Investigating metabolite levels and stress responses of *Drosophila melanogaster* populations selected for increased dispersal

A thesis submitted to **Indian Institute of Science Education and Research Pune** in partial fulfilment of the requirements for the **BS-MS Dual Degree Programme**

by

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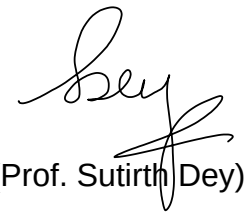
Certificate

This is to certify that this dissertation entitled “Investigating metabolite levels and stress responses of *Drosophila melanogaster* populations selected for increased dispersal” towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Krishna Chaitanya Sattaru at Indian Institute of Science Education and Research under the supervision of Prof. Sutirth Dey, Professor, Department of Biology, during the academic year 2022-2023.



(Prof. Sutirth Dey)

Committee:

Prof. Sutirth Dey

Dr. Anand Krishnan

This thesis is

dedicated to

My family

Father (Bayyan Naidu),

Mother (Madhavi)

&

Sister (Susmitha)

Declaration

I hereby declare that the matter embodied in the report entitled “Investigating metabolite levels and stress responses of *Drosophila melanogaster* populations selected for increased dispersal” are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Education and Research, Pune, under the supervision of Prof. Sutirth Dey and the same has not been submitted elsewhere for any other degree

(Krishna Chaitanya Sattaru)

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Abstract

Dispersal is an energy-intensive process. Therefore, selection for greater dispersal might lead to changes in the body composition, resulting in altered stress responses of the organisms. However, it is not known how dispersal selection can alter the storage of metabolites like protein, lipid, and glycogen across age. In this study, we investigated how the levels of lipid, protein, and glycogen change across age and how that correlates with stress responses in *D. melanogaster* populations selected for increased dispersal. We found that dispersal selection at an early age led to an increase in the glycogen and triglyceride levels of the organism at later ages. However, there were no discernible patterns in the variation of protein content across age. We also found that increased dispersal increased the chance of survival after a heat shock and lowered the desiccation resistance of the organism in both males and females. Increased dispersal also decreased the starvation resistance and increased the chill coma recovery time in males; however, the patterns were not so clear for the females. We also found that dispersal selected populations show a sexual dimorphism for survival after a cold shock.

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Contributions

Contributor name	Contributor role
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Krishna Chaitanya Sattaru, Anish Koner	Software
Krishna Chaitanya Sattaru, Anish Koner	Validation
Krishna Chaitanya Sattaru, Anish Koner	Formal analysis
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Krishna Chaitanya Sattaru, Prof. Sutirth Dey, Anish Koner, Vibishan B, Akshay Malwade	Visualization
Prof. Sutirth Dey	Supervision
Anish Koner	Project administration
Prof. Sutirth Dey	Funding acquisition

This contributor syntax is based on the Journal of Cell Science CRediT Taxonomy[1].

[1] https://journals.biologists.com/jcs/pages/author-contributions

Chapter 1 Introduction

Dispersal can be defined as “any movement of individuals or propagules with potential consequences for gene flow across space” (Ronce, 2007). Many organisms enhance their chances of survival by tracking environmental conditions favorable to them through dispersal (Travis *et al.*, 2013). Dispersal might benefit organisms when faced with unfavorable situations such as lower quality and quantity of resources (Mathieu *et al.*, 2010). It could also help to reduce overcrowding and kin competition (Clobert, 2012). It would also help increase genetic diversity by reducing the effect of inbreeding and facilitating gene flow (Ronce, 2007; Clobert, 2012). Dispersal can also help reduce local extinctions of small populations because of genetic drift (Brown and Kodric-Brown, 1977). At the same time, dispersal might also be disadvantageous to the organism as there is no guarantee that the location that the organisms are dispersing towards will have better resources. They might also risk increased predation or competition during dispersal through an unhospitable landscape.

There have been rapid changes in the climate in the past few decades because of anthropogenic effects such as global warming. Since dispersal is most often the first strategy adopted by any organism when faced with such stressful situations (Bell and Gonzalez, 2011), it is likely that many natural populations are under severe selection pressure for dispersal. That is the reason why the causes and effects of dispersal evolution have been investigated thoroughly in ecology and evolution for the last two decades (reviewed in J Clobert et al., 2001; Clobert, 2012)

The results of selection for dispersal have been equivocal: some studies have shown that selection can lead to dispersal evolution (Fronhofer *et al.*, 2014; Ochocki and Miller, 2017; Tung *et al.*, 2018b), while other studies have failed to get any evolutionary response to selection (Bitume *et al.*, 2011; Tien *et al.*, 2011). Some authors have studied dispersal using correlated traits such as wing polymorphism (Denno, 1994), while others have only looked at a single aspect of dispersal, such as propensity (Yano and Takafuji, 2002). However, most of these studies have been short-term (5-10

generations) and used small population sizes (thus leading to a lot of genetic drift) (although see Tung *et al.*, 2018b). Therefore, to better understand the effects of dispersal evolution, one would need to study populations of larger sizes that have undergone selection for a longer time.

To study the evolution of dispersal in organisms, we have selected outbred populations of *Drosophila melanogaster* for higher dispersal propensity and ability in early life (Tung *et al.*, 2018b). As a correlated response, we have seen an increase in the locomotor activity of the selected populations (Tung *et al.*, 2018a). Selected populations were also observed to be more aggressive as compared to the controls (Tung *et al.*, 2018a). Similar results were also seen in wild populations of Trinidad killifish (*Rivulus hartii*) (Fraser *et al.*, 2001). We have also observed that the selected populations tended to be more explorative as compared to the controls (Tung *et al.*, 2018a). This positive correlation of dispersal with explorative behavior was also observed in wild populations of great tits (Dingemanse *et al.*, 2003). We have also seen that the effects of increased dispersal are not limited to the age at which selection acts upon them, as the selected populations had shown an increase in their locomotor activity across age as compared to the control group (manuscript under preparation). The selected populations also had lower fecundity and longevity than the control populations; however, these trade-offs were not visible when assayed previously after 67 generations of selection, rather they only became apparent after 166 generations of selection (Tung *et al.*, 2018a; manuscript under preparation). These results suggest that their energetic budget might have started to become a limiting factor under the notion that dispersal is an energy-intensive process.

When resources available to an organism are limited, increased dispersal will likely require more resources to be allocated towards dispersal-related traits, thus leading to a change in the body composition of the organism. Changes in the body composition could also lead to changes in the metabolic profile and metabolic rate of the organism. Indeed, we have seen an increase in the respiration and cellular respiration rate in dispersal selected populations, suggesting an increase in their metabolic rates (Tung *et al.*, 2018a; manuscript under preparation). This could, in principle, change the levels of

various macromolecules like glycogen and lipid in the body of the dispersal-selected populations, which in turn could affect their responses to various stresses. For example, in *Drosophila melanogaster*, desiccation, and starvation resistance are known to be positively correlated with glycogen and lipid content, respectively (Graves *et al.*, 1992). This correlation between starvation resistance and lipid content was also seen in a study that compared 43 independent outbred populations selected for different stresses (Djawdan *et al.*, 1998). However, this correlation did not appear when starvation resistance and lipid content were compared in isofemale strains of *Drosophila melanogaster* derived from wild flies collected in temperate Tasmania and tropical northern Queensland (although a positive correlation between lipid content and cold resistance was noted) (Hoffmann *et al.*, 2001). Triglyceride content is also known to be positively correlated with heat resistance in both laboratory and field populations of *Corythucha ciliata* (Ju *et al.*, 2014). However, the effect of selection for increased dispersal on levels of macromolecules such as glycogen, triglycerides, and proteins has not been studied previously. Understanding how the macromolecular profile of the organism can change across age would also help us understand the various trade-offs at different ages that might appear because of increased dispersal.

In the current study, I have looked at how the metabolic profile of the populations selected for increased dispersal has varied across age in comparison with their controls. I have looked at the variations in the whole-body protein, glycogen, and triglyceride content across age in these populations. I have also looked at how selection for increased dispersal has affected their response to various stresses compared to their controls. I have looked at desiccation and starvation resistance, chill coma recovery, cold shock, and heat shock survival of these populations.

Chapter 2 Materials and Methods

2.1. Stock populations:

The populations used for the experiments are derived from four large laboratory-bred populations of *Drosophila melanogaster* (DB_{1-4}), whose ancestry can be traced back to the wild flies (IV lines) caught at South Amherst, MA, USA by Ives in 1970 (Ives, 1970). From each DB_i, two populations, VB_i (selected for higher dispersal propensity and ability in early life) and VBC_i (respective control population with no selection pressure), were derived. Each VB_i and VBC_i are considered as a single block (Block-i) because of their common ancestry to DB_i (Tung *et al.*, 2018b).

2.2. Maintenance Regime:

In each generation, VB_{1-4} are subjected to selection for higher dispersal propensity and ability on the 12th-day post egg collection. On the day of the selection, the adults are transferred into a source, connected to a sink through a pipe of a certain length (dispersal distance). To prevent the flies from experiencing any desiccation stress during this process, the sink is provided with wet cotton. VBCs are also provided with damp cotton after three hours or when 25 % of VBs reach the sink, whichever happens earlier, to maintain similar conditions for VBs and VBCs. The individuals that reach the sink by the end of six hours or the first 50 % to reach the sink, whichever happens earlier, are selected and transferred into plexiglass cages (25 cm x 20 cm x 15 cm). VBC_{1-4} are transferred into the cage without any selection pressure. Each cage is maintained at a population density of ~2400 individuals and is provided with banana-jaggery food. Each population is provided with banana-jaggery food supplemented with live yeast on the thirteenth-day post egg collection to boost fecundity. On the night of the following day, each population is provided with a banana-jaggery medium whose edges are cut to provide a vertical substrate for laying eggs. The eggs are collected into bottles (160 mm Dia x 140 mm Ht Laxbro autoclavable plastic bottles) containing ~50 ml of banana jaggery food after 12 hours to form the next generation. Each bottle is maintained at a density of 350-400 eggs. All the populations are maintained under uniform lighting conditions at 25 ^{O}C. We initially started with a dispersal distance of 2 m,

and the dispersal distance has been gradually increasing across generations. VB_{1-4} were selected for higher dispersal propensity and ability for 177 generations, and the dispersal distance had been increased to 70 m by the time the last assay was performed. More details about the maintenance regime and the selection protocol can be found at (Tung *et al.*, 2018a).

All populations that are part of a single block were always assayed together. All four blocks were relaxed under similar conditions for one generation without any selection pressure before performing the assay to avoid non-genetic parental effects.

2.3. Biochemical Assays:

Because of logistical constraints, the biochemical assays were only performed for a single block. We have used Block-4 for this study since the same was used previously when an untargeted metabolomics study was done on these populations so that the results can be compared if needed. From each relaxed population, 350-400 eggs were collected in a bottle containing 50 ml of banana jaggery food, and egg collection was performed for eighteen such bottles. The flies eclosing from these eggs were then transferred into plexiglass cages on the 12th-day post egg collection, making it three cages per population. For every experiment, flies were collected randomly from all three cages to avoid any bias.

On the 15th-day post egg collection, flies were collected from the cages and sorted by sex under CO_2. The sorted flies were transferred into 2 ml microcentrifuge tubes and flash-frozen with Liquid Nitrogen. They were then stored at -80 ^oC until the day of the assay. This process was repeated at four different time points, with each time point being seven days apart from the previous one, and considering day 15 post egg collection as the first time point. VB_4 was selected for higher dispersal propensity and ability for 170 generations, and the dispersal distance has been increased to 68m by the time of the assays.

2.3.1. Dry Bodyweight:

At a given time point, for each population and sex, we had five replicates of 20 flies each for measuring the dry body weight. The flies were dried at 60 ^{o}C for 72 Hrs to remove any water content in them. Their dry body weight was then measured by using a precision weighing balance (Mettler Toledo ME104; d = 0.1 mg). This was repeated for all four time points. These data were then used for normalizing the biochemical content of the samples before comparison across populations and time points.

2.3.2. Protein estimation (Bradford Assay):

Bradford reagent from Sigma Aldrich (B6916-500ML) was used for this assay. In a basic medium, the Coomassie brilliant blue G-250 dye (Bradford reagent) forms a protein-dye complex in the presence of proteins, changing its wavelength of maximum absorbance from 470 nm to 595 nm. This will make the absorbance of the sample at 595 nm to be proportional to the amount of protein present in the sample.

Bovine serum albumin (HiMedia MB083-5G) solution of concentrations 1000 µg/ml, 500 µg/ml, and 250 µg/ml were prepared by serial dilutions with MilliQ water to get the absorbance values for the standard curve. MilliQ water was used as a blank. For samples, we had ten replicates per sex from each population. For males, each replicate contained 5 flies, whereas for females, each replicate contained 3 flies. These flies were then dried at 30 ^{o}C for 25 Hrs. Each sample was then homogenized in 360 µl of lysis buffer (100mM KH2PO4, 1mM EDTA, 1mM DTT, pH= 7.4) in a 2ml microcentrifuge tube using a micro pestle. The samples were then centrifuged at 2000 rpm at 4 ^{o}C for 13 min, and the supernatant was collected.

5 µl of each standard was added to a 96-well plate. For each sample, 5 µl of supernatant was added to the 96-well plate. 250 µl of Bradford reagent was added to the standards and the samples. The plate was then incubated at room temperature, protected from light for 15-20 mins using aluminum foil. After the incubation, the absorbance of the standards and samples was measured at 595 nm.

The absorbance values of the standards and samples were standardized by subtracting the absorbance value of the blank from them. A standard curve of absorbance vs. protein concentration was plotted using the corrected absorbance values of the standards. Using the standard curve, the whole-body protein content of each of the samples was calculated from their corrected absorbance values. These values were then normalized with the mean dry body weight of the respective population and sex.

2.3.3. Glycogen estimation:

Glycogen Assay Kit by Sigma Aldrich (MAK016) was used for this assay, and the assays were carried out according to the manufacturer's specifications. The kit works by breaking down the glycogen into glucose using a hydrolysis enzyme mix. The amount of glucose present in the sample is then measured using a coupled enzymatic reaction which gives a colorimetric product with its absorbance proportional to the amount of glucose present in the sample.

The 2 mg/ml glycogen standard solution provided in the kit was diluted with the hydrolysis buffer to concentrations of 0.008 mg/ml, 0.016 mg/ml, 0.024 mg/ml, 0.032 mg/ml, and 0.04 mg/ml to get the absorbance values for the standard curve. The hydrolysis buffer was used as a blank. For samples, we had ten replicates per sex from each population at the first time point, whereas we had eight replicates per sex from each population at the later time points. For both sexes, each replicate contained 3 flies. Each sample was homogenized in 1000 µl of MilliQ water in a 1.5 ml microcentrifuge tube using a micro pestle. The samples were then incubated at 100 ^{o}C on a thermomixer for 5 min without shaking to inactivate the enzymes and centrifuged at 10000 rpm at 4 ^{o}C for 5 min, and the supernatant was collected.

25 µl of each standard was added to a 96-well plate. For each sample, 5 µl of supernatant was added to the 96-well plate. The sample volumes were then adjusted to 25 µl using the hydrolysis buffer. 1 µl of hydrolysis enzyme mix was added to the standards and the samples. This was followed by an incubation period of 30 min at room temperature to convert the glycogen into glucose. For each sample, a sample blank was also set up in which hydrolysis enzyme mix is not added to account for the

already existing glucose molecules in the samples. 25 µl of Master Mix (A mixture of development buffer, development enzyme mix, and fluorescent peroxidase substrate in a 23:1:1 ratio) was then added to the standards, samples, and sample blanks. The plate was then incubated at room temperature protected from light for 30 mins. After the incubation, the absorbance of the standards and samples was measured at 570 nm.

The absorbance values of the standards were standardized by subtracting the absorbance value of the blank from them. A standard curve of absorbance vs. glycogen concentration was plotted using the corrected absorbance values of the standards. The absorbance values of the samples were standardized by subtracting the absorbance value of the corresponding sample blank from them. Using the standard curve, the whole-body glycogen content of each of the samples was calculated from their corrected absorbance values. These values were then normalized with the mean dry body weight of the respective population and sex.

2.3.4. Triglyceride estimation:

Adipogenesis Assay Kit by Sigma Aldrich (MAK040) was used for this assay, and the assays were carried out according to the manufacturer's specifications. The kit works by converting triglycerides into glycerol and fatty acids using lipase. The amount of glycerol in the sample is then measured using a coupled enzymatic reaction which gives a colorimetric product with its absorbance proportional to the amount of triglycerides present in the sample.

The 1 mM triglyceride standard solution provided in the kit was diluted with the assay buffer to concentrations of 0.04 mM, 0.08 mM, 0.12 mM, 0.16 mM, and 0.2 mM to get the absorbance values for the standard curve. The assay buffer was used as a blank. For samples, we had ten replicates per sex from each population. For males, each replicate contained 5 flies, whereas for females, each replicate contained 3 flies. Each sample was homogenized in 1000 µl of MilliQ water in a 1.5 ml microcentrifuge tube using a micro pestle. 100 µl of lipid extraction buffer was then added to each sample. The samples were then incubated at 95 ^{o}C on a thermomixer at 300 rpm for 30 min and centrifuged at 13500 rpm at 4 ^{o}C for 15 min, and the supernatant was collected.

25 µl of each standard was added to a 96-well plate. For males, 15 µl of supernatant was added to the 96-well plate, whereas for females, 10 µl of supernatant was added to the 96-well plate. The sample volumes were then adjusted to 25 µl using the assay buffer. 1 µl of lipase was then added to the standards and the samples. This was followed by an incubation period of 10 min at room temperature to convert the triglycerides to glycerol and fatty acids. 25 µl of Master Mix (A mixture of assay buffer, probe, and enzyme mix in a 23:1:1 ratio) was then added to the standards, and the samples and the plate was incubated at 37 ^{o}C protected from light for 30 mins using aluminum foil. After the incubation, the absorbance of the standards and samples was measured at 570 nm.

The absorbance values of the standards and samples were standardized by subtracting the absorbance value of the blank from them. A standard curve of absorbance vs. triglyceride concentration was plotted using the corrected absorbance values of the standards. Using the standard curve, the whole-body triglyceride content of each of the samples was calculated from their corrected absorbance values. These values were then normalized with the mean dry body weight of the respective population and sex.

2.4. Stress Response Assays:

From each relaxed population, ~50 eggs were collected in a plastic vial (Laxbro Culture tube Cat No. BT-30PS) containing 6 ml of banana jaggery food, and egg collection was performed for 40 such vials. The flies eclosing from these eggs were used for the experiments. The flies were transferred to vials containing fresh food on Day-12 post egg collection. For the setup of every assay, flies were pooled together from multiple vials for each population. For all the assays, flies were transferred via aspiration and without CO_2 to avoid any stress due to CO_2.

Because of logistical constraints, each block was assayed separately. Block-1, Block-2, Block-3, and Block-4 were assayed after 174, 175, 176, and 177 generations of selection, respectively.

2.4.1. Desiccation Resistance:

On Day-12 post egg collection, groups of 10 flies were transferred into 10 empty plastic vials for each population and sex. Survival was checked every two hours until all the flies were dead.

2.4.2. Starvation Resistance:

On Day-12 post egg collection, groups of 10 flies were transferred into 10 plastic vials for each population and sex. Each plastic vial was provided with ~4 ml of 1.3% agar solution to avoid any stress due to desiccation. Survival was checked every four hours until at least 90% of flies had died for every population and sex, after which the survival was checked every twelve hours until all the flies were dead. All the flies, including the dead ones, were transferred into fresh agar vials three days after the setup. This was done to avoid any effect of larval activity.

2.4.3. Chill Coma Recovery:

On Day-14 post egg collection, 40 flies were individually transferred into 1.5 ml microcentrifuge tubes for each population and sex. A small wet/moist cotton piece was stuck in the lid of each microcentrifuge tube to avoid any stress due to desiccation. These microcentrifuge tubes were then placed on melting ice (0 ^{o}C) for 6 Hrs. After the shock, the flies were transferred onto lids of Petri plates (55 mm Dia x 15 mm Ht Laxbro Tissue culture Dish Disposable Cat no. PD-55 TC), and the recovery period was scored. A fly was considered as recovered when it could stand on all its six legs without any external support. If a fly could not recover within 90 mins after the shock, it was considered an outlier and was not used for analysis.

2.4.4. Cold Shock Survival:

On Day-14 post egg collection, groups of 10 flies were transferred into 10 empty glass vials for each population and sex. The glass vials were plugged with wet/moist cotton plugs to avoid any stress due to desiccation. The glass vials were then placed on melting ice (0 ^{o}C) for 16 Hrs. After the shock, the flies were transferred into plastic vials

containing 6 ml of banana jaggery food to recover. The vials were placed horizontally during the first 24 Hrs of the recovery period making sure that none of the flies were on the food to avoid any accidental deaths because of the wings getting stuck on the food while unconscious. Survival was checked after 48 Hrs of recovery.

2.4.5. Heat Shock Survival:

On Day-13 post egg collection, groups of 10 flies were transferred into 10 – 12 empty glass vials for each population and sex. The glass vials were plugged with wet/moist cotton plugs to avoid any stress due to desiccation. The glass vials were then placed in a water bath maintained at a temperature of 38.3 ^{o}C for 1 hour. After the shock, the flies were transferred into plastic vials containing 6 ml of banana jaggery food to recover. The vials were placed horizontally during the first 24 Hrs of the recovery period making sure that none of the flies were on the food to avoid any accidental deaths because of the wings getting stuck in the food while unconscious. Survival was checked after 48 Hrs of recovery.

2.5. Data Analysis:

Males and females were analyzed separately for all assays.

For the biochemical assays, three different sets of comparisons were made. (i) VBs and VBCs were compared at each time point, (ii) VBs were compared across different time points, and (iii) VBCs were compared across different time points. A t-test was performed for each of the comparisons, followed by a Holm Šidák step down over each of the three sets of comparisons to determine the comparisons that were significant, considering $\alpha = 0.05$. For dry body weight, the mean dry body weight of each sample was used as a unit of analysis. For protein, glycogen, and triglyceride estimation, the respective metabolite content in each sample normalized with the mean dry body weight of the respective population and sex was used as the unit of analysis.

For desiccation resistance, starvation resistance, and chill coma recovery, VBs and VBCs were compared using a two-factor mixed model ANOVA. Selection (2-levels, VB-

VBC) was used as a fixed factor, and Block (4-levels, Block 1-4) was used as a random factor, considering $\alpha = 0.05$. For desiccation and starvation resistance, the mean survival time of the flies in each vial is used as a unit of analysis. For chill coma recovery, the recovery time of each individual replicate is used as a unit of analysis.

For heat shock and cold shock survival, the survival data were modeled using a generalized linear mixed model (GLMM) with binomial error distribution and LOGIT as the link function. The number of live flies in each vial at the time of the check was taken as an indicator of the number of successes. The number of dead flies in each vial at the time of the check was taken as an indicator of the number of failures. Selection (2-levels, VB-VBC) was used as a fixed factor, and Block (4-levels, Block 1-4) was used as a random factor. This was followed by a Type-III Wald Chi-square test to test for the significance of the effect of selection, considering $\alpha = 0.05$.

Effect sizes were computed using Effect size generator ver 2.3.0 by ClinTools (Devilly, 2004). Mixed model ANOVA analyses were performed using STATISTICA ver 8.0 by StatSoft (Stat Soft, Inc, 2007). Generalized linear mixed model analyses were performed using lmerTest, lme4, and car packages on R ver 4.2.2. All the graphs were plotted using dplyr and ggplot2 packages on R ver 4.2.2.

Chapter 3 Results

3.1. Body weight was not affected by selection for dispersal

There was no significant difference in the dry body weights of VBs and VBCs at any given time point (Figure 1A, 1B; Table 1.1). In both VBs and VBCs, the dry body weight of females increased from T1 to T2, followed by a decrease from T2 to T3, which increased again by T4 (Figure 1A; Table 1.2). There was no change in the dry body weight of males across age in both VBs and VBCs (Figure 1B; Table 1.2).

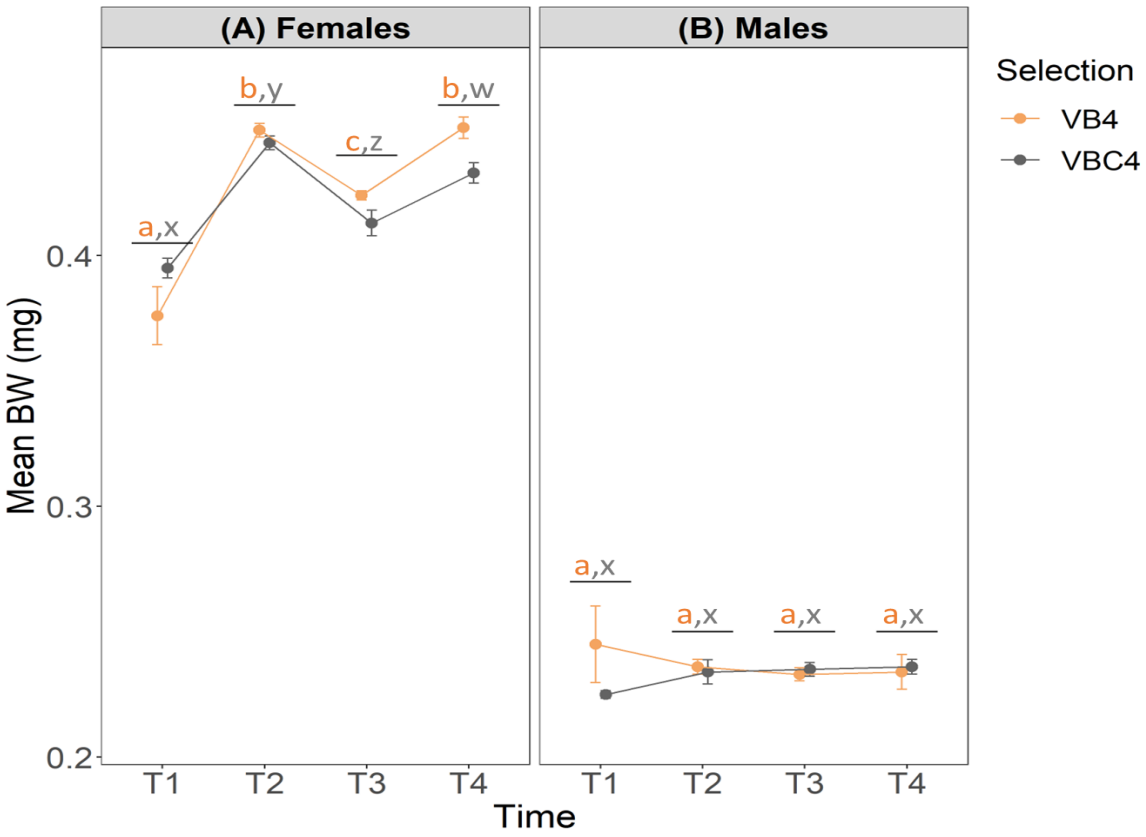

Figure 1: Dry body weight of selected and control populations in (A) females and (B) males across age

All the time points represent age from the day of egg collection. N = 5 for both VB and VBC at all time points. The points represent the mean body weight of the population at that given time point, and the error bars represent the standard error. * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$ after a Holm-Sidak step down). a, b, c and w, x, y, z represent how VBs and VBCs varied across age, respectively; different alphabets across any two time points indicate that the values are different at those two time points (i.e., $p < 0.05$ after a Holm-Sidak step down).

Sex	Population	Comparison	p-value	Holm Sidak corrected p-value	Effect size (d_{Cohen})
Females	VB	T1-T2	2.51E-04	**0.001**	3.941
	VB	T1-T3	0.003	**0.007**	2.594
	VB	T1-T4	2.95E-04	**0.001**	3.847
	VB	T2-T3	5.05E-05	**0.001**	4.958
	VB	T2-T4	0.849	-	0.124
	VB	T3-T4	4.25E-04	**0.001**	3.641
	VBC	T1-T2	5.72E-06	**3.43E-05**	6.667
	VBC	T1-T3	0.023	**0.047**	1.767
	VBC	T1-T4	1.42E-04	**0.001**	4.282
	VBC	T2-T3	5.82E-04	**0.002**	3.471
	VBC	T2-T4	0.040	**0.047**	1.549
	VBC	T3-T4	0.016	**0.047**	1.929
Males	VB	T1-T2	0.578	-	0.367
	VB	T1-T3	0.460	-	0.490
	VB	T1-T4	0.530	-	0.168
	VB	T2-T3	0.461	-	0.490
	VB	T2-T4	0.780	-	0.168
	VB	T3-T4	0.896	-	0.085
	VBC	T1-T2	0.116	-	1.116
	VBC	T1-T3	0.013	-	2.000
	VBC	T1-T4	0.011	0.062	2.098
	VBC	T2-T3	0.862	-	0.114
	VBC	T2-T4	0.733	-	0.224
	VBC	T3-T4	0.809	-	0.158

Table 1.1: Effect sizes and p-values after a Holm Sidak step down for comparisons of dry body weights between different time points for each population. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

Sex	Time point	Comparison	p-value	Holm Sidak corrected p-value	Effect size (d_{Cohen})
Females	T1	VB-VBC	0.158	-	0.986
	T2	VB-VBC	0.233	-	0.816
	T3	VB-VBC	0.079	-	1.270
	T4	VB-VBC	0.016	0.062	1.924
Males	T1	VB-VBC	0.228	-	0.825
	T2	VB-VBC	0.733	-	0.224
	T3	VB-VBC	0.608	-	0.338
	T4	VB-VBC	0.780	-	0.168

Table 1.2: Effect sizes and p-values after a Holm Sidak step down for comparisons of dry body weights of VB and VBC at each time point. α for statistical significance is considered to be 0.05.

3.2. Selection for dispersal affected the protein content of females but not males

In females, VBs had higher whole-body protein content than VBCs at T1 (Figure 2A; Table 2.1). The protein content of VBs had decreased from T1 to T2, causing the difference between VBs and VBCs to disappear (Figure 2A; Table 2.1, 2.2). There was a spike in the protein content of VBCs at T3, causing the difference between VBs and VBCs to be significant again at T3 before converging by T4 (Figure 2A; Table 2.1, 2.2).

In males, there was no significant difference in the whole-body protein content between VBs and VBCs at any time point (Figure 2B; Table 2.1). The protein content of VBCs gradually decreased with age, while the protein content of VBs did not vary from T1 to T2 but decreased sharply from T2 to T3, with no significant change from T3 to T4 (Figure 2B; Table 2.2).

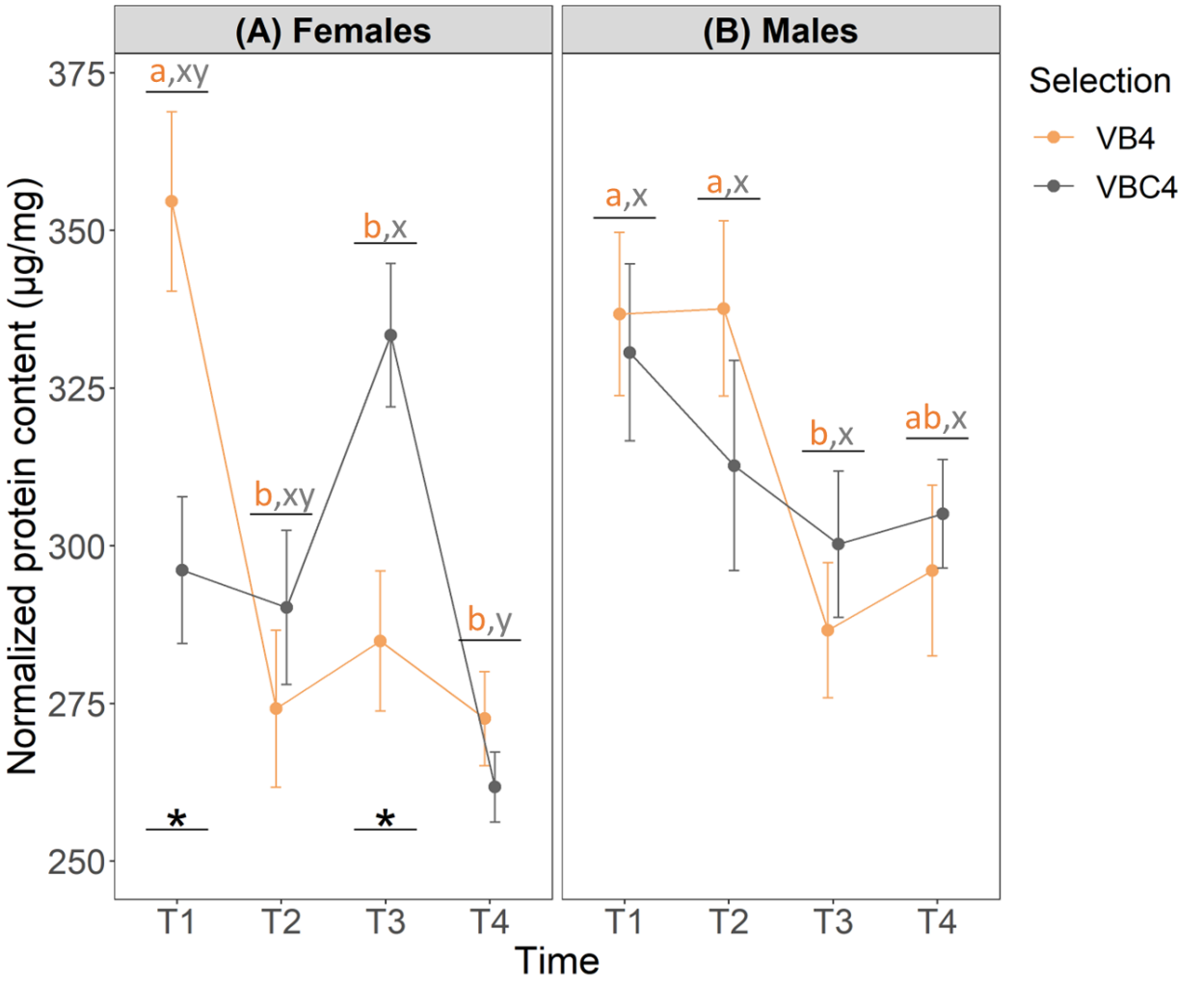

Figure 2: Whole-body protein content of selected and control populations in (A) females and (B) males across age

All the time points represent age from the day of egg collection. The protein content was normalized with the mean dry body weight of the respective population and sex at that age. N = 10 for both VB and VBC at all time points. The points represent the mean body weight of the population at that given time point, and the error bars represent the standard error. * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$ after a Holm-Sidak step down). a, b, c and w, x, y, z represent how VBs and VBCs varied across age, respectively; different alphabets across any two time points indicate that the values are different at those two time points (i.e., $p < 0.05$ after a Holm-Sidak step down).

Sex	Population	Comparison	p-value	Holm Sidak corrected p-value	Effect size (d_{Cohen})
Females	VB	T1-T2	4.78E-4	**0.002**	1.902
	VB	T1-T3	0.001	**0.005**	1.727
	VB	T1-T4	7.42E-05	**0.001**	2.283
	VB	T2-T3	0.528	-	0.288
	VB	T2-T4	0.914	-	0.049
	VB	T3-T4	0.369	-	0.412
	VBC	T1-T2	0.730	-	0.157
	VBC	T1-T3	0.034	-	1.024
	VBC	T1-T4	0.015	0.075	1.195
	VBC	T2-T3	0.019	-	1.156
	VBC	T2-T4	0.048	-	0.950
	VBC	T3-T4	2.26E-05	**0.001**	2.533
Males	VB	T1-T2	0.964	-	0.020
	VB	T1-T3	0.008	**0.047**	1.335
	VB	T1-T4	0.043	0.163	0.972
	VB	T2-T3	0.009	**0.047**	1.301
	VB	T2-T4	0.046	-	0.958
	VB	T3-T4	0.589	-	0.246
	VBC	T1-T2	0.420	-	0.369
	VBC	T1-T3	0.112	-	0.747
	VBC	T1-T4	0.138	-	0.694
	VBC	T2-T3	0.547	-	0.274
	VBC	T2-T4	0.689	-	0.182
	VBC	T3-T4	0.742	-	0.150

Table 2.1: Effect sizes and p-values after a Holm Sidak step down for comparisons of whole-body protein content between different time points for each population. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

Sex	Time point	Comparison	p-value	Holm Sidak corrected p-value	Effect size (d_{Cohen})
Females	T1	VB-VBC	0.005	**0.020**	1.423
	T2	VB-VBC	0.369	-	0.412
	T3	VB-VBC	0.007	**0.021**	1.364
	T4	VB-VBC	0.259	-	0.521
Males	T1	VB-VBC	0.753	-	0.143
	T2	VB-VBC	0.265	-	0.514
	T3	VB-VBC	0.398	-	0.387
	T4	VB-VBC	0.581	-	0.251

Table 2.2: Effect sizes and p-values after a Holm Sidak step down for comparisons of whole-body protein content of VB and VBC at each time point. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

3.3. Selection for dispersal led to an increase in glycogen content at later ages

In females, whole-body glycogen content had decreased sharply from T1 to T2, followed by an increase from T2 to T3, with no significant difference between VBs and VBCs (Figure 3A; Table 3.1, 3.2). Glycogen content had increased from T3 to T4 in VBs, while it decreased from T3 to T4 in VBCs, resulting in VBs having higher glycogen content than VBCs at T4 (Figure 3A; Table 3.1, 3.2).

In males, whole-body glycogen content decreased sharply from T1 to T2, with no significant difference between VBs and VBCs (Figure 3B; Table 3.1,3.2). Even though the variation of glycogen content from T2 to T4 is not statistically significant in either VBs or VBCs, the glycogen content of VBs gradually increased from T2 to T4, while it gradually decreased in VBCs, resulting in VBs having higher glycogen content than VBCs at T3 and T4.

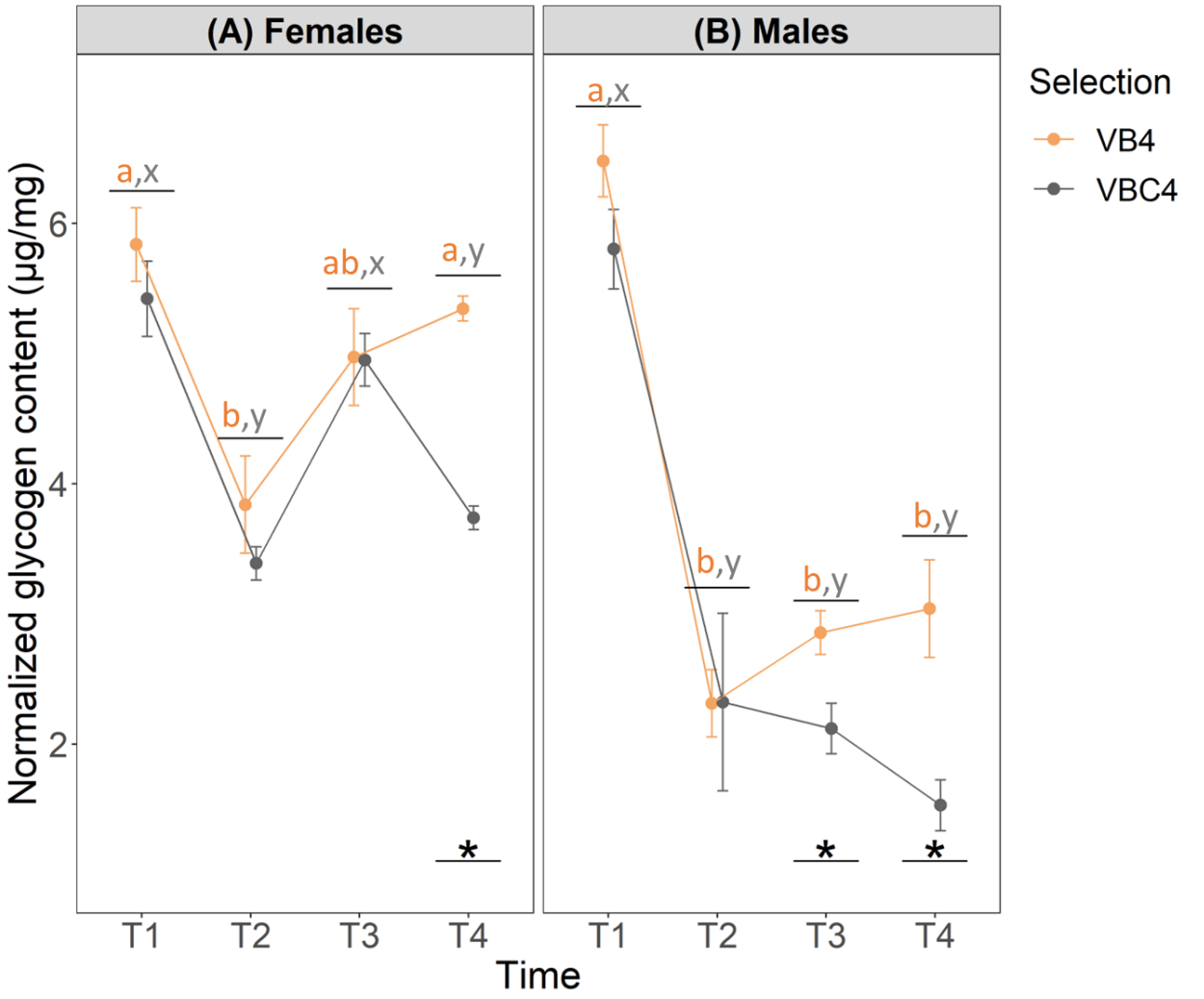

Figure 3: Whole-body glycogen content of selected and control populations in (A) females and (B) males across age

All the time points represent age from the day of egg collection. The glycogen content was normalized with the mean dry body weight of the respective population and sex at that age. N = 10 for both VB and VBC at T1, and N = 8 for both VB and VBC at T2, T3, and T4. The points represent the mean body weight of the population at that given time point, and the error bars represent the standard error. * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$ after a Holm-Sidak step down). a, b, c and w, x, y, z represent how VBs and VBCs varied across age, respectively; different alphabets across any two time points indicate that the values are different at those two time points (i.e., $p < 0.05$ after a Holm-Sidak step down).

Sex	Population	Comparison	p-value	Holm Sidak corrected p-value	Effect size (d_{Cohen})
Females	VB	T1-T2	4.99E-4	**0.003**	2.062
	VB	T1-T3	0.077	-	0.896
	VB	T1-T4	0.155	-	0.709
	VB	T2-T3	0.049	0.183	1.077
	VB	T2-T4	0.002	**0.008**	1.955
	VB	T3-T4	0.3466	-	0.487
	VBC	T1-T2	2.25E-05	**1.12E-04**	2.797
	VBC	T1-T3	0.225	-	0.599
	VBC	T1-T4	1.26E-4	**3.80E-04**	2.379
	VBC	T2-T3	1.32E-05	**7.92E-05**	3.268
	VBC	T2-T4	0.043	0.084	1.115
	VBC	T3-T4	8.28E-05	**3.31E-04**	2.734
Males	VB	T1-T2	9.26E-09	**5.56E-08**	5.125
	VB	T1-T3	1.36E-08	**6.80E-08**	4.987
	VB	T1-T4	1.14E-06	**4.56E-06**	3.588
	VB	T2-T3	0.099	-	0.882
	VB	T2-T4	0.132	-	0.799
	VB	T3-T4	0.663	-	0.223
	VBC	T1-T2	1.29E-4	**5.19E-04**	2.374
	VBC	T1-T3	4.75E-08	**2.37E-07**	4.559
	VBC	T1-T4	6.20E-09	**3.72E-08**	5.272
	VBC	T2-T3	0.777	-	0.144
	VBC	T2-T4	0.282	-	0.560
	VBC	T3-T4	0.050	-	1.072

Table 3.1: Effect sizes and p-values after a Holm Sidak step down for comparisons of whole-body glycogen content between different time points for each population. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

Sex	Time point	Comparison	p-value	Holm Sidak corrected p-value	Effect size (d_{Cohen})
Females	T1	VB-VBC	0.317	-	0.461
	T2	VB-VBC	0.273	-	0.571
	T3	VB-VBC	0.962	-	0.024
	T4	VB-VBC	7.64E-09	**3.06E-08**	6.095
Males	T1	VB-VBC	0.117	-	0.737
	T2	VB-VBC	0.989	-	0.007
	T3	VB-VBC	0.012	**0.036**	1.439
	T4	VB-VBC	0.003	**0.012**	1.786

Table 3.2: Effect sizes and p-values after a Holm Sidak step down for comparisons of whole-body glycogen content of VB and VBC at each time point. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

3.4. Selection for dispersal led to an increase in triglyceride content at later ages

In females, there was no significant difference in the whole-body triglyceride content of VBs and VBCs at T1 (Figure 4A; Table 4.1). The triglyceride content increased gradually with age in VBs while it decreased with age in VBCs, resulting in VBs having higher triglyceride content than VBCs from T2 (Figure 4A; Table 4.1, 4.2).

In males, there was no difference in the whole-body triglyceride content of VBs and VBCs at T1 (Figure 4B; Table 4.1). The triglyceride content of VBCs had decreased from T1 to T2, resulting in VBs having higher triglyceride content than VBCs from T2 (Figure 4B; Table 4.1, 4.2). There was an increase in the triglyceride content from T2 to T3 in both VBs and VBCs, which decreased again by T4 (Figure 4B; Table 4.2).

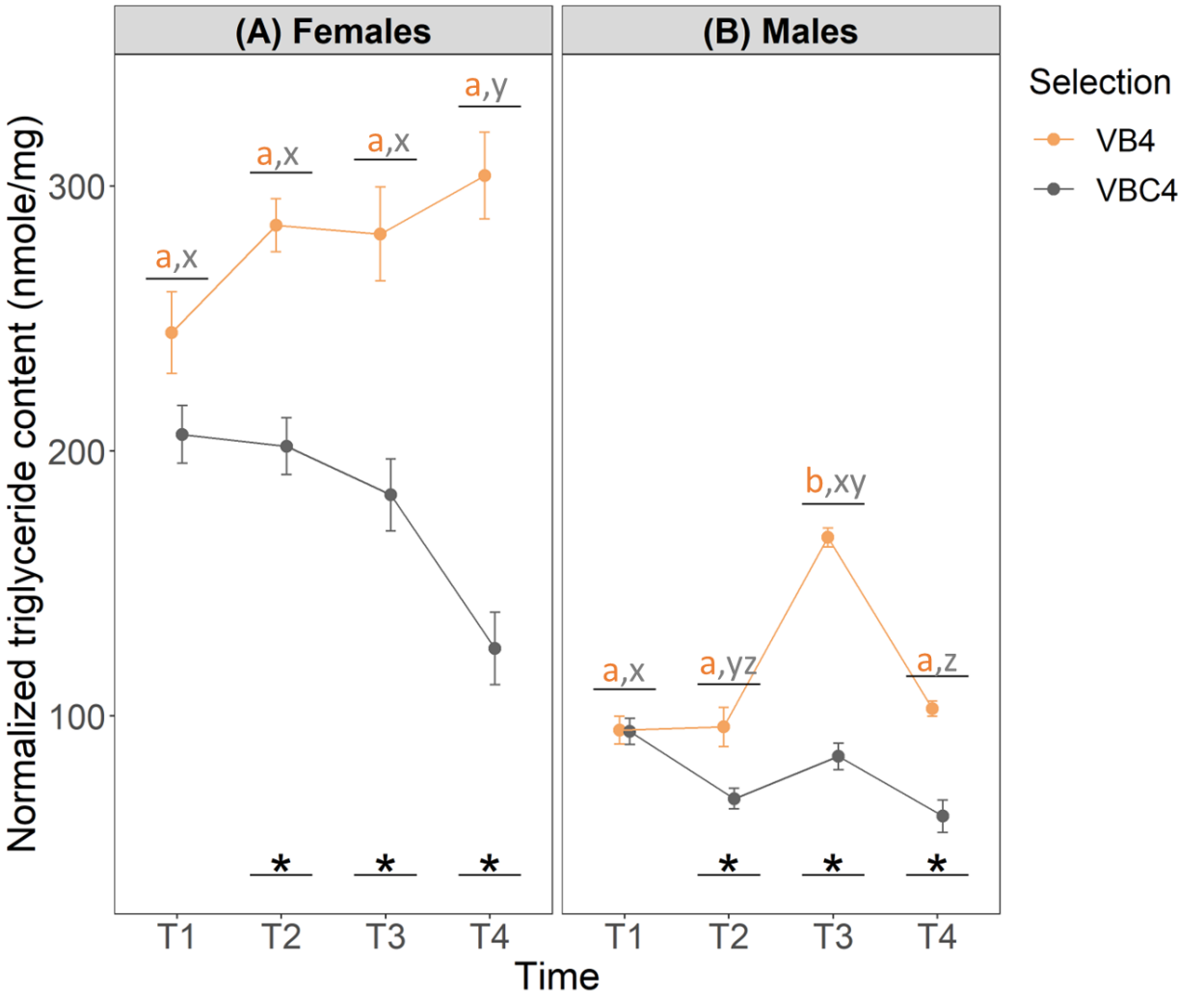

Figure 4: Whole-body triglyceride content of selected and control populations in (A) females and (B) males across age

All the time points represent age from the day of egg collection. The triglyceride content was normalized with the mean dry body weight of the respective population and sex at that age. N=10 for both VB and VBC at all time points. The points represent the mean body weight of the population at that given time point, and the error bars represent the standard errors around the mean (SEM). * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$ after a Holm-Sidak step down). a, b, c and w, x, y, z represent how VBs and VBCs varied across age, respectively; different alphabets across any two time points indicate that the values are different at those two time points (i.e., $p < 0.05$ after a Holm-Sidak step down).

Sex	Population	Comparison	p-value	Holm Sidak corrected p-value	Effect size (d_{Cohen})
Females	VB	T1-T2	0.041	-	0.986
	VB	T1-T3	0.129	-	0.711
	VB	T1-T4	0.017	0.096	1.181
	VB	T2-T3	0.876	-	0.071
	VB	T2-T4	0.339	-	0.440
	VB	T3-T4	0.372	-	0.410
	VBC	T1-T2	0.773	-	0.131
	VBC	T1-T3	0.206	-	0.587
	VBC	T1-T4	2.07E-04	**0.001**	2.071
	VBC	T2-T3	0.302	-	0.475
	VBC	T2-T4	3.47E-04	**0.002**	1.967
	VBC	T3-T4	0.008	**0.030**	1.341
Males	VB	T1-T2	0.897	-	0.059
	VB	T1-T3	1.03E-09	**5.15E-09**	5.132
	VB	T1-T4	0.188	-	0.612
	VB	T2-T3	6.30E-08	**2.52E-07**	3.929
	VB	T2-T4	0.388	-	0.396
	VB	T3-T4	3.41E-11	**2.05E-10**	6.327
	VBC	T1-T2	8.20E-04	**0.004**	1.794
	VBC	T1-T3	0.197	-	0.599
	VBC	T1-T4	7.27E-04	**0.004**	1.818
	VBC	T2-T3	0.022	0.065	1.121
	VBC	T2-T4	0.373	-	0.408
	VBC	T3-T4	0.010	**0.041**	1.278

Table 4.1: Effect sizes and p-values after a Holm Sidak step down for comparisons of whole-body triglyceride content between different time points for each population. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

Sex	Time point	Comparison	p-value	Holm Sidak corrected p-value	Effect size (d_{Cohen})
Females	T1	VB-VBC	0.056	-	0.913
	T2	VB-VBC	2.11E-05	**6.33E-05**	2.547
	T3	VB-VBC	3.34E-04	**0.001**	1.974
	T4	VB-VBC	1.26E-07	**5.04E-07**	3.747
Males	T1	VB-VBC	0.945	-	0.031
	T2	VB-VBC	4.39E-04	**0.009**	1.456
	T3	VB-VBC	7.91E-11	**3.16E-10**	6.014
	T4	VB-VBC	1.08E-05	**3.24E-05**	2.693

Table 4.2: Effect sizes and p-values after a Holm Sidak step down for comparisons of whole-body triglyceride content of VB and VBC at each time point. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

3.5. Selection for dispersal led to a lower desiccation resistance

VBs had lower desiccation resistance than VBCs in both males and females (Figure 5A, 5B; Table 5.1, 5.2).

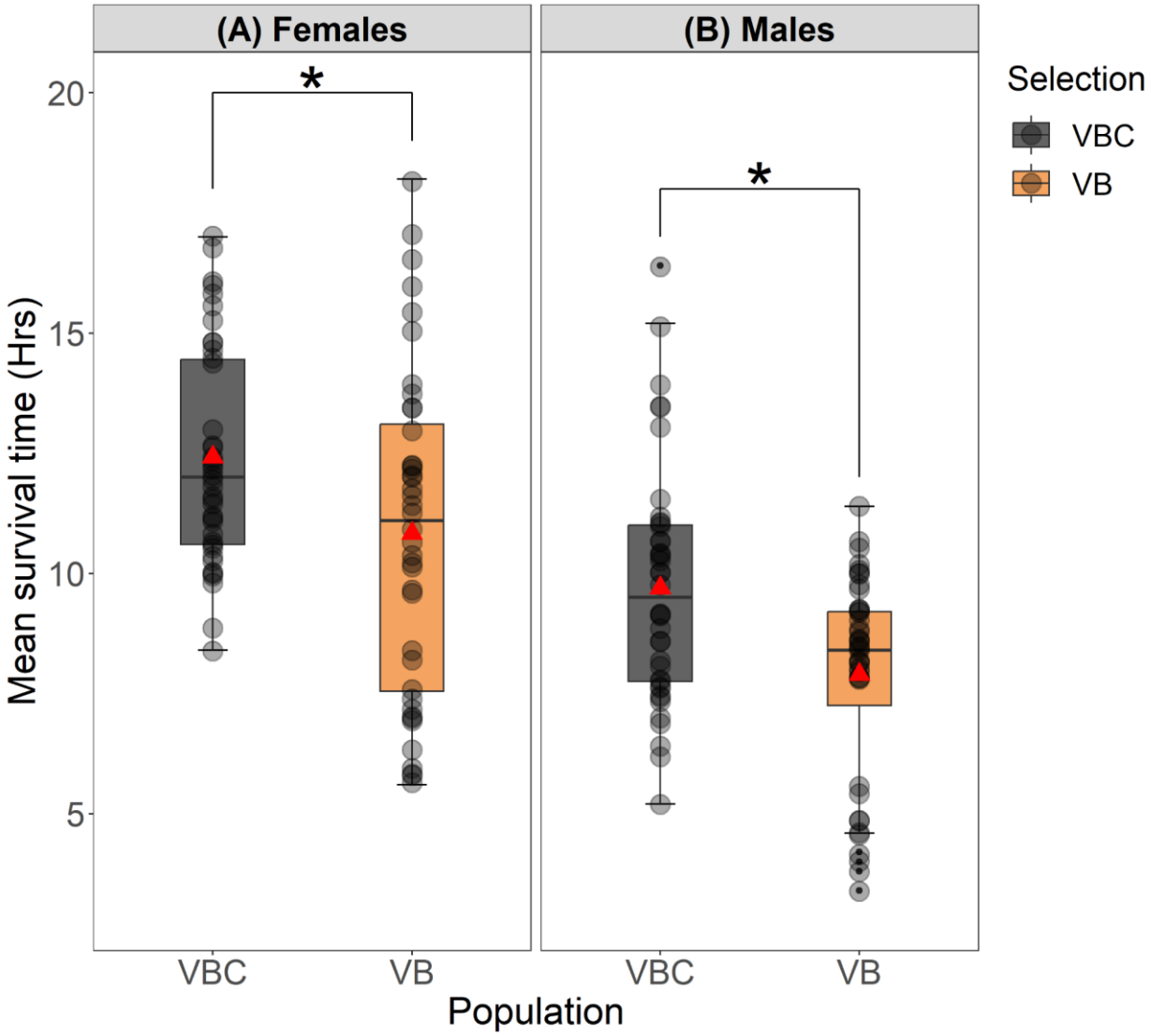

Figure 5: Desiccation resistance of selected and control populations in (A) females and (B) males

N = 40 for both VB and VBC for males and females. The points represent the pooled data for all the replicates of VB and VBC populations. The edges of the box denote the 25th and 75th percentiles, while the red triangle and the solid line represent the mean and median, respectively. The upper and lower whiskers extend from the box to the largest and smallest values no further than 1.5*(Inter quartile range) from the box, and * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$).

Trait	Effect	Deg. of freedom	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p	Effect size (d_{Cohen})
Desiccation resistance (Females)	Sel	1	50.2	75	2.835	17.72	**0.000***	0.564
	Block	3	148.4	75	2.835	52.34	**0.000***	-

Table 5.1: Results from ANOVA of desiccation resistance of females with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

Trait	Effect	Deg. of freedom	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p	Effect size (d_{Cohen})
Desiccation resistance (Males)	Sel	1	65.16	75	2.113	30.84	**0.000***	0.770
	Block	3	90.17	75	2.113	42.68	**0.000***	-

Table 5.2: Results from ANOVA of desiccation resistance of males with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

3.6. Selection for dispersal led to a lower starvation resistance in males but not in females

VBs had lower starvation resistance than VBCs in males (Figure 6.1B; Table 6.2). There was no difference in the starvation resistance of females between VBs and VBCs when all the blocks were grouped together (Figure 6.1A; Table 6.1). However, when the data for each block were analyzed separately, it was observed that VB females had higher starvation resistance than VBC females in Block-1 and Block-4 (Figure 6.2A, 6.2D; Table 6.3), while VBC females had higher starvation resistance than VB females in Block-3 (Figure 6.2C; Table 6.3).

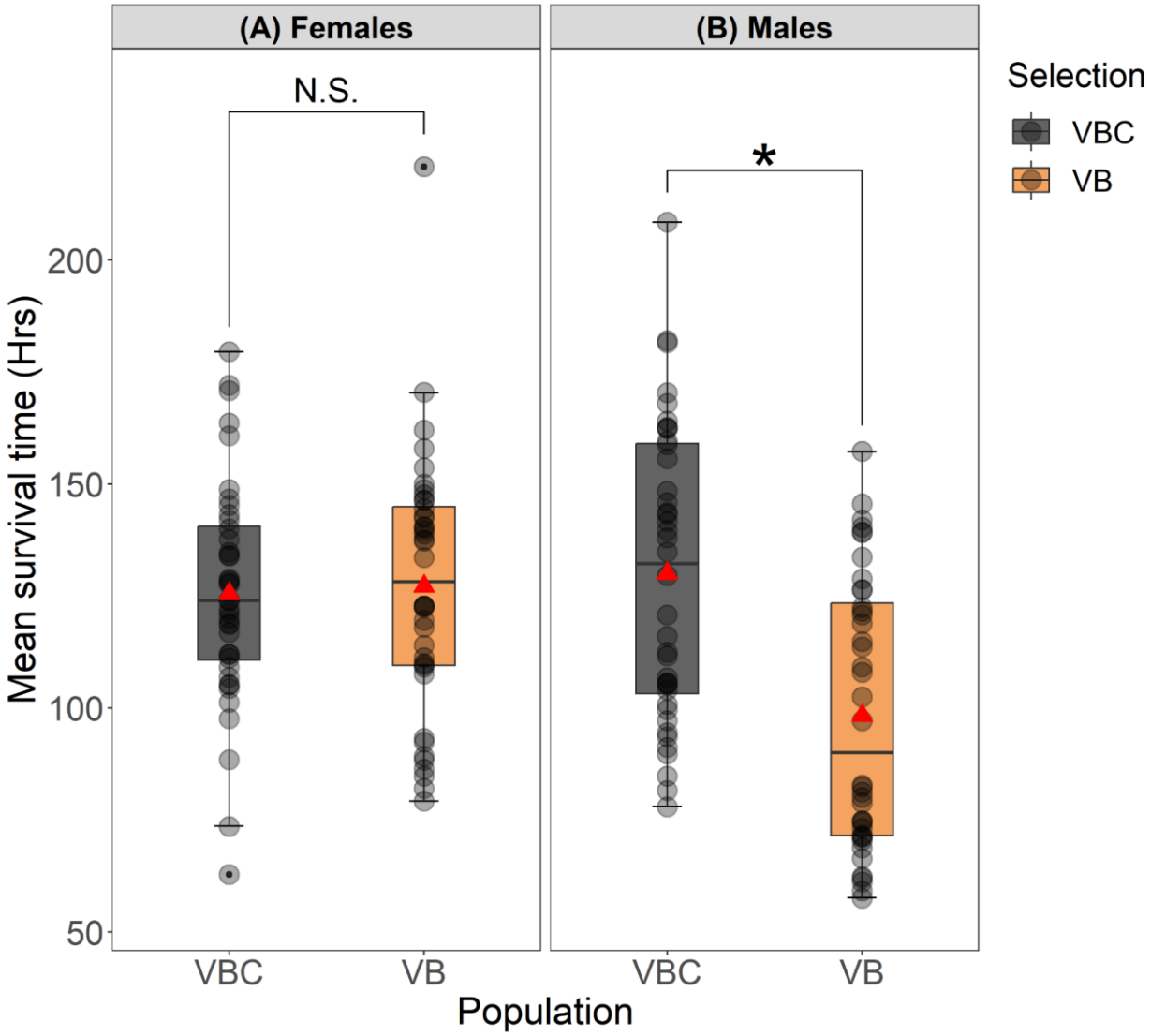

Figure 6.1: Starvation resistance of selected and control populations in (A) females and (B) males

N = 40 for both VB and VBC for males and females. The points represent the pooled data for all the replicates of VB and VBC populations. The edges of the box denote the 25th and 75th percentiles, while the red triangle and the solid line represent the mean and median, respectively. The upper and lower whiskers extend from the box to the largest and smallest values no further than 1.5*(Inter quartile range) from the box, and * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$).

Trait	Effect	Deg. of freedom	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p	Effect size (d_{Cohen})
Starvation resistance (Females)	Sel	1	54	75	428.7	0.13	0.723	0.061
	Block	3	8230	75	428.7	19.2	**0.000***	-

Table 6.1: Results from ANOVA of starvation resistance of females with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

Trait	Effect	Deg. of freedom	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p	Effect size (d_{Cohen})
Starvation resistance (Males)	Sel	1	2.01E+04	75	232.6	86.36	**0.000***	1.007
	Block	3	1.99E+04	75	232.6	85.71	**0.000***	-

Table 6.2: Results from ANOVA of starvation resistance of males with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

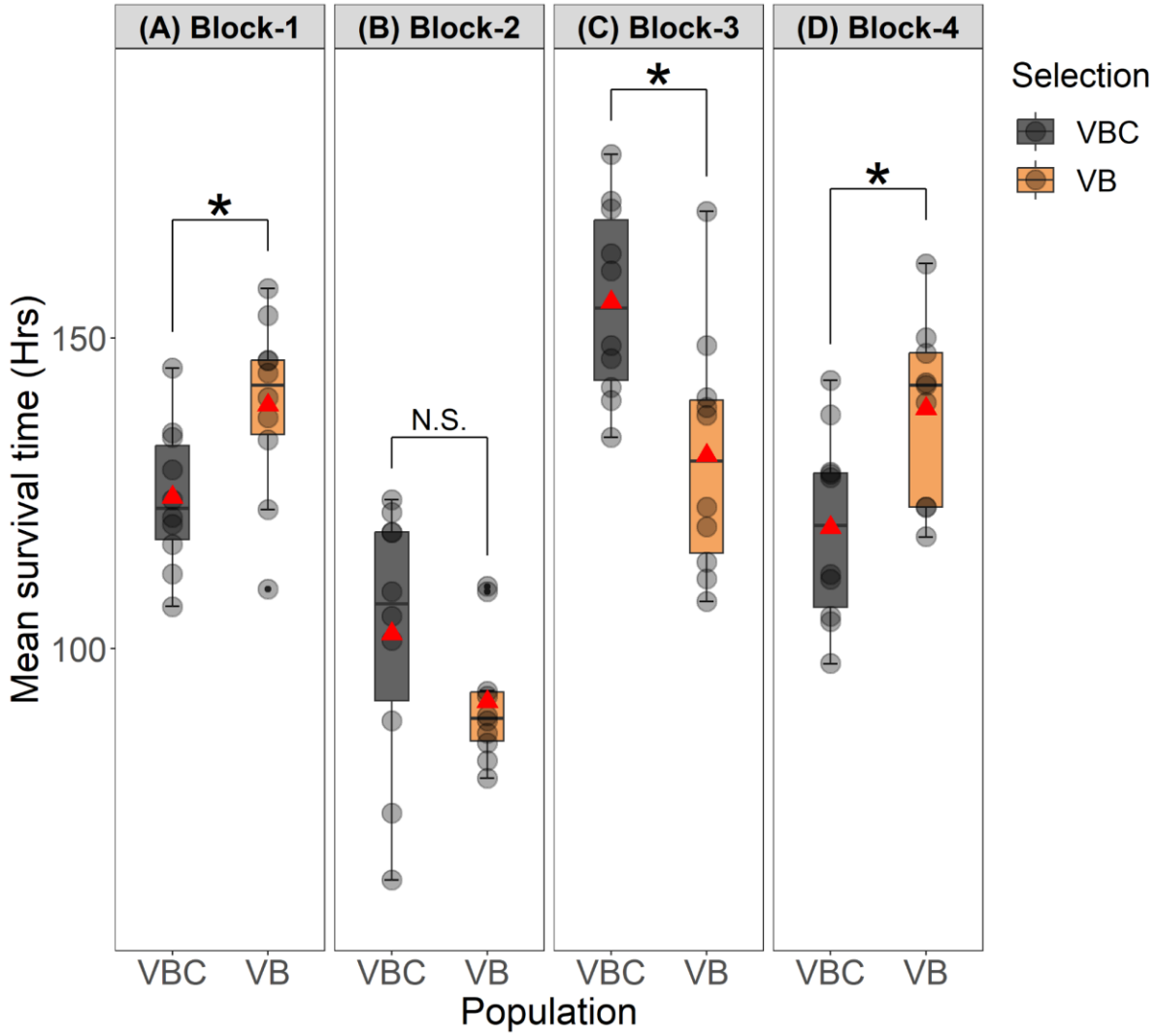

Figure 6.2: Starvation resistance of females of selected and control populations in (A) Block-1, (B) Block-2, (C) Block-3, and (D) Block-4

N = 10 for both VB and VBC for all blocks. The points represent the pooled data for all the replicates of VB and VBC populations. The edges of the box denote the 25th and 75th percentiles, while the red triangle and the solid line represent the mean and median, respectively. The upper and lower whiskers extend from the box to the largest and smallest values no further than 1.5*(Inter quartile range) from the box, and * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$).

Trait	Effect	Block	SS	Deg. of freedom	MS	F	p	Effect size (d_{Cohen})
Starvation resistance (Females)	Sel	Block-1	1101	1	1101	6.414	**0.021***	1.085
		Block-2	596.2	1	596.2	2.138	0.161	0.626
		Block-3	3051	1	3051	9.681	**0.006***	1.333
		Block-4	3743	1	3743	6.785	**0.018***	1.116

Table 6.3: Results from ANOVA of starvation resistance of females for each individual block with selection as a fixed factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

3.7. Selection for dispersal led to an increase in chill coma recovery time in males but not in females

VBs had taken longer to recover from the chill coma when compared to VBCs in males (Figure 7.1B; Table 7.2). There was no difference in the time taken to recover from a chill coma in females between VBs and VBCs when all the blocks were grouped together (Figure 7.1A; Table 7.1). However, when the data for each block were analyzed separately, it was observed that VB females took longer time to recover from chill coma than VBC females in Block-1 (Figure 7.2A; Table 7.3), while VBC females took longer time to recover than VB females in Block-3 (Figure 7.2C, Table 7.3).

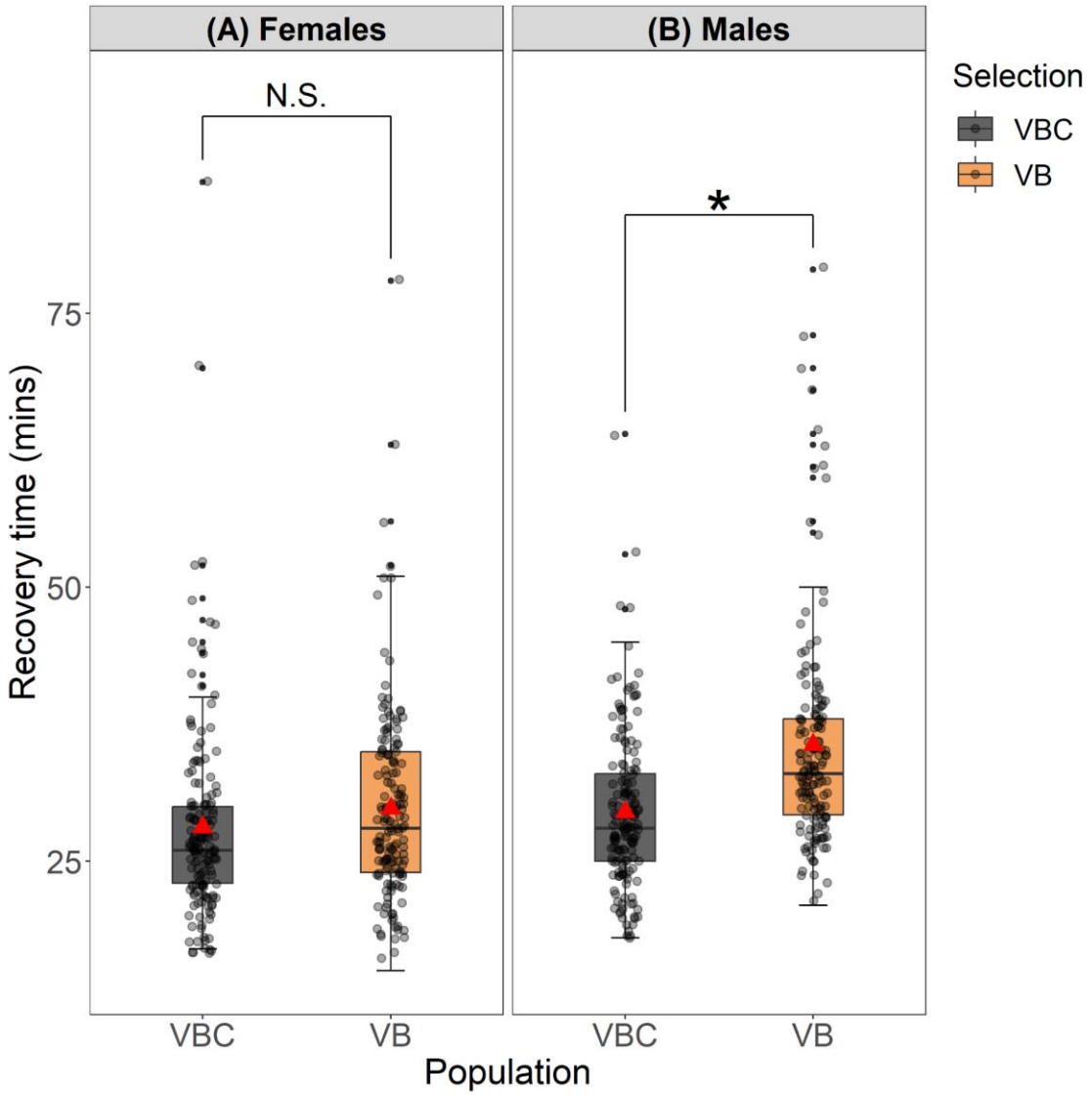

Figure 7.1: Recovery time from a chill coma for selected and control populations in (A) females and (B) males

Recovery time was measured after a cold shock of 0 ^{o}C for 6 Hrs. N = 157 for VB females. N = 160 for VBC females, N = 154 for VB males, and N = 160 for VBC males. The points represent the pooled data for all the replicates of VB and VBC populations. The edges of the box denote the 25th and 75th percentiles, while the red triangle and the solid line represent the mean and median, respectively. The upper and lower whiskers extend from the box to the largest and smallest values no further than 1.5*(Inter quartile range) from the box, and * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$).

Trait	Effect	Deg. of freedom	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p	Effect size (d_{Cohen})
Chill coma recovery (Females)	Sel	1	297.2	312	78.61	3.78	0.053	0.208
	Block	3	322.4	312	78.61	4.101	**0.007***	-

Table 7.1: Results from ANOVA of chill coma recovery time of females with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

Trait	Effect	Deg. of freedom	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p	Effect size (d_{Cohen})
Chill coma recovery (Males)	Sel	1	3025	309	68.06	44.45	**0.000***	0.709
	Block	3	789	309	68.06	11.59	**0.000***	-

Table 7.2: Results from ANOVA of chill coma recovery time of males with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

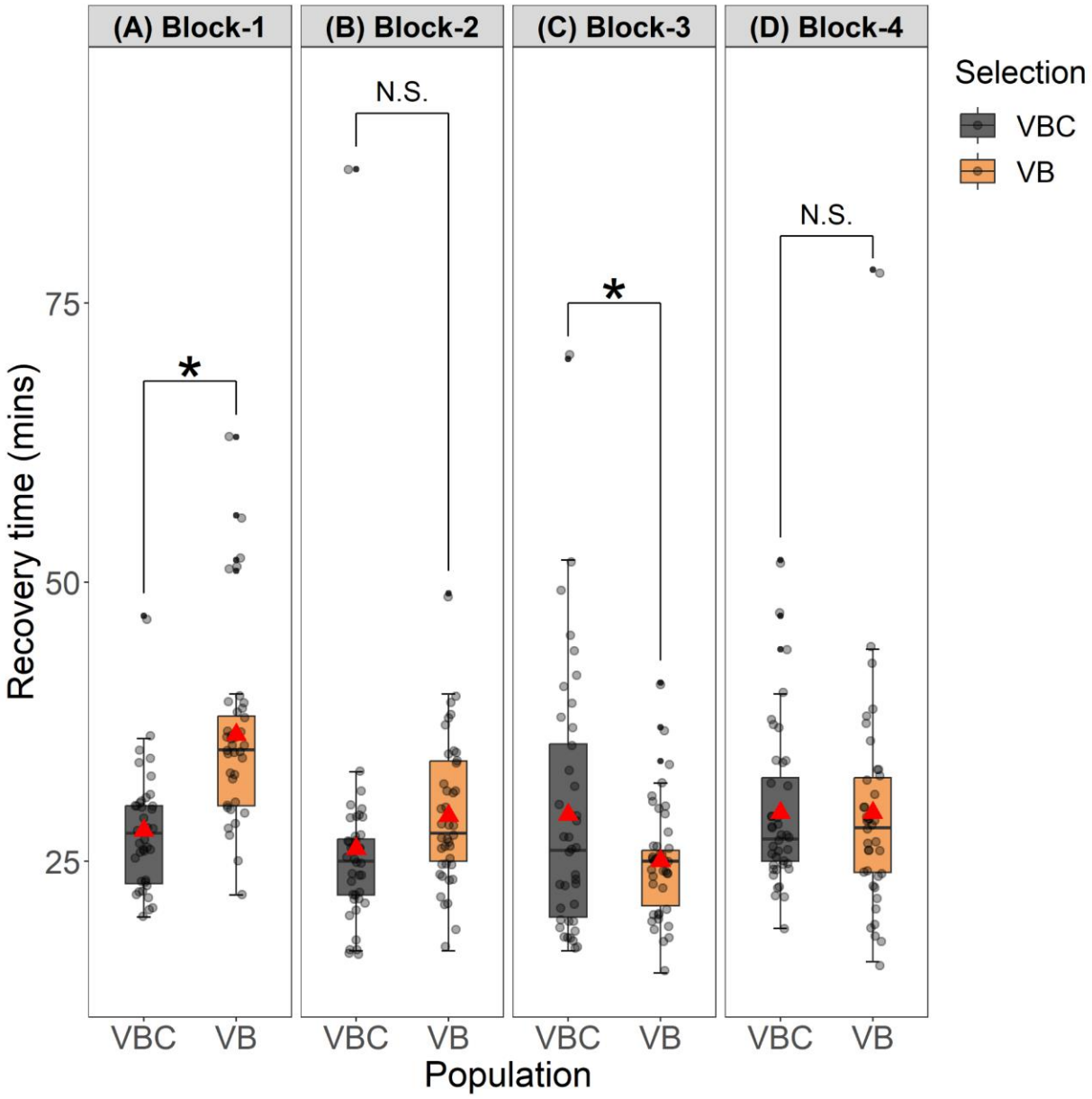

Figure 7.2: Recovery time from a chill coma for females of selected and control populations in (A) Block-1, (B) Block-2, (C) Block-3, and (D) Block-4

N = 37 for VB and N = 40 for VBC in Block-1, N= 40 for both VB and VBC in Block-2, N = 41 for VB and N = 40 for VBC in Block-3, and N = 39 for VB and N = 40 for VBC in Block-4. The points represent the pooled data for all the replicates of VB and VBC populations. The edges of the box denote the 25th and 75th percentiles, while the red triangle and the solid line represent the mean and median, respectively. The upper and lower whiskers extend from the box to the largest and smallest values no further than 1.5*(Inter quartile range) from the box, and * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$).

Trait	Effect	Block	SS	Deg. of freedom	MS	F	p	Effect size (d_{Cohen})
Chill coma recovery (Females)	Sel	Block-1	1654	1	1654	30.56	**0.000***	1.240
		Block-2	174	1	174	2.216	0.141	0.330
		Block-3	348.9	1	348.9	4.339	**0.040***	0.457
		Block-4	0.002	1	0.002	0	0.996	0.001

Table 7.3: Results from ANOVA of chill coma recovery time of females with selection as a fixed factor for each individual block and their effect sizes. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

3.8. Selection for dispersal had a sex-specific effect on cold shock resistance

Survival after experiencing a cold shock was higher in VB females as compared to VBC females (Figure 8A; Table 8.1). However, in males, the survival of VBCs was higher as compared to VBs (Figure 8B; Table 8.2).

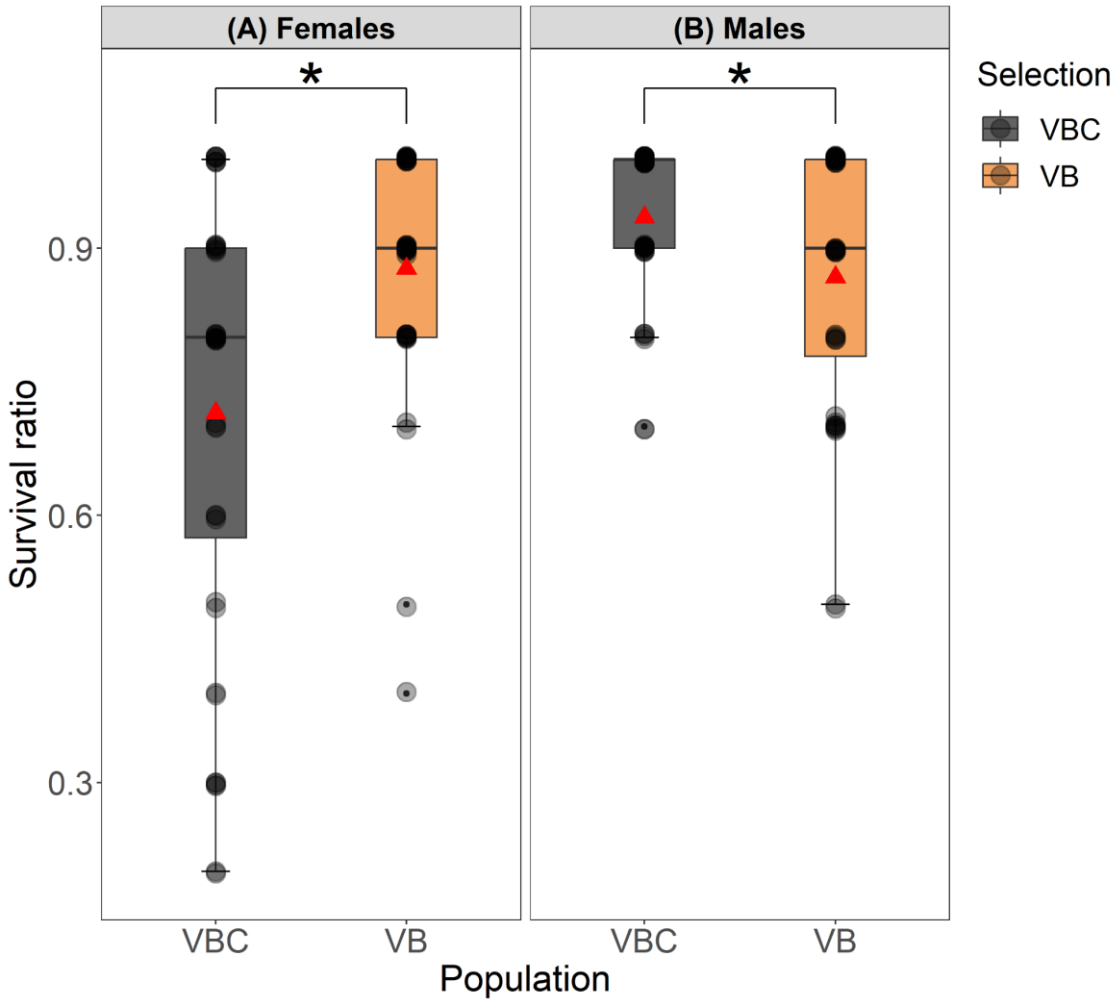

Figure 8: Survival after a cold shock for selected and control populations in (A) females and (B) males

Survival ratio refers to Number of flies alive/ Total number of flies in a replicate. Survival was measured after recovering for 48 Hrs from a cold shock of 0 ^{o}C for 16 Hrs, followed by a recovery period of 48 Hrs. N = 40 for both VB and VBC for males and females. The points represent the pooled data for all the replicates of VB and VBC populations. The edges of the box denote the 25th and 75th percentiles, while the red triangle and the solid line represent the mean and median, respectively. The upper and lower whiskers extend from the box to the largest and smallest values no further than 1.5*(Inter quartile range) from the box, and * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$).

Distribution: BINOMIAL			Link function: LOGIT		
Analysis of Deviance Table (Type III Wald chi-square tests)					
Trait	Effect	Chisq	Deg. of freedom	Pr(>Chisq)	Effect size
Cold shock survival (Females)	(Intercept)	1.901	1	0.168	-
	Sel	4.471	1	**0.034 ***	0.807

Table 8.1: Results of generalized linear mixed model analysis of the cold shock survival data of females with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

Distribution: BINOMIAL			Link function: LOGIT		
Analysis of Deviance Table (Type III Wald chi-square tests)					
Trait	Effect	Chisq	Deg. of freedom	Pr(>Chisq)	Effect size
Cold shock survival (Males)	(Intercept)	14.309	1	**1.55E-04 *** **	-
	Sel	10.506	1	**0.001 ** **	0.565

Table 8.2: Results of generalized linear mixed model analysis of the cold shock survival data of males with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

3.9. Selection for dispersal led to an increased heat shock resistance

Survival after experiencing a heat shock was higher in VBs as compared to VBCs for both males and females (Figure 9A, 9B; Table 9.1, 9.2).

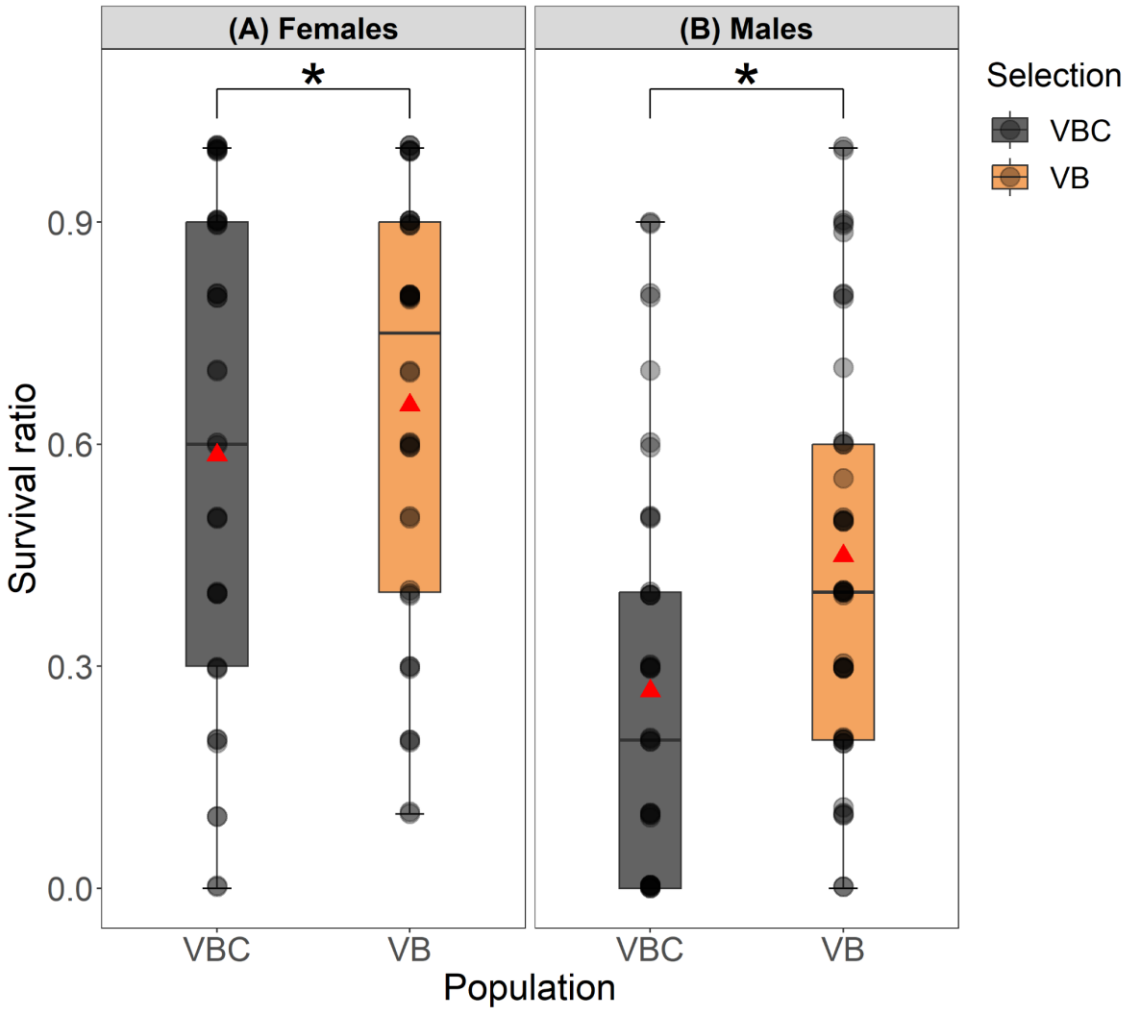

Figure 9: Survival after a heat shock for selected and control populations in (A) females and (B) males

Survival ratio refers to Number of flies alive/ Total number of flies in a replicate. Survival was measured after recovering for 48 Hrs from a heat shock of 38.3 ^{o}C for 1 Hr. N = 40 for VB females. N = 41 for VBC females, N = 44 for VB males, and N = 42 for VBC males. The points represent the pooled data for all the replicates of VB and VBC populations. The edges of the box denote the 25th and 75th percentiles, while the red triangle and the solid line represent the mean and median, respectively. The upper and lower whiskers extend from the box to the largest and smallest values no further than 1.5*(Inter quartile range) from the box, and * denotes that VBs and VBCs have significantly different values at that time point (i.e., $p < 0.05$).

Distribution: BINOMIAL			Link function: LOGIT		
Analysis of Deviance Table (Type III Wald chi-square tests)					
Trait	Effect	Chisq	Deg. of freedom	Pr(>Chisq)	Effect size
Heat shock survival (Females)	(Intercept)	1.901	1	0.168	-
	Sel	4.471	1	**0.034***	0.211

Table 9.1: Results of generalized linear mixed model analysis of the heat shock survival data of females with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

Distribution: BINOMIAL			Link function: LOGIT		
Analysis of Deviance Table (Type III Wald chi-square tests)					
Trait	Effect	Chisq	Deg. of freedom	Pr(>Chisq)	Effect size
Heat shock survival (Males)	(Intercept)	0.331	1	0.565	-
	Sel	31.996	1	**1.54E-08***	0.655

Table 9.2: Results of generalized linear mixed model analysis of the heat shock survival data of males with selection as a fixed factor and block as a random factor. α for statistical significance is considered to be 0.05. Bold face denotes statistically significant p-values.

	VBs wrt VBCs			
Trait	**T1 (Day-15)**	**T2 (Day-22)**	**T3 (Day-29)**	**T4 (Day-36)**
Dry body weight	~	~	~	~
Whole-body protein content	↑	~	↓	~
Whole-body glycogen content	~	~	~	↑
Whole-body triglyceride content	~	↑	↑	↑

Table 10: Summary of results of the biochemical assays for VBs as compared to VBCs in females. The arrows indicate the increase and decrease in the trait value, while ~ represents that there is no significant difference between VBs and VBCs.

	VBs wrt VBCs			
Trait	**T1 (Day-15)**	**T2 (Day-22)**	**T3 (Day-29)**	**T4 (Day-36)**
Dry body weight	~	~	~	~
Whole-body protein content	~	~	~	~
Whole-body glycogen content	~	~	↑	↑
Whole-body triglyceride content	~	↑	↑	↑

Table 11: Summary of results of the biochemical assays for VBs as compared to VBCs in males. The arrows indicate the increase and decrease in the trait value, while ~ represents that there is no significant difference between VBs and VBCs.

	VBs wrt VBCs	
Trait	**Females**	**Males**
Desiccation resistance	↓	↓
Starvation resistance	~	↓
Chill Coma Recovery time	~	↑
Cold Shock Survival	↑	↓
Heat Shock Survival	↑	↑

Table 12: Summary of results of the stress responses of VBs as compared to VBCs in females and males. The arrows indicate the increase and decrease in the trait value, while ~ represents that the direction of change was not the same for all blocks.

Chapter 4 Discussion

Dispersal selection did not affect the dry body weight of the flies

Larger organisms are expected to have higher dispersal ability since they can store more resources. Indeed, Several studies have previously shown that there is a positive correlation between body size and dispersal ability (Dingle *et al.*, 1980; Gu and Danthanarayana, 1992; although see Sutherland *et al.*, 2000). However, we have not seen any significant difference in the dry body weight of the flies (Figure 1), which could be taken as a proxy for their body size (Sequeira and MacKauer, 1993; MacMillan *et al.*, 2009). This observation suggests that VBs and VBCs have a similar amount of resources that make up their dry body weight. So, VBs might have evolved a more efficient way of using their existing resources to keep up with the higher locomotor activity. It might also be possible that even though the total amount of resources stored has not varied between VBs and VBCs, VBs might have evolved to allocate these resources differently. To check the possibility that allocation patterns of the major macromolecules have changed, we looked at the stored metabolites of the selected population and their controls.

Dispersal selection led to an increase in the glycogen and triglyceride content of the flies

Glycogen and triglycerides are the primary stores of energy in an organism (Arrese and Soulages, 2010). Since dispersal is an energy-intensive process, one would expect an increase in glycogen and triglyceride levels in the populations selected for increased dispersal. As expected, VBs have higher glycogen and triglyceride levels than VBCs. However, this difference was only visible at later time points. VBs and VBCs have similar glycogen and triglyceride levels at the initial time period T1, which is the closest time point to the age at which selection acts in our populations (Figure 3,4). This observation is counterintuitive as one would expect the difference in triglyceride and

glycogen content to be maximum around the time the flies are expected to disperse in their selection regime.

To explain this observation, we note that a previous study conducted in our lab showed that VBs have higher locomotor activity than VBCs across their age (Tung *et al.*, 2018a; manuscript under preparation). However, this difference in locomotor activity between VBs and VBCs is maximum during early life and decreases with age. VBs are also known to have higher respiration rate and cellular respiration rate than VBCs (Tung *et al.*, 2018a; manuscript under preparation). Therefore, it is possible that the VBs might need more glucose than the VBCs to maintain the increased locomotor activity, particularly during the early period of their life. However, it is also known that the amount of glucose readily available to an organism is limited (Bender and Mayes, 2016). Thus, it is possible that although the VBs have evolved higher amounts of glycogen and triglyceride, they are forced to use up this excess glycogen and triglyceride at early time points as they need more energy because of their higher locomotor activity. This could have caused this difference to disappear, leading to the net glycogen and triglyceride content being similar between VBs and VBCs at the early time point. Since the locomotor activity of VBs decreases with age, they might not be using all the additional glycogen and triglycerides at later ages, making the difference appear at later ages.

Dispersal selection led to a decrease in the desiccation resistance of the flies

In *Drosophila melanogaster*, desiccation resistance is positively correlated with the amount of glycogen stored (Graves *et al.*, 1992). Since both VBs and VBCs have similar glycogen levels remaining in their body at T1, one would expect no difference between the desiccation resistance of VBs and VBCs (measured at day 12 after egg collection). However, we have seen that VBs have lower desiccation resistance than VBCs (Figure 5), which is consistent with a previous study conducted on this population (Mishra *et al.*, 2018). This could be because the flies could not replenish their glycogen content once the desiccation resistance assay was set up. Since VBs use more glycogen because of

their increased locomotor activity, VBs might be exhausting their glycogen reserves faster than VBCs. This could have led to VBs having lower desiccation resistance than VBCs.

Dispersal selection led to a decrease in the starvation resistance and an increase in the chill coma recovery time of the flies

Studies have shown a positive correlation between lipid content and starvation resistance (Graves *et al.*, 1992; Djawdan *et al.*, 1998; although see Hoffmann *et al.*, 2001). In insects, lipids are primarily stored as triglycerides (Arrese and Soulages, 2010). Given that VBs and VBCs exhibit similar levels of triglyceride content at T1, they might be expected to display similar levels of resistance to starvation. However, we observed that VB males exhibit lower starvation resistance than VBC males (Figure 6.1). This could be due to the fact that the flies were unable to replenish their triglyceride levels after the setup of the starvation resistance assay. As a result of their increased locomotor activity, VB males may consume their triglyceride reserves faster than VBC males, leading to a more rapid depletion of these resources and ultimately lower starvation resistance in VB males. Interestingly, we also found that VB males recovered slower from chill coma when compared with VBC males (Figure 7.1). If recovery from a chill coma were also dependent on their resource levels, then VBs would be expected to take a similar time for recovery as VBCs. However, the physiological processes behind recovery from a chill coma in *Drosophila melanogaster* are not yet understood, and more studies need to be done in this direction.

In females, we have seen a variation in starvation resistance between different blocks. VB females have higher starvation resistance than VBC females in Block-1 and Block-4, whereas VBC females have higher starvation resistance than VB females in Block-3 (Figure 6.2). Similar variation was also seen across blocks in their time to recover from a chill coma (Figure 7.2). This could be due to the fact that these populations have not been directly selected for any of these resistance traits. So, while trying to attain higher dispersal propensity and ability, each block might have followed a different trajectory of

evolution as they are maintained independently and therefore experienced different trade-off patterns.

It should, however, be noted that starvation resistance and chill coma recovery followed similar trends. VB males had lower starvation resistance and took longer to recover from chill coma than VBC males (Figure 6.1, 7.1). Similarly, VB1 females had higher starvation resistance and took less time to recover from chill coma than VBC1 females, whereas VB3 females had lower starvation resistance and took longer to recover from chill coma than VBC3 females (Figure 6.2, 7.2). This suggests that there might be some common mechanisms underlying starvation resistance and chill coma recovery.

Dispersal selection led to an increase in heat resistance in both sexes and sexual dimorphism in cold resistance

Studies have shown that in both sexes, survival after heat shock and cold shock shows opposite trends when compared between flies reared on carbohydrate-rich and protein-rich diets (Andersen *et al.*, 2010; Sisodia and Singh, 2012). We observed that, as compared to VBC males, VB males had higher survival after heat shock but lower survival after cold shock. However, we have also noticed that this antagonistic relationship between heat and cold shock resistance did not appear in females (Figure 8, 9). Those previous studies have only considered the nutrient content of the diet and not the amount of metabolites being stored in the flies. This suggests that the relationship between metabolite content and resistance to thermal stress could be more complex and needs to be studied in more detail.

Glycerol is known to act as a cryoprotectant in arctiid moths (Layne *et al.*, 1999; Marshall and Sinclair, 2011). If the selected populations are metabolizing triglycerides at a higher rate, they might have more free glycerol in their hemolymph compared to the controls, leading to increased survival after a cold shock in VB males. This is a testable prediction that can be verified in these flies in the future.

Conclusions

One takeaway from this study is that selection for increased dispersal at an early age has led to a change in the metabolic profile of the organism, causing an increase in the amount of glycogen and triglycerides stored. However, the change was not apparent in early life, when the force of selection was acting. This could probably be because of their higher locomotor activity which caused the difference to become apparent only in later life. One could test this hypothesis by checking if VBs have a higher amount of glycogen and triglycerides than VBCs at the time of eclosion. We have also looked at the variation in the protein content due to dispersal selection but did not find any specific pattern.

Selection for increased dispersal also led to changes in the stress responses of the organisms as compared to the controls. The selected populations have experienced trade-offs in their response to desiccation stress in both males and females and starvation stress in males. The males from the selected populations also took longer to recover from a chill coma. However, we have seen that selected populations had increased survival after heat shock. We have also seen a dimorphism in their survival after a cold shock, with selected females showing an increase in survival, while the males displayed a decrease in survival.

Taken together, these results show that dispersal selection alters the stress resistance patterns as well as the metabolite profile of the selected populations. Several natural populations are likely to be under intense selection pressure to increase dispersal. Our results suggest that such organisms might be worse off in facing stresses like starvation and desiccation. More crucially, since the levels of metabolites like lipids, proteins, and glycogens might change, the dispersal selected organisms might be more susceptible to a suite of metabolic diseases (Abdel-Maksoud and Hokanson, 2002; Lissner and Schneider, 2018), which could have further effects on their fitness under natural conditions. While it is impossible to predict which natural populations will show the same evolutionary response as our laboratory-reared populations, it is also not possible to assert that no natural population will do the same. Thus, our results suggest that it is

essential to exercise caution when predicting the effects of climate change on dispersal, as these changes could have significant impacts on the ability of organisms to adapt and survive in changing environments

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