 1998) at 25°C, with constant light. On the 12th day from the day of egg collection, the VB_{1-4} populations were subjected to a three-compartment source-path-destination dispersal selection setup, and only the most dispersive half of the population (the first 50% of the population reaching the destination) were allowed to lay eggs for the next generation. VBC_{1-4} populations were not subjected to selection for dispersal, but the rest of the rearing conditions were identical to that of the VB_{1-4} populations.

Experiments

Experiment 1: For investigating population level effect

Prior to this experiment, selection pressure for dispersal was relaxed for eight generations i.e. VB_{1-4} and VBC_{1-4} populations were maintained identically without selection pressure to minimize non-genetic parental effects. ~300 eggs were collected in drosophila-bottles (Laxbro® #FLBT20) containing 50mL banana-jaggery food. Eight such replicates were made for each population. On 11th day from egg collection, all flies from eight bottles of same population were pooled into a single cage (25 cm × 20 cm × 15 cm) and maintained with fresh food being provided every alternate day. This was done for each of the eight

populations (VB_{1-4} and VBC_{1-4}). From each of these population cages, 32 flies of each sex were randomly sampled at predetermined age-points (see "Assay Details" below). Overall, locomotor activity of 2048 flies (32 flies × 2 sexes × 8 populations × 4 age-points) was measured for this experiment. Figure S1 shows a schematic diagram of the experimental design for investigating population level effect.

Experiment 2: For tracking individual flies

In experiment 2, we collected longitudinal data as opposed to the cross-sectional data gathered in experiment 1. Since the lifespans of individuals can vary greatly within a population, cross-sectional activity data can be affected by survivorship bias, which can influence the interpretation of results. Therefore, experiment 2 was designed as a proof-of-principle validation of the findings from experiment 1, and was conducted using a specific set of selected and control populations, chosen at random.

This experiment was performed on the dispersal selected populations (VB_2), after relaxation of selection pressure for dispersal over nine generations, and its ancestry matched control (VBC_2). In order to generate experimental flies for each population, ~300 eggs were collected in drosophila-bottles (Laxbro® #FLBT20) containing 50mL banana-jaggery food. Three such replicates were made for each population. From the three bottles, 30 individuals of each sex were chosen at random during the P13-P15 stage of pupa and hosted individually in glass vials containing ~4mL of food and incubated at 25°C. On eclosion, these 120 virgin adult flies (*i.e.* 30 individuals per sex per population) were checked daily for their viability until death, and their age-dependent locomotor activity was tracked by measuring the trait at predetermined age-points (see "Assay Details" below). The flies were provided fresh food every alternate day, while strictly maintaining their vial identity. Figure S2 shows a schematic diagram of the experimental design for tracking individual flies.

Assay Details

Locomotor activity assay (used for experiments 1 and 2)

Locomotor activity was measured as the number of times per hour that each fly present in a glass tube (diameter: 5 mm, length: 8 cm) crossed the middle of the tube. This was done using Drosophila Activity Monitoring (DAM2) data collection system (Trikinetics Inc, Waltham, MA) which recorded the number of times each fly would cross the infrared beams at the centre of the glass tube every 30 seconds using a standard protocol (Chiu *et al.* 2010).

For experiment 1, we tracked locomotor activity of four independent but identically maintained, large, outbred, dispersal-selected populations VB_{1-4} across age and contrasted the same against corresponding four ancestry-matched control populations VBC_{1-4}. To do this, we randomly sampled 32 flies of each sex from each of these eight populations (~2400 adults on the 12th day in each population) on the 12th, 26th, 40th, and 47th day from the day of egg collection and measured their locomotor activity. On the specified days of activity measurement, flies were collected from population cages and introduced into clean glass tubes (diameter: 5 mm, length: 8 cm), without CO2 anaesthesia. Each glass tube was secured with cotton plugs on both sides. Activity was recorded for 2h and 5mins, and the initial 5 min was excluded to minimize the effect of disturbance caused at the time of setup. At the end of the recording, mortality was recorded and all the flies in the glass tubes were discarded. Overall, for experiment 1, locomotor activity of 2048 flies (32 flies × 2 sexes × 8 populations × 4 age-points) was measured.

For experiment 2, we repeatedly measured locomotor activity of same set of 30 male and 30 female flies each of VB_2 and VBC_2 populations on the 12th, 26th, 33rd, 40th, and 47th day from the day of egg collection. On the specified days of activity measurement, flies were introduced into individually labelled (to maintain identity of each fly), clean glass tubes

(diameter: 5 mm, length: 8 cm), without CO_2 anaesthesia. Each glass tube contained 1.5% w/vol agar, and was sealed with parafilm on one end and secured with cotton plug on the other. Activity was recorded for 4h and 5mins, and the initial 5 min was excluded to minimize the effect of disturbance caused at the time of setup. At the end of the recording, the flies were transferred back to the corresponding individually labelled glass vials (to maintain identity of each fly) with food. Overall, for experiment 2, 120 flies (30 flies × 2 sexes × 2 populations) were individually tracked by maintaining in labelled containers and their locomotor activity was measured at five pre-determined age-points.

Longevity assay (used for experiment 2)

Longevity of each fly was assessed as the number of days it was alive. Each day, we manually looked at each of the flies that were being individually tracked (from experiment 2) until all flies were dead. Complete absence of movement of limbs, abdomen and proboscis was recorded as death.

Statistical Analysis

Locomotor activity assay of experiment 1

Activity was measured for a total of 2 hours and 5 min. We excluded the initial 5 min to eliminate the artifact in activity data caused due to setup. We disregarded the flies which were found dead at the end of the recording. For all the remaining flies, out of the 2 hours of recording, we only considered the first 1 hour of data. We did this to minimize the effect of potential artifacts generated by inactivity due to death and hyperactivity due to starvation (Yu *et al.* 2016). From the data obtained after these corrections, we calculated the average of one hour locomotor activity of 32 flies for each of 16 treatment groups (2 sexes × 8 populations) across 4 age-points.

To test how evolution of greater dispersal affects locomotor activity of dispersal selected population with age, we performed a 2-way repeated measure ANOVA with Selection (VB_{1-4} vs. VBC_{1-4}) and Sex (male vs. female) as fixed factors, and Age (12, 26, 40, and 47) as the repeated measure on mean activity levels calculated (as mentioned above) at each age-point for both sexes of each population. When the interaction effect was significant, post-hoc was performed using Tukey's HSD test.

We further calculated the slope of the average activity between every consecutive time-points for both sexes of each population. To test whether the rate of age-dependent decline of activity changes due to selection for dispersal, we subjected the above calculated slopes to a full-factorial 2-way fixed factor repeated measure ANOVA with Selection (VB vs. VBC) and Sex (male vs. female) as fixed factors, and rate of change of activity (slope) over three time-intervals as the repeated measure.

Locomotor activity assay of experiment 2

Activity was measured for a total of 4 hours and 5 min. For the analysis, we excluded the activity data of the first 5mins and the subsequent 4h data was used to calculate per hour activity. Then this data was subjected to 2-way repeated measure ANOVA with Selection (VB_2 vs. VBC_2) and Sex (male vs. female) as fixed factors, and Age (12, 26, 33, 40, and 47) as the repeated measure to test how evolution of greater dispersal affects locomotor activity of individual flies (*i.e.* here, we measure longitudinal data as opposed to the cross-sectional data obtained from experiment 1) with age.

We further calculated the slope of the activity levels between every consecutive time-points for all the flies that lived at least until the second age-point. To test whether the rate of age-dependent decline of activity changes due to selection for dispersal, we subjected the above calculated slopes to a full-factorial 2-way fixed factor repeated measure ANOVA with

Selection (VB_2 vs. VBC_2) and Sex (male vs. female) as fixed factors, and rate of change of activity (slope) over 4 time-intervals as the repeated measure.

Longevity assay of experiment 2

To check whether there is change in lifespan due to evolution of greater dispersal, we performed a full-factorial 2-way fixed factor ANOVA on longevity data (measured as mention in "Assay Details" section above) with Selection (VB_2 vs. VBC_2) and Sex (male vs. female) as fixed factors.

All the statistical tests were performed using STATISTICA® v5 (StatSoft. Inc., Tulsa, Oklahoma) and graphs are generated using *matplotlib* (Version: 3.5.1) module in Python 3.8.3 (https://www.python.org/).

Results

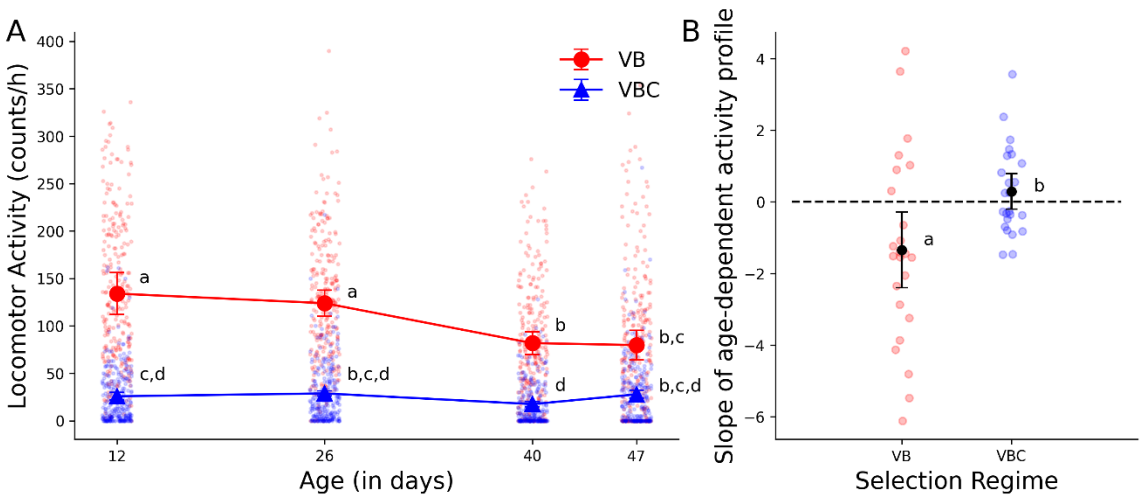


Figure 1. Age-dependent locomotor activity profile from cross-sectional data of Experiment 1. (A) Mean (± 95% CI) locomotor activity of populations from dispersal selected VB_{1-4} (red circle) and its ancestry-matched control VBC_{1-4} (blue triangle) populations are plotted against age, measured in days from the time of egg collection. Activity level of each individual at the tested age-point of VB_{1-4} and VBC_{1-4} populations are represented as red and blue scatter points respectively. Some of the error bars are not visible due to their small size. (B) Mean (± 95% CI) of the slopes of locomotor activity between consecutive age-points for dispersal selected VB_{1-4} and its ancestry-matched control VBC_{1-4} are plotted. The scatter points denote the slopes of the line between each consecutive age-points for VB_{1-4} (red) and VBC_{1-4} (blue) populations. Different lower-case alphabets denote statistically significant differences ($p<0.05$).

Dispersal evolution leads to greater rate of functional senescence

In the cross-sectional study (experiment 1) of locomotor activity across age, we found that activity declines faster in the dispersal selected populations as compared to their ancestry-matched controls (Figure 1, Table S1 and Table S2). This led to significant Age × Selection

interaction in experiment 1 (Figure 1A and Table S3; Age × Selection $F_{3,36} = 7.52$, $p = 0.0005$) and steeper slope (Figure 1B and Table S2; $F_{1,24} = 7.63$, $p = 0.017$). This observation is further validated, when we see that locomotor activity of dispersal selected flies also declines at a greater rate in the longitudinal study i.e., experiment 2 (Figure 2A, Table S4 and Table S6; Age × Selection $F_{4,240} = 12.2$, $p < 0.00001$). This is furthermore clear from the slope values which clearly show steeper decline in activity across age of the dispersal selected flies in experiment 2 (Figure 2B and Table S5; $F_{1,180} = 31.63$, $p = 0.000001$).

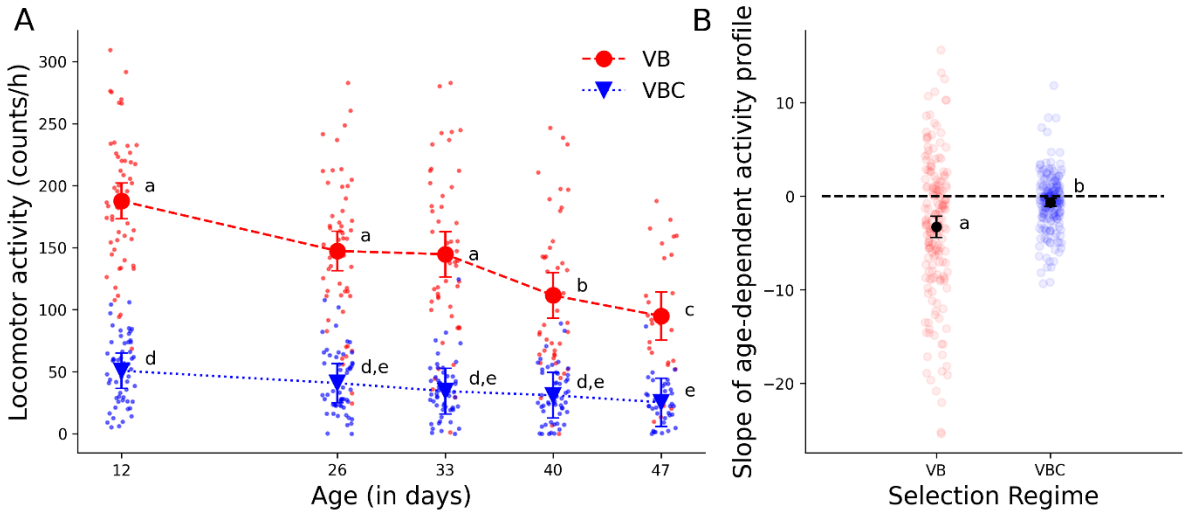


Figure 2. Age-dependent locomotor activity profile from longitudinal data of Experiment 2. (A) Mean (± 95% CI) locomotor activity of individuals from dispersal selected population VB_2 (red circle) and its ancestry-matched control population VBC_2 (blue triangle) are plotted against age, measured in days from the time of egg collection. Activity level of each individual at the tested age-point of VB_2 and VBC_2 are represented as red and blue scatter points respectively. (B) Mean (± 95% CI) of the slopes of locomotor activity of individual flies between consecutive age-points for dispersal selected VB_2 and its ancestry-matched control VBC_2 are plotted. The scatter points denote the slopes of the line for each fly between each consecutive age-points for VB_2 (red) and VBC_2 (blue)

populations. Different lower-case alphabets denote statistically significant differences ($p<0.05$). Some of the error bars are not visible due to their small size.

Despite elevated functional senescence, dispersal selected flies have high locomotor activity across age

We see that despite dispersal evolution causing greater rate of decline in locomotor activity with age (as mentioned above), the locomotor activity of the dispersal selected flies does not drop below the activity level of their ancestry-matched control flies even at late age in both cross-sectional (experiment 1, Figure 1A and Table S3) and longitudinal (experiment 2, Figure 2A and Table S6) studies. A comprehensive table containing mean activity levels and *p* values for experiment 1 and experiment 2 can be found in Table S1, Table S3, Table S4 and Table S6.

Selection for greater dispersal properties trades-off with lifespan

In experiment 2, as we tracked each fly individually, we were able to measure the lifespan of these flies. From this data, we see that the dispersal selected flies live significantly shorter (Figure 3 and Table S7; $F_{1,107} = 4.52$, $p = 0.036$) than their ancestry-matched controls. However, the difference in longevity between the dispersal selected and their ancestry-matched control flies in comparable in both males and females (Selection × Sex $F_{1,107} = 0.76$, $p = 0.4$).

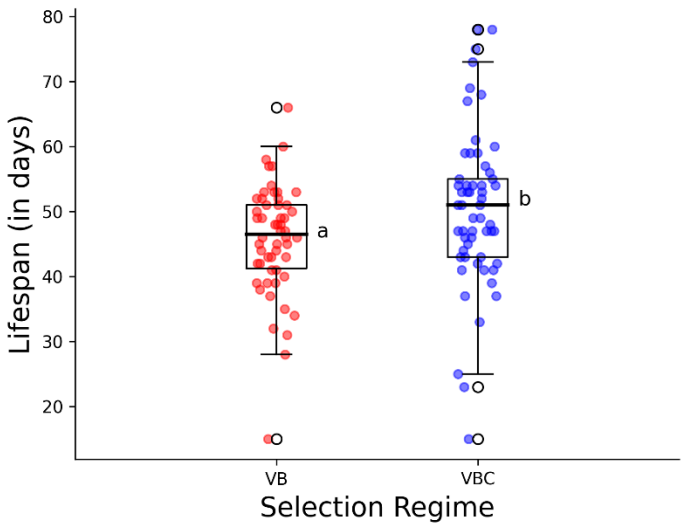


Figure 3. Lifespan of dispersal selected (VB_2) and control (VBC_2) populations. In the box plot, the middle line, lower and upper edge of the box, lower and upper end of the whiskers represent mean, 25th, 75th, 5th, 95th percentile of the longevity data respectively. The scatter points denote longevity of each individual of the dispersal selected VB_2 (red) and VBC_2 (blue) populations. Different lower-case alphabets denote statistically significant differences ($p<0.05$).

Discussion

Active dispersal is one of the primary ways organisms cope with the eminent threat to their survival and proliferation in the context of contemporary rapid climate change and habitat degradation (Berg *et al.* 2010). In the longer timeframe, this provides the premise for evolution of dispersal properties, such as dispersal propensity, ability and speed, in organisms. Previously several research, both theoretically and empirically, have demonstrated that indeed dispersal properties of a species can evolve rather rapidly under such congenial conditions (McPeek & Holt 1992; Hutson *et al.* 2003; Fronhofer *et al.* 2014; Tung *et al.* 2018b). This evolution of greater dispersal properties can lead to a species becoming invasive, as is the case with Cane toads in Australia (Phillips *et al.* 2008; Shine *et al.* 2021) and dwarf spiders in Europe (Narimanov *et al.* 2022), and pose a threat to native ecosystems. In this study, we investigated whether the evolution of greater dispersal at an early stage of life has an impact on the later stages of the organisms. The rationale behind this is that dispersal is an energy intensive process (Bonte *et al.* 2012) and it is known already that at the sub-organismal level, dispersal evolution alters several metabolic pathways involving energy homeostasis (Tung *et al.* 2018a). But how these will manifest themselves at the late life was yet unknown.

In this study, we have used four large (n ~2500) outbred populations of *Drosophila melanogaster*, which were selected for greater dispersal at their early life. In these dispersal-evolved populations, we quantified the status of functional senescence in locomotor activity of both male and female flies, and contrasted the data against their ancestry matched control populations, which haven’t undergone dispersal evolution. Interestingly, we found that dispersal evolution leads to faster decline in locomotor activity with age (Figure 1) indicating that evolving greater dispersal at early life, indeed, has adverse effect in the late life stages

resulting in accelerated functional senescence. Further, in order to demonstrate that the above result is not an artefact of cross-sectional experiment involving population level averages, as a proof-of-principle, we tracked individual flies of one randomly chosen population throughout their lifespan and measured locomotor activity at predetermined age-points. This experiment supported the above insight by yielding the same results (Figure 2).

Moreover, we also found that despite faster age-dependent decline in locomotor activity in dispersal evolved flies, their locomotor activity does not drop below the activity level of their ancestry-matched control flies at any given age (Figure 1A and Figure 2A). It is further noteworthy that this elevated locomotor activity in the dispersal selected flies persisted even in the absence dispersal selection for over nine generations. Together, these imply that the dispersal evolved individuals continue to carry and exhibit corelated traits of dispersal evolution like increased locomotor activity even after they reach their destination, and it can last over multiple generations long after the selection pressure for dispersal ceases. At the mechanistic level, these observations indicate that the metabolic remodelling as a correlated response to dispersal evolution proposed in previous study (Tung *et al.* 2018a) is likely to have become constitutive in the dispersal selected flies leading to persistent elevated expression of locomotor activity in the selected flies throughout their life. Elevated locomotor activity in the more dispersive individuals throughout their life have important ecological implications as well. Prior research has shown that higher locomotor activity promotes mating success due to an elevated courtship behaviour (Kyriacou 1981; Cobb *et al.* 1987) as well as aids females to find suitable oviposition sites (Ferguson *et al.* 2015). Thus, taken together, all else being equal, elevated locomotor activity in the dispersal-selected populations will provide them greater lifelong reproductive output, and thereby, superior colonization success in natural settings.

Furthermore, we observed that faster functional senescence in dispersal-selected flies is accompanied by shorter lifespan (Figure 3). This could potentially be a negative consequence of constitutively elevated expression of locomotor activity in the selected flies throughout their life (Figure 1A and Figure 2A). Although we do not have direct evidence for a mechanistic explanation behind these observations, here we provide three likely mechanisms that could have led to this observation. Firstly, the dispersal-selected flies were reported to have evolved an elevated level of metabolite 3-HK (Tung *et al.* 2018a), which is associated with age-dependent neural degeneration in *Drosophila* (Savvateeva *et al.* 2000) and hence the greater rate of neuromuscular impairment, leading to deterioration of locomotor activity and overall reduced lifespan. Secondly, studies have shown that elevated physical activity can lead to the activation of stress-responsive pathways such as the MAPK and NF-κB pathways (Kramer & Goodyear 2007; Egan & Zierath 2013), which can lead to increased oxidative stress and inflammation. Similarly, the selected files were found to have a greater level of cellular respiration resulting in an elevated level of ATP in these flies (Tung *et al.* 2018a). It is also known that free radicals are produced during ATP production via oxidative phosphorylation in mitochondria (Cadenas & Davies 2000). These responses can ultimately lead to the activation of pro-aging pathways and the observed acceleration of aging-associated declines in physiological function in the selected populations. Thirdly, *Drosophila* insulin-TOR signalling pathways are known to affect cardiac functional aging, and thereby shorten lifespan (Wessells *et al.* 2009). Systemic glucose level is reported to be higher in dispersal-selected flies (Tung *et al.* 2018a), which can lead to relatively greater activation of insulin-TOR signalling pathways in these flies resulting in faster senescence.

While further targeted experiments are necessary to fully understand the underlying mechanisms, this study presents the first empirical evidence that aging evolves as a direct consequence of dispersal evolution, and offers a potential explanation for the previously

observed correlation between higher dispersal rates and decreased longevity in neotropical butterflies (Tufto *et al.* 2012). From the existing literature, we know that often dispersal, particularly in severely patchy habitats, results in populations with small effective population size, which leads to strong random genetic drift in these population (Polechová 2022). In a study with water fleas, *Daphnia magna*, it was shown that genetic drift can drive reduced lifespan and accelerated aging (Lohr *et al.* 2014). However, in a natural setting, it is difficult to dissect out whether reduced lifespan and accelerated senescence is due to dispersal or due to genetic drift caused by habitat fragmentation and/or isolation. Whereas in our study, using large outbred populations we show that, independent of genetic drift route, dispersal evolution can have direct negative impact on the physiological function of organisms at late life and their lifespan.

Moreover, it is worth noting that in a previous study on the same populations (Tung *et al.* 2018b), the difference between the selected and control populations in terms of dispersal ability and propensity was apparent as early as after 10 generations of selection. However, the lifespan of the dispersal-selected and control populations were not significantly different, although there was a trend (Figure 3C in ref Tung *et al.* 2018a). Whereas, the same trade-off became apparent after 153 generations of dispersal selection. This is an excellent demonstration of the fact that the rate of evolution of the correlated traits can differ drastically compared to the traits which are under direct selection. Further, it also highlights that the trait correlations observed in the short-term can change over a longer evolutionary timescale.

Finally, our findings demonstrate that although the trade-off between dispersal and longevity logically indicates a rapid decline of dispersal and associated traits such as locomotor activity upon relaxation of selection for greater dispersal, it may not occur as per the expectations in

all circumstances. Specifically, we observed elevated levels of locomotor activity in our dispersal-evolved flies even after nine generations of relaxation of selection pressure. This apparent discrepancy can be explained by the fact that these flies are maintained in a 15-day discrete-generation lifecycle, and therefore, they do not experience their full lifespan in these laboratory conditions. This suggests that the trade-off may be avoided in natural settings where organisms reproduce rapidly at an early stage of life. This hypothesis can be tested in future research using natural populations. Furthermore, the effect of faster aging and shortened lifespan on other life-history and physiological traits is an important avenue for future research to explore.

In summary, evolutionary history has an impact on age-dependent locomotor activity in fruit flies. The flies that evolved greater dispersal at their early life suffer from steeper age-dependent decline in locomotor activity, and have shorter lifespan. This pattern of trade-off between dispersal and senescence is observed in both male and females. The exact molecular mechanism behind this antagonistic effect (shorter lifespan as a result of selection for dispersal in early life) remains to be discovered. Additionally, we found that a correlated increase in locomotor activity due to dispersal selection does not disappear even after nine generations of removal of selection pressure and the increased locomotor activity remains higher than that of the control populations until later ages. In the current global scenario, where habitat degradation and destruction, and rapid climate change are rampant, species that are better at dispersing have a distinct evolutionary advantage. This highlights the importance of gaining a complete understanding of the interplay between dispersal evolution and senescence in the dispersing individuals. As senescence greatly affects the performance of an organism, such knowledge can inform us about the distribution and interactions of species with their environment, and have far-reaching implications for various eco-evolutionary processes. For example, it can help us predict the success of species in colonizing new areas,

invading new territories, expanding their range, and spreading diseases, as well as the stability of populations with strong dispersal abilities. Thus, this understanding is pertinent and relevant to the research community, especially those working in the fields of evolution, ecology, and conservation.

References

Alex Perkins, T., Phillips, B.L., Baskett, M.L. & Hastings, A. (2013). Evolution of dispersal and life history interact to drive accelerating spread of an invasive species. *Ecol. Lett.*, 16, 1079–1087.

Andrade-Restrepo, M., Champagnat, N. & Ferrière, R. (2019). Local adaptation, dispersal evolution, and the spatial eco-evolutionary dynamics of invasion. *Ecol. Lett.*, 22, 767–777.

Berg, M.P., Kiers, E.T., Driessen, G., Van Der HEIJDEN, M., Kooi, B.W., Kuenen, F., *et al.* (2010). Adapt or disperse: understanding species persistence in a changing world. *Glob. Change Biol.*, 16, 587–598.

Bonte, D. & Dahirel, M. (2017). Dispersal: a central and independent trait in life history. *Oikos*, 126, 472–479.

Bonte, D., Van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M., *et al.* (2012). Costs of dispersal. *Biol. Rev.*, 87, 290–312.

Cadenas, E. & Davies, K.J. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic. Biol. Med.*, 29, 222–230.

Chiu, J.C., Low, K.H., Pike, D.H., Yildirim, E. & Edery, I. (2010). Assaying Locomotor Activity to Study Circadian Rhythms and Sleep Parameters in Drosophila. *JoVE J. Vis. Exp.*, e2157.

Cobb, M., Connolly, K. & Burnet, B. (1987). The relationship between locomotor activity and courtship in the melanogaster species sub-group of Drosophila. *Anim. Behav.*, 35, 705–713.

Dean, R.L., Scozzafava, J., Goas, J.A., Regan, B., Beer, B. & Bartus, R.T. (1981). Age-related differences in behavior across the life span of the C57BL/6J mouse. *Exp. Aging Res.*, 7, 427–451.

Deyo, R.A., Straube, K.T., Moyer, J.R. & Disterhoft, J.F. (1989). Nimodipine ameliorates aging-related changes in open-field behaviors of the rabbit. *Exp. Aging Res.*, 15, 169–175.

van Dijken, F.R., van Sambeek, M.P.J.W. & Scharloo, W. (1979). Divergent selection on locomotor activity in Drosophila melanogaster. III. Genetic analysis. *Behav. Genet.*, 9, 563–570.

Egan, B. & Zierath, J.R. (2013). Exercise Metabolism and the Molecular Regulation of Skeletal Muscle Adaptation. *Cell Metab.*, 17, 162–184.

Emborg, M.E., Ma, S.Y., Mufson, E.J., Levey, A.I., Taylor, M.D., Brown, W.D., *et al.* (1998). Age-related declines in nigral neuronal function correlate with motor impairments in rhesus monkeys. *J. Comp. Neurol.*, 401, 253–265.

Ferguson, C.T.J., O'Neill, T.L., Audsley, N. & Isaac, R.E. (2015). The sexually dimorphic behaviour of adult Drosophila suzukii: elevated female locomotor activity and loss of siesta is a post-mating response. *J. Exp. Biol.*, 218, 3855–3861.

Fernández, J.R., Grant, M.D., Tulli, N.M., Karkowski, L.M. & McClearn, G.E. (1999). Differences in locomotor activity across the lifespan of Drosophila melanogaster☆. *Exp. Gerontol.*, 34, 621–631.

Fjerdingstad, E.J., Schtickzelle, N., Manhes, P., Gutierrez, A. & Clobert, J. (2007). Evolution of dispersal and life history strategies – Tetrahymena ciliates. *BMC Evol. Biol.*, 7, 133.

Fronhofer, E.A., Stelz, J.M., Lutz, E., Poethke, H.J. & Bonte, D. (2014). SPATIALLY CORRELATED EXTINCTIONS SELECT FOR LESS EMIGRATION BUT LARGER DISPERSAL DISTANCES IN THE SPIDER MITE TETRANYCHUS URTICAE. *Evolution*, 68, 1838–1844.

Garant, D., Forde, S.E. & Hendry, A.P. (2007). The Multifarious Effects of Dispersal and Gene Flow on Contemporary Adaptation. *Funct. Ecol.*, 21, 434–443.

Goodrick, C.L. (1971). Free Exploration and Adaptation Within an Open Field as a Function of Trials and Between-Trial-Interval for Mature-Young, Mature-Old, and Senescent Wistar Rats. *J. Gerontol.*, 26, 58–62.

Greenwood, P.J. & Harvey, P.H. (1982). The Natal and Breeding Dispersal of Birds. *Annu. Rev. Ecol. Syst.*, 13, 1–21.

Grotewiel, M.S., Martin, I., Bhandari, P. & Cook-Wiens, E. (2005). Functional senescence in Drosophila melanogaster. *Ageing Res. Rev.*, 4, 372–397.

Hargreaves, A.L. & Eckert, C.G. (2014). Evolution of dispersal and mating systems along geographic gradients: implications for shifting ranges. *Funct. Ecol.*, 28, 5–21.

Huang, F., Peng, S., Chen, B., Liao, H., Huang, Q., Lin, Z., *et al.* (2015). Rapid evolution of dispersal-related traits during range expansion of an invasive vine Mikania micrantha. *Oikos*, 124, 1023–1030.

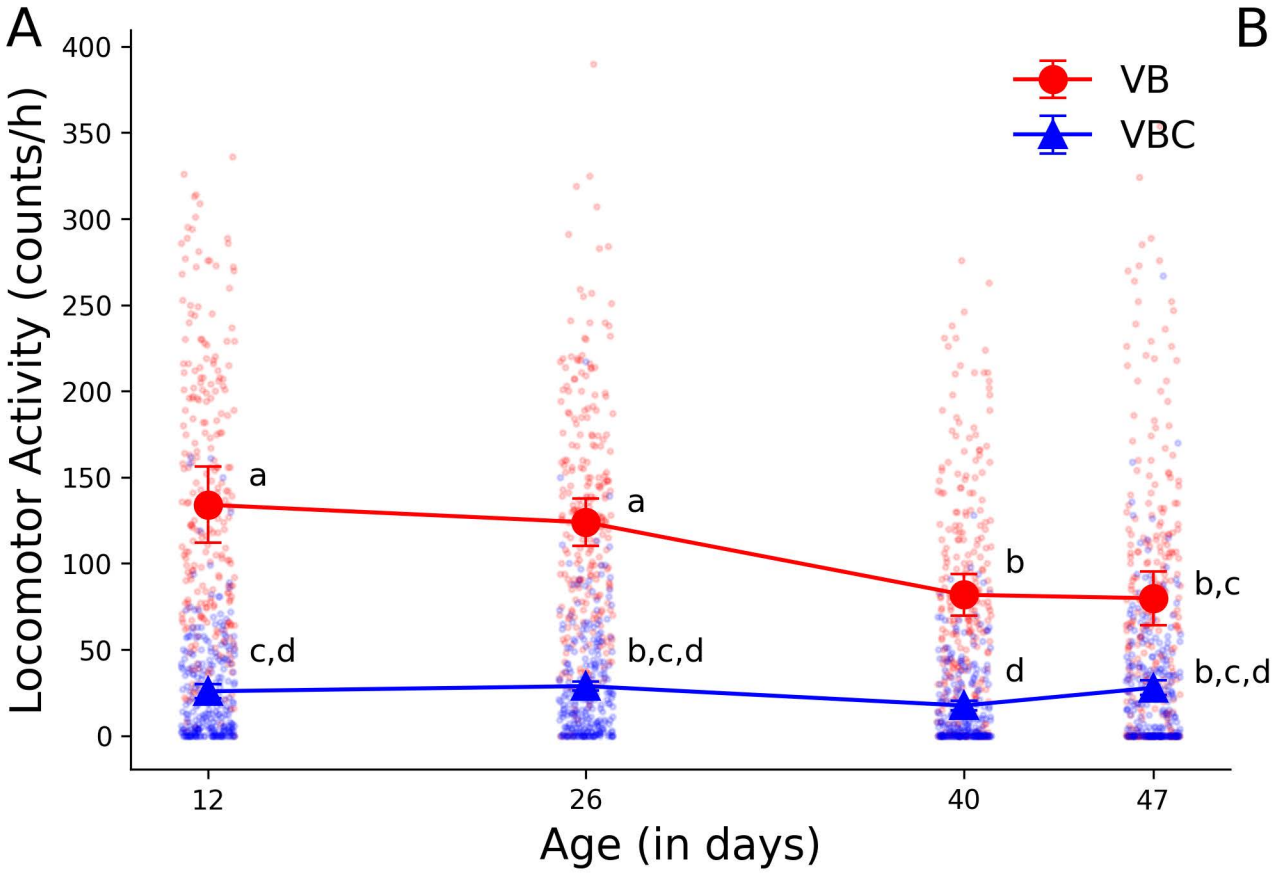
Hutson, V., Martinez, S., Mischaikow, K. & Vickers, G.T. (2003). The evolution of dispersal. *J. Math. Biol.*, 47, 483–517.

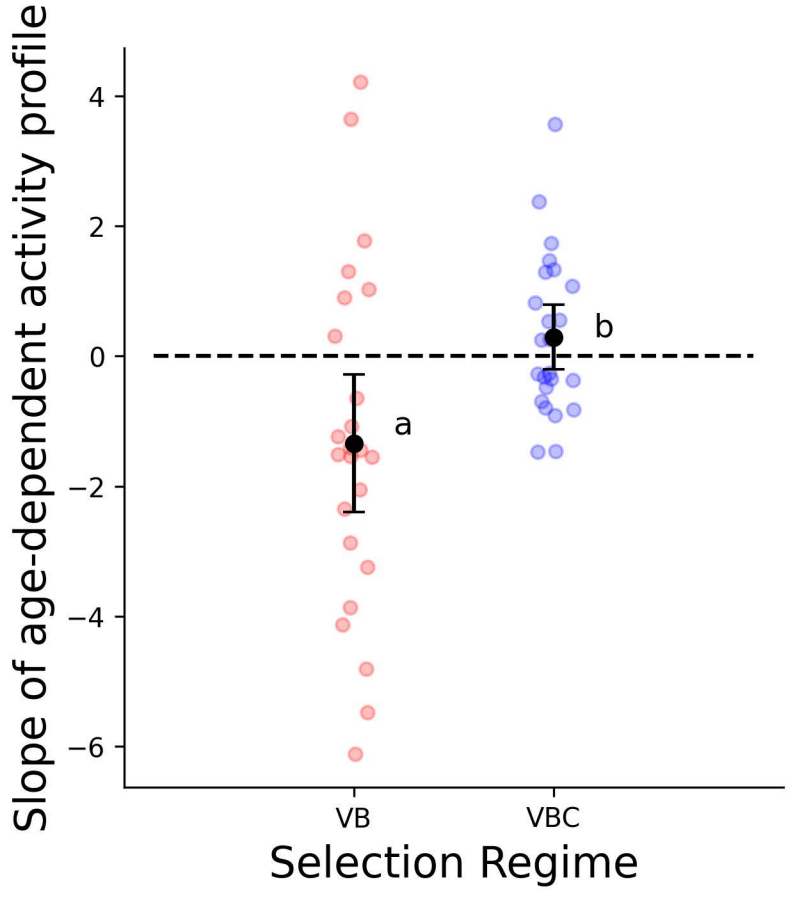
Kramer, H.F. & Goodyear, L.J. (2007). Exercise, MAPK, and NF-κB signaling in skeletal muscle. *J. Appl. Physiol.*, 103, 388–395.

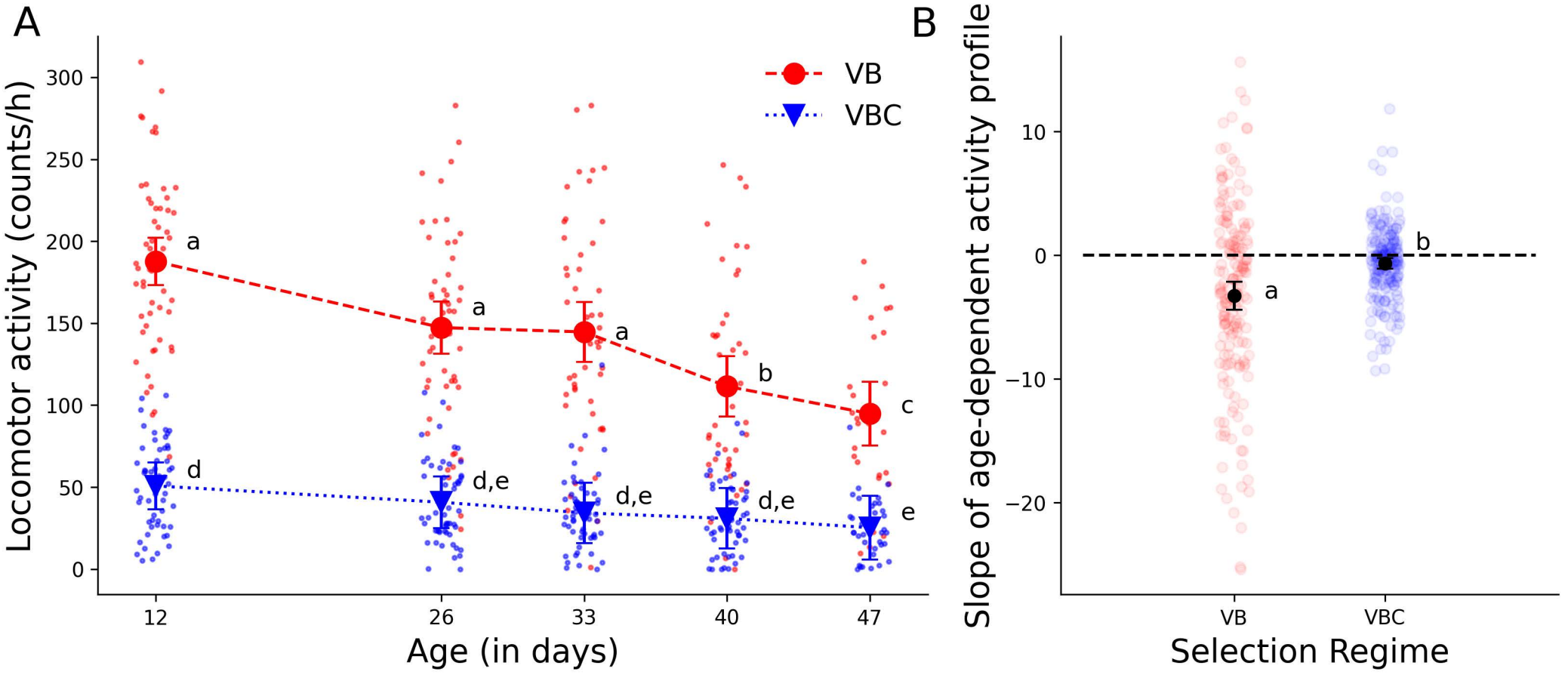
Kyriacou, C.P. (1981). The relationship between locomotor activity and sexual behaviour in ebony strains of Drosophila melanogaster. *Anim. Behav.*, 29, 462–471.

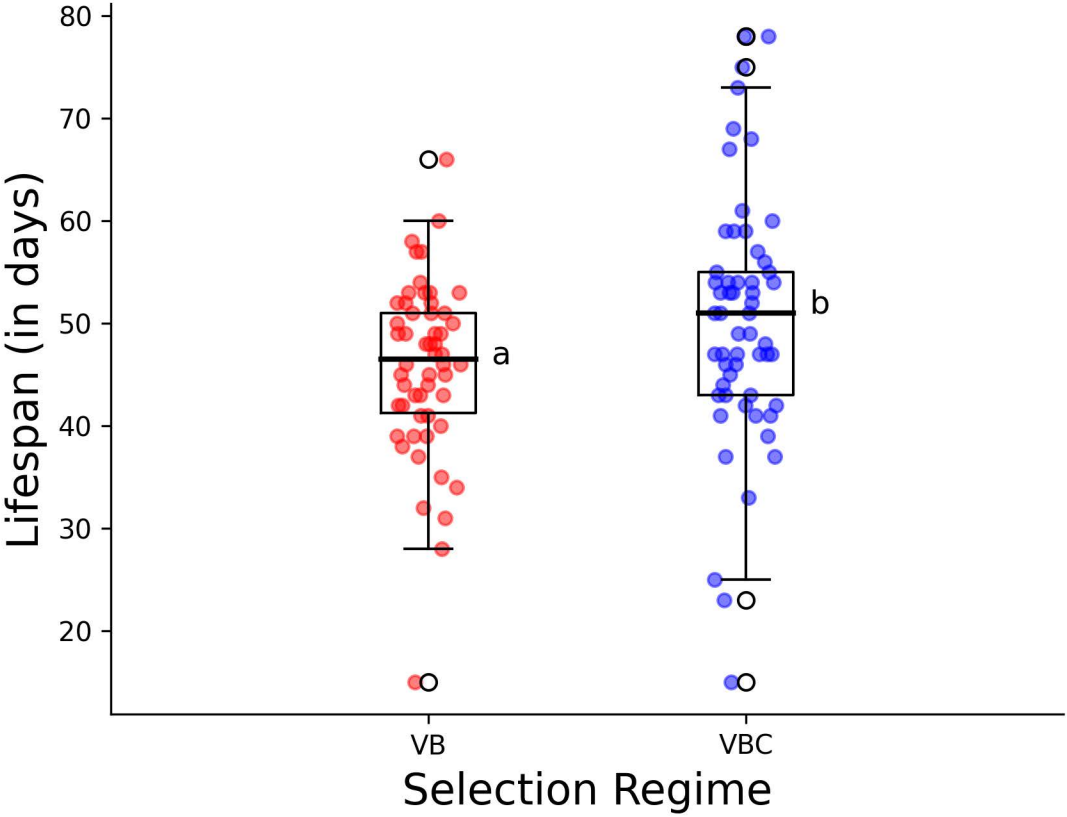
Lambrinos, J.G. (2004). How Interactions Between Ecology and Evolution Influence Contemporary Invasion Dynamics. *Ecology*, 85, 2061–2070.

Le Bourg, E. (1987). The rate of living theory. Spontaneous locomotor activity, aging and longevity in Drosophila melanogaster. *Exp. Gerontol.*, 22, 359–369.
Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends Ecol. Evol.*, 17, 183–189.
Levine, M.S., Lloyd, R.L., Fisher, R.S., Hull, C.D. & Buchwald, N.A. (1987). Sensory, motor and cognitive alterations in aged cats. *Neurobiol. Aging*, 8, 253–263.
Lohr, J.N., David, P. & Haag, C.R. (2014). Reduced lifespan and increased ageing driven by genetic drift in small populations: low genetic diversity reduces lifespan. *Evolution*, 68, 2494–2508.
McGhee, K.E., Barbosa, A.J., Bissell, K., Darby, N.A. & Foshee, S. (2021). Maternal stress during pregnancy affects activity, exploration and potential dispersal of daughters in an invasive fish. *Anim. Behav.*, 171, 41–50.
McGibbon, C.A. & Krebs, D.E. (2004). Discriminating age and disability effects in locomotion: neuromuscular adaptations in musculoskeletal pathology. *J. Appl. Physiol.*, 96, 149–160.
McPeek, M.A. & Holt, R.D. (1992). The Evolution of Dispersal in Spatially and Temporally Varying Environments. *Am. Nat.*, 140, 1010–1027.
Michelangeli, M., Smith, C.R., Wong, B.B.M. & Chapple, D.G. (2017). Aggression mediates dispersal tendency in an invasive lizard. *Anim. Behav.*, 133, 29–34.
Miyamoto, M., Kiyota, Y., Yamazaki, N., Nagaoka, A., Matsuo, T., Nagawa, Y., *et al.* (1986). Age-related changes in learning and memory in the senescence-accelerated mouse (SAM). *Physiol. Behav.*, 38, 399–406.
Nagahara, A.H. & Handa, R.J. (1997). Age-related changes in c-fos mRNA induction after open-field exposure in the rat brain. *Neurobiol. Aging*, 18, 45–55.
Narimanov, N., Bauer, T., Bonte, D., Fahse, L. & Entling, M.H. (2022). Accelerated invasion through the evolution of dispersal behaviour. *Glob. Ecol. Biogeogr.*, 31, 2423–2436.
Ochocki, B.M. & Miller, T.E.X. (2017). Rapid evolution of dispersal ability makes biological invasions faster and more variable. *Nat. Commun.*, 8, 14315.
Phillips, B.L., Brown, G.P., Travis, J.M.J., Shine, R. & Price, S.E.T.D. (2008). Reid's Paradox Revisited: The Evolution of Dispersal Kernels during Range Expansion. *Am. Nat.*, 172, S34–S48.
Polechová, J. (2022). The costs and benefits of dispersal in small populations. *Philos. Trans. R. Soc. B Biol. Sci.*, 377, 20210011.
Renault, D., Laparie, M., McCauley, S.J. & Bonte, D. (2018). Environmental Adaptations, Ecological Filtering, and Dispersal Central to Insect Invasions. *Annu. Rev. Entomol.*, 63, 345–368.
Savvateeva, E., Popov, A., Kamyshev, N., Bragina, J., Heisenberg, M., Senitz, D., *et al.* (2000). Age-dependent memory loss, synaptic pathology and altered brain plasticity in the Drosophila mutant cardinal accumulating 3-hydroxykynurenine. *J. Neural Transm.*, 107, 581–601.
Searcy, C.A., Gilbert, B., Krkošek, M., Rowe, L. & McCauley, S.J. (2018). Positive correlation between dispersal and body size in Green Frogs (Rana clamitans) naturally colonizing an experimental landscape. *Can. J. Zool.*, 96, 1378–1384.
Sheeba, V. & Joshi, A. (1998). A test of simple models of population growth using data from very small populations of Drosophila melanogaster. *Curr. Sci.*, 75, 1406–1410.
Shine, R., Alford, R.A., Blennerhasset, R., Brown, G.P., DeVore, J.L., Ducatez, S., *et al.* (2021). Increased rates of dispersal of free-ranging cane toads (Rhinella marina) during their global invasion. *Sci. Rep.*, 11, 23574.
Studds, C.E., Kyser, T.K. & Marra, P.P. (2008). Natal dispersal driven by environmental conditions interacting across the annual cycle of a migratory songbird. *Proc. Natl. Acad. Sci.*, 105, 2929–2933.
Tufto, J., Lande, R., Ringsby, T.-H., Engen, S., Sæther, B.-E., Walla, T.R., *et al.* (2012). Estimating Brownian motion dispersal rate, longevity and population density from spatially explicit mark–recapture data on tropical butterflies. *J. Anim. Ecol.*, 81, 756–769.
Tung, S., Mishra, A., Gogna, N., Aamir Sadiq, M., Shreenidhi, P.M., Shree Sruti, V.R., *et al.* (2018a). Evolution of dispersal syndrome and its corresponding metabolomic changes. *Evolution*, 72, 1890–1903.
Tung, S., Mishra, A., Shreenidhi, P.M., Sadiq, M.A., Joshi, S., Sruti, V.R.S., *et al.* (2018b). Simultaneous evolution of multiple dispersal components and kernel. *Oikos*, 127, 34–44.
Wahlström, L.K. (1994). The significance of male-male aggression for yearling dispersal in roe deer (Capreolus capreolus). *Behav. Ecol. Sociobiol.*, 35, 409–412.
Wessells, R., Fitzgerald, E., Piazza, N., Ocorr, K., Morley, S., Davies, C., *et al.* (2009). d4eBP acts downstream of both dTOR and dFoxo to modulate cardiac functional aging in Drosophila. *Aging Cell*, 8, 542–552.
Wu, N.C. & Seebacher, F. (2022). Physiology can predict animal activity, exploration, and dispersal. *Commun. Biol.*, 5, 1–11.
Yu, Y., Huang, R., Ye, J., Zhang, V., Wu, C., Cheng, G., *et al.* (2016). Regulation of starvation-induced hyperactivity by insulin and glucagon signaling in adult Drosophila. *eLife*, 5, e15693.


A
Locomotor Activity (counts/h)
400
350
300
250
200
150
100
50
0
VB
VBC
a
a
b
b,c
c,d
b,c,d
d
b,c,d
12
26
40
47
Age (in days)


B
Slope of age-dependent activity profile
4
2
0
−2
−4
−6
a
b
VB
VBC
Selection Regime


A
B
VB
VBC
Locomotor activity (counts/h)
Age (in days)
Slope of age-dependent activity profile
Selection Regime
300
250
200
150
100
50
0
12
26
33
40
47
10
0
−10
−20
a
a
a
b
c
d
d,e
d,e
d,e
e
a
b


Lifespan (in days)
80
70
60
50
40
30
20
a
b
VB
VBC
Selection Regime