Variation in plasticity in response to combined thermal-nutritional stress is genetically independent between sexes and across traits

Yeuk Man Movis Choy[#], Greg M Walter, Christen Mirth, and Carla M Sgrò

School of Biological Sciences, Monash University, Melbourne, 3800, Victoria, Australia

[#] Corresponding author: yeuk.choy@monash.edu

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Abstract:

Phenotypic plasticity helps animals to buffer the effects of increasing thermal and nutritional stress created by climate change. Studies have shown that plastic responses to single and combined stressors can vary among genetically diverged populations. However, less is known about how plasticity in response to combined stress varies among individuals within a population or whether such variation changes across life-history traits. This is important because individual variation within populations shapes population level responses to environmental change. Here, we used isogenic lines of *Drosophila melanogaster* to assess plasticity of egg-adult viability and sex-specific body size for combinations of two temperatures (25°C or 28°C) and three diets (standard diet, low caloric diet, or low protein:carbohydrate ratio diet). Our results reveal substantial within-population genetic variation in plasticity for egg-to-adult viability and wing size in response to combined thermal-nutritional stress. This genetic variation in plasticity was a result of cross-environment genetic correlations that were generally < 1 rather than changes in the expression of genetic variation across environments. Cross-sex genetic correlations for body size were weaker when the sexes were reared in different conditions, suggesting wing size has the potential to evolve independently across sexes. Further, our results suggest that plasticity in egg-adult viability is genetically independent from plasticity in body size. By quantifying plasticity in response to combined stress between sexes and across traits, our study aims to develop a more nuanced understanding of the potential for populations to adapt to ongoing climate change.

Introduction

Rises in temperatures and increased frequency of extreme weather events caused by global change are placing many organisms at a higher risk of extinction (Diffenbaugh and Field 2013; Wiens 2016; Midgley and Hannah 2019). In addition to exposing organisms to more stressful thermal environments, climate change is also creating shifts in precipitation, which in combination with the increasing CO_2 concentration, are affecting the distribution, abundance (Van Der Putten *et al.* 2010; Pecl *et al.* 2017), and nutritional composition of plants (Kreuzwieser and Gessler 2010; Lukac *et al.* 2010; Jin *et al.* 2019). This will expose herbivorous and frugivorous animals to greater nutritional stress. In particular, ectothermic

herbivores and frugivores will be at a greater risk as they rely on ambient temperatures to maintain their metabolism (Hawlena and Schmitz 2010; Walters *et al.* 2012). As one of the largest groups of primary consumers, small changes in the population dynamics of these ectotherms could affect the whole food chain and disturb ecosystem function (Rosenblatt and Schmitz 2016). Understanding how ectothermic frugivores respond to combined thermal and nutritional stress will be crucial in predicting how species and ecosystems will respond to ongoing climate change.

When an environment becomes stressful, organisms can initially mitigate stress via phenotypic plasticity (Huey *et al.* 2002) – the ability of a single genotype to express different phenotypes in different environments. Life-history traits can respond plastically to environmental variation, acting act as a short-term buffer to help organisms survive and reproduce in the face of environmental change (Via *et al.* 1995; Schilthuizen and Kellermann 2014; Urban *et al.* 2014; Seebacher *et al.* 2015; Sgrò *et al.* 2016; Abram *et al.* 2017). For instance, in insects, body size (French *et al.* 1998; Bochdanovits and De Jong 2003), development time (Schou *et al.* 2017), and reproductive success (Janowitz and Fischer 2011; Wang *et al.* 2013) have been shown to decrease with increasing temperature. Pre-adult viability (Min *et al.* 2007; Jang and Lee 2018), development time (Economos and Lints 1984), body size (Min *et al.* 2007; Jang and Lee 2018), and reproductive success (Bader and Williams 2012) also shift plastically in response to reductions in food quality and quantity. Although both temperature and nutrition can individually cause plastic shifts in key life-history traits (Hoffmann *et al.* 2003, 2013; Zhang *et al.* 2015), wild animals are exposed to multiple stressors simultaneously (Stillwell *et al.* 2007; Rosenblatt and Schmitz 2016), which makes it important to understand plastic shifts in response to combinations of environmental stressors predicted to occur under climate change.

More recently, research has focussed on understanding plastic responses to combinations of stressors (Polak *et al.* 2004; Bubliy *et al.* 2012; Kutz *et al.* 2019; Chakraborty *et al.* 2020). Studies examining plastic responses to combined thermal-nutritional stress in insects reveal wide-ranging effects on life-history traits including viability, developmental time, body size, and lifespan (Tun-Lin *et al.* 2000; Couret *et al.* 2014; Clissold and Simpson 2015; Rho and Lee 2017; Kutz *et al.* 2019). For example, in adult mealworm beetles (*Tenebrio molitor*), higher

temperatures significantly reduced survival on sub-optimal diets, whereas diet did not impact survival at a lower temperature (Rho and Lee 2017). Similarly, in *Drosophila melanogaster*, the negative impacts of sub-optimal diets on viability were exacerbated at higher temperatures (Kutz *et al.* 2019). These studies suggest that temperature and nutrition may interact to pose greater levels of stress than they do individually.

Plastic responses to environmental stress can vary among populations and between individuals within populations, quantified as genotype by environment interactions (G × E) (Via and Lande 1985; Dreyer *et al.* 2016; Frankino *et al.* 2019; Chakraborty *et al.* 2022). Several studies have looked at among-population variation in plasticity along latitudinal gradients (Schmidt *et al.* 2005; Sgrò *et al.* 2010; Sisodia and Singh 2010; Chakraborty *et al.* 2020), while fewer studies have quantified genetic variation in plasticity within a population or between individual genotypes (Mossman *et al.* 2016; Wang *et al.* 2017; Lafuente *et al.* 2018; Frankino *et al.* 2019; Chakraborty *et al.* 2022). Understanding the distribution of plastic responses between individuals of a population is vital for determining whether populations will persist with climate change, given that within-population variation in plasticity shapes variation in plasticity at the population level and the response of the population to selection (Ghalambor *et al.* 2007; Frankino *et al.* 2019).

A powerful approach for measuring within-population variation in plasticity is to create isogenic lines – inbred lines that are initiated from a natural population with each line representing a fixed genotype (Ørsted *et al.* 2018; Franěk *et al.* 2020) – and then expose them to different environments. Variation among isogenic lines within environments represent genetic variation of the sampled population. By measuring differences in their response to multiple environments and calculating slopes of reaction norms for all genotypes, we can quantify variation in plasticity among genotypes (Scheiner 1993; Via and Conner 1995). Variation in plasticity among genotypes (or significant G × E) can be caused by significant variation in genes regulating the expressed phenotype (quantified by changes in genetic variance across environments), or a significant genetic correlation between two trait characters that is less than one (Via and Conner 1995), or both. Understanding within-population variation, and decoupling its cause, is essential for predicting a population's adaptability to long-term environmental stress (Ghalambor *et al.* 2007).

107

108 In addition to plasticity varying among genotypes within a population, plasticity can also
109 potentially vary between sexes. However, evidence for sex-specific plasticity is mixed.
110 Studies of a range of life-history and stress resistance traits have failed to find evidence for
111 sex-specific plasticity. In contrast, males and females differ in their plastic responses to
112 developmental conditions for body size (Pétavy *et al.* 2001; Teder and Tammaru 2005;
113 Stillwell and Fox 2007) and cold resistance (Pottier *et al.* 2021; Hangartner *et al.* 2022).
114 Although size differences between sexes (sexual size dimorphism) is common across insect
115 taxa (Teder and Tammaru 2005; Blanckenhorn *et al.* 2006; Stillwell *et al.* 2010a), no studies
116 have looked at sex-specific plasticity in size response to combined thermal-nutritional
117 stress. Sex-specific variation may further complicate the prediction of a population's
118 response and its dynamics under climate change (Hangartner *et al.* 2022), making it crucial
119 to measure sex-specific plasticity in life-history traits in response to combined stress.
120

121 In this study, we used a set of newly derived isogenic lines of *D. melanogaster* initiated from
122 a population collected along the east coast of Australia. These lines allow us to extend upon
123 previous studies that have examined variation in plasticity between populations (Sgrò *et al.*
124 2010; Cockerell *et al.* 2014; van Heerwaarden *et al.* 2016; Chakraborty *et al.* 2020).
125 Specifically, we used these lines to quantify sex-specific variation in plasticity in response to
126 combined thermal and nutritional stress. We subjected developing larvae to six
127 combinations of thermal and nutritional conditions that included two temperatures: 25°C
128 and 28°C, and three diets: standard, low caloric, and low protein to carbohydrate (P:C) diets.
129 These conditions were chosen to best simulate climate change, which is expected to cause a
130 3°C increase in average temperature (Australian Government 2014), and to reduce protein
131 and vary carbohydrate contents in plants (DaMatta *et al.* 2010; Gowik and Westhoff 2011;
132 IPCC 2018). We assessed plasticity of egg-to-adult viability and adult wing centroid size,
133 which have previously shown sensitivity to combined thermal and nutritional stress (Kutz *et*
134 *al.* 2019; Chakraborty *et al.* 2020). Given that *D. melanogaster* shows sexual size
135 dimorphism, and that the sexes may vary in their plasticity (Stillwell *et al.* 2010b; Shingleton
136 *et al.* 2017; Pottier *et al.* 2021; Hangartner *et al.* 2022), we assessed size plasticity separately
137 for males and females. By quantifying the genetic and sex-specific variation in plasticity

138 within a population in response to combinations of environmental stressors, our study
139 sheds new insight into how animal populations may respond to ongoing climate change.
140
141 **Methods**
142
143 *Fly stocks - isogenic lines*
144
145 We used isogenic lines that were established by Chakraborty *et al.* (2022). Briefly, two
146 hundred field-inseminated female *D. melanogaster* were collected in Duranbah, New South
147 Wales, Australia (28.3° S, 153.5° E) in 2018. From each of these females, two independent
148 iso-female lines were established and bred into four hundred lines. Full-sibling mating was
149 performed in each of these four hundred lines for twenty generations following MacKay *et*
150 *al.* (2012), resulting in 72 isogenic lines. Four copies of each isogenic line were reared at
151 25°C on standard diets (1:3 P:C ratio and 320 kcal/ L) under 12:12 light:dark cycle for twelve
152 generations prior to the experiment, as described below. The standard diet was prepared by
153 mixing water with potato flakes (18.18 g/ L), yeast (36.36 g/ L), agar (6.36 g/ L), and dextrose
154 (27.27 g/ L), with addition of nipagin and propionic acid to prevent bacterial and fungal
155 growth (Holleley *et al.* 2008).
156
157 *Developmental treatment conditions and choice of traits*
158
159 We used six thermal-nutritional conditions to simulate the possible shifts in temperature
160 (IPCC 2018) and nutrition (DaMatta *et al.* 2010; Gowik and Westhoff 2011; IPCC 2018) under
161 climate change. They included combinations of two temperatures: 25°C and 28°C, and three
162 diets: standard diet (1:3 P:C ratio and 320 kcal/L), low caloric diet (1:3 P:C and 80kcal/L), and
163 low P:C diet (1:12 P:C and 320kcal/L). 25°C represents the average summer temperature in
164 south-east Australia, whilst 28°C represents the 3°C temperature increase projected in
165 twenty years under climate change (Australian Government 2014). The low caloric diet and
166 the low P:C diets simulate a reduction of protein and variation of carbohydrates in plants
167 composition projected under climate change (DaMatta *et al.* 2010). These conditions have
168 been previously shown to successfully induce plastic responses in our traits of interest, egg-
169 to-adult viability and wing size (Kutz *et al.* 2019; Chakraborty *et al.* 2020).

Egg-to-adult viability and wing size were chosen because they are fitness-related developmental traits known to be sensitive to nutrition and diet individually and in combination (Kutz *et al.* 2019; Chakraborty *et al.* 2020). Egg-to-adult viability is a direct indication of an individual's ability to survive to the reproductive adult stage (Kristensen *et al.* 2015), and larger adult body size often correlates with greater fitness in ectotherms, including *Drosophila* (Kingsolver and Huey 2008). Assessing variation in plasticity within a population using these fitness-related developmental traits will allow us to better understand how well species survive under stressful environments at the population level.

Experimental set up - Egg lays to obtain experimental flies

Adults from isogenic lines were transferred to laying plates containing apple juice, agar, nipagin, and a surface layer of autoclaved yeast to encourage oviposition. Laying plates were maintained at 25°C for 18 hours to allow egg laying.

Egg-to-adult viability assay

Eggs were collected from laying plates and transferred to 7.2 ml vials containing their respective diets. Three replicate vials were included for each treatment and line combination, with a density of twenty eggs per vial (Agnew *et al.* 2002). Vials containing fresh eggs were kept at their respective treatment temperature at a 12:12 hour light: dark cycle until adult eclosion. Egg-to-adult viability was calculated as the number of eclosed adults relative to the initial number of eggs deposited into each vial (20 eggs).

Adult body size measurement

Wing size was used as a proxy for body size (Partridge *et al.* 1994). After eclosing, adults were sexed and preserved in a 70% ethanol-30% glycerol solution for wing dissection. Left wings of each sex were removed with fine forceps, transferred to glass slides, and suspended in 70% ethanol-30% glycerol solution for imaging. To measure the wing centroid size, landmarks were positioned at eight vein intersections as indicated in Fig. 1, and the X-

202 and Y- coordinates of landmarks were determined using the software package tpsDIG (Rohlf
203 2015). Using the software CoorDGen8 (Sheets 2014), wing area was calculated as square
204 root of the sum of the squared distances between each landmark.
205
206 *Statistical analysis*
207
208 *Testing for overall genotype-by-environment (G × E) interactions*
209
210 R was used to perform all data analysis and visualization (version 3.4.3) (R Core Team,
211 2017). To test for significant G × E, we used the *lme4* package (Bates *et al.* 2015), data of
212 egg-to-adult viability and wing size were fitted using linear mixed effects models. As both
213 traits had normal error distributions, they were fitted with a Gaussian error distribution. For
214 both traits, temperature and diet were treated as fixed effects, and line and block as
215 random effects. Both intercepts and slopes were allowed to vary in all models, since
216 differences in the slope of the reaction norm of each isogenic line would reflect significant G
217 × E. Wing size was analysed separately for each sex.
218
219 Models were fitted separately for each trait. Random effects of block, line, and
220 temperature-by-diet-by-line were added sequentially (see equations 1-3) and the
221 significance of each variable was tested using log-likelihood ratio tests from the *lrtest*
222 package (Zeileis and Hothorn, 2002).
223
224 Specifically, the first model (equation 1) included diet and temperature as the fixed effects,
225 and block as a random effect. This estimates the overall population response to
226 combinations of temperature and diet, while accounting for the differences in egg-to-adult
227 viability/wing size among blocks.
228
229 $$\mathrm{y} \sim 1 + \mathrm{Temperature} * \mathrm{Diet} + (1|\mathrm{Block}) \qquad \text{Eqn. 1}$$
230
231 In the second model (equation 2), line was added as an additional random effect to test for
232 differences in egg-to-adult viability/wing size across the isogenic lines.
233

$$y \sim 1 + \text{Temperature} * \text{Diet} + (1|\text{Block}) + (1|\text{Line}) \qquad \text{Eqn. 2}$$

In the third model (equation 3), the diet-by-temperature interaction was added to the random line term to test for overall G × E, allowing for random intercepts and random slopes.

$$y \sim 1 + \text{Temperature} * \text{Diet} + (1|\text{Block}) + (1 + \text{Diet:Temperature}|\text{Line}) \qquad \text{Eqn. 3}$$

Quantifying genetic (co)variance

To determine whether significant G × E was due to changes in genetic variance across environments and/or genetic correlations that are less than one, genetic (co)variance matrices for egg-to-adult viability and wing size were estimated across treatments, and across sexes (the latter only for wing size) (Via and Lande 1985). We treated each combination of diet (low calorie and low P:C), temperature (25°C and 28°C), and sex (only for wing size) as separate traits, resulting in six traits for viability, and twelve traits for wing size across both sexes following Falconer (1952) and Via and Lande (1985). Our analyses were performed on the raw data.

Specifically, we used the *MCMCglmm* package (Hadfield, 2010) to implement linear mixed effects models, fitted using Markov Chain Monte Carlo (MCMC), to estimate genetic (co)variances and genetic correlations. For each model, substantial burn-in (100,000 iterations) and thinning intervals (500 iterations) were included to ensure model convergence. Effective sample sizes were checked to ensure more than 85% of samples were saved. Uninformative parameter-expanded priors were used for variance components. We checked their sensitivities by changing scale parameters to ensure no changes in the posterior distribution. Each model was fitted with a Gaussian error distribution. A thousand iterations were kept from each model, which provided the posterior distribution for each parameter estimated.

Quantifying genetic (co)variance in egg-to-adult viability

To estimate genetic (co)variance in egg-to-adult viability within and across environments, we used this equation

$$y_{jklm} = T_j + l_{k(j)} + b_{l(jk)} + e_{m(jkl)}\ , \qquad \text{Eqn. 4}$$

where we included viability as the response variable y_{jklm} and treatment T_j as the only fixed effect. Random effects included k^{th} isogenic line $l_{k(j)}$ and l^{th} block $b_{l(j)}$ within the treatments, to remove any variance due to differences between experimental blocks. The term $e_{m(jkl)}$ represents residual variance. For the isogenic line component, random intercepts of each treatment and random slopes between treatments were estimated. This respectively calculates the genetic variance in egg-to-adult viability in each treatment and genetic covariance between treatments, resulting in a 6 × 6 genetic (co)variance matrix. Genetic correlations between treatments were calculated from the covariance matrix (Walter *et al.* 2020).

Quantifying genetic (co)variance in wing size

To estimate genetic (co)variance in wing size within and across environments, we used this equation

$$y_{ijklm} = T_i + S_j + l_{k(ij)} + b_{l(ijk)} + e_{m(ijkl)} \qquad \text{Eqn. 5}$$

where we included wing size as the response variable y_{ijklm}, and treatment T_i and sex S_j as fixed effects. Random effects included k^{th} isogenic line $l_{k(ij)}$ and l^{th} block $b_{l(ijk)}$ within the treatments, to remove any variance due to differences between experimental blocks. The term $e_{m(ijkl)}$ represents residual variance. The random intercepts of each treatment and sex, as well as the random slopes between treatments and sex, were estimated. These values represent genetic variances of wing size among isogenic lines in each sex and treatment, and genetic covariances between sex and treatment respectively, which were then arranged into a 12 × 12 (co)variance matrix. Genetic correlations of wing size within and between the sexes and treatments were further calculated from the (co)variance matrix (Walter *et al.* 2020).

Genetic correlations between egg-to-adult viability and wing size

297 To estimate genetic correlations between egg-to-adult viability and wing size across
298 treatments, we applied equation 4 but included both egg-to-adult viability and wing size as
299 a bivariate response variable (note that wing size was averaged over both sexes and
300 estimated without sex as a fixed effect). As egg-to-adult viability and wing size are measured
301 on different scales, we standardised each trait by dividing trait values by their grand mean
302 (Houle 1992). Treatment (the six thermal-nutritional conditions) was initially included as a
303 fixed effect, however, this returned inflated (co)variance estimates. To obtain more
304 conservative estimates of genetic (co)variance, equation 4 was applied separately within
305 each treatment, and only included isogenic line and block as random effects. Random slopes
306 and intercepts were estimated, representing the genetic variance in viability and wing size,
307 and the genetic covariance between viability and wing size respectively. This produced six 2
308 × 2 genetic (co)variance matrices. Genetic correlations between traits for each treatment
309 were then calculated from each (co)variance matrix.
310
311 **Results**
312
313 We aimed to characterise genetic variation (G × E) in plasticity for both egg-to-adult viability
314 and sex-specific wing size in response to combined stressors within a population. We
315 subjected *D. melanogaster* isogenic lines to six different thermal-nutritional conditions
316 (25°C—standard diet, 25°C—low caloric diet, 25°C—low P:C diet, 28°C—standard diet,
317 28°C—low caloric diet, and 28°C—low P:C diet) and tested for significant genetic variation in
318 plasticity in egg-to-adult viability and wing size. We then calculated genetic (co)variances
319 and genetic correlations between traits within and across environments to understand the
320 causes of the significant G × E observed for each trait.
321
322 *Genotype-by-environment interactions in egg-to-adult viability*
323
324 Temperature and diet had significant individual effects on egg-to-adult viability (Fig. 2A and
325 Table S1). On average, viability was higher at 25°C compared to 28°C, and higher on
326 standard diets compared to low caloric and low P:C diets (Fig. 2A). The best fit model
327 included the random effect of temperature-by-diet-by-line interactions (Table 1), indicating

328 significant genetic variation in plasticity for viability (G × E) in response to combinations of
329 temperature and diet, captured by non-parallel individual reaction norms among lines (Fig.
330 2A).
331
332 *Genetic (co)variances in egg-to-adult viability*
333
334 We estimated genetic (co)variance matrices to determine whether significant G × E for
335 viability was caused by differences in the distribution of genetic variance across
336 environments and/or cross-environment genetic correlations that are less than one (Via and
337 Lande 1985). We found that the estimates of genetic variance did not differ across
338 treatments (Fig. 2B and Fig. S1), evidenced by the overlapping 95% highest posterior density
339 (HPD) intervals across treatments (Fig 2B). This indicates that significant G × E in viability
340 was not due to differences in genetic variance across environments.
341
342 Genetic correlations between treatment pairs were all positive and significantly greater
343 than zero, but mostly less than one (Fig. 2C and Fig. S2). This suggests that significant G × E
344 for viability results from differences in how isogenic lines respond to environments
345 (Falconer 1952; Via and Lande 1985).
346
347 *Genotype-by-environment interactions in wing size*
348
349 Initially, we analysed wing size separately for males and females. Temperature and diet
350 were found to significantly affect both male and female wing size (Table S2 and Table S3).
351 Wing sizes in both sexes were larger at 25°C compared to 28°C, and when larvae were
352 reared on standard diets compared to low caloric and low P:C diets (Fig. 3A and Fig. 3B). As
353 evidence for genetic variance in plasticity for wing size in males and females, we found the
354 best fit models included the random effect of temperature-by-diet-by-line interaction (Table
355 2). Such significant G × E in male and female wing size within the population is visualised as
356 non-parallel reaction norms (Fig. 3A and Fig. 3B).
357
358 *Genetic (co)variances in wing size*

We found significant genetic variance in wing size for all treatments within each sex; however, the distribution of genetic variance did not differ across treatments (Fig. 3C and Fig. S3). Thus, significant G × E in wing size in both males and females was not caused by the differences in the distribution of genetic variance across the environments.

Genetic correlations in wing size across the six thermal-nutritional treatments within each sex were all positive, significantly greater than zero, and mostly less than 1 (Fig. 3D, Fig. S4 and Fig. S5). This suggests that, similar to what we found for viability, significant G × E in wing size of both males and females was caused by differences in the alleles contributing to the response across environments (Falconer 1952; Via and Lande 1985).

Cross-sex genetic correlations for wing size

To determine whether wing size has a similar genetic bases in both sexes, we estimated the cross-sex genetic correlation for wing size within and between all thermal-nutritional treatments and sexes. This helps us to understand the potential for size to evolve independently in each sex and in each environment (Falconer 1952; Via and Lande 1985).

Cross-sex correlations within treatments (indicated in red in Fig. 4, Fig S.6) were significantly greater than zero, and large, but less than one, indicating that even though sexes share some genetic basis for these traits, there is also some degree of genetic independence. Furthermore, cross-sex genetic correlations were smaller when males and females developed in different treatments. Specifically, the correlation between males from 28°C standard diet condition and females from 25°C low P:C diet condition (indicated in blue in Fig. 4) was significantly lower than correlations within 25°C standard diet and 28°C standard diet conditions. This suggests that males and females share less of a genetic basis for wing size when reared in different environments, which could allow sexes to evolve more independently as conditions become more variable.

Genetic correlations between egg-to-adult viability and wing size

Finally, we estimated genetic correlations between egg-to-adult viability and wing size (sexes combined) across all thermal-nutritional treatments to determine whether these traits shared similar genetic basis, and whether they can evolve independently in different environments (Falconer 1952; Via and Lande 1985). Correlations between viability and wing size were not significantly different from zero in all treatments (Fig. 5 and Fig. S7), indicating that they are genetically independent.

Discussion

Climate change is simultaneously imposing thermal and nutritional stress on animals (Van Der Putten *et al.* 2010; Pecl *et al.* 2017), and is expected to negatively affect biodiversity in the long term (Walters *et al.* 2012; Rosenblatt and Schmitz 2016; IPCC 2018). Phenotypic plasticity will be an important means by which animals will mitigate the increasing stress under climate change (Urban *et al.* 2014; Seebacher *et al.* 2015; Sgrò *et al.* 2016). Single-population studies have shown that there are shifts in plastic responses of life-history traits to changes in temperature (French *et al.* 1998; Hoffmann *et al.* 2003; Schou *et al.* 2017) and nutrition individually (Min *et al.* 2007; Bader and Williams 2012; Jang and Lee 2018). Temperature has also been shown to modify plastic responses of life history traits to nutritional stress (Lee and Roh 2010; Kutz *et al.* 2019; Chakraborty *et al.* 2020). While these studies improve our understanding of how animals may respond to combined stress resulting from climate change, they have not accounted for genetic variation in plasticity within a population.

Individuals within a population can also show substantial variation in plasticity (Mossman *et al.* 2016; Wang *et al.* 2017; Lafuente *et al.* 2018; Frankino *et al.* 2019). In particular, understanding within-population variation matters because it may shape the capacity for populations to adapt and persist in the face of ongoing environmental change (Lande 2009; Chevin *et al.* 2010; Dreyer *et al.* 2016; Frankino *et al.* 2019).

Consistent with previous studies performed at the population level (Petersen *et al.* 2000; Stillwell *et al.* 2007; Lee and Roh 2010; Kutz *et al.* 2019; Chakraborty *et al.* 2020), we found that low quality diets exacerbated the negative effects of elevated temperatures on two life

423 history traits, egg-adult viability and adult body size. Specifically, both viability and wing size
424 were reduced on low quality diets; this effect was greater at 28°C compared to 25°C. This
425 suggests that exposure to combined thermal-nutritional stress, projected under climate
426 change, may negatively impact population persistence (DaMatta *et al.* 2010; Gowik and
427 Westhoff 2011; IPCC 2018; Kutz *et al.* 2019). Vitally, we found that individual genotypes
428 within a population can also vary in the extent and direction of plasticity for both traits.
429
430 Previous studies investigating a range of taxa have reported extensive within-population
431 variation in plastic responses to temperature and nutrition individually, for a range of life-
432 history traits and stress resistance traits (Mossman *et al.* 2016; Wang *et al.* 2017; Lafuente
433 *et al.* 2018; Frankino *et al.* 2019), including viability (Duun Rohde *et al.* 2016) and body size
434 (Barker and Krebs 1995; Imasheva *et al.* 1999; Lafuente *et al.* 2018). We have found that
435 populations also harbour significant genetic variation in plasticity for both viability and body
436 size in response to combinations of temperature and diet.
437
438 The extent to which genotypes within a population vary in their reaction norm will
439 determine how the population will respond to selection imposed by environmental change
440 (Chevin *et al.* 2010; Dreyer *et al.* 2016; Frankino *et al.* 2019). As a result, populations with
441 similar mean reaction norms may vary markedly in the distribution of their genotype-
442 specific reaction norms, and this would cause them to respond differently to selection
443 (Dreyer *et al.* 2016; Frankino *et al.* 2019). Predictions of long-term evolutionary responses to
444 environmental changes using population-level reaction norms, could be inaccurate, unless
445 the genotype-specific reaction norms are also considered (Reed *et al.* 2010; Dreyer *et al.*
446 2016; Frankino *et al.* 2019).
447
448 We found significant genetic variance for viability across all combinations of diet and
449 temperature. However, the expression of genetic variance did not vary across
450 environments. Bubliy *et al.* (2001), and Bubliy and Loeschcke (2002) both used half-sib
451 analyses to investigate genetic variance in plasticity for viability to changes in temperature.
452 Consistent with the present study, Bubliy and Loeschcke (2002) found no significant
453 difference in the expression of genetic variance for viability between 13°C and 25°C. In
454 contrast, Bubliy *et al.* (2001) observed a significant increase in genetic variance for viability

455 at 32°C compared to 25°C. Perhaps if we had increased the rearing temperature above 28°C,
456 we might have observed differences in expression of genetic variance. Instead, we found
457 that significant G × E interactions for viability was a result of genetic correlations across
458 environments that were above zero and often less than one. This indicates that viability
459 across some thermal-nutritional conditions is controlled by different alleles or differently by
460 the same alleles (Via and Lande 1985), meaning that viability has some capacity to evolve
461 independently across different thermal-nutritional environments (Via and Lande 1987).
462
463 Although body size plasticity has been extensively shown to vary within a population in
464 response to temperature (Barker and Krebs 1995; Lafuente *et al.* 2018) and nutrition
465 (Imasheva *et al.* 1999), the source of G × E differed across studies. De Moed *et al.* (1997)
466 found that the significant G × E for body size was due to differences in the expression of
467 genetic variance across environments. In contrast, we found that the significant G × E for
468 body size for both sexes was due to genetic correlations across treatments that were
469 significantly different from zero but often less than one. Similarly, Barker and Krebs (1995)
470 and Imasheva *et al.* (1999) have also reported the same source of G × E for body size
471 plasticity to changes in temperature and dietary yeast concentration respectively. These
472 differences across studies are likely to arise due to differences in the source populations and
473 experimental conditions used.
474
475 As expected from previous studies, we found that wing size exhibited sexual dimorphism
476 (females were larger than males), and there were strong and positive cross-sex genetic
477 correlations within treatments (Cowley *et al.* 1986; Cowley and Atchley 1990; Reeve and
478 Fairbairn 1996; Lasne *et al.* 2018). Strong and positive cross-sex genetic correlations indicate
479 that the genetic basis of plasticity in wing size is largely shared in males and females, though
480 because these correlations are less than one there is some potential for independent
481 evolution of size plasticity between the sexes (Lande 1980; Via and Lande 1987). Cross-sex
482 genetic correlations were reduced when males and females developed in different
483 environments. Thus, there is a greater possibility of independent evolution in wing size
484 plasticity when the sexes experience markedly different nutritional-thermal environments.
485 Delcourt *et al.* (2009) and Berger *et al.* (2014) have also reported changes in magnitude of

486 cross-sex genetic correlation of fitness when rearing males and females in different
487 environments. This matters because, in the wild, individual *Drosophila* are often found in
488 different microhabitats – different temperatures due to daily thermal fluctuations (Noer *et*
489 *al.* 2020) and different nutrients due to different niches, resources (Diepenbrock and
490 Burrack 2017), and nutritional requirements (Lee *et al.* 2013; Camus *et al.* 2018; Duxbury
491 and Chapman 2021). Estimating cross-sex genetic correlation between males and females
492 that developed in different environments may help improve the accuracy when estimating
493 the individual evolvability between sexes.
494
495 Finally, genetic correlations between viability and wing size across all six thermal-nutritional
496 treatments were not different from zero. This means that these two traits do not share a
497 similar genetic basis, and are able to evolve independently (Via and Lande 1985). Our
498 findings differ from earlier studies. For instance, Teuschl *et al.* (2007) artificially selected
499 dung flies with different body sizes – small, control, and large – and assessed their pre-adult
500 viability in combinations of high temperature and limited food. Interestingly, larger males
501 had higher pre-adult mortality, suggesting body size and viability might be negatively
502 genetically correlated. Dingle (1992) also observed a similar phenomenon in milkweed bugs
503 under food shortage, such that higher pre-adult survival was traded off with smaller body
504 size, which again indicates a negative genetic correlation between these two traits (Via and
505 Lande 1985). The fact that we found no correlation between viability and wing size suggests
506 that the genetic correlation between viability and body size may be species- and condition-
507 specific.
508
509 In summary, we explicitly assessed within-population variation in plasticity of egg-to-adult
510 viability and sex-specific wing size in *D. melanogaster*, in response to combined thermal-
511 nutritional conditions. We demonstrated that, even though combined thermal-nutritional
512 stress reduced viability and wing size (i.e., reduced performance) on average, individual
513 genotypes still varied in their plastic responses, such that some genotypes had increased
514 viability/wing size under increasingly stressful conditions. Additionally, our results suggest
515 that the independent evolution of wing size between the sexes may be possible when the
516 sexes experience different environmental conditions, and that viability and wing size are
517 also genetically independent. Our research sheds light on the importance of measuring the

extent of plasticity of fitness-related life-history traits in response to combined stressors, as well as measuring cross-sex genetic correlation between treatments. This will be crucial for making accurate predictions of how species may respond to climate change.

Results – Tables and graphs

Table 1. Linear models testing for genotype (Line) by environment interactions in egg-to-adult viability in response to combined developmental temperature and diet conditions.

Model number	Egg-to-adult viability (y) model	Likelihood ratio test	P-value
1.1	y ~ 1 + Temperature * Diet + (1|Block)	-	-
1.2	y ~ 1 + Temperature * Diet + (1|Block) + (1|Line)	1.2 to 1.1	<0.001
1.3	y ~ 1 + Temperature * Diet + (1|Block) + (1|Line) + (Temperature:Diet|Line)	1.3 to 1.2	<0.001

Table 2. Linear models testing for genotype (Line) by environment interactions in wing size of both sexes in response to combined developmental temperature and diet conditions.

Model number	Male wing size (y) model	Likelihood ratio test	P-value
2.1	y ~ -1 + Temperature * Diet + (1|Block)	-	-
2.2	y ~ -1 + Temperature * Diet + (1|Block) + (1|Line)	2.2 to 2.1	<0.001
2.3	y ~ -1 + Temperature * Diet + (1|Block) + (Temperature:Diet|Line)	2.3 to 2.2	<0.001
	Female wing size (y) model		
3.1	y ~ -1 + Temperature * Diet + (1|Block)	-	-
3.2	y ~ -1 + Temperature * Diet + (1|Block) + (1|Line)	3.2 to 3.1	<0.001
3.3	y ~ -1 + Temperature * Diet + (1|Block) + (Temperature:Diet|Line)	3.3 to 3.2	<0.001

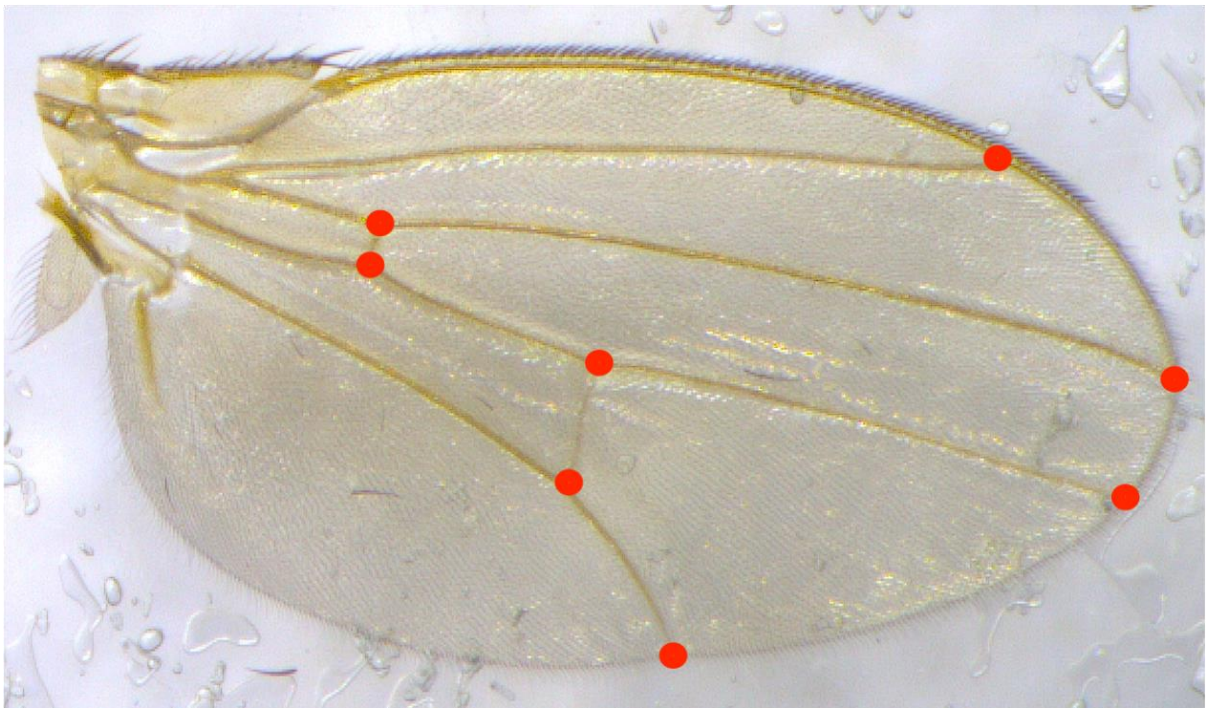


Fig. 1. Landmakrs used for calculating wing centroid size, indicted by red dots.

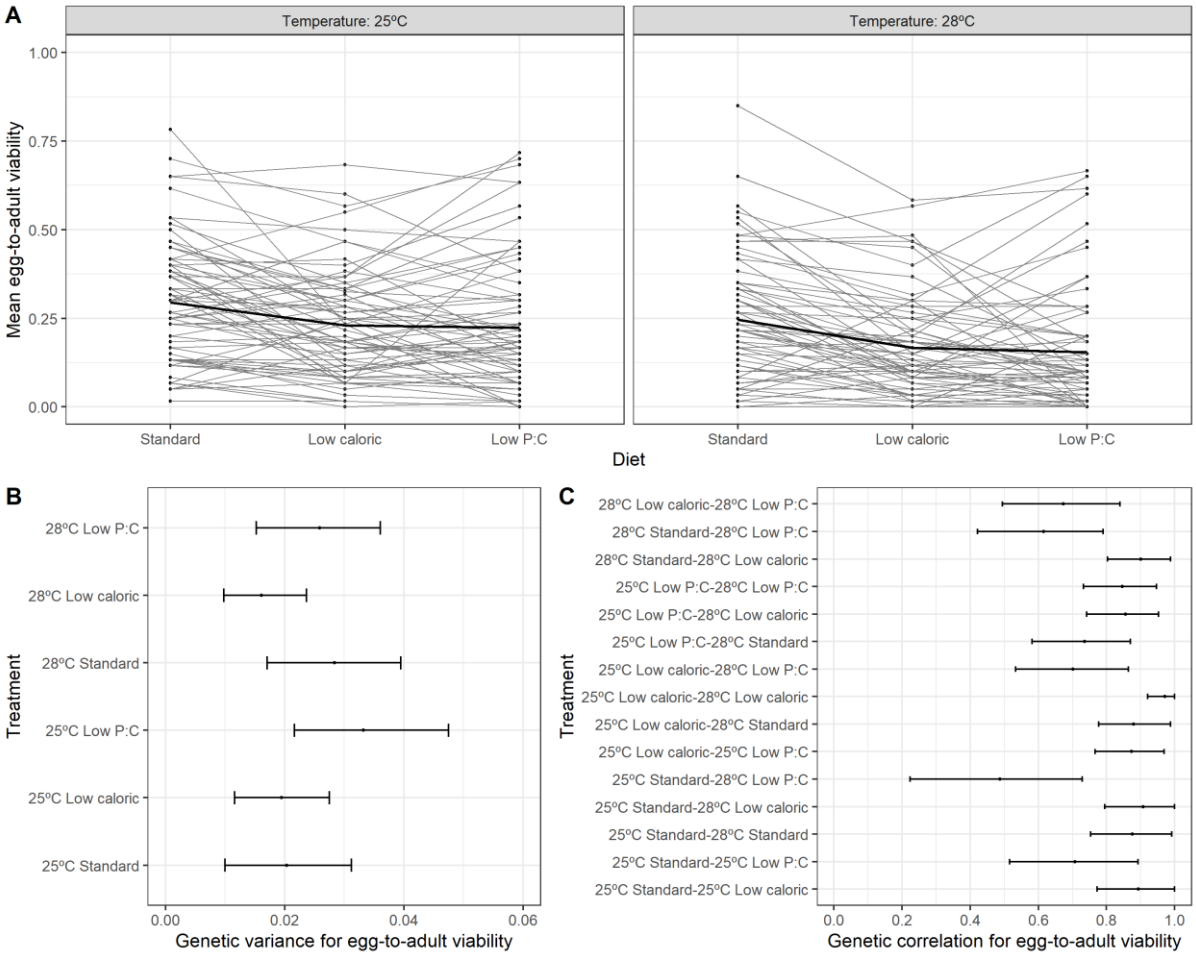
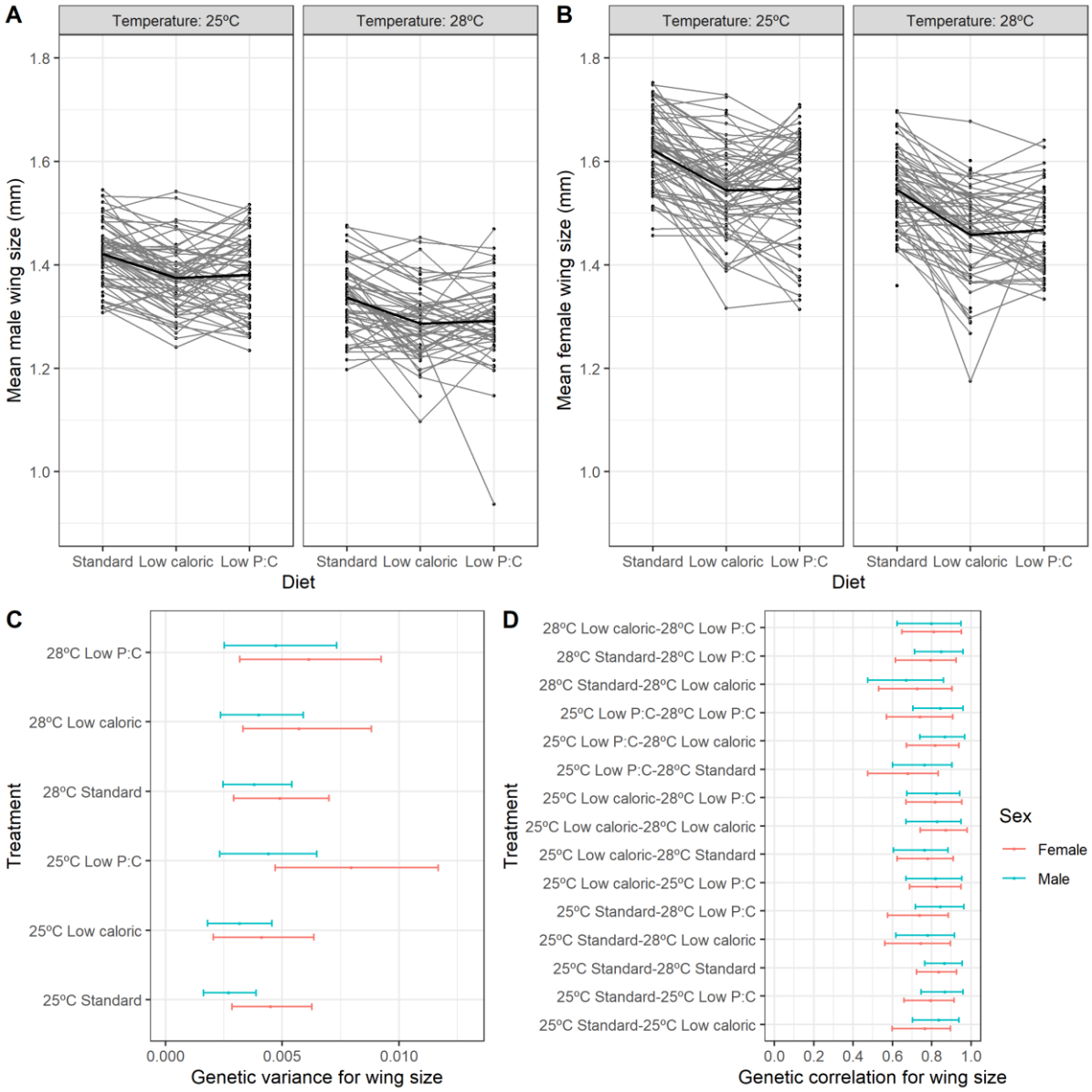


Fig. 2. Mean egg-to-adult viability of each isogenic lines (**A**), estimates of its genetic variance in response to six combined thermal-nutritional treatments, (**B**) and the genetic correlation within and across environments for viability (**C**). Black line presents the population mean in

536 panel A. Error bars shows 95% upper and lower highest posterior density (HPD) intervals in
537 panels B and C.



538
539 **Fig. 3.** Mean wing size of (**A**) males and (**B**) females of isogenic lines, estimates of their (**C**)
540 genetic variance, and (**D**) the genetic correlation within and across six combined thermal-
541 nutritional treatments. Black line presents the population mean in panels A and B. Error bars
542 shows 95% upper and lower HPD intervals in panels C and D.

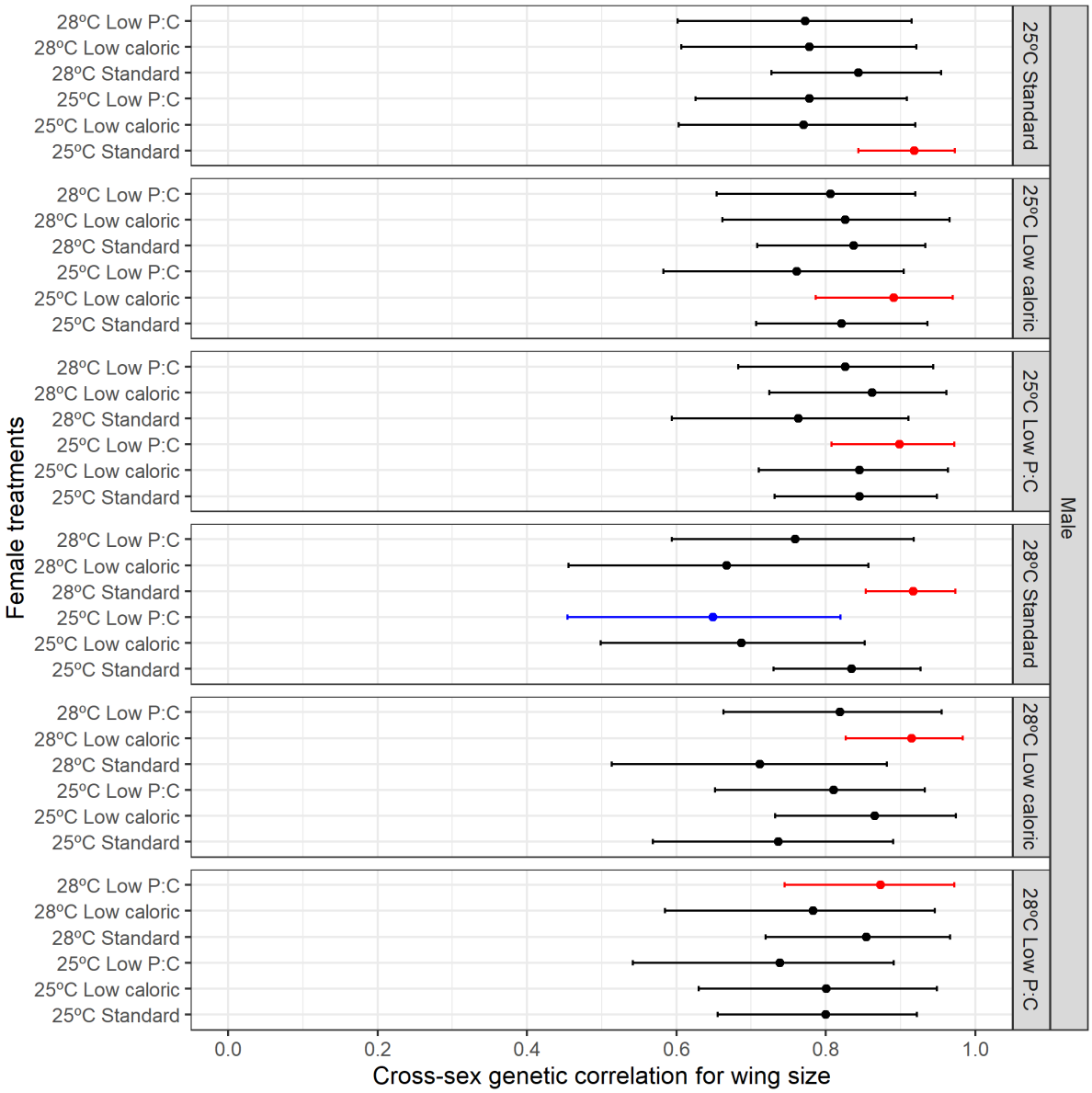


Fig. 4. Cross-sex genetic correlation for wing size between six combined thermal-nutritional treatments. Error bars shows 95% upper and lower HPD intervals. Red error bars indicate cross-sex genetic correlation within treatments. The blue error bar shows a significantly smaller cross-sex genetic correlation between females from 25°C low P:C diet and males from 28°C standard diet, compared to cross-sex genetic correlations within 25°C standard diet and 28°C standard diet.

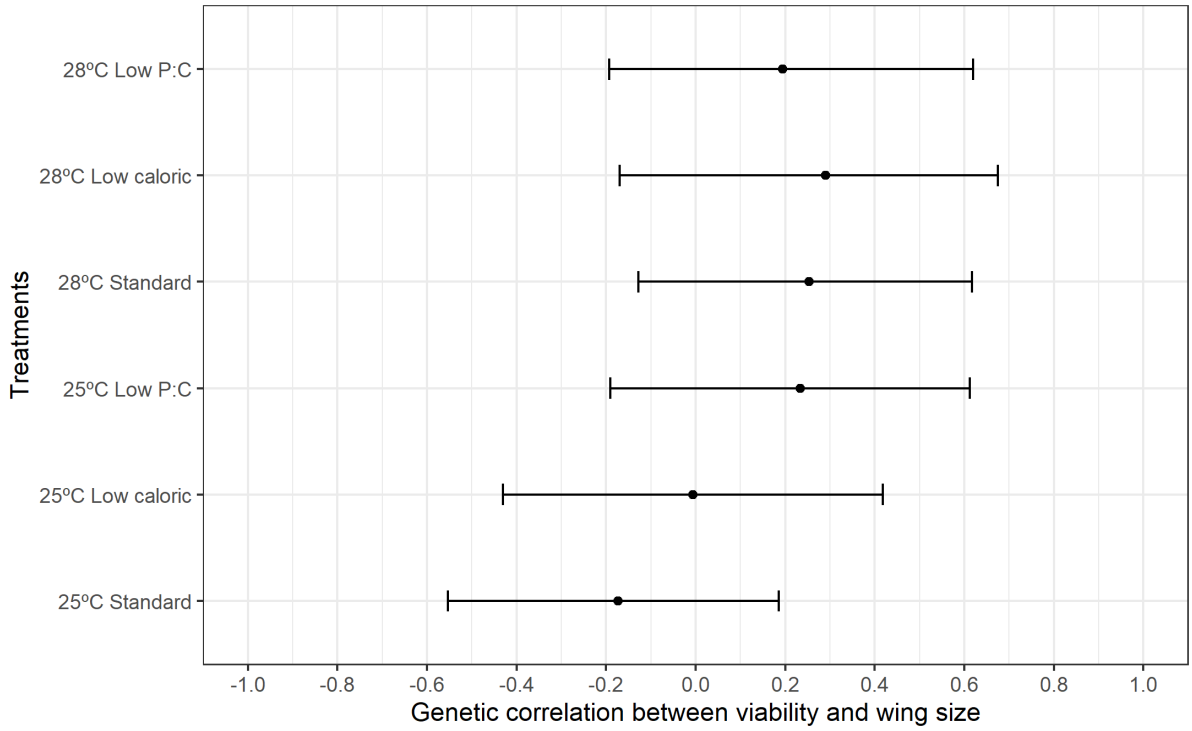


Fig. 5. Genetic correlation between egg-to-adult viability and wing size within each of six combined thermal-nutritional treatments. Wing size of both sexes was combined for estimating the genetic correlation between egg-to-adult viability and wing size. Error bars shows 95% upper and lower HPD intervals.

556 **Bibliography**

557

558 Abram PK, Boivin G, Moiroux J, Brodeur J (2017) 'Behavioural effects of temperature on
559 ectothermic animals: unifying thermal physiology and behavioural plasticity' *Biological*
560 *Reviews* **92**, 1859–1876. doi:10.1111/brv.12312
561 Agnew P, Hide M, Sidobre C, Michalakis Y (2002) 'A minimalist approach to the effects of
562 density-dependent competition on insect life-history traits' *Ecological Entomology* **27**,
563 396–402. doi:10.1046/j.1365-2311.2002.00430.x
564 Australian Government (2014) 'Climate change and extremes' Available at
565 http://www.bom.gov.au/climate/change/#tabs=Tracker&tracker=timeseries
566 Bader CA, Williams CR (2012) 'Mating, ovariole number and sperm production of the
567 dengue vector mosquito Aedes aegypti (L.) in Australia: Broad thermal optima provide
568 the capacity for survival in a changing climate' *Physiological Entomology* **37**, 136–144.
569 doi:10.1111/j.1365-3032.2011.00818.x
570 Barker JSF, Krebs RA (1995) 'Genetic variation and plasticity of thorax length and wing
571 length in Drosophila aldrichi and D. buzzatii' *Journal of Evolutionary Biology* **8**, 689–
572 709. doi:10.1046/j.1420-9101.1995.8060689.x
573 Bates D, Mächler M, Bolker BM, Walker SC (2015) 'Fitting linear mixed-effects models using
574 lme4' *Journal of Statistical Software* **67**,. doi:10.18637/jss.v067.i01
575 Berger D, Grieshop K, Lind MI, Goenaga J, Maklakov AA, Arnqvist G (2014) 'Intralocus sexual
576 conflict and environmental stress' *Evolution* **68**, 2184–2196. doi:10.1111/evo.12439
577 Blanckenhorn WU, Stillwell RC, Young KA, Fox CW, Ashton KG (2006) 'When Rensch Meets
578 Bergmann: Does Sexual Size Dimorphism Change Systematically With Latitude?'
579 *Evolution* **60**, 2004. doi:10.1554/06-110.1
580 Bochdanovits Z, De Jong G (2003) 'Experimental evolution in Drosophila melanogaster:
581 Interaction of temperature and food quality selection regimes' *Evolution* **57**, 1829–
582 1836. doi:10.1111/j.0014-3820.2003.tb00590.x
583 Bubliy OA, Kristensen TN, Kellermann V, Loeschcke V (2012) 'Plastic responses to four
584 environmental stresses and cross-resistance in a laboratory population of Drosophila
585 melanogaster' *Functional Ecology* **26**, 245–253. doi:10.1111/j.1365-2435.2011.01928.x
586 Bubliy OA, Loeschcke V (2002) 'Effect of low stressful temperature on genetic variation of
587 five quantitative traits in Drosophila melanogaster' *Heredity* **89**, 70–75.

588 doi:10.1038/sj.hdy.6800104
589 Bubliy OA, Loeschcke V, Imasheva AG (2001) 'Genetic variation of morphological traits in
590 Drosophila melanogaster under poor nutrition: isofemale lines and offspring-parent
591 regression' *Heredity* **86**, 363–369. doi:10.1046/j.1365-2540.2001.00837.x
592 Camus MF, Huang CC, Reuter M, Fowler K (2018) 'Dietary choices are influenced by
593 genotype, mating status, and sex in Drosophila melanogaster' *Ecology and Evolution* **8**,
594 5385–5393. doi:10.1002/ece3.4055
595 Chakraborty A, Sgrò CM, Mirth CK (2020) 'Does local adaptation along a latitudinal cline
596 shape plastic responses to combined thermal and nutritional stress?' *Evolution* **74**,
597 2073–2087. doi:10.1111/evo.14065
598 Chakraborty A, Walter GM, Monro K, Alves AN, Mirth CK, Sgrò CM (2022) 'Within-
599 population variation in body size plasticity in response to combined nutritional and
600 thermal stress is partially independent from variation in development time' *Journal of*
601 *Evolutionary Biology* 1–16. doi:10.1111/jeb.14099
602 Chevin LM, Lande R, Mace GM (2010) 'Adaptation, plasticity, and extinction in a changing
603 environment: Towards a predictive theory' *PLoS Biology* **8**,.
604 doi:10.1371/journal.pbio.1000357
605 Clissold FJ, Simpson SJ (2015) 'Temperature, food quality and life history traits of
606 herbivorous insects' *Current Opinion in Insect Science* **11**, 63–70.
607 doi:10.1016/j.cois.2015.10.011
608 Cockerell FE, Sgrò CM, McKechnie SW (2014) 'Latitudinal clines in heat tolerance, protein
609 synthesis rate and transcript level of a candidate gene in Drosophila melanogaster'
610 *Journal of Insect Physiology* **60**, 136–144. doi:10.1016/j.jinsphys.2013.12.003
611 Couret J, Dotson E, Benedict MQ (2014) 'Temperature, larval diet, and density effects on
612 development rate and survival of Aedes aegypti (Diptera: Culicidae)' *PLoS ONE* **9**,.
613 doi:10.1371/journal.pone.0087468
614 Cowley DE, Atchley WR (1990) 'Development and quantitative genetics of correlation
615 structure among body parts of Drosophila melanogaster' *The American Naturalist* **135**,
616 242–268. doi:10.1086/285041
617 Cowley DE, Atchley WR, Rutledge JJ (1986) 'Quantitative genetics of drosophila
618 melanogaster . I. Sexual dimorphism in genetic parameters for wing traits' *Genetics*
619 **114**, 549–566. doi:10.1093/genetics/114.2.549

620 DaMatta FM, Grandis A, Arenque BC, Buckeridge MS (2010) 'Impacts of climate changes on
621 crop physiology and food quality' *Food Research International* **43**, 1814–1823.
622 doi:10.1016/j.foodres.2009.11.001
623 Delcourt M, Blows MW, Rundle HD (2009) 'Sexually antagonistic genetic variance for fitness
624 in an ancestral and a novel environment' *Proceedings of the Royal Society B: Biological*
625 *Sciences* **276**, 2009–2014. doi:10.1098/rspb.2008.1459
626 Diepenbrock LM, Burrack HJ (2017) 'Variation of within-crop microhabitat use by Drosophila
627 suzukii (Diptera: Drosophilidae) in blackberry' *Journal of Applied Entomology* **141**, 1–7.
628 doi:10.1111/jen.12335
629 Diffenbaugh NS, Field CB (2013) 'Changes in ecologically critical terrestrial climate
630 conditions' *Science* **341**, 486–492. doi:10.1126/science.1237123
631 Dingle H (1992) 'Food level reaction norms in size-selected milkweed bugs Oncopeltus
632 fasciatus' *Ecological Entomology* **17**, 121–126. doi:10.1111/j.1365-
633 2311.1992.tb01168.x
634 Dreyer AP, Saleh Ziabari O, Swanson EM, Chawla A, Frankino WA, Shingleton AW (2016)
635 'Cryptic individual scaling relationships and the evolution of morphological scaling'
636 *Evolution* **70**, 1703–1716. doi:10.1111/evo.12984
637 Duun Rohde P, Krag K, Loeschcke V, Overgaard J, Sørensen P, Nygaard Kristensen T (2016) 'A
638 quantitative genomic approach for analysis of fitness and stress related traits in a
639 Drosophila melanogaster model population' *International Journal of Genomics* **2016**,.
640 doi:10.1155/2016/2157494
641 Duxbury EML, Chapman T (2021) 'Sex-specific responses of life span and fitness to variation
642 in developmental versus adult diets in drosophila melanogaster' *Journals of*
643 *Gerontology - Series A Biological Sciences and Medical Sciences* **75**, 1431–1438.
644 doi:10.1093/GERONA/GLZ175
645 Economos AC, Lints FA (1984) 'Growth rate and life span in Drosophila. I. Methods and
646 mechanisms of variation of growth rate' *Mechanisms of Ageing and Development* **27**,
647 1–13. doi:10.1016/0047-6374(84)90078-2
648 Falconer DS (1952) 'The problem of environment and selection' *The American Naturalist* **86**,
649 293–298. doi:10.1086/281736
650 Franěk R, Baloch AR, Kašpar V, Saito T, Fujimoto T, Arai K, Pšenička M (2020) 'Isogenic lines
651 in fish – a critical review' *Reviews in Aquaculture* **12**, 1412–1434.

652 doi:10.1111/raq.12389
653 Frankino WA, Bakota E, Dworkin I, Wilkinson GS, Wolf JB, Shingleton AW (2019) 'Individual
654 cryptic scaling relationships and the evolution of animal form' *Integrative and*
655 *Comparative Biology* **59**, 1411–1428. doi:10.1093/icb/icz135
656 French V, Feast M, Partridge L (1998) 'Body size and cell size in Drosophila: The
657 developmental response to temperature' *Journal of Insect Physiology* **44**, 1081–1089.
658 doi:10.1016/S0022-1910(98)00061-4
659 Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) 'Adaptive versus non-adaptive
660 phenotypic plasticity and the potential for contemporary adaptation in new
661 environments' *Functional Ecology* **21**, 394–407. doi:10.1111/j.1365-2435.2007.01283.x
662 Gowik U, Westhoff P (2011) 'The Path from C3 to C4 photosynthesis' *Plant Physiology* **155**,
663 56–63. doi:10.1104/pp.110.165308
664 Hangartner S, Sgrò CM, Connallon T, Booksmythe I (2022) 'Sexual dimorphism in phenotypic
665 plasticity and persistence under environmental change: An extension of theory and
666 meta-analysis of current data' *Ecology Letters* 1–16. doi:10.1111/ele.14005
667 Hawlena D, Schmitz OJ (2010) 'Herbivore physiological response to predation risk and
668 implications for ecosystem nutrient dynamics' *Proceedings of the National Academy of*
669 *Sciences of the United States of America* **107**, 15503–15507.
670 doi:10.1073/pnas.1009300107
671 van Heerwaarden B, Kellermann V, Sgrò CM (2016) 'Limited scope for plasticity to increase
672 upper thermal limits' *Functional Ecology* **30**, 1947–1956. doi:10.1111/1365-2435.12687
673 Hoffmann AA, Chown SL, Clusella-Trullas S (2013) 'Upper thermal limits in terrestrial
674 ectotherms: How constrained are they?' *Functional Ecology* **27**, 934–949.
675 doi:10.1111/j.1365-2435.2012.02036.x
676 Hoffmann AA, Sørensen JG, Loeschcke V (2003) 'Adaptation of Drosophila to temperature
677 extremes: Bringing together quantitative and molecular approaches' *Journal of*
678 *Thermal Biology* **28**, 175–216. doi:10.1016/S0306-4565(02)00057-8
679 Holleley CE, Hocking AD, Schubert TL, Whitehead MR (2008) 'Control of Penicillium
680 roqueforti (Thom) infection in cultures of Drosophila melanogaster (Meigen) (Diptera:
681 Drosophilidae)' *Australian Journal of Entomology* **47**, 149–152. doi:10.1111/j.1440-
682 6055.2007.00630.x
683 Houle D (1992) 'Comparing evolvability and variability of quantitative traits' *Genetics* **130**,

195–204. doi:10.1093/genetics/130.1.195

Huey RB, Carlson M, Crozier L, Frazier M, Hamilton H, Harley C, Hoang A, Kingsolver JG (2002) 'Plants versus animals: Do they deal with stress in different ways?' *Integrative and Comparative Biology* **42**, 415–423. doi:10.1093/icb/42.3.415

Imasheva AG, Bosenko D V., Bubli OA (1999) 'Variation in morphological traits of Drosophila melanogaster (fruit fly) under nutritional stress' *Heredity* **82**, 187–192. doi:10.1038/sj.hdy.6884660

IPCC (2018) 'IPCC Special Report on the impacts of global warming of 1.5°C.' Available at https://report.ipcc.ch/sr15/pdf/sr15_spm_final.pdf%0Ahttp://www.ipcc.ch/report/sr15/

Jang T, Lee KP (2018) 'Comparing the impacts of macronutrients on life-history traits in larval and adult Drosophila melanogaster: The use of nutritional geometry and chemically defined diets' *Journal of Experimental Biology* **221**,. doi:10.1242/jeb.181115

Janowitz SA, Fischer K (2011) 'Opposing effects of heat stress on male versus female reproductive success in Bicyclus anynana butterflies' *Journal of Thermal Biology* **36**, 283–287. doi:10.1016/j.jtherbio.2011.04.001

Jin J, Armstrong R, Tang C (2019) 'Impact of elevated CO2 on grain nutrient concentration varies with crops and soils – A long-term FACE study' *Science of the Total Environment* **651**, 2641–2647. doi:10.1016/j.scitotenv.2018.10.170

Kingsolver JG, Huey RB (2008) 'Size, temperature, and fitness: Three rules' *Evolutionary Ecology Research* **10**, 251–268.

Kreuzwieser J, Gessler A (2010) 'Global climate change and tree nutrition: Influence of water availability' *Tree Physiology* **30**, 1221–1234. doi:10.1093/treephys/tpq055

Kristensen TN, Overgaard J, Lassen J, Hoffmann AA, Sgrò C (2015) 'Low evolutionary potential for egg-to-adult viability in Drosophila melanogaster at high temperatures' *Evolution* **69**, 803–814. doi:10.1111/evo.12617

Kutz TC, Sgrò CM, Mirth CK (2019) 'Interacting with change: Diet mediates how larvae respond to their thermal environment' *Functional Ecology* **33**, 1940–1951. doi:10.1111/1365-2435.13414

Lafuente E, Duneau D, Beldade P (2018) 'Genetic basis of thermal plasticity variation in Drosophila melanogaster body size' *PLoS Genetics* **14**, 1–24. doi:10.1371/journal.pgen.1007686

716 Lande R (1980) ‘Sexual dimorphism, sexual selection, and adaptation in polygenic
717 characters’ *Evolution* **34**, 292. doi:10.2307/2407393
718 Lande R (2009) ‘Adaptation to an extraordinary environment by evolution of phenotypic
719 plasticity and genetic assimilation’ *Journal of Evolutionary Biology* **22**, 1435–1446.
720 doi:10.1111/j.1420-9101.2009.01754.x
721 Lasne C, Hangartner SB, Connallon T, Sgrò CM (2018) ‘Cross-sex genetic correlations and the
722 evolution of sex-specific local adaptation: insights from classical trait clines in
723 Drosophila melanogaster’ *Evolution* **72**, 1317–1327. doi:10.1111/evo.13494
724 Lee KP, Kim JS, Min KJ (2013) ‘Sexual dimorphism in nutrient intake and life span is mediated
725 by mating in Drosophila melanogaster’ *Animal Behaviour* **86**, 987–992.
726 doi:10.1016/j.anbehav.2013.08.018
727 Lee KP, Roh C (2010) ‘Temperature-by-nutrient interactions affecting growth rate in an
728 insect ectotherm’ *Entomologia Experimentalis et Applicata* **136**, 151–163.
729 doi:10.1111/j.1570-7458.2010.01018.x
730 Lukac M, Calfapietra C, Lagomarsino A, Loreto F (2010) ‘Global climate change and tree
731 nutrition: Effects of elevated CO2 and temperature’ *Tree Physiology* **30**, 1209–1220.
732 doi:10.1093/treephys/tpq040
733 MacKay TFC, Richards S, Stone EA, Barbadilla A, Ayroles JF, Zhu D, Casillas S, Han Y, Magwire
734 MM, Cridland JM, Richardson MF, Anholt RRH, Barrón M, Bess C, Blankenburg KP,
735 Carbone MA, Castellano D, Chaboub L, Duncan L, Harris Z, Javaid M, Jayaseelan JC,
736 Jhangiani SN, Jordan KW, Lara F, Lawrence F, Lee SL, Librado P, Linheiro RS, Lyman RF,
737 MacKey AJ, Munidasa M, Muzny DM, Nazareth L, Newsham I, Perales L, Pu LL, Qu C,
738 Ràmia M, Reid JG, Rollmann SM, Rozas J, Saada N, Turlapati L, Worley KC, Wu YQ,
739 Yamamoto A, Zhu Y, Bergman CM, Thornton KR, Mittelman D, Gibbs RA (2012) ‘The
740 Drosophila melanogaster Genetic Reference Panel’ *Nature* **482**, 173–178.
741 doi:10.1038/nature10811
742 Midgley G, Hannah L (2019) ‘Extinction risk from climate change’ *Biodiversity and Climate*
743 *Change: Transforming the Biosphere* 294–296. doi:10.2307/j.ctv8jnzw1.37
744 Min KJ, Flatt T, Kulaots I, Tatar M (2007) ‘Counting calories in Drosophila diet restriction’
745 *Experimental Gerontology* **42**, 247–251. doi:10.1016/j.exger.2006.10.009
746 De Moed GH, De Jong G, Scharloo W (1997) ‘Environmental effects on body size variation in
747 Drosophila melanogaster and its cellular basis’ *Genetical Research* **70**, 35–43.

748 doi:10.1017/S0016672397002930
749 Mossman JA, Biancani LM, Zhu CT, Rand DM (2016) 'Mitonuclear epistasis for development
750 time and its modification by diet in Drosophila' *Genetics* **203**, 463–484.
751 doi:10.1534/genetics.116.187286
752 Noer NK, Pagter M, Bahrndorff S, Malmendal A, Kristensen TN (2020) 'Impacts of thermal
753 fluctuations on heat tolerance and its metabolomic basis in Arabidopsis thaliana,
754 Drosophila melanogaster, and Orchesella cincta' *PLoS ONE* **15**, 1–20.
755 doi:10.1371/journal.pone.0237201
756 Ørsted M, Rohde PD, Hoffmann AA, Sørensen P, Kristensen TN (2018) 'Environmental
757 variation partitioned into separate heritable components' *Evolution* **72**, 136–152.
758 doi:10.1111/evo.13391
759 Partridge L, Barrie B, Fowler K, French V (1994) 'Evolution and development of body size and
760 cell size in Drosophila melanogaster in response to temperature' *Evolution* **48**, 1269–
761 1276. doi:10.1111/j.1558-5646.1994.tb05311.x
762 Pecl GT, Araújo MB, Bell JD, Blanchard J, Bonebrake TC, Chen I-C, Clark TD, Colwell RK,
763 Danielsen F, Evengård B, Falconi L, Ferrier S, Frusher S, Garcia RA, Griffis RB, Hobday AJ,
764 Janion-Scheepers C, Jarzyna MA, Jennings S, Lenoir J, Linnetved HI, Martin VY,
765 McCormack PC, McDonald J, Mitchell NJ, Mustonen T, Pandolfi JM, Pettorelli N, Popova
766 E, Robinson SA, Scheffers BR, Shaw JD, Sorte CJB, Strugnell JM, Sunday JM, Tuanmu M-
767 N, Vergés A, Villanueva C, Wernberg T, Wapstra E, Williams SE (2017) 'Biodiversity
768 redistribution under climate change: Impacts on ecosystems and human well-being'
769 *Science* **355**, 1–9. doi:10.1126/science.aai9214
770 Pétavy G, Moreteau B, Gibert P, Morin JP, David JR (2001) 'Phenotypic plasticity of body size
771 in Drosophila: Effects of a daily periodicity of growth temperature in two sibling
772 species' *Physiological Entomology* **26**, 351–361. doi:10.1046/j.0307-6962.2001.00255.x
773 Petersen C, Woods HA, Kingsolver JG (2000) 'Stage-specific effects of temperature and
774 dietary protein on growth and survival of Manduca sexta caterpillars' *Physiological*
775 *Entomology* **25**, 35–40. doi:10.1046/j.1365-3032.2000.00163.x
776 Polak M, Kroeger DE, Cartwright IL, Ponce DeLeon C (2004) 'Genotype-specific responses of
777 fluctuating asymmetry and of preadult survival to the effects of lead and temperature
778 stress in Drosophila melanogaster' *Environmental Pollution* **127**, 145–155.
779 doi:10.1016/S0269-7491(03)00238-0

780 Pottier P, Burke S, Drobniak SM, Lagisz M, Nakagawa S (2021) 'Sexual (in)equality? A meta-
781 analysis of sex differences in thermal acclimation capacity across ectotherms.'
782 doi:10.1111/1365-2435.13899
783 Van Der Putten WH, Macel M, Visser ME (2010) 'Predicting species distribution and
784 abundance responses to climate change: Why it is essential to include biotic
785 interactions across trophic levels' *Philosophical Transactions of the Royal Society B:*
786 *Biological Sciences* **365**, 2025–2034. doi:10.1098/rstb.2010.0037
787 Reed TE, Robin SW, Schindler DE, Hard JJ, Kinnison MT (2010) 'Phenotypic plasticity and
788 population viability: The importance of environmental predictability' *Proceedings of the*
789 *Royal Society B: Biological Sciences* **277**, 3391–3400. doi:10.1098/rspb.2010.0771
790 Reeve JP, Fairbairn DJ (1996) 'Sexual size dimorphism as a correlated response to selection
791 on body size: an empirical test of the quantitative genetic model' *Evolution* **50**, 1927–
792 1938. doi:10.1111/j.1558-5646.1996.tb03580.x
793 Rho MS, Lee KP (2017) 'Temperature-driven plasticity in nutrient use and preference in an
794 ectotherm' *Oecologia* **185**, 401–413. doi:10.1007/s00442-017-3959-4
795 Rohlf FJ (2015) 'The tps series of software' *Hystrix* **26**, 1–4. doi:10.4404/hystrix-26.1-11264
796 Rosenblatt AE, Schmitz OJ (2016) 'Climate change, nutrition, and bottom-up and top-down
797 food web processes' *Trends in Ecology and Evolution* **31**, 965–975.
798 doi:10.1016/j.tree.2016.09.009
799 Scheiner SM (1993) 'Genetics and evolution of phenotypic plasticity' *Annual Review of*
800 *Ecology and Systematics* **24**, 35–68. doi:10.1146/annurev.es.24.110193.000343
801 Schilthuizen M, Kellermann V (2014) 'Contemporary climate change and terrestrial
802 invertebrates: Evolutionary versus plastic changes' *Evolutionary Applications* **7**, 56–67.
803 doi:10.1111/eva.12116
804 Schmidt PS, Matzkin L, Ippolito M, Eanes WF (2005) 'Geographic variation in diapause
805 incidence, life-history traits, and climatic adaptation in Drosophila melanogaster'
806 *Evolution* **59**, 1721–1732. doi:10.1111/j.0014-3820.2005.tb01821.x
807 Schou MF, Kristensen TN, Pedersen A, Göran Karlsson B, Loeschcke V, Malmendal A (2017)
808 'Metabolic and functional characterization of effects of developmental temperature in
809 Drosophila melanogaster' *American Journal of Physiology - Regulatory Integrative and*
810 *Comparative Physiology* **312**, R211–R222. doi:10.1152/ajpregu.00268.2016
811 Seebacher F, White CR, Franklin CE (2015) 'Physiological plasticity increases resilience of

812 ectothermic animals to climate change' *Nature Climate Change* **5**, 61–66.
813 doi:10.1038/nclimate2457
814 Sgrò CM, Overgaard J, Kristensen TN, Mitchell KA, Cockerell FE, Hoffmann AA (2010) 'A
815 comprehensive assessment of geographic variation in heat tolerance and hardening
816 capacity in populations of Drosophila melanogaster from Eastern Australia' *Journal of*
817 *Evolutionary Biology* **23**, 2484–2493. doi:10.1111/j.1420-9101.2010.02110.x
818 Sgrò CM, Terblanche JS, Hoffmann AA (2016) 'What can plasticity contribute to Insect
819 responses to climate change?' *Annual Review of Entomology* **61**, 433–451.
820 doi:10.1146/annurev-ento-010715-023859
821 Sheets HD (2014) 'PCAGen8. Integrated Morphometrics Package Suite (IMP) 8'
822 Shingleton AW, Masandika JR, Thorsen LS, Zhu Y, Mirth CK (2017) 'The sex-specific effects of
823 diet quality versus quantity on morphology in Drosophila melanogaster' *Royal Society*
824 *Open Science* **4**,. doi:10.1098/rsos.170375
825 Sisodia S, Singh BN (2010) 'Resistance to environmental stress in Drosophila ananassae:
826 Latitudinal variation and adaptation among populations' *Journal of Evolutionary*
827 *Biology* **23**, 1979–1988. doi:10.1111/j.1420-9101.2010.02061.x
828 Stillwell RC, Blanckenhorn WU, Teder T, Davidowitz G, Fox CW (2010a) 'Sex differences in
829 phenotypic plasticity affect variation in sexual size dimorphism in insects: From
830 physiology to evolution' *Annual Review of Entomology* **55**, 227–245.
831 doi:10.1146/annurev-ento-112408-085500
832 Stillwell RC, Blanckenhorn WU, Teder T, Davidowitz G, Fox CW (2010b) 'Sex Differences in
833 Phenotypic Plasticity Affect Variation in Sexual Size Dimorphism in Insects: From
834 Physiology to Evolution' *Annual Review of Entomology* **55**, 227–245.
835 doi:10.1146/annurev-ento-112408-085500
836 Stillwell RC, Fox CW (2007) 'Environmental effects on sexual size dimorphism of a seed-
837 feeding beetle' *Oecologia* **153**, 273–280. doi:10.1007/s00442-007-0724-0
838 Stillwell RC, Wallin WG, Hitchcock LJ, Fox CW (2007) 'Phenotypic plasticity in a complex
839 world: Interactive effects of food and temperature on fitness components of a seed
840 beetle' *Oecologia* **153**, 309–321. doi:10.1007/s00442-007-0748-5
841 Teder T, Tammaru T (2005) 'Sexual size dimorphism within species increases with body size
842 in insects' *Oikos* **108**, 321–334. doi:10.1111/j.0030-1299.2005.13609.x
843 Teuschl Y, Reim C, Blanckenhorn WU (2007) 'Correlated responses to artificial body size

844 selection in growth, development, phenotypic plasticity and juvenile viability in yellow
845 dung flies' *Journal of Evolutionary Biology* **20**, 87–103. doi:10.1111/j.1420-
846 9101.2006.01225.x
847 Tun-Lin W, Burkot TR, Kay BH (2000) 'Effects of temperature and larval diet on development
848 rates and survival of the dengue vector Aedes aegypti in north Queensland, Australia'
849 *Medical and Veterinary Entomology* **14**, 31–37. doi:10.1046/j.1365-2915.2000.00207.x
850 Urban MC, Richardson JL, Freidenfelds NA (2014) 'Plasticity and genetic adaptation mediate
851 amphibian and reptile responses to climate change' *Evolutionary Applications* **7**, 88–
852 103. doi:10.1111/eva.12114
853 Via S, Conner J (1995) 'Evolution in heterogeneous environments: Genetic variability within
854 and across different grains in Tribouum castaneum' *Heredity* **74**, 80–90.
855 doi:10.1038/hdy.1995.10
856 Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH (1995)
857 'Adaptive phenotypic plasticity: consensus and controversy' *Trends in Ecology &*
858 *Evolution* **10**, 212–217. doi:10.1016/S0169-5347(00)89061-8
859 Via S, Lande R (1985) 'Genotype-environment interaction and the evolution of phenotypic
860 plasticity.' *Evolution* **39**, 505–522. doi:10.1111/j.1558-5646.1985.tb00391.x
861 Via S, Lande R (1987) 'Evolution of genetic variability in a spatially heterogeneous
862 environment: Effects of genotype–environment interaction' *Genetical Research* **49**,
863 147–156. doi:10.1017/S001667230002694X
864 Walter GM, Clark J, Terranova D, Cozzolino S, Cristaudo A, Hiscock SJ, Bridle JR (2020)
865 'Hidden genetic variation in plasticity increases the potential to adapt to novel
866 environments' *bioRxiv* 1–32.
867 Walters RJ, Blanckenhorn WU, Berger D (2012) 'Forecasting extinction risk of ectotherms
868 under climate warming: An evolutionary perspective' *Functional Ecology* **26**, 1324–
869 1338. doi:10.1111/j.1365-2435.2012.02045.x
870 Wang JB, Lu HL, St. Leger RJ (2017) 'The genetic basis for variation in resistance to infection
871 in the Drosophila melanogaster genetic reference panel' *PLoS Pathogens* **13**, 1–33.
872 doi:10.1371/journal.ppat.1006260
873 Wang S, Tan XL, Guo XJ, Zhang F (2013) 'Effect of temperature and photoperiod on the
874 development, reproduction, and predation of the predatory ladybird Cheilomenes
875 sexmaculata (Coleoptera: Coccinellidae)' *Journal of Economic Entomology* **106**, 2621–

2629. doi:10.1603/EC13095

Wiens JJ (2016) ‘Climate-related local extinctions are already widespread among plant and animal species’ *PLoS Biology* **14**, 1–18. doi:10.1371/journal.pbio.2001104

Zhang W, Rudolf VHW, Ma C Sen (2015) ‘Stage-specific heat effects: timing and duration of heat waves alter demographic rates of a global insect pest’ *Oecologia* **179**, 947–957. doi:10.1007/s00442-015-3409-0