Insights into desiccation resistance and related traits in *Drosophila melanogaster*: sex-specific genetic architecture and selection

Callum Levett

Thesis submitted to the University of Ottawa
in partial Fulfillment of the requirements for the
Master of Science in biology

Department of Biology
Faculty of Science
University of Ottawa

Acknowledgments: It is difficult to express how it feels to be here at the final steps of my degree: anyone who has worked on a project this large I'm sure can relate. Part of me can't believe it's over while another part is immensely proud that my hard work has resulted in something truly mine; a piece of work that I can celebrate, a goal accomplished and evidence of the skills I have cultivated which I will carry with me into my future. The final part of me feels so deeply grateful, as I would never have been able to complete this without the support of those around me.

Firstly, to my supervisors Vincent Careau and Howard Rundle, who have spent the last two years supporting me through my academic development, thank you for the opportunity to complete this work. Vincent, thank you for seeing potential in me and for the allowing me the space to prove myself. Thank you for fostering a welcoming environment where I could discuss ideas with you and my peers with mutual respect: I always felt that you took my opinions seriously no matter how much we disagreed on a topic. Howard, thank you for looking past my quiet first impressions and allowing me to open up. Thank you for all your guidance, your help has been so valuable, and I appreciate that you challenged me to improve; my writing now far exceeds what I thought I was capable of. I am so grateful for you both and your influence has allowed me to grow so much.

To Heath MacMillan and Charles Darveau, thank you for assisting me in improving my thesis. I found the discussions we had during my defence to be rewarding and provided me with a new perspective. I am grateful for your time and wisdom throughout the process, and my writing benefitted greatly.

To all the members of my lab thank you for accepting me, for your friendship and for the opportunity to learn from you. Wyeth, thank you for being my go-to person to bother in the lab, I appreciate the help you provided, both in completing my work and avoiding it. Nico, thank you for putting up with me and Wyeth and for your supportive nature, I always knew who to go to when I needed reassurance. Kenny thank you for teaching me everything I know about fly handling and for not making me come in early to learn it. Max, thank you for going through the accelerated program before me, then letting me bother you with questions about it, and for being so welcoming and friendly. Paige thank you for bringing me to volleyball and for respecting my perspective even when we disagree. Mark thank you for laughing with me when we were stuck counting flies. I am better for having known each of you.

To my parents thank you for the love and support you always gave me, I appreciate you helping me through a second degree (even though you wished I would only do one). You're both so foundational to who I am as a person, and I am forever grateful.

My friends are so important to me and without them I'm not sure I could have finished this degree. Thank you all for encouraging me and cheering me up over this last year. I will always miss our Wednesdays.

They say it takes a village and without my village the following thesis would never have been possible.

Abstract: Desiccation resistance is a key determinant of insect survival in terrestrial environments. While desiccation resistance often differs between the sexes, its sex-specific quantitative genetic architecture remains incompletely understood. Prior work, either explicitly or as a function of experimental design, tend to focus on females, and quantitative genetic studies, using individual-based measures, are rare. Using a pedigreed population of *Drosophila melanogaster*, I took many individual measurements of desiccation resistance (survival under extreme desiccation stress) alongside three traits that may underlie variation in this – metabolic rate, locomotory activity, and dry body mass. This allowed sex-specific additive genetic (co)variances for these traits to be estimated using quantitative genetic animal models, as well as phenotypic selection gradients. All traits were heritable in both sexes, with evidence for sex differences in additive genetic variance and covariance structure. Genetic correlations among traits differed in magnitude and sometimes in sign between males and females, but the uncertainty around individual estimates were too large to infer statistical significance. Cross-sex within-trait genetic correlations were generally close to one, except for locomotor activity, suggesting some potential for independent evolutionary responses in males and females. Using survival time under desiccation as a proxy for fitness, I found pronounced sex differences in viability selection. Selection differed significantly between the sexes overall, driven primarily by differences in selection on metabolic rate and body mass. In both sexes, survival under desiccation stress was associated with lower activity and larger body mass. In males, survival under desiccation was also associated with lower metabolic rate, while there was no evidence of selection on metabolic rate in females. Correlational selection also differed between sexes, with males exhibiting a more complex fitness surface. These results show that desiccation resistance is a multivariate trait shaped by sex-specific genetic architecture and sex-specific selection, emphasizing the importance of incorporating sex and individual-level variation when studying desiccation resistance and its evolution.

Résumé : La résistance à la dessiccation est un facteur déterminant de la survie des insectes en milieu terrestre, mais son architecture génétique spécifique au sexe reste encore mal comprise. Les travaux antérieurs, que ce soit explicitement ou indirectement par biais expérimental, tendent à se concentrer sur les femelles, et les études de génétique quantitative basées sur des mesures individuelles sont rares. À partir d'une population *Drosophila melanogaster*, j'ai pris une grande quantité de mesures individuelles de la résistance à la dessiccation (survie en conditions de dessiccation extrême) ainsi que trois caractères potentiellement sous-jacents : le métabolisme, l'activité locomotrice et la masse corporelle. Ceci a permis d'estimer les (co)variances génétiques spécifiques au sexe pour ces caractères, ainsi que les gradients de sélection phénotypique. Tous les caractères étaient héréditaires chez les deux sexes, avec des différences liées au sexe dans la variance et la structure de covariance génétiques additives. Les corrélations génétiques entre les caractères différaient en amplitude et parfois en signe entre les mâles et les femelles, mais l'incertitude entourant les estimations était trop importante pour détecter des différences significatives. Les corrélations génétiques entre les sexes étaient généralement proches de 1, à l'exception de l'activité locomotrice, suggérant un potentiel de réponses évolutives indépendantes chez les mâles et les femelles. En utilisant le temps de survie en conditions de dessiccation comme indicateur de la valeur sélective, j'ai observé des différences marquées entre les sexes en matière de sélection. Chez les mâles, la survie en condition de dessiccation était associée à un métabolisme plus faible, une activité réduite et une masse corporelle plus importante, avec des signes de sélection disruptive sur le métabolisme et l'activité. Chez les femelles, la survie en conditions de dessiccation était positivement corrélée à la masse corporelle et négativement à l'activité, sans sélection détectable sur le métabolisme. La sélection corrélationnelle différait également entre les sexes, les mâles présentant une surface de valeur sélective plus complexe. Ces résultats montrent que la résistance à la dessiccation est un caractère multivarié façonné par une architecture génétique et une sélection spécifique au sexe, soulignant l'importance d'intégrer la variation liée au sexe et à l'individu lors de la prédiction des réponses évolutives à l'augmentation du stress hydrique induite par le changement climatique.

Table of Contents

Introduction

One of the major challenges faced by insects living in terrestrial environments is water loss and the resulting desiccation stress it induces. As opposed to other terrestrial animals, insects are particularly susceptible to water loss due to their high surface-area-to-volume ratio and their respiratory physiology: thus desiccation stress can greatly impact their survival (Wang et al. 2021). Despite the problem imposed by desiccation, insects can be found in a wide range of environments, including those with relatively low humidity. The ability to survive in a low humidity environment is referred to as desiccation resistance. Insect species vary in desiccation resistance primarily due to variation in rates of water loss and total available water storage, as the amount of tolerable water loss varies little among species (Gibbs et al. 2003). Water loss in insects occurs through three pathways: excretion, respiration, and evaporation (Wang et al. 2021), although excretion does not appear to be an important source of water loss for all species (Gibbs et al. 2003). The dynamics of water loss may be impacted by sex (Chippindale et al. 1998; Nervo et al. 2021; Wang et al. 2019) and underlying physiological, behavioural and morphological traits, some of which have been observed to differ between low and high desiccation resistance populations (Wang et al. 2021). In the context of evolutionary physiology, a major aim is to understand the past evolution and future adaptive potential of desiccation resistance while accounting for any sex-based differences therein. This endeavour may become more critical as anthropogenic climate change accelerates and extreme climatic events, including droughts, become more frequent (Masson-Delmotte and Intergovernmental Panel on Climate Change 2023).

Metabolic rate is a measure of the energy expenditure of an individual over time which, in turn, impacts the resources an individual must acquire from its environment. Metabolic rate is

profoundly important in understanding ecology and physiology as it sets the pace of, and limits to, many biological processes (Brown et al. 2004). In insects, metabolic rate may be associated with desiccation resistance through metabolism of macronutrients which releases useable water (Chippindale et al. 1998) and through respiratory water loss. Metabolic rate in insects has often been studied in connection with desiccation resistance because higher metabolic rate requires more frequent opening of spiracles for gas exchange, exposing the respiratory system to increased water loss (Hadley and Quinlan 1993; Quinlan and Hadley 1993; Lighton et al. 1993). While respiratory water loss may not be a large contributor to total water loss during periods of low metabolic rate (Gibbs et al. 2003), as metabolic rate rises respiratory water loss becomes an increasingly large contributor to an insects' total water loss (Hadley 1994). Thus, behaviour and desiccation are likely linked, at least in part, via the metabolic effects of behaviour: behaviours involve muscle contractions, the energetic costs of which increase metabolic demands. Consequently, rates of respiratory water loss are known to increase substantially during energetically expensive behaviours (Chown and Nicolson 2004).

While the link between metabolic rate, behaviour and desiccation resistance appears intuitive, the contribution of body size is less clear. Larger body size decreases the surface area to volume ratio and provides increased capacity for water storage. Thus, all else being equal, larger size should decreases relative evaporative loss (Hadley 1994) and enhance desiccation resistance. Empirically, however, this relationship is not so straightforward. In some species of ants, for example, desiccation resistance does increase with dry body mass, but not as quickly as surface-area-to-volume ratio would predict (Hood and Tschinkel 1990). In a study of dung beetle species where males are dimorphic for size and females are the same size as the larger males, small vs. large males did not differ substantially in desiccation resistance and females had substantially

higher resistance than all males (Nervo et al. 2021). Additionally, in two species of lepidoptera, body size was shown to have very little effect on larval ability to resist desiccation (Woods and Singer 2001). These, and other results, imply sex-specific effects of body size on desiccation resistance.

Drosophilae are a cosmopolitan group that appear to have originated in sub-Saharan Africa and have subsequently adapted to many varied environments (Lachaise et al. 1988); desiccation has been a challenge faced, and overcome, by members of this family across its evolutionary history. In *Drosophila*, desiccation resistance has been studied extensively through comparative and genetic approaches, as well as in artificial selection studies, with results indicating that genetic variance for desiccation resistance appears to be common (Hoffmann and Parsons 1989; Rajpurohit et al. 2016), but not ubiquitous (Hoffmann et al. 2003). This, combined with the knowledge that in *Drosophila melanogaster* desiccation resistance is clearly a complex quantitative trait (e.g., over 1000 desiccation related genes have been identified in it and its sister species (Wang et al. 2021)), positions this species as a powerful model for a quantitative genetics study of desiccation resistance and traits that may contribute to it.

Of the three possible routes of water loss, evaporation and respiration are known to be the most import in *Drosophila* (Williams and Bradley 1998; Gibbs and Matzkin 2001), with excretion accounting for less than 6% of total water loss (Gibbs et al. 2003). In line with this, metabolic rate and water loss are strongly correlated among xeric insect species, with metabolic rate tending to be lower in desert-adapted *Drosophila* (Gibbs et al. 2003). Evidence for correlated evolution of metabolic rate in response to desiccation stress comes from artificial selection experiments and comparative studies (Blows and Hoffmann 1993; Hoffmann and Parsons 1993; Marron et al. 2003). Within several *Drosophila* species, metabolic rate has been

shown to decrease under selection for increased desiccation resistance. For instance, Blows and Hoffmann (1993) showed that metabolic rate was ~17% lower in *D. melanogaster* populations artificially selected for increased desiccation resistance, as compared to control populations. The association between metabolic rate and desiccation resistance is also likely affected by behaviour.

As noted above, behaviour is likely linked to desiccation through metabolic costs of movement, therefore examining energetically demanding components of behaviour may shed light on the relationship between behaviour and desiccation resistance. In this case, locomotory activity (hereafter referred to simply as activity) is an energetically expensive component of many more complex behaviours including foraging, predator avoidance and mate acquisition, making it relevant to understanding the connection between behaviour and desiccation resistance. Previous studies in *Drosophila* have generally shown activity under desiccation stress to be negatively associated with desiccation resistance (Hoffmann and Parsons 1989, 1993; Gibbs et al. 2003). In one artificial selection experiment by Hoffmann and Parsons (1989), activity under desiccation was found to be ~18% lower in populations selected for higher desiccation resistance. While activity in dry environments could be beneficial for escaping (i.e. finding less stressful microclimates), the disadvantages can be intuitively understood: activity in low humidity conditions will directly increase respiratory water loss. One may therefore expect that individuals would reduce their activity when exposed to severe desiccation stress. Studies on the consistency of animal behaviour, however, suggest that phenotypic plasticity can, in some cases, be limited (Sih et al. 2004) such that individuals may be restricted in the extent to which activity can be reduced in low humidity. As such, activity under ambient humidity may be

constrained by adaptation to desiccation resistance. Examining how general activity (in ambient humidity) relates to desiccation resistance presents an interesting avenue of exploration.

Body size has also been studied in relation to desiccation resistance in *Drosophila* and results are varied. Some studies have found that individuals with higher desiccation resistance were larger than those with lower resistance (Telonis-Scott et al. 2006; Rajpurohit et al. 2016), while others did not find any association (Hoffmann and Parsons 1989, 1993; Blows and Hoffmann 1993). In a selection experiment, Chippindale et al. (1998) found that *D. melanogaster* populations were sexually dimorphic for desiccation resistance, and this was associated in part with females being larger. While both sexes' average dry body mass increased when selected for greater desiccation resistance, selected females had substantially greater gains in desiccation resistance than selected males (Chippindale et al. 1998). The effect of body mass on desiccation resistance may therefore be sex-specific, but few studies have explored this.

Even though much work has been done on desiccation resistance in *D. melanogaster*, there are several shortcomings that suggest key gaps to be addressed. First, previous studies often employed experimental designs and/or analyses that ignored, or did not fully account for, sex-based differences, despite well-established sexual dimorphism in desiccation resistance. Indeed, some selection studies focused only on females (Hoffmann and Parsons 1989, 1993), while others selected both sexes together, resulting in most (if not all) males dying during selection (Djawdan et al. 1998; Chippindale et al. 1998; Williams et al. 1998; Folk and Bradley 2003; Rajpurohit et al. 2016). The result is that males were unintentionally selected for early breeding rather than for desiccation resistance (Chippindale et al. 1998). Some comparative studies have also focused on only one sex, for example one study on geographically disparate *D. melanogaster* populations only examined females (Parkash et al. 2009). Considering that key

genes associated with desiccation resistance may be sex specific (Fanara et al. 2024), that males and females often have different fitness optima for shared traits, and that genes benefiting one sex can sometimes be costly when expressed in the other (Cox and Calsbeek 2009), understanding sex-based differences may provide important context on how adaptation to desiccation is realized in populations.

A second shortcoming of many previous studies (both comparative and quantifying selection), is that desiccation resistance, metabolic rate, and/or activity, were measured on groups of flies rather than on individuals (Hoffmann and Parsons 1989, 1993; Parkash et al. 2009; Ferveur et al. 2018; Rajpurohit et al. 2018). This is likely because individual measures of metabolic rate are methodologically difficult in *D. melanogaster* given their small size and low CO_2 enrichment, (Burggren et al. 2017). Group measures of activity and desiccation are also convenient, requiring less laborious or sophisticated monitoring. Group measures, however, limit the avenues in which trait variation can be explored; only pre-determined groups can be compared, and within-group dynamics are impossible to examine. Additionally, social behaviour can greatly impact metabolic rate (and likely desiccation resistance) in *Drosophila* (Burggren et al. 2017). For example, aggregation behaviour may improve desiccation resistance through maintenance of microclimates (Chown and Nicolson 2004). Thus, group measurements of activity, metabolic rate, and desiccation may represent trait dynamics unique to the specific context (i.e., population density and sex-ratio) they are measured in. Finally, group measurements complicate, and often prohibit, estimation of additive genetic (co)variance as well as attempts to quantify selection.

With the above in mind, desiccation resistance is clearly a complex trait that is likely to reflect the combined outcome of many (possibly genetically correlated) phenotypes, the effects

of which may be sex specific. While studies have investigated associations among some of these, they have largely ignored the role of sex and have tended to employ group measurements. To address these issues, here I use *D. melanogaster* to quantify desiccation resistance (survival time under severe desiccation stress) and some key traits that are likely to impact it in. Building on past work, I phenotype individual male and female flies (i.e., avoiding group measurements), and my analyses account for possible differences between the sexes. This permits associations among traits to be quantified at the additive-genetic level, which is appropriate to understanding trait evolution. Additionally, by considering survival time under desiccation stress as a component of fitness, I quantify how selection acts on key traits impacting desiccation resistance in a sex-specific manner

Materials and Methods

Stock population

I used an outbred, laboratory-adapted population of *D. melanogaster* that was created approximately 40 generations earlier by pooling equal numbers of individuals from 15 replicate populations, described in the evolution experiment of Yun et al. (2018, 2021). Prior to pooling, these populations had evolved for >120 generations in the presence of mate competition that occurred each generation over a six-day period in 1.65 L cylindrical plastic Ziploc food storage containers (labeled $MC_{complex}$ in Yun et al. (2018, 2021)). These 'complex' mating containers had five separate patches of food (three 20 mL and two 10 mL cups) with subdivided surfaces, along with two coiled pipe cleaners protruding from the interior of the lid. Evidence suggests that these containers increased female control over mating interactions because, compared to standard

laboratory environments (e.g., *Drosophila* vials), sexual interactions and mating were less frequent and the expression of male harm was reduced or eliminated (Yun et al. 2017, 2019, 2021). Adaptation to this mating environment also resulted in the evolution of less harmful males in this or more standard lab-environments (i.e. vials; (Yun et al. 2021)). The populations were also subjected to a novel rearing environment that altered the food and the temperature they experienced as larvae (three different larval environments), as described in Yun et al. (2018).

After pooling the 15 populations, the novel rearing environments were eliminated and the new population was maintained on a cornmeal/yeast medium (9 g/L agar, 30 g/L yeast, 62 g/L cornmeal, 130 g/L sugar, 6 mL/L propionic acid), matching that of the ancestor of the evolution experiment, at standard and constant temperature (25°C), relative humidity (50%), and photoperiod (12L:12D). The complex mating environment was maintained each generation, with 35 males and 35 females being placed in each of eight containers to interact and mate for six days, with food sources replaced after three days to reduce mortality resulting from flies becoming lodged in deteriorating food. At the end of the six days, flies were anesthetized, males were discarded, and 120 females were randomly chosen and divided among eight standard *Drosophila* vials. Females were allowed to lay eggs for 24 hours and then discarded; once offspring emerged from these vials, they were pooled, and eight groups of 35 males and 35 females were randomly chosen with each group and allocated to one of eight new containers as the beginning of the next generation. To facilitate the current experiment, a copy of this stock population was derived from it and maintained following the same procedure but on a schedule offset by one week.

Experimental design

A breeding design was performed to create replicate parent-offspring families, consisting of both parents and two offspring (one male and one female). Data were collected from parents and offspring for activity, metabolic rate under desiccation stress, desiccation resistance, and dry body mass (hereafter referred to as body mass), providing the necessary information to estimate additive genetic variances (V_A) and covariances (COV_A) for these traits (i.e., the **G** matrix) using an 'animal model' (Kruuk 2004; de Villemereuil et al. 2013). Here metabolic rate refers to whole animal CO_2 emissions as a proxy for metabolic rate, measured on individuals under very low (~0%) humidity, who do not have their movement or food intake restricted. By taking measurements on related individuals (parents and offspring) that do not share an environment, I maximized the statistical power to estimate V_A and hence the narrow-sense heritability (h^2). I also explored sex-based differences in the genetic (co)variances.

The parents of each family were collected within approximately six to eight hours after eclosing to ensure that they were unmated (virgin). These flies were anesthetized with CO_2, then single male-female pairs were randomly assigned and placed separately into standard *Drosophila* vials with 10 mL of media. In all instances of CO_2 anesthetization steps were taken to ensure that exposure to CO_2 was minimized, reducing any possible impacts on behaviour or physiology. The lowest possible dose rate was used during handling, exposure time was limited, CO_2 was humidified prior to use and flies were only anesthetized when necessary. After 2-3 days, sires were removed and phenotyped while the dams remained one day longer, after which they were also removed and phenotyped. The larvae were then left to mature and subsequently eclose as adults, at which time two male-female offspring pairs per family were moved to holding vials (two pairs were created to ensure at least one male and one female were healthy and alive for

testing). To match the age, social environment and breeding status of their parents, offspring pairs were left for approximately two days. One of the two males was then randomly chosen (avoiding any obvious defects) to be phenotyped, followed by one female chosen and phenotyped the next day. For each block, approximately 80 families were created, with 64 of those that produced sufficient offspring haphazardly chosen for use. All parents in each block were collected during the same 24 h window to control for parental age, although age was somewhat confounded with sex as females were always one day older than their male counterparts when phenotyped.

All flies were phenotyped individually as follows. First, flies were anaesthetized using CO_2 and loaded into a monitor where they underwent 24 hours of activity recording. Subsequently, flies were transferred to individual flow-through respirometry chambers and had their metabolic rate and activity monitored for 20 hours at near-zero humidity. All flies died at some point during this period, most likely from severe desiccation stress (if the air stream is re-humidified, mortality is not observed over ~24h). Finally, after respirometry, dead flies were transferred to labelled Eppendorf tubes and dried prior to body mass measurements. Tubes had holes in the top and the flies were left in a sealed container with 'Drierite' (W.A. Hammond Drierite Co. Ltd., Xenia, Ohio) for at least five days (preliminary tests determined that mass decreased steadily until this point, after which it remained stable). Body mass was measured using an mx5 microbalance (Mettler, Toledo, Columbus, Ohio) to the nearest µg, and flies were then frozen for possible future use.

Activity measurements

Activity under ambient humidity was measured using *Drosophila* activity monitors (DAMs; Trikinetics, Waltham, Massachusetts). Each DAM has the capability to concurrently monitor the activity of 32 separate flies, each in their own 'chamber', and each experimental block used two monitors, allowing a total of 64 individuals to be measured. The monitors were housed within a dedicated incubator that maintained the same temperature, humidity and light/dark cycle as the stock population, and it was not opened during a 24 h trial. Flies were randomly assigned a monitor and chamber number, dosed with CO_2 then placed into 5 mm polycarbonate tubes (the 'chamber') with cotton at one end and standard *Drosophila* food at the other. An infrared beam bisects the center of each tube and is received by a photo transistor. Activity is measured as the total number of interruptions to this beam caused by fly locomotion, compiled into five-minute bins. The monitors were connected to a computer outside of the incubator that was actively running DAM system 303X software (Trikinetics). The flies were loaded into the activity monitor between 4 – 6 pm, depending on the block. After the 24-hour activity measurement period was completed, flies were removed from the DAM and transferred directly into the flow-through respirometry system without the use of CO_2.

Measurements Under Desiccation Stress

All measurements under desiccation stress were taken using one of four Multiple Animal Versatile Energetics (MAVEn) flow-through units (Sable Systems, North Las Vegas, Nevada). For each MAVEn unit a single stream of air is fed into the reference (A) channel of an external gas analyser (LiCor LI-7000) and then is split, providing separate regulated air flow into each of

the 16 chambers and a baseline; each flow is then routed to the measurement (B) channel of the analyser in successive order. A total of four MAVEn units were housed together in an incubator with temperature control set at 25°C. Building compressed air was first routed through a purge gas generator, then through a mixing tank and two chemical scrubbing units containing soda-lime and Drierite® to remove any remaining CO_2 and water vapor. During standard procedures for quantifying metabolic rate, the air is then re-humidified before entering the chambers to avoid desiccation stress (e.g., Videlier et al. 2019). In the current experiment however, the dry air was routed directly to the chambers, generating severe desiccation stress and causing the flies to die much more rapidly. Metabolic rate of each fly was monitored for 90 seconds every 26 minutes (90 s per chamber for 16 chambers, plus 120s baseline monitoring per cycle), with the analyzer recording the CO_2 level, flow rate, temperature, and H_2O. Flies were not immobilized during metabolic rate measures: locomotory activity under desiccation stress was constantly monitored in every chamber via an internal MAVEn activity board that uses three evenly spaced proximity infrared sensors.

Flies were randomly assigned to a MAVEn unit and chamber and loaded into their separate tubes without CO_2 anaesthesia. Recording started once the tubes were connected to the air flow, and the flies remained in the MAVEn for 20 hours receiving a constant flow of dry air, corresponding to 47 sample periods per chamber. While the incubator was set to maintain a temperature of 25°C, each MAVEn unit was on a different shelf and temperature varied somewhat across shelves (presumably because the units restrict the internal air flow, see Husain et al. (2024)). Data from each MAVEn unit were collected after the trial and processed using Expedata software (Sable Systems, North Las Vegas, Nevada). For each individual, metabolic rate under desiccation stress was determined as the average of the first six 90-second

measurements (corresponding to the first three hours of the metabolic run), and their average activity under desiccation stress was also extracted over this period. Six cycles were chosen to get the same number of measurements from each fly before the least desiccation-resistant flies began to die.

To quantify time to death under desiccation stress, two methods were explored. First, death was estimated as the point at which an individual's metabolic rate dropped below a threshold of 5×10^{-6} ml/min CO_2. There is necessarily bias in these estimates because of the post-mortem release of CO_2 (Lighton and Turner 2004), which was apparent in my data and caused an overestimation of desiccation resistance. Moreover, measuring time of death using CO_2 production is imprecise and will overestimate resistance because the air stream was multiplexed, and each chamber was monitored (for a 90 s window) only once every 26 minutes. The second, more precise, method of determining time of death used the continuous activity data. In this method, adapted from Lighton and Turner (2004), a linear regression is performed on the cumulative sum of activity over time, and the residuals are extracted. The highest peak of these residuals is used to determine when, in seconds, there was a sudden reduction in activity followed by no subsequent movement, thus indicating time of death (which is followed by a post-mortem release of CO_2). The two measurements of time to death (from activity or CO_2) were strongly correlated ($r = 0.96$). Individual replicates were also manually checked when the two methods yielded substantially different results. Method 2 (based on activity) was used in subsequent analyses because of its greater accuracy and precision.

Statistical analyses

I attempted to phenotype 1,536 individuals (6 blocks × 64 families/block × 2 offspring/family × 2 parents/family). However, due to accidental deaths, escapes, and minor equipment issues (e.g., some chambers lost pressure during trials resulting in unusable data and the MAVEn activity monitoring system failed in a few instances), the final sample size was 1,366 individuals. Statistical analyses used a combination of ASReml-R version 4.2 (Butler et al. 2023) for animal models and least squares regression models for the selection analysis (Lande and Arnold 1983). All continuous traits were standardized to a mean of 0 and a variance of 1, allowing for estimates extracted from models to be comparable both within model and to other studies (Schielzeth 2010). Sex was represented by a binary variable where females = 0 and males = 1.

In the initial phase of analysis, the parameters of interest were extracted from several multivariate animal models that used pedigree information to construct a relatedness matrix, which was included (as its inverse) as a random effect to estimate the additive genetic (co)variance and correlation matrix between the traits of interest. All animal models included four responses variables: activity, body mass, metabolic rate under desiccation stress, and desiccation resistance. Initially, to test significance of sex-based differences in genetic architecture, additive genetic variance was partitioned between sexes in a single model, the fit of which was compared to a reduced model where estimates were forced to the same value in each sex. This method, however, proved problematic for two reasons: 1) identical models, one with a correlational structure and one with a (co)variance structure, had equivalent log-likelihoods when unrestricted but did not when estimates were forced to be equal cross-sex, giving different results as to whether modelling separately by sex significantly improved the fit of the model. Although the log-likelihoods could be reconciled by changing starting values of the reduced model, the

initial differences suggest the existence of some local optima in the likelihood surface that one or both models were converging upon. 2) These types of models in ASReml-R assume that cross-sex within-trait correlations are zero when variances are partitioned between the sexes, and one when the model is reduced. More complex models that explicitly estimate these cross-sex within-trait correlations suggest these assumptions are inaccurate. Given these concerns, I instead opted to fit separate sex-specific models and perform likelihood ratio tests (LRTs) with reduced models in which each model's estimates were fixed to the values corresponding to the other sex's estimates, as well as to a full pooled-sex model. In this method, reduced models had equivalent log-likelihoods across correlation and (co)variance structures. The fit of both sex's model was significantly worsened when additive genetic (co)variance or correlations were fixed to values from the other sex, and the male model's fit was significantly worsened when values were fixed to those from (male-female) pooled estimates. It is important to note that this method is somewhat anti-conservative, since fixing a model to point estimates fails to account for error around the fixed values.

Given evidence of sex differences in genetic architecture, all phenotypes were treated as different traits in males and females, opening two avenues for estimating genetic parameters: 1) fitting 4×4 models separately for each sex ($\mathbf{G}_{male}$ and $\mathbf{G}_{female}$), or 2) fitting a single 8×8 model. While option #2 provides additional information in the form of the **B** matrix (the matrix of cross-sex genetic covariances/correlations), this asks a lot of the data, requiring estimation of 16 additional parameters at the additive genetic level. Considering the size of my data set, precision of these estimates was low and hence I opted to proceed primarily via the 1st approach, although following this I do present estimates from the 2nd approach, focussing interpretation on within-trait intersex correlations as these are of particular interest (see below).

Each block consisted of four distinct sets of measurements for each phenotype, these sets corresponded to groups of all the sires, dams, daughters, and sons measured separately within a block. To correct for mean differences among blocks, and among sets within blocks, a fixed effect representing the interaction of block and set was included for all traits. Importantly, mean differences were non-trivial between block-sets for most traits; calibration of the MAVEn (affecting metabolic rate), rearing density (affecting body mass and potentially desiccation resistance), and differences in stock preparation (food, incubators, etc.) created differences in the trait means among sets, both within and among blocks; because parents are not measured in the same block-set as their offspring, correcting for these differences was vital for detecting genetic signals. Given that males and females were never measured in the same block-set, the block-set effect is at some level confounded with sex: mean effects of block-set are, however, corrected for as described above likely rendering this confound minimal. A fixed effect that identified MAVEn unit was included where relevant (although its effect was non-significant) accounting for differences among units including the temperature differences between shelves. A fixed effect identifying DAM monitor was also included where relevant.

Multivariate animal models were fit in ASReml-R (Butler et al. 2023) using either correlation or covariance structures at the genetic and residual levels. From the correlation structure models, the narrow-sense heritability (h^2) of each trait was estimated as V_A divided by the sum of V_A and residual variance for that trait, and the associated standard errors were calculated using the delta method via the vpredict() function in ASReml-R (Butler et al. 2023). To test significance of sex differences in specific parameters (e.g. V_A in mass, or COV_A, mass-metabolic rate), I used the same approach as above for testing overall differences in **G** (i.e. the value in one sex was constrained at the estimate for the other and a LRT was used to test whether

this worsened the fit of the model via a χ^2 distribution with one degree of freedom) differences were considered significant only if both female and male model fit was significantly worsened. To test significant differences between cross-sex cross-trait estimates in the **B** matrix (i.e. does $\boldsymbol{r_A}$ between male mass and female activity differ from $\boldsymbol{r_A}$ between female mass and male activity) I used LRTs comparing the full model with a reduced version that forced estimates of interest to a joint value, via a χ^2 distribution with one degree of freedom. The significance of each r_A and V_A was determined via a LRT comparing the full model with a reduced version that constrained the parameter of interest to zero or one, via a χ^2 distribution with 0.5 degrees of freedom (Dominicus et al. 2006).

To gain additional insight into how male and female genetic architecture differs, I characterized the major axes of differentiation between $\mathbf{G_{male}}$ and $\mathbf{G_{female}}$ via an eigenanalysis of the difference matrix, following Jarvis et al. (2025). Subtracting $\mathbf{G_{female}}$ from $\mathbf{G_{male}}$ creates a new matrix, $\mathbf{G_{diff}}$. An eigenanalysis of this new matrix reveals the linear combination of traits for which variance between males and females differ the most (Sztepanacz and Rundle 2012; Walter et al. 2018). This procedure is quantitatively equivalent to an eigentensor analysis (Sztepanacz and Rundle 2012; Aguirre et al. 2014), as described in Hine et al. (2009). Although the eigentensor approach can characterize differences in more than two **G** matrices (Sztepanacz and Rundle 2012), I am only interested in differences between $\mathbf{G_{male}}$ and $\mathbf{G_{female}}$, and hence I chose to use the $\mathbf{G_{diff}}$ approach as it is more straightforward to implement and interpret.

Separate analyses were performed to quantify selection, using survival time under desiccation stress as a fitness proxy for an individual's ability to survive a less extreme instance of desiccation stress (i.e., all individuals died in our assay, but I assume those that took longer to do so have a higher likelihood of surviving a shorter, or less severe, bout of desiccation).

Relative fitness of an individual was calculated by dividing its survival time under desiccation by the population average. Using ordinary least-squares regression models (Lande and Arnold 1983), variance standardized phenotypic values of each trait (i.e., locomotor activity under non-desiccation conditions, metabolic rate under desiccation stress, and body mass) were regressed on relative fitness to estimate linear (β) and non-linear (γ) selection gradients on these traits. Models were run separately for linear and non-linear selection. A model that allowed selection to vary between the sexes was compared, using a partial *F*-test, to a reduced model where selection was estimated jointly for males and females (Chenoweth et al. 2013). To test whether each individual selection gradient significantly differed between males and females, a model where all selection was allowed to vary between males and females was compared, using a partial *F*-test, to a model where each trait's gradient was estimated jointly (Chenoweth et al. 2013). Male and female data were then split, trait values were re-standardized, and relative fitness was re-estimated, separately within each sex. Sex-specific ordinary least-square models were then fit to estimate selection gradients in each sex separately. Significance of the selection gradients was estimated using two tailed *t*-tests. Finally, selection was visualized using thin-plate splines to approximate the fitness surface for pairwise combinations of traits (Blows et al. 2003). Thin-plate splines were created using the Tps function of the fields package in R (Nychka et al. 2014).

Results

Quantitative genetic architecture

Divergence in the underlying genetic architecture of the four traits was tested, separately by sex, by comparing an 'unconstrained' model that estimated **G** for that sex, to two different 'reduced

models', one in which estimates were fixed to the point estimate from the opposite sex and one in which they were fixed to point estimates from a pooled-sex model. In males, the unconstrained model was a significantly better fit compared to both a reduced model fixed to female estimates (LRT: $\chi^2_{10} = 100.09$, $P < 0.001$) and to pooled estimates (LRT: $\chi^2_{10} = 24.01$, $P = 0.008$). In females, the unconstrained model was a significantly better fit than one fixing to the male estimates (LRT: $\chi^2_{10} = 16.37$, $P = 0.037$), but not when fixing to pooled estimates (LRT: $\chi^2_{10} = 9.47$, $P = 0.488$). When estimated separately by sex, all traits had significant V_A and were heritable in both males and females (Fig. 1). A visual examination reveals higher V_A, and h^2, of three traits in females compared to males.

Males and females also differed substantially in the point estimates of several within-sex genetic correlations (r_A's; Fig. 1). For instance, r_A between activity and desiccation resistance was strongly negative in males and approached significantly different from zero (LRT: $\chi^2_{0.5} = 2.30$, $P = 0.054$) whereas it was weakly positive in females. Desiccation resistance and body mass were also negatively genetically correlated in males while weakly and positively correlated in females. Although metabolic rate was correlated positively with body mass in both males and females, the correlation was substantially stronger in males and significantly different from zero in both sexes (males: LRT: $\chi^2_{0.5} = 3.64$, $P = 0.022$, females: LRT: $\chi^2_{0.5} = 2.89$, $P = 0.036$). The r_A between body mass and activity in males and females had opposing signs: males had a weak negative correlation between body mass and activity, while females had a weak positive correlation. Despite marked differences in several correlations, and the significance of sex-based differences in **G** matrices, uncertainties (±se) for individual estimates were quite large meaning the origin of sex-based differences are difficult to ascertain from visual comparisons between $\mathbf{G}_{male}$ and $\mathbf{G}_{female}$.

To help characterize the differences between the $\mathbf{G}_{male}$ and $\mathbf{G}_{female}$ I performed an eigenanalysis of their difference matrix, $\mathbf{G}_{diff}$. This revealed the linear combination of traits for which genetic variance in males and females differed the most (the eigenvectors) (Table 1). Three of the four eigenvectors had substantial eigenvalues and, as such, are relevant, with the sign of the eigenvector indicating the sex with more variance in that trait combination (negative denoting greater variance in females, positive greater variance in males). The first eigenvector (e_1) indicated that females had more variance for a linear combination of traits which mainly contrasted desiccation resistance and metabolic rate. The second eigenvector (e_2) indicated that males had more variance for a combination of traits which contrasted activity to all others. The final eigenvector (e_3) had similar contributions from all traits and reflected the overall pattern of greater variance in females (e_2 reversing this for activity, the one trait for which males had greater variance).

Finally, for completeness I further examined sex-specific additive (co)variation and cross-sex cross-trait correlations through the estimation of the **B** matrix, which provides tentative information on how underlying genetic architecture interacts across sex (Fig. 2). Although no significant cross-sex cross-trait correlations emerged, likely due to the large data demands of this type of model, it is noteworthy that male metabolic rate was positively correlated with female desiccation resistance ($r_A = 0.286 \pm 0.286$), but the opposite was true for female metabolic rate and male desiccation resistance ($r_A = -0.538 \pm 0.610$), although this difference was not significant (LRT, $\chi^2_1 = 2.55$, $P = 0.11$). The main diagonal of **B** comprises the cross-sex correlations for each trait, which provide important information on the extent to which the genetic basis of a trait is shared between males and females. These estimates were close to (or

bounded to) one for all traits except activity (Fig. 2), which was relatively weak and significantly different from one (LRT: $\chi^2_{0.5} = 3.65$, $P = 0.028$).

Phenotypic selection

Partial F-tests indicated that linear and non-linear models allowing selection to vary by sex were a significantly better fit than models that estimated selection in a pooled manner (linear: $F_{3,1112,} = 27.61$, $P < 0.0001$; non-linear: $F_{6,1100} = 8.73$, $P < 0.0004$). Therefore, all analysis below proceeded in a sex-separated manner.

Linear selection on body mass and metabolic rate differed significantly between males and females, and differences in linear selection on activity approached significance (Table 2). Directional selection was concordant in males and females for body mass, favouring higher values in both sexes, and activity, favouring lower values in both sexes, although selection on body mass was substantially stronger in males than females (Fig. 3). There was significant selection favouring lower metabolic rate in males, but no detectable selection in females. In males, body mass had the largest linear selection gradient; the same was true in females, although selection was about half as strong (Fig. 3).

Patterns of non-linear selection also differed between males and females, although only differences in quadratic selection on metabolic rate, and correlational selection between metabolic rate and body mass, were significant (differences in correlational selection between activity and body mass also approached significance; (Table 2).) In males, there was significant stabilizing selection on body mass, but significant disruptive selection on activity and metabolic rate (Fig. 4). In females, as with males, there was significant stabilizing selection on body mass

but no significant quadratic selection on activity or metabolic rate (Fig. 4). Quadratic selection on activity appeared visually similar between males and females despite the lack of significance in females (Fig. 4c). In males, there was significant correlational selection between body mass and metabolic rate and between body mass and activity, while no significant correlational selection was detected in females (Table 3).

The overall shapes of male and female fitness surfaces are generally congruent with results from the parametric linear and non-linear selection gradients above (Fig. 5). Males appear to have a peak survival time at just above mean body mass in both bivariate surfaces containing it (Fig. 5a, c), aligning with selection for higher body mass and stabilizing selection on body mass discussed above. Correlational selection between body mass and metabolic rate in males is also well represented, with survival time peaking at slightly above average body mass and low metabolic rate (Fig. 5a). Splines depicting activity in males are also consistent with estimated selection gradients. Male fitness surfaces are consistent with disruptive selection favouring low activity, as survival time peaks at high and low activity with the higher peak being at lower activity (Fig. 5b, c). The spline depicting male metabolic rate and activity together does show some possible correlated selection (Fig. 5b), the gradient of which only approached significance in males (Table 3). Splines depicting male metabolic rate are consistent with selection favouring low metabolic rate: survival time peaked at low metabolic rate. The splines however appear to depict stabilizing selection rather than disruptive, as survival time decreases at higher metabolic rate across body mass and activity ranges; although some local peaks can be seen (specifically at high body mass) (Fig 5a, b).

Female splines reflect positive linear selection gradients for body mass, and the lack of significant correlational selection detected between body mass and the other traits; survival time

also increases with body mass, across both metabolic rate and activity ranges (Fig. 5d, f). However, the fitness surface does not clearly represent the significant stabilizing selection on body mass detected in females: there is only a small peak at higher body mass ranges when modeled with activity (Fig. 5f). Female fitness surfaces depicting activity are consistent with selection weakly favouring low activity (Fig. 5e, f). Female splines are consistent with very weak selection on metabolic rate, as survival time appears mostly consistent across the measured metabolic rate range (Fig 5d, e). Some evidence for selection favouring high metabolic rate in females can be seen as fitness is greatest at high metabolic rate across activity ranges, although this effect is small (Fig. 5e).

Discussion

Desiccation stress resulting from water loss is a challenge faced by all terrestrial organisms, one that is particularly impactful to insects given their small surface-area-to-volume ratio and respiratory physiology (Hadley 1994). Many studies have examined desiccation resistance in *D. melanogaster*, although they have often focussed on only one sex and have tended to employ group-based measurements. Individual measurements, taken from related individuals of both sexes, allow for analysis of underlying genetic architecture and fitness surfaces in a sex-specific manner. Here, I estimated genetic (co)variances of desiccation resistance, metabolic rate under desiccation stress, activity under ambient humidity, and body mass, as well as selection coefficients of the latter three using survival time under desiccation stress as a proxy for fitness, in male and female *D. melanogaster*. Significant V_A was detected in all four traits in females and males, with some interesting patterns of covariance emerging both across and within sexes,

although uncertainty in these estimates was often wide and point estimates were generally non-significant. Females and males differed significantly in their overall genetic architecture, owing to complex differences that included discrepancies in V_A. Aspects of linear, quadratic and correlational selection all differed significantly between males and females, suggesting the potential for sexual conflict in adaptation to desiccation stress in this species.

Quantitative genetic architecture

Quantitative genetic studies of desiccation resistance are quite rare, likely owing to several difficulties in measuring a sufficiently large number of individuals. As such, the genetic architecture underlying desiccation resistance remains largely unknown (Fanara et al. 2024). Sex-specific aspects are particularly poorly understood because most studies focus only on one sex, either deliberately or as a consequence of experimental designs that caused selection to differ between the sexes (Hoffmann and Parsons 1993; Djawdan et al. 1998; Folk and Bradley 2003; Blows et al. 2003; Rajpurohit et al. 2016; etc.). Using multivariate animal models, I was able to detect significant differences in genetic architecture underlying desiccation resistance between male and female *D. melanogaster*. This is consistent with one previous study which found evidence of sex-specific genes associated with desiccation resistance (Fanara et al. 2024). The presence of significant differences in $\mathbf{G}_{\mathbf{male}}$ and $\mathbf{G}_{\mathbf{female}}$ indicates, generally, that adaptation to low humidity environments may manifest differently between the sexes, contrary to assumptions made in many previous studies. Additionally, although uncertainty in the **B** matrix was high for most point estimates, cross-sex within trait r_A for metabolic rate, body mass, and desiccation resistance were all high and not significantly different from one (P = 0.465, 1, and 0.9

respectively), implying a genetic constraint on sex-specific adaptation to desiccation stress and the possibility of intra-locus sexual conflict if there is sex-specific selection acting on these shared traits.

The exact nature of sex-based differences in genetic architecture is somewhat difficult to ascertain given the large uncertainties of the point estimates, however the eigenanalysis of $\mathbf{G}_{\mathbf{diff}}$ allows for some inferences to be made. For example, e_1 (the linear combination of traits for which genetic variance differs most), contrasts metabolic rate and desiccation resistance, with females having more variance for this trait. Greater additive genetic variance for desiccation resistance in females is evident from comparing male and female V_A for the trait: females however have higher V_A for metabolic rate. This indicates that males and females differ substantially in covariances involving these traits in addition to the obvious V_A differences. Additionally, e_2 reflects males having higher V_A than females for activity and lower V_A for all other traits, while e_3 has similar contributions from activity, metabolic rate and body mass, some of which conflict with the trait combinations in larger eigenvectors. Overall, the eigenanalysis of $\mathbf{G}_{\mathbf{diff}}$ makes clear that male and female genetic architecture differences are not limited simply to V_A but also encompassed more complex covariance differences.

Interpretation of differences in r_A between males and females is somewhat limited by large uncertainties in point estimates: differences between V_A in males and females are, however, more easily interpretable, with males have substantially less V_A for three of the four traits examined including desiccation resistance, indicating that males are likely to respond less readily to selection on those traits. A history of consistent sexual selection has been shown effective in reducing additive genetic variance (Hine et al. 2009) males then, may have experienced historic sexual selection which depleted variance for these traits in a sex-specific manner.

Consistent sexual selection on males can result from both male-male competition and female mate choice (Andersson 1994) both of which have been observed in *D. melanogaster* (Ferveur and Sureau 1996; Yun et al. 2021). There is some evidence that there may be a trade-off between desiccation resistance and reproductive success in *D. melanogaster* which affects males more than females (Kwan et al. 2008). In males, the need for costly behaviours related to courtship and mating may prevent males from achieving their desiccation resistance optimum causing long term directional selection on traits related to desiccation to persist. Consistent sexual selection on desiccation resistance may be a result of cuticular hydrocarbons (CHCs). Insects, including *D. melanogaster,* use CHCs to waterproof the cuticle, making them vital to desiccation resistance (Ferveur et al. 2018). CHCs are also used in chemical communication and are a target of female mate choice (Ferveur and Sureau 1996; Grillet et al. 2012); the persistent sexual selection which results from this may reduce variance in male CHCs, and therefore in desiccation resistance as a by-product. Behaviours could also be affected, as sexual selection has been shown to decrease variability of male behaviour in *D. melanogaster* (Brand et al. 2024). Although sexual selection offers an intuitive explanation for lower V_A, two caveats exist to this interpretation; first, males had higher V_A for activity than females, but more active males have higher reproductive success (Long and Rice 2007) and therefore we would expect the same depletion of V_A. Consider though that ambulatory locomotion is only one component of various behaviours which may not play a large role in mate competition and would thus not be under indirect selection. Second, selection is not the only evolutionary process that could act here; it is somewhat reasonable to assume genetic drift does not affect males and females differently as they share the same autosomal genome, but it is not possible to rule out genetic drift or mutation entirely.

Phenotypic selection

To examine the potential role of selection in shaping adaptation to desiccation stress, I used survival time under extremely dry (and fatal) conditions as a proxy for fitness, with the assumption that individuals that die rapidly would be less likely to survive (and subsequently reproduce) when naturally exposed to bouts of desiccation stress. Under this assumption, overall linear, quadratic and correlational selection differed significantly between males and females, indicating that fitness optima were not the same between the sexes. This, in combination with differences in genetic architecture, provides evidence that adaptation to desiccation stress in *D. melanogaster* could result in different outcomes in males and females.

Differences in selection on metabolic rate were perhaps the most striking. Female survival time remained largely unaffected by metabolic rate under stress, showing only non-significant and weak linear, quadratic and correlational selection coefficients. By contrast, in males there was strong and significant negative directional and disruptive selection on metabolic rate, in addition to correlational selection with body mass. The selection on metabolic rate in males is consistent with prior artificial selection studies in which selection for increased desiccation resistance resulted in a correlated response of lower metabolic rate (Hoffmann and Parsons 1989, 1993). An apparent contradiction, however, is that artificial selection studies were primarily focussed on female flies, but I did not detect linear selection on metabolic rate in females. One possible explanation for the sex differences in selection on metabolic rate is body size: larger individuals can both store more water (Gibbs et al. 1997) and have less relative surface area to lose water over, which could mean metabolic rate has a negligible impact on survival time in females (the larger sex), but metabolic rate is relatively more important in males (the smaller sex).

Why then do other studies find metabolic rate decreases when selecting for increased desiccation resistance? My results suggest that metabolic rate and desiccation resistance are strongly negatively correlated at the genetic level, meaning that indirect selection could occur on metabolic rate in females. It is difficult to imagine however, that reduced metabolic rate is simply coincidental given that water loss rates represent the primary reason for differences in resistance across species, and that reduction of respiratory water loss is a major strategy to reduce overall water loss (Gibbs et al. 2003). This may come down to differences in metabolic rate that are not captured in the present study; desert-adapted *Drosophila* species have shown cyclic CO_2 release (Gibbs et al. 2003), something that *D. melanogaster* is also capable of (Williams and Bradley 1998) and could present as reduced metabolic rate (although respiratory patterns can change independent of metabolic rate). Additionally, a major difference in the present vs previous studies is the use of individual measures: social dynamics have been shown to alter metabolic rate in *D. melanogaster* (Burggren et al. 2017), so decreased metabolic rate in response to selection in studies taking group measures, could be a function of adaptive female social dynamics (i.e., grouping). Under the conditions tested, I found little evidence that female metabolic rate affects survival in a bout of extreme desiccation stress. Males also had significant disruptive selection on metabolic rate, although this pattern may be influenced by the significant correlational selection with body mass: a small peak in relative survival time was present in the male fitness surface when both body mass and metabolic rate were high (Fig. 5).

Both sexes experienced positive directional selection and stabilizing selection on body mass (Fig. 4), with the apparent peak for each sex occurring just below the limit of measured ranges. Given the decline in relative fitness at higher body mass, very large males may not perform as well as females of the same size; this decline may be, at least in males, the result of

correlational selection with metabolic rate, or of factors not studied here such as macronutrient storage differences (Djawdan et al. 1998). Overall, this may be consistent with the assertion that larger body mass allows for greater water storage relative to surface area (Gibbs et al. 1997) but offers some confirmation that male and female differences in desiccation resistance are not purely a function of size.

The significant correlational selection I found between body mass and metabolic rate in males seems particularly important considering the long-lasting interest in the evolution of metabolic scaling (White and Kearney 2014; Careau and Glazier 2022). White et al. (2019) proposed that sublinear metabolic scaling allometry is due to correlational selection favouring small and large individuals with, respectively, high and low body mass-specific metabolic rates. When some combinations of metabolic rate and body mass are advantageous over others, such correlational selection should, over time, alter trait covariances (Sinervo and Svensson 2002) and as a result the allometry exponent (Careau and Glazier 2022). I am aware of only three studies that have tested for correlational selection on body mass and metabolic rate (Artacho et al. 2015; White et al. 2019; Videlier et al. 2021) of which, the former two also found evidence for correlated selection between body mass and metabolic rate. In my study, the observed negative correlational selection between metabolic rate and body mass would suggest that over time, correlational selection should change trait covariance towards the observed sublinear allometry exponent. Obviously, more research is needed on this topic but results from my study suggests that forms of selection associated with desiccation stress might play a role in the metabolic scaling of insects.

Selection for activity was also relatively similar between males and females with lower values favoured in both. This is consistent with previous findings in selection and comparative

studies which found activity to be lower in flies with higher desiccation resistance (Hoffmann and Parsons 1989, 1993; Gibbs et al. 2003). Behavioural plasticity has previously been shown to be limited (DeWitt et al. 1998; Sih et al. 2004) and, given that I measured activity in ambient humidity, these results suggest that flies may not be capable of fully altering their behaviour in different conditions: activity in ambient conditions should (presumably) have no direct effect on desiccation resistance.

In males, the effect of activity on survival time is somewhat more complicated as male activity has significant (but weak) disruptive selection and significant correlational selection with body mass. Correlational selection with body mass may be the basis of disruptive selection as high activity improves fitness only at median body mass ranges (Fig. 5). How then could high activity improve male fitness and why does the effect appear body mass specific? One possible explanation involves energy storage and nutrient metabolism. Several studies have found that increased glycogen storage and/or metabolism is associated with higher desiccation resistance (Djawdan et al. 1998; Chippindale et al. 1998; Marron et al. 2003), and that metabolism of glycogen releases usable water (Marron et al. 2003). While in the DAM flies do have access to food, but activity is measured through beam breaks meaning, that higher activity individuals may spend less time eating (i.e. breaking the beam requires leaving the provided food). Then, during desiccation testing, flies are subjected to a small starvation stress; more active individuals may thus be more susceptible to starving during desiccation testing. This becomes relevant as some insect species have been shown to preferentially use carbohydrate stores when subjected to starvation (Jutsum and Goldsworthy 1975; Auerswald and Gäde 2000); while available evidence does not suggest this is the case in *D. melanogaster* (Marron et al. 2003), Marron et al. (2003) found that *D. melanogaster* do metabolize a substantial amount of glycogen when starved.

Potentially then, individuals who move around more (and eat less) are more affected by the light starvation and therefore benefit from increased glycogen metabolism.

High activity may also be associated with longer survival due to condition: longevity has been shown to increase with selection for desiccation resistance (Rose et al. 1992), indicating that being "healthy" may be beneficial to surviving longer in low humidity. Selecting for longevity has also been associated with increased activity (Vermeulen et al. 2006). Thus, individuals with high activity may be more long-lived (i.e. higher condition/ healthier) which may provide benefits under desiccation stress. In males specifically, high activity is also associated with higher reproductive success under mate competition (Long and Rice 2007); strong and healthy males are likely those with an advantage competing for mates (male-male competition, mate guarding, etc.) and therefore high activity may be an indication of health. Although this trend does not hold true for the largest and smallest males. Overall, selection is likely to favour males with either high or low and females with low activity in ambient humidity.

Conclusion

Through individual measurements on related flies of both sexes, I gained insights into the underlying sex-specific genetic architecture of metabolic rate under desiccation stress, activity in ambient humidity, dry body mass and desiccation resistance. Males and females differed significantly for their genetic architecture indicating the possibility of sexual conflict in the evolution of desiccation resistance. I also gained insights into how selection during bouts of desiccation may affect metabolic rate, body mass, and activity in a sex-specific manner. This analysis illuminated the possible sexual conflict that could occur as males and females did not

necessarily share the same fitness optima. Adaptation to desiccation stress is likely to be limited by intra-locus sexual conflict, especially considering the strong cross-sex within-trait additive genetic correlations between three out of four traits examined. Overall, this study highlights the importance of considering the genetic architecture underlying desiccation resistance and selection acting on related traits in a sex-specific manner. An important goal for future studies would be to attempt to gain more insight into specific genetic covariances through increased sample size. Investigation of different components of fitness could also provide more context to the adaptive potential of desiccation resistance.

Tables

Table 1. Eigenvectors associated eigenvalues and trait loadings from the eigen analysis of $\mathbf{G}_{diff}$ (the difference matrix from the subtraction of $\mathbf{G}_{female}$ from $\mathbf{G}_{male}$). Click here to return to first in-text mention of this table.

		Trait Loadings			
Eigenvector	Eigenvalue	Activity	Metabolic Rate	Body mass	Desiccation Resistance
e_1	-0.164	-0.197	0.529	-0.048	-0.824
e_2	0.122	0.877	-0.238	-0.228	-0.349
e_3	-0.104	-0.417	-0.560	-0.681	-0.220
e_4	-0.018	0.134	0.591	-0.694	0.388

Table 2. Results of partial *F*-tests, testing for significant differences between male and female selection gradients, boldness denotes significant differences. Click here to return to first in-text mention of this table.

	F	a	*b*	*P*
Linear selection				
Activity	3.188	1	1374	0.074
Metabolic rate	**79.790**	**1**	**1374**	**<0.001**
Dry body mass	**27.056**	**1**	**1374**	**<0.001**
Quadratic selection				
Activity	0.266	1	1362	0.606
Metabolic rate	**22.229**	**1**	**1362**	**<0.001**
Dry body mass	1.201	1	1362	0.273
Correlational selection				
Activity × metabolic rate	0.741	1	1362	0.082
Activity × dry body mass	3.034	1	1362	0.389
Dry body mass × metabolic rate	**4.300**	**1**	**1362**	**0.038**

Table 3. Linear and non-linear selection coefficients from sex-specific multiple regression models of relative fitness as function of activity, dry body mass, and metabolic rate. Coefficients were estimated from sex-specific models where relative fitness was calculated from survival time under desiccation stress (individually for each sex). Boldness denotes significance. Click here to return to first in-text mention of this figure.

	Males			Females		
	Estimate	± SE	*P*	Estimate	± SE	*P*
Linear selection						
Activity	**-0.019**	**± 0.007**	**0.013**	**-0.028**	**± 0.007**	**<0.001**
Metabolic rate	**-0.133**	**± 0.011**	**<0.001**	0.007	± 0.01	0.458
Dry body mass	**0.146**	**± 0.011**	**<0.001**	**0.075**	**± 0.01**	**<0.001**
Quadratic selection						
Activity	**0.009**	**± 0.004**	**0.036**	0.008	± 0.005	0.102
Metabolic rate	**0.052**	**± 0.008**	**<0.001**	-0.007	± 0.006	0.246
Dry body mass	**-0.035**	**± 0.014**	**0.015**	**-0.025**	**± 0.010**	**0.010**
Correlational selection						
Activity × metabolic rate	0.017	± 0.011	0.099	0.005	± 0.009	0.619
Activity × dry body mass	**-0.028**	**± 0.011**	**0.009**	-0.004	± 0.009	0.701
Dry body mass × metabolic rate	**-0.061**	**± 0.021**	**0.004**	-0.001	± 0.013	0.910

Figures

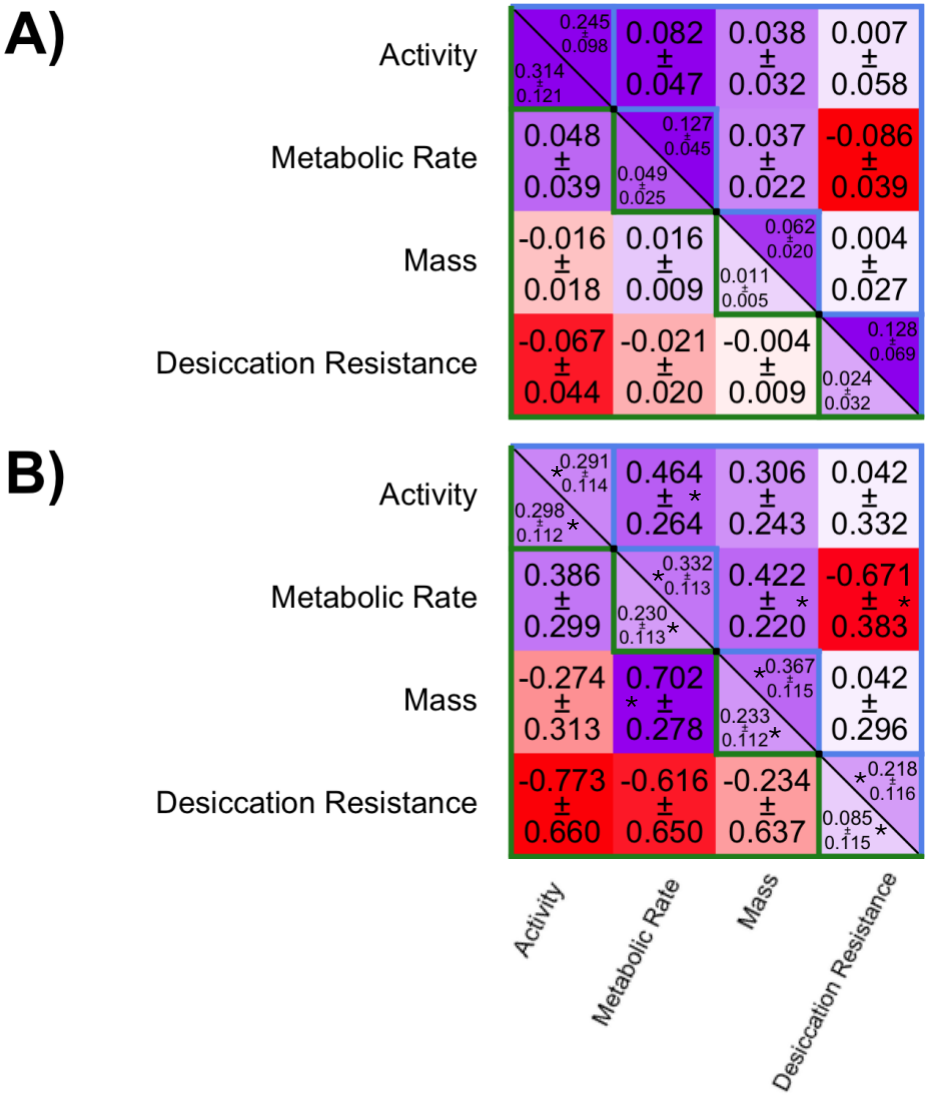

Figure 1. (A) Sex-specific additive genetic variance (V_A) (±se, diagonals) and covariances (±se, off-diagonals) in locomotor activity, metabolic rate under desiccation stress, dry body mass, and desiccation resistance for male (lower left) and female (upper right) *D. melanogaster*. (B) Sex-specific narrow-sense heritabilities (±se; diagonals) and additive genetic correlations (r_A) (±se, males: lower left, females: upper right). Negative estimates are in red and positive are in purple, with intensity proportional to the magnitude of the estimate. An * denotes significant difference from 0. Click here to return to first in-text mention of this figure.

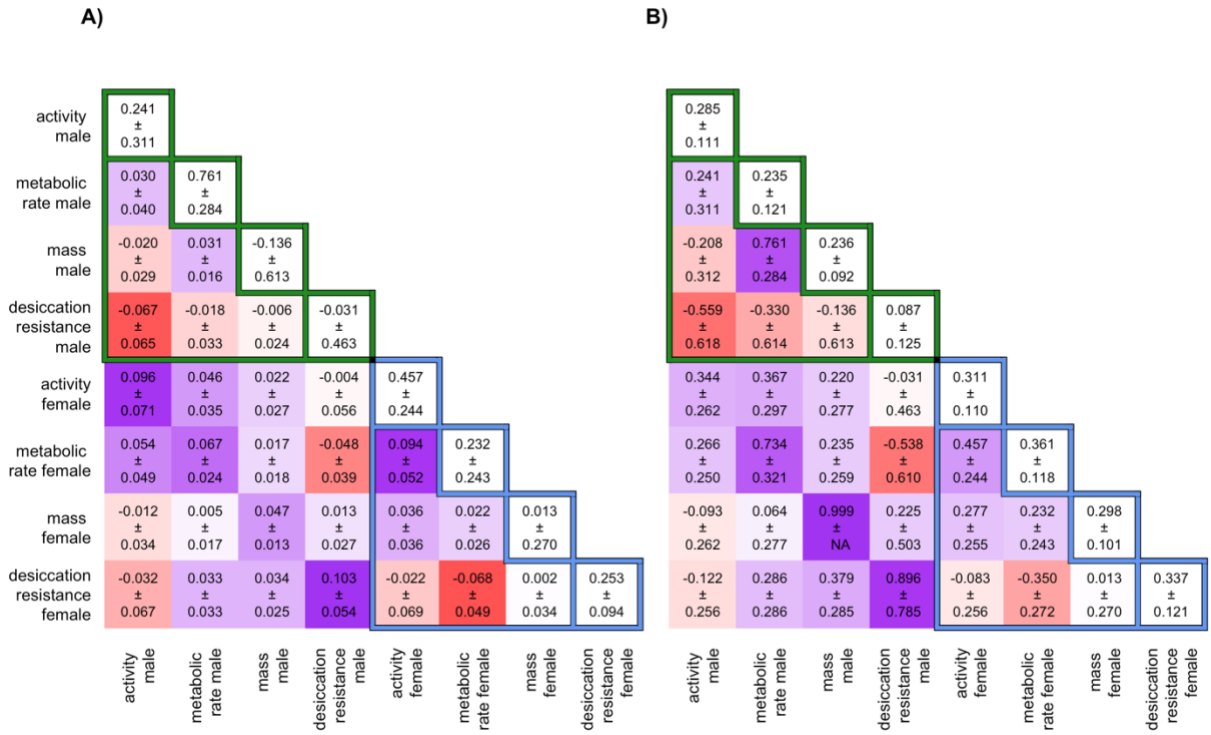

Figure 2. (A) Additive genetic covariance matrices (**G**) between activity, metabolic rate under desiccation stress, dry body mass, and desiccation for male (outlined in green) and female (outlined in blue) *D. melanogaster* and cross-sex cross-trait (co)variance matrix (**B**) generated from a multivariate animal model. Additive genetic covariances (±se) are displayed on the off diagonals with cross sex (co)variances displayed in the bottom left 4×4 matrix. (B) Additive genetic correlation matrices from a model with matching fixed and random effects but with corgh structure. Additive genetic correlations are displayed on the off diagonals and sex-specific narrow sense heritability (±se) of each trait is displayed on the diagonals. In the lower square, cross-sex correlations (±se) are displayed on the diagonals and cross-sex cross-trait correlations are displayed on the off-diagonals. Negative covariances/correlations are in red and positive are in purple, with intensity proportional to the magnitude of the estimate. Click here to return to first in-text mention of this figure.

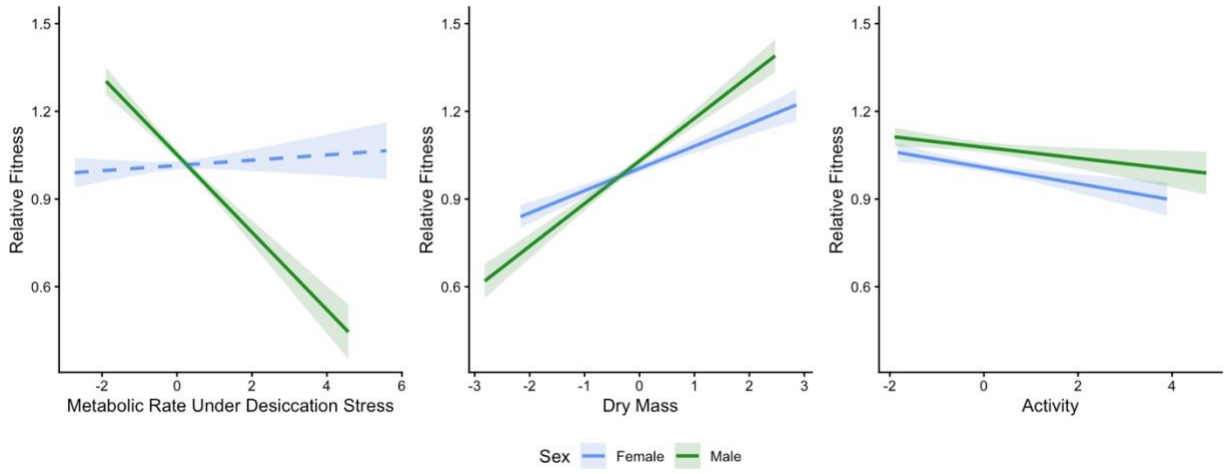

Figure 3: Linear phenotypic selection slopes for metabolic rate under desiccation stress, dry mass, and locomotor activity in male (green, $n = 687$) and female (blue, $n = 679$) *Drosophila melanogaster*, as estimated from separate multivariate least square regression models. Solid lines represent significant linear selection gradients. Relative fitness was computed separately for each sex from survival time under desiccation stress. Click here to return to first in-text mention of this figure.

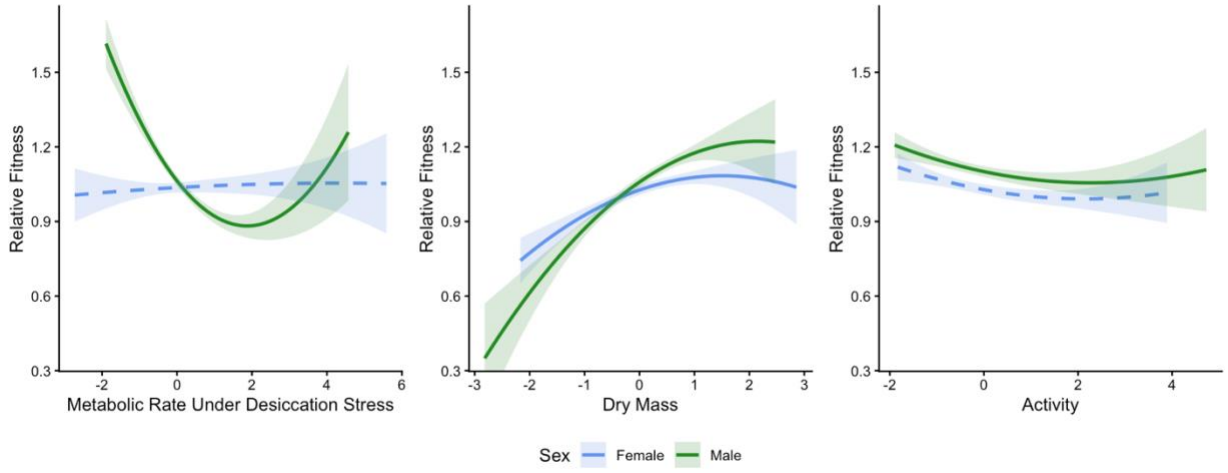

Figure 4: Quadratic phenotypic selection curves for metabolic rate under desiccation stress, dry mass, and locomotor activity separated by sex in male (green, n = 687) and female (blue, n = 679) *Drosophila melanogaster*, as estimated from separate least square regression models. Solid lines represent non-linear significant selection gradients. Relative fitness was computed separately for each sex from survival time under desiccation stress. Click here to return to first in-text mention of this figure.

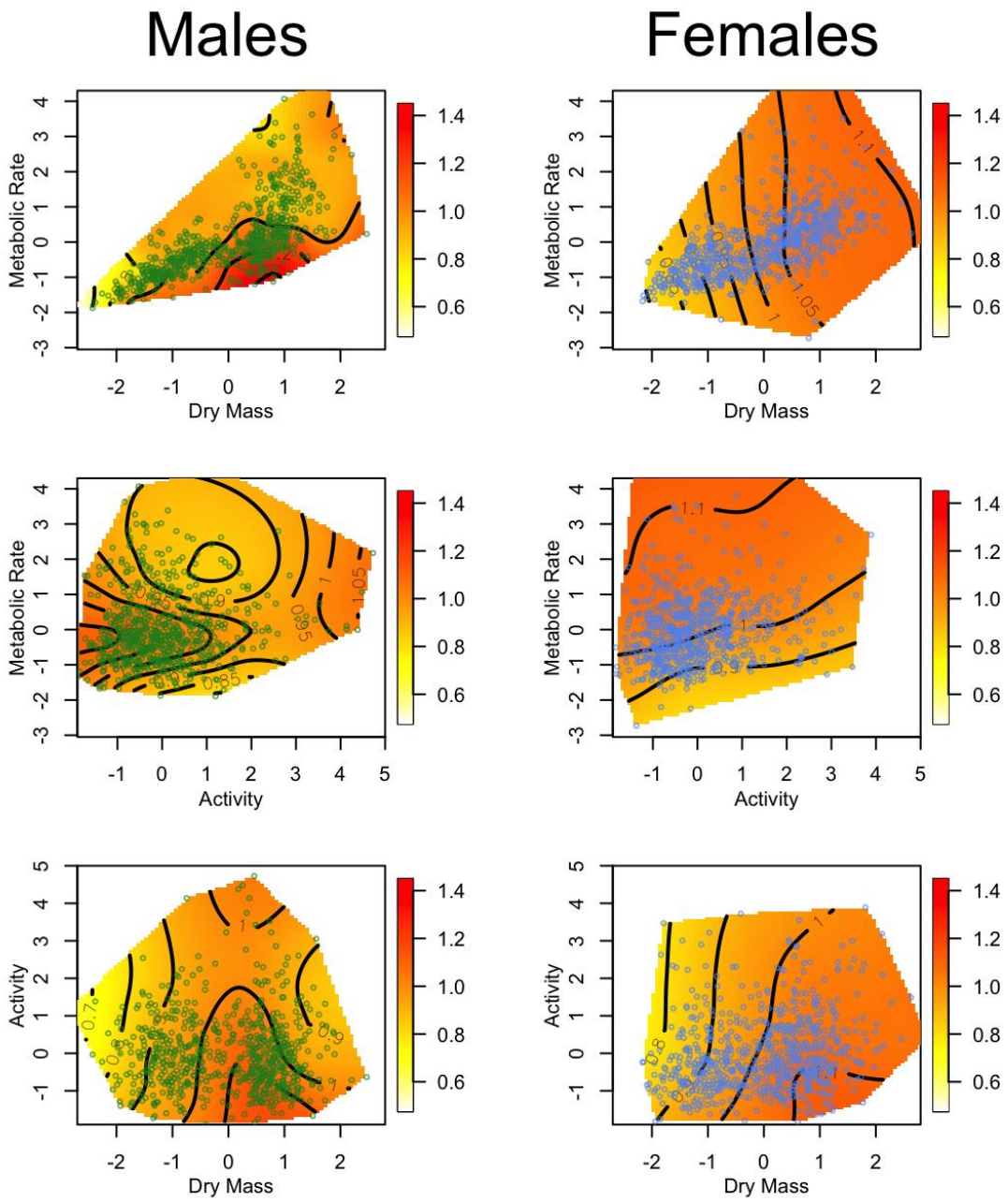

Figure 5: Contour map visualization (using thin-plate splines) of fitness surfaces for metabolic rate under desiccation stress, dry body mass, and locomotor activity in male (left panels, $n = 687$) and female (right panels, $n = 679$) *D. melanogaster*. Relative fitness is computed separately for each sex from survival time under desiccation stress. Open circles represent individuals. Click here to return to first in-text mention of this figure.

References

Aguirre, J.D., Hine, E., McGuigan, K., and Blows, M.W. 2014. Comparing G: multivariate analysis of genetic variation in multiple populations. Heredity **112**(1): 21–29. doi:10.1038/hdy.2013.12.

Andersson, M. 1994. Sexual Selection. Princeton University Press. doi:10.2307/j.ctvs32s1x.

Artacho, P., Saravia, J., Ferrandière, B.D., Perret, S., and Le Galliard, J.-F. 2015. Quantification of correlational selection on thermal physiology, thermoregulatory behavior, and energy metabolism in lizards. Ecol. Evol. **5**(17): 3600–3609. doi:10.1002/ece3.1548.

Auerswald, L., and Gäde, G. 2000. Metabolic changes in the African fruit beetle, Pachnoda sinuata, during starvation. J. Insect Physiol. **46**(3): 343–351. doi:10.1016/S0022-1910(99)00187-0.

Blows, M.W., Brooks, R., and Kraft, P.G. 2003. Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. Evolution **57**(7): 1622–1630. doi:10.1111/j.0014-3820.2003.tb00369.x.

Blows, M.W., and Hoffmann, A.A. 1993. The genetics of central and marginal populations of *Drosophila serrata* I. genetic variation for stress resistance and species borders. Evolution **47**(4): 1255–1270. doi:10.1111/j.1558-5646.1993.tb02151.x.

Brand, J.A., Aich, U., Yee, W.K.W., Wong, B.B.M., and Dowling, D.K. 2024. Sexual Selection Increases Male Behavioral Consistency in *Drosophila melanogaster*. Am. Nat. **203**(6): 713–725. doi:10.1086/729600.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., and West, G.B. 2004. Toward a Metabolic Theory of Ecology. Ecology **85**(7): 1771–1789. doi:10.1890/03-9000.

Burggren, W., Souder, B.M., and Ho, D.H. 2017. Metabolic rate and hypoxia tolerance are affected by group interactions and sex in the fruit fly (*Drosophila melanogaster*): new data and a literature survey. Biol. Open: bio.023994. doi:10.1242/bio.023994.

Butler, D.G., B.R., C., A.R, G., B.G., G., and R., T. 2023. ASReml-R Reference Manual Version 4.2. VSN International Ltd., Hemel Hempstead, HP2 4TP,.

Careau, V., and Glazier, D.S. 2022. A quantitative genetics perspective on the body-mass scaling of metabolic rate. J. Exp. Biol. **225**(Suppl_1): jeb243393. doi:10.1242/jeb.243393.

Chenoweth, S.F., Hunt, J., and Rundle, H.D. 2013. Analyzing and Comparing the Geometry of Individual Fitness Surfaces. *In* The Adaptive Landscape in Evolutionary Biology. *Edited by* E. Svensson and R. Calsbeek. Oxford University Press. pp. 126–149. doi:10.1093/acprof:oso/9780199595372.003.0009.

Chippindale, A.K., Gibbs, A.G., Sheik, M., Yee, K.J., Djawdan, M., Bradley, T.J., and Rose, M.R. 1998. Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. Evolution **52**(5): 1342–1352. doi:10.1111/j.1558-5646.1998.tb02016.x.

Chown, S.L., and Nicolson, S. (*Editors*). 2004. References. *In* Insect Physiological Ecology: Mechanisms and Patterns. Oxford University Press. p. 0. Available from

https://doi.org/10.1093/acprof:oso/9780198515494.001.0001 [accessed 18 May 2026].
Cox, R.M., and Calsbeek, R. 2009. Sexually Antagonistic Selection, Sexual Dimorphism, and the Resolution of Intralocus Sexual Conflict. Am. Nat. **173**(2): 176–187. doi:10.1086/595841.
DeWitt, T.J., Sih, A., and Wilson, D.S. 1998. Costs and limits of phenotypic plasticity. Trends Ecol. Evol. **13**(2): 77–81. doi:10.1016/S0169-5347(97)01274-3.
Djawdan, M., Chippindale, A.K., Rose, M.R., and Bradley, T.J. 1998. Metabolic Reserves and Evolved Stress Resistance in Drosophila melanogaster.
Dominicus, A., Skrondal, A., Gjessing, H.K., Pedersen, N.L., and Palmgren, J. 2006. Likelihood Ratio Tests in Behavioral Genetics: Problems and Solutions. Behav. Genet. **36**(2): 331–340. doi:10.1007/s10519-005-9034-7.
Fanara, J.J., Sassi, P.L., Goenaga, J., and Hasson, E. 2024. Genetic basis and repeatability for desiccation resistance in Drosophila melanogaster (Diptera: Drosophilidae). Genetica **152**(1): 1–9. doi:10.1007/s10709-023-00201-0.
Ferveur, J.-F., Cortot, J., Rihani, K., Cobb, M., and Everaerts, C. 2018. Desiccation resistance: effect of cuticular hydrocarbons and water content in *Drosophila melanogaster* adults. PeerJ **6**: e4318. doi:10.7717/peerj.4318.
Ferveur, J.-F., and Sureau, G. 1996. Simultaneous influence on male courtship of stimulatory and inhibitory pheromones produced by live sex-mosaic *Drosophila melanogaster*. Proc. R. Soc. Lond. B Biol. Sci. **263**(1373): 967–973. doi:10.1098/rspb.1996.0143.
Folk, D.G., and Bradley, T.J. 2003. Evolved patterns and rates of water loss and ion regulation in laboratory-selected populations of Drosophila melanogaster. J. Exp. Biol. **206**(16): 2779–2786. doi:10.1242/jeb.00498.
Gibbs, A.G., Chippindale, A.K., and Rose, M.R. 1997. Physiological Mechanisms of Evolved Desiccation Resistance in Drosophila Melanogaster. J. Exp. Biol. **200**(12): 1821–1832. doi:10.1242/jeb.200.12.1821.
Gibbs, A.G., Fukuzato, F., and Matzkin, L.M. 2003. Evolution of water conservation mechanisms in Drosophila. J. Exp. Biol. **206**(7): 1183–1192. doi:10.1242/jeb.00233.
Gibbs, A.G., and Matzkin, L.M. 2001. Evolution of water balance in the genus Drosophila. J. Exp. Biol. **204**(13): 2331–2338. doi:10.1242/jeb.204.13.2331.
Grillet, M., Everaerts, C., Houot, B., Ritchie, M.G., Cobb, M., and Ferveur, J.-F. 2012. Incipient speciation in Drosophila melanogaster involves chemical signals. Sci. Rep. **2**(1): 224. Nature Publishing Group. doi:10.1038/srep00224.
Hadley, N.F. 1994. Ventilatory Patterns and Respiratory Transpiration in Adult Terrestrial Insects. Physiol. Zool. **67**(1): 175–189. [University of Chicago Press, Division of Comparative Physiology and Biochemistry, Society for Integrative and Comparative Biology].
Hadley, N.F., and Quinlan, M.C. 1993. Discontinuous Carbon Dioxide Release in the Eastern Lubber Grasshopper *Romalea Guttata* and its Effect on Respiratory Transpiration. J. Exp. Biol. **177**(1): 169–180. doi:10.1242/jeb.177.1.169.

Hine, E., Chenoweth, S.F., Rundle, H.D., and Blows, M.W. 2009. Characterizing the evolution of genetic variance using genetic covariance tensors. Philos. Trans. R. Soc. B Biol. Sci. **364**(1523): 1567–1578. doi:10.1098/rstb.2008.0313.

Hoffmann, A.A., and Parsons, P.A. 1989. An integrated approach to environmental stress tolerance and life-history variation: desiccation tolerance in Drosophila. Biol. J. Linn. Soc. **37**(1–2): 117–136. doi:10.1111/j.1095-8312.1989.tb02098.x.

Hoffmann, A.A., and Parsons, P.A. 1993. Direct and correlated responses to selection for desiccation resistance: a comparison of *Drosophila melanogaster* and *D. simulans*. J. Evol. Biol. **6**(5): 643–657. doi:10.1046/j.1420-9101.1993.6050643.x.

Hoffmann, Ary.A., Hallas, R.J., Dean, J.A., and Schiffer, M. 2003. Low Potential for Climatic Stress Adaptation in a Rainforest *Drosophila* Species. Science **301**(5629): 100–102. doi:10.1126/science.1084296.

Hood, W.G., and Tschinkel, W.R. 1990. Desiccation resistance in arboreal and terrestrial ants. Physiol. Entomol. **15**(1): 23–35. doi:10.1111/j.1365-3032.1990.tb00489.x.

Husain, M., Rundle, H.D., and Careau, V. 2024. Among- and Within-Individual Variance in Metabolic Thermal Reaction Norms. Ecol. Evol. Physiol. **97**(1): 64–70. doi:10.1086/729925.

Jarvis, W.M.C., Careau, V., and Rundle, H.D. 2025. Divergence in genetic (co)variances and the alignment of gmax with phenotypic divergence. Evolution **79**(4): 597–610. doi:10.1093/evolut/qpaf009.

Jutsum, A.R., and Goldsworthy, G.J. 1975. Some problems of homeostasis of haemolymph metabolites in the locust. J. Insect Physiol. **21**(12): 1899–1908. doi:10.1016/0022-1910(75)90221-8.

Kruuk, L.E.B. 2004. Estimating genetic parameters in natural populations using the 'animal model.' Philos. Trans. R. Soc. Lond. B. Biol. Sci. **359**(1446): 873–890. doi:10.1098/rstb.2003.1437.

Kwan, L., Bedhomme, S., Prasad, N.G., and Chippindale, A.K. 2008. Sexual conflict and environmental change: trade-offs within and between the sexes during the evolution of desiccation resistance. J. Genet. **87**(4): 383–394. doi:10.1007/s12041-008-0061-z.

Lachaise, D., Cariou, M.-L., David, J.R., Lemeunier, F., Tsacas, L., and Ashburner, M. 1988. Historical Biogeography of the Drosophila melanogaster Species Subgroup. *In* Evolutionary Biology. *Edited by* M.K. Hecht, B. Wallace, and G.T. Prance. Springer US, Boston, MA. pp. 159–225. doi:10.1007/978-1-4613-0931-4_4.

Lande, R., and Arnold, S.J. 1983. The measurement of selection on correlated characters. Evolution **37**(6): 1210–1226. doi:10.1111/j.1558-5646.1983.tb00236.x.

Lighton, J.R.B., Fukushi, T., and Wehner, R. 1993. Ventilation in Cataglyphis bicolor: Regulation of carbon dioxide release from the thoracic and abdominal spiracles. J. Insect Physiol. **39**(8): 687–699. doi:10.1016/0022-1910(93)90074-2.

Lighton, J.R.B., and Turner, R.J. 2004. Thermolimit respirometry: an objective assessment of critical thermal maxima in two sympatric desert harvester ants, *Pogonomyrmex rugosus* and *P. californicus*. J. Exp. Biol. **207**(11): 1903–1913. doi:10.1242/jeb.00970.

Long, T.A.F., and Rice, W.R. 2007. Adult locomotory activity mediates intralocus sexual conflict in a laboratory-adapted population of Drosophila melanogaster. Proc. R. Soc. B Biol. Sci. **274**(1629): 3105–3112. doi:10.1098/rspb.2007.1140.

Marron, M.T., Markow, T.A., Kain, K.J., and Gibbs, A.G. 2003. Effects of starvation and desiccation on energy metabolism in desert and mesic Drosophila. J. Insect Physiol. **49**(3): 261–270. doi:10.1016/S0022-1910(02)00287-1.

Masson-Delmotte, V. and Intergovernmental Panel on Climate Change (*Editors*). 2023. Climate change 2021: the physical science basis: Working Group I contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Nervo, B., Roggero, A., Chamberlain, D., Rolando, A., and Palestrini, C. 2021. Dung beetle resistance to desiccation varies within and among populations. Physiol. Entomol. **46**(3–4): 230–243. doi:10.1111/phen.12366.

Nychka, D., Furrer, R., and Sain, S. 2014. fields: Tools for spatial data. R package version 7.1. Available from http://CRAN.R-project.org/package=fields.

Parkash, R., Rajpurohit, S., and Ramniwas, S. 2009. Impact of Darker, Intermediate and Lighter Phenotypes of Body Melanization on Desiccation Resistance in *Drosophila melanogaster*. J. Insect Sci. **9**(49): 1–10. doi:10.1673/031.009.4901.

Quinlan, M.C., and Hadley, N.F. 1993. Gas Exchange, Ventilatory Patterns, and Water Loss in Two Lubber Grasshoppers: Quantifying Cuticular and Respiratory Transpiration. Physiol. Zool. **66**(4): 628–642. doi:10.1086/physzool.66.4.30163812.

Rajpurohit, S., Gefen, E., Bergland, A.O., Petrov, D.A., Gibbs, A.G., and Schmidt, P.S. 2018. Spatiotemporal dynamics and genome-wide association analysis of desiccation tolerance in *Drosophila melanogaster*. Mol. Ecol. **27**(17): 3525–3540. doi:10.1111/mec.14814.

Rajpurohit, S., Peterson, L.M., Orr, A.J., Marlon, A.J., and Gibbs, A.G. 2016. An Experimental Evolution Test of the Relationship between Melanism and Desiccation Survival in Insects. PLOS ONE **11**(9): e0163414. doi:10.1371/journal.pone.0163414.

Rose, M.R., Vu, L.N., Park, S.U., and Graves, J.L. 1992. Selection on stress resistance increases longevity in Drosophila melanogaster. Exp. Gerontol. **27**(2): 241–250. doi:10.1016/0531-5565(92)90048-5.

Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. Methods Ecol. Evol. **1**(2): 103–113. doi:10.1111/j.2041-210X.2010.00012.x.

Sih, A., Bell, A., and Johnson, J.C. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol. Evol. **19**(7): 372–378. doi:10.1016/j.tree.2004.04.009.

Sinervo, B., and Svensson, E. 2002. Correlational selection and the evolution of genomic architecture. Heredity **89**(5): 329–338. doi:10.1038/sj.hdy.6800148.

Sztepanacz, J.L., and Rundle, H.D. 2012. Reduced genetic variance among high fitness individuals: inferring stabilizing selection on male sexual displays in *Drosophila serrata*. Evolution **66**(10): 3101–3110. doi:10.1111/j.1558-5646.2012.01658.x.

Telonis-Scott, M., Guthridge, K.M., and Hoffmann, A.A. 2006. A new set of laboratory-selected *Drosophila melanogaster* lines for the analysis of desiccation resistance:

response to selection, physiology and correlated responses. J. Exp. Biol. **209**(10): 1837–1847. doi:10.1242/jeb.02201.

Vermeulen, C.J., Cremers, T.I.F.H., Westerink, B.H.C., Van De Zande, L., and Bijlsma, R. 2006. Changes in dopamine levels and locomotor activity in response to selection on virgin lifespan in Drosophila melanogaster. Mech. Ageing Dev. **127**(7): 610–617. doi:10.1016/j.mad.2006.02.004.

Videlier, M., Careau, V., Wilson, A.J., and Rundle, H.D. 2021. Quantifying selection on standard metabolic rate and body mass in *Drosophila melanogaster*. Evolution **75**(1): 130–140. doi:10.1111/evo.14126.

Videlier, M., Rundle, H.D., and Careau, V. 2019. Sex-Specific Among-Individual Covariation in Locomotor Activity and Resting Metabolic Rate in *Drosophila melanogaster*. Am. Nat. **194**(6): E164–E176. doi:10.1086/705678.

de Villemereuil, P., Gimenez, O., and Doligez, B. 2013. Comparing parent–offspring regression with frequentist and Bayesian animal models to estimate heritability in wild populations: a simulation study for Gaussian and binary traits. Methods Ecol. Evol. **4**(3): 260–275. John Wiley & Sons, Ltd. doi:10.1111/2041-210X.12011.

Walter, G.M., Aguirre, J.D., Blows, M.W., and Ortiz, -Barrientos Daniel. 2018. Evolution of Genetic Variance during Adaptive Radiation. Am. Nat. **191**(4): E108–E128. The University of Chicago Press. doi:10.1086/696123.

Wang, C.-Y., Bong, L.-J., and Neoh, K.-B. 2019. Adult Paederus fuscipes (Coleoptera: Staphylinidae) Beetles Overcome Water Loss With Increased Total Body Water Content, Energy Metabolite Storage, and Reduced Cuticular Permeability: Age, Sex-Specific, and Mating Status Effects on Desiccation. Environ. Entomol. **48**(4): 911–922. doi:10.1093/ee/nvz065.

Wang, Y., Ferveur, J.-F., and Moussian, B. 2021. Eco-genetics of desiccation resistance in Drosophila. Biol. Rev. **96**(4): 1421–1440. doi:10.1111/brv.12709.

White, C.R., and Kearney, M.R. 2014. Metabolic Scaling in Animals: Methods, Empirical Results, and Theoretical Explanations. *In* Comprehensive Physiology, 1st edition. *Edited by* R. Terjung. Wiley. pp. 231–256. doi:10.1002/cphy.c110049.

White, C.R., Marshall, D.J., Alton, L.A., Arnold, P.A., Beaman, J.E., Bywater, C.L., Condon, C., Crispin, T.S., Janetzki, A., Pirtle, E., Winwood-Smith, H.S., Angilletta, M.J., Chenoweth, S.F., Franklin, C.E., Halsey, L.G., Kearney, M.R., Portugal, S.J., and Ortiz-Barrientos, D. 2019. The origin and maintenance of metabolic allometry in animals. Nat. Ecol. Evol. **3**(4): 598–603. doi:10.1038/s41559-019-0839-9.

Williams, A.E., and Bradley, T.J. 1998. The effect of respiratory pattern on water loss in desiccation-resistant Drosophila melanogaster. J. Exp. Biol. **201**(21): 2953–2959. doi:10.1242/jeb.201.21.2953.

Williams, A.E., Rose, M.R., and Bradley, T.J. 1998. Using laboratory selection for desiccation resistance to examine the relationship between respiratory pattern and water loss in insects. J. Exp. Biol. **201**(21): 2945–2952. doi:10.1242/jeb.201.21.2945.

Woods, H.A., and Singer, M.S. 2001. Contrasting Responses to Desiccation and Starvation by Eggs and Neonates of Two Lepidoptera. Physiol. Biochem. Zool. **74**(4): 594–606. doi:10.1086/322169.

Yun, L., Agrawal, A.F., and Rundle, H.D. 2021. On Male Harm: How It Is Measured and How It Evolves in Different Environments. Am. Nat. **198**(2): 219–231. doi:10.1086/715038.

Yun, L., Bayoumi, M., Yang, S., Chen, P.J., Rundle, H.D., and Agrawal, A.F. 2019. Testing for local adaptation in adult male and female fitness among populations evolved under different mate competition regimes. Evolution **73**(8): 1604–1616. doi:10.1111/evo.13787.

Yun, L., Chen, P.J., Kwok, K.E., Angell, C.S., Rundle, H.D., and Agrawal, A.F. 2018. Competition for mates and the improvement of nonsexual fitness. Proc. Natl. Acad. Sci. **115**(26): 6762–6767. doi:10.1073/pnas.1805435115.

Yun, L., Chen, P.J., Singh, A., Agrawal, A.F., and Rundle, H.D. 2017. The physical environment mediates male harm and its effect on selection in females. Proc. R. Soc. B Biol. Sci. **284**(1858): 20170424. doi:10.1098/rspb.2017.0424.