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

Key words: plasticity, temperature, nutrition, sex-specific plasticity, wing size, isogenic lines, genetic variance, genetic correlation, reaction norm, *Drosophila melanogaster*

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 $\times$ 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

97 Conner 1995). Variation in plasticity among genotypes (or significant G × E) can be caused
98 by significant variation in genes regulating the expressed phenotype (quantified by changes
99 in genetic variance across environments), or a significant genetic correlation between two
100 trait characters that is less than one (Via and Conner 1995), or both. Understanding within-
101 population variation, and decoupling its cause, is essential for predicting a population's
102 adaptability to long-term environmental stress (Ghalambor *et al.* 2007).
103
104 In addition to plasticity varying among genotypes within a population, plasticity can also
105 potentially vary between sexes. However, evidence for sex-specific plasticity is mixed.
106 Studies of a range of life-history and stress resistance traits have failed to find evidence for
107 sex-specific plasticity. In contrast, males and females differ in their plastic responses to
108 developmental conditions for body size (Pétavy *et al.* 2001; Teder and Tammaru 2005;
109 Stillwell and Fox 2007) and cold resistance (Pottier *et al.* 2021; Hangartner *et al.* 2022).
110 Although size differences between sexes (sexual size dimorphism) is common across insect
111 taxa (Teder and Tammaru 2005; Blanckenhorn *et al.* 2006; Stillwell *et al.* 2010a), no studies
112 have looked at sex-specific plasticity in size response to combined thermal-nutritional
113 stress. Sex-specific variation may further complicate the prediction of a population's
114 response and its dynamics under climate change (Hangartner *et al.* 2022), making it crucial
115 to measure sex-specific plasticity in life-history traits in response to combined stress.
116
117 In this study, we used a set of newly derived isogenic lines of *D. melanogaster* initiated from
118 a population collected along the east coast of Australia. These lines allow us to extend upon
119 previous studies that have examined variation in plasticity between populations (Sgrò *et al.*
120 2010; Cockerell *et al.* 2014; van Heerwaarden *et al.* 2016; Chakraborty *et al.* 2020).
121 Specifically, we used these lines to quantify sex-specific variation in plasticity in response to
122 combined thermal and nutritional stress. We subjected developing larvae to six
123 combinations of thermal and nutritional conditions that included two temperatures: 25°C
124 and 28°C, and three diets: standard, low caloric, and low protein to carbohydrate (P:C) diets.
125 These conditions were chosen to best simulate climate change, which is expected to cause a
126 3°C increase in average temperature (Australian Government 2014), and to reduce protein
127 and vary carbohydrate contents in plants (DaMatta *et al.* 2010; Gowik and Westhoff 2011;
128 IPCC 2018). We assessed plasticity of egg-to-adult viability and adult wing centroid size,

129 which have previously shown sensitivity to combined thermal and nutritional stress (Kutz *et*
130 *al.* 2019; Chakraborty *et al.* 2020). Given that *D. melanogaster* shows sexual size
131 dimorphism, and that the sexes may vary in their plasticity (Stillwell *et al.* 2010b; Shingleton
132 *et al.* 2017; Pottier *et al.* 2021; Hangartner *et al.* 2022), we assessed size plasticity separately
133 for males and females. By quantifying the genetic and sex-specific variation in plasticity
134 within a population in response to combinations of environmental stressors, our study
135 sheds new insight into how animal populations may respond to ongoing climate change.
136
137 **Methods**
138
139 *Fly stocks - isogenic lines*
140
141 We used isogenic lines that were established by Chakraborty *et al.* (2022). Briefly, two
142 hundred field-inseminated female *D. melanogaster* were collected in Duranbah, New South
143 Wales, Australia (28.3° S, 153.5° E) in 2018. From each of these females, two independent
144 iso-female lines were established and bred into four hundred lines. Full-sibling mating was
145 performed in each of these four hundred lines for twenty generations following MacKay *et*
146 *al.* (2012), resulting in 72 isogenic lines. Four copies of each isogenic line were reared at
147 25°C on standard diets (1:3 P:C ratio and 320 kcal/ L) under 12:12 light:dark cycle for twelve
148 generations prior to the experiment, as described below. The standard diet was prepared by
149 mixing water with potato flakes (18.18 g/ L), yeast (36.36 g/ L), agar (6.36 g/ L), and dextrose
150 (27.27 g/ L), with addition of nipagin and propionic acid to prevent bacterial and fungal
151 growth (Holleley *et al.* 2008).
152
153 *Developmental treatment conditions and choice of traits*
154
155 We used six thermal-nutritional conditions to simulate the possible shifts in temperature
156 (IPCC 2018) and nutrition (DaMatta *et al.* 2010; Gowik and Westhoff 2011; IPCC 2018) under
157 climate change. They included combinations of two temperatures: 25°C and 28°C, and three
158 diets: standard diet (1:3 P:C ratio and 320 kcal/L), low caloric diet (1:3 P:C and 80kcal/L), and
159 low P:C diet (1:12 P:C and 320kcal/L). 25°C represents the average summer temperature in
160 south-east Australia, whilst 28°C represents the 3°C temperature increase projected in

161 twenty years under climate change (Australian Government 2014). The low caloric diet and
162 the low P:C diets simulate a reduction of protein and variation of carbohydrates in plants
163 composition projected under climate change (DaMatta *et al.* 2010). These conditions have
164 been previously shown to successfully induce plastic responses in our traits of interest, egg-
165 to-adult viability and wing size (Kutz *et al.* 2019; Chakraborty *et al.* 2020).
166
167 Egg-to-adult viability and wing size were chosen because they are fitness-related
168 developmental traits known to be sensitive to nutrition and diet individually and in
169 combination (Kutz *et al.* 2019; Chakraborty *et al.* 2020). Egg-to-adult viability is a direct
170 indication of an individual's ability to survive to the reproductive adult stage (Kristensen *et*
171 *al.* 2015), and larger adult body size often correlates with greater fitness in ectotherms,
172 including *Drosophila* (Kingsolver and Huey 2008). Assessing variation in plasticity within a
173 population using these fitness-related developmental traits will allow us to better
174 understand how well species survive under stressful environments at the population level.
175
176 *Experimental set up - Egg lays to obtain experimental flies*
177
178 Adults from isogenic lines were transferred to laying plates containing apple juice, agar,
179 nipagin, and a surface layer of autoclaved yeast to encourage oviposition. Laying plates
180 were maintained at 25°C for 18 hours to allow egg laying.
181
182 *Egg-to-adult viability assay*
183
184 Eggs were collected from laying plates and transferred to 7.2 ml vials containing their
185 respective diets. Three replicate vials were included for each treatment and line
186 combination, with a density of twenty eggs per vial (Agnew *et al.* 2002). Vials containing
187 fresh eggs were kept at their respective treatment temperature at a 12:12 hour light: dark
188 cycle until adult eclosion. Egg-to-adult viability was calculated as the number of eclosed
189 adults relative to the initial number of eggs deposited into each vial (20 eggs).
190
191 *Adult body size measurement*
192

193 Wing size was used as a proxy for body size (Partridge *et al.* 1994). After eclosing, adults
194 were sexed and preserved in a 70% ethanol-30% glycerol solution for wing dissection. Left
195 wings of each sex were removed with fine forceps, transferred to glass slides, and
196 suspended in 70% ethanol-30% glycerol solution for imaging. To measure the wing centroid
197 size, landmarks were positioned at eight vein intersections as indicated in Fig. 1, and the X-
198 and Y- coordinates of landmarks were determined using the software package tpsDIG (Rohlf
199 2015). Using the software CoorDGen8 (Sheets 2014), wing area was calculated as square
200 root of the sum of the squared distances between each landmark.
201
202 *Statistical analysis*
203
204 *Testing for overall genotype-by-environment (G × E) interactions*
205
206 R was used to perform all data analysis and visualization (version 3.4.3) (R Core Team,
207 2017). To test for significant G × E, we used the *lme4* package (Bates *et al.* 2015), data of
208 egg-to-adult viability and wing size were fitted using linear mixed effects models. As both
209 traits had normal error distributions, they were fitted with a Gaussian error distribution. For
210 both traits, temperature and diet were treated as fixed effects, and line and block as
211 random effects. Both intercepts and slopes were allowed to vary in all models, since
212 differences in the slope of the reaction norm of each isogenic line would reflect significant G
213 × E. Wing size was analysed separately for each sex.
214
215 Models were fitted separately for each trait. Random effects of block, line, and
216 temperature-by-diet-by-line were added sequentially (see equations 1-3) and the
217 significance of each variable was tested using log-likelihood ratio tests from the *lrtest*
218 package (Zeileis and Hothorn, 2002).
219
220 Specifically, the first model (equation 1) included diet and temperature as the fixed effects,
221 and block as a random effect. This estimates the overall population response to
222 combinations of temperature and diet, while accounting for the differences in egg-to-adult
223 viability/wing size among blocks.
224

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

In the second model (equation 2), line was added as an additional random effect to test for differences in egg-to-adult viability/wing size across the isogenic lines.

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

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

257 iterations were kept from each model, which provided the posterior distribution for each
258 parameter estimated.
259
260 *Quantifying genetic (co)variance in egg-to-adult viability*
261
262 To estimate genetic (co)variance in egg-to-adult viability within and across environments,
263 we used this equation

264 $$y_{jklm} = T_j + l_{k(j)} + b_{l(jk)} + e_{m(jkl)}\ , \qquad \text{Eqn. 4}$$

265 where we included viability as the response variable y_{jklm} and treatment T_j as the only
266 fixed effect. Random effects included k^{th} isogenic line $l_{k(j)}$ and l^{th} block $b_{l(j)}$ within the
267 treatments, to remove any variance due to differences between experimental blocks. The
268 term $e_{m(jkl)}$ represents residual variance. For the isogenic line component, random
269 intercepts of each treatment and random slopes between treatments were estimated. This
270 respectively calculates the genetic variance in egg-to-adult viability in each treatment and
271 genetic covariance between treatments, resulting in a 6 × 6 genetic (co)variance matrix.
272 Genetic correlations between treatments were calculated from the covariance matrix
273 (Walter *et al.* 2020).
274
275 *Quantifying genetic (co)variance in wing size*
276
277 To estimate genetic (co)variance in wing size within and across environments, we used this
278 equation

279 $$y_{ijklm} = T_i + S_j + l_{k(ij)} + b_{l(ijk)} + e_{m(ijkl)} \qquad \text{Eqn. 5}$$

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

288 and between the sexes and treatments were further calculated from the (co)variance matrix
289 (Walter *et al.* 2020).
290
291 *Genetic correlations between egg-to-adult viability and wing size*
292
293 To estimate genetic correlations between egg-to-adult viability and wing size across
294 treatments, we applied equation 4 but included both egg-to-adult viability and wing size as
295 a bivariate response variable (note that wing size was averaged over both sexes and
296 estimated without sex as a fixed effect). As egg-to-adult viability and wing size are measured
297 on different scales, we standardised each trait by dividing trait values by their grand mean
298 (Houle 1992). Treatment (the six thermal-nutritional conditions) was initially included as a
299 fixed effect, however, this returned inflated (co)variance estimates. To obtain more
300 conservative estimates of genetic (co)variance, equation 4 was applied separately within
301 each treatment, and only included isogenic line and block as random effects. Random slopes
302 and intercepts were estimated, representing the genetic variance in viability and wing size,
303 and the genetic covariance between viability and wing size respectively. This produced six 2
304 × 2 genetic (co)variance matrices. Genetic correlations between traits for each treatment
305 were then calculated from each (co)variance matrix.
306
307 **Results**
308
309 We aimed to characterise genetic variation (G × E) in plasticity for both egg-to-adult viability
310 and sex-specific wing size in response to combined stressors within a population. We
311 subjected *D. melanogaster* isogenic lines to six different thermal-nutritional conditions
312 (25°C—standard diet, 25°C—low caloric diet, 25°C—low P:C diet, 28°C—standard diet,
313 28°C—low caloric diet, and 28°C—low P:C diet) and tested for significant genetic variation in
314 plasticity in egg-to-adult viability and wing size. We then calculated genetic (co)variances
315 and genetic correlations between traits within and across environments to understand the
316 causes of the significant G × E observed for each trait.
317
318 *Genotype-by-environment interactions in egg-to-adult viability*
319

320 Temperature and diet had significant individual effects on egg-to-adult viability (Fig. 2A and
321 Table S1). On average, viability was higher at 25°C compared to 28°C, and higher on
322 standard diets compared to low caloric and low P:C diets (Fig. 2A). The best fit model
323 included the random effect of temperature-by-diet-by-line interactions (Table 1), indicating
324 significant genetic variation in plasticity for viability (G × E) in response to combinations of
325 temperature and diet, captured by non-parallel individual reaction norms among lines (Fig.
326 2A).
327
328 *Genetic (co)variances in egg-to-adult viability*
329
330 We estimated genetic (co)variance matrices to determine whether significant G × E for
331 viability was caused by differences in the distribution of genetic variance across
332 environments and/or cross-environment genetic correlations that are less than one (Via and
333 Lande 1985). We found that the estimates of genetic variance did not differ across
334 treatments (Fig. 2B and Fig. S1), evidenced by the overlapping 95% highest posterior density
335 (HPD) intervals across treatments (Fig 2B). This indicates that significant G × E in viability
336 was not due to differences in genetic variance across environments.
337
338 Genetic correlations between treatment pairs were all positive and significantly greater
339 than zero, but mostly less than one (Fig. 2C and Fig. S2). This suggests that significant G × E
340 for viability results from differences in how isogenic lines respond to environments
341 (Falconer 1952; Via and Lande 1985).
342
343 *Genotype-by-environment interactions in wing size*
344
345 Initially, we analysed wing size separately for males and females. Temperature and diet
346 were found to significantly affect both male and female wing size (Table S2 and Table S3).
347 Wing sizes in both sexes were larger at 25°C compared to 28°C, and when larvae were
348 reared on standard diets compared to low caloric and low P:C diets (Fig. 3A and Fig. 3B). As
349 evidence for genetic variance in plasticity for wing size in males and females, we found the
350 best fit models included the random effect of temperature-by-diet-by-line interaction (Table

351 2). Such significant G × E in male and female wing size within the population is visualised as
352 non-parallel reaction norms (Fig. 3A and Fig. 3B).
353
354 *Genetic (co)variances in wing size*
355
356 We found significant genetic variance in wing size for all treatments within each sex;
357 however, the distribution of genetic variance did not differ across treatments (Fig. 3C and
358 Fig. S3). Thus, significant G × E in wing size in both males and females was not caused by the
359 differences in the distribution of genetic variance across the environments.
360
361 Genetic correlations in wing size across the six thermal-nutritional treatments within each
362 sex were all positive, significantly greater than zero, and mostly less than 1 (Fig. 3D, Fig. S4
363 and Fig. S5). This suggests that, similar to what we found for viability, significant G × E in
364 wing size of both males and females was caused by differences in the alleles contributing to
365 the response across environments (Falconer 1952; Via and Lande 1985).
366
367 *Cross-sex genetic correlations for wing size*
368
369 To determine whether wing size has a similar genetic bases in both sexes, we estimated the
370 cross-sex genetic correlation for wing size within and between all thermal-nutritional
371 treatments and sexes. This helps us to understand the potential for size to evolve
372 independently in each sex and in each environment (Falconer 1952; Via and Lande 1985).
373
374 Cross-sex correlations within treatments (indicated in red in Fig. 4, Fig S.6) were significantly
375 greater than zero, and large, but less than one, indicating that even though sexes share
376 some genetic basis for these traits, there is also some degree of genetic independence.
377 Furthermore, cross-sex genetic correlations were smaller when males and females
378 developed in different treatments. Specifically, the correlation between males from 28°C
379 standard diet condition and females from 25°C low P:C diet condition (indicated in blue in
380 Fig. 4) was significantly lower than correlations within 25°C standard diet and 28°C standard
381 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 history traits, egg-adult viability and adult body size. Specifically, both viability and wing size were reduced on low quality diets; this effect was greater at 28°C compared to 25°C. This suggests that exposure to combined thermal-nutritional stress, projected under climate change, may negatively impact population persistence (DaMatta *et al.* 2010; Gowik and Westhoff 2011; IPCC 2018; Kutz *et al.* 2019). Vitally, we found that individual genotypes within a population can also vary in the extent and direction of plasticity for both traits.

Previous studies investigating a range of taxa have reported extensive within-population variation in plastic responses to temperature and nutrition individually, for a range of life-history traits and stress resistance traits (Mossman *et al.* 2016; Wang *et al.* 2017; Lafuente *et al.* 2018; Frankino *et al.* 2019), including viability (Duun Rohde *et al.* 2016) and body size (Barker and Krebs 1995; Imasheva *et al.* 1999; Lafuente *et al.* 2018). We have found that populations also harbour significant genetic variation in plasticity for both viability and body size in response to combinations of temperature and diet.

The extent to which genotypes within a population vary in their reaction norm will determine how the population will respond to selection imposed by environmental change (Chevin *et al.* 2010; Dreyer *et al.* 2016; Frankino *et al.* 2019). As a result, populations with similar mean reaction norms may vary markedly in the distribution of their genotype-specific reaction norms, and this would cause them to respond differently to selection (Dreyer *et al.* 2016; Frankino *et al.* 2019). Predictions of long-term evolutionary responses to environmental changes using population-level reaction norms, could be inaccurate, unless the genotype-specific reaction norms are also considered (Reed *et al.* 2010; Dreyer *et al.* 2016; Frankino *et al.* 2019).

444 We found significant genetic variance for viability across all combinations of diet and
445 temperature. However, the expression of genetic variance did not vary across
446 environments. Bubliy *et al.* (2001), and Bubliy and Loeschcke (2002) both used half-sib
447 analyses to investigate genetic variance in plasticity for viability to changes in temperature.
448 Consistent with the present study, Bubliy and Loeschcke (2002) found no significant
449 difference in the expression of genetic variance for viability between 13°C and 25°C. In
450 contrast, Bubliy *et al.* (2001) observed a significant increase in genetic variance for viability
451 at 32°C compared to 25°C. Perhaps if we had increased the rearing temperature above 28°C,
452 we might have observed differences in expression of genetic variance. Instead, we found
453 that significant G × E interactions for viability was a result of genetic correlations across
454 environments that were above zero and often less than one. This indicates that viability
455 across some thermal-nutritional conditions is controlled by different alleles or differently by
456 the same alleles (Via and Lande 1985), meaning that viability has some capacity to evolve
457 independently across different thermal-nutritional environments (Via and Lande 1987).
458
459 Although body size plasticity has been extensively shown to vary within a population in
460 response to temperature (Barker and Krebs 1995; Lafuente *et al.* 2018) and nutrition
461 (Imasheva *et al.* 1999), the source of G × E differed across studies. De Moed *et al.* (1997)
462 found that the significant G × E for body size was due to differences in the expression of
463 genetic variance across environments. In contrast, we found that the significant G × E for
464 body size for both sexes was due to genetic correlations across treatments that were
465 significantly different from zero but often less than one. Similarly, Barker and Krebs (1995)
466 and Imasheva *et al.* (1999) have also reported the same source of G × E for body size
467 plasticity to changes in temperature and dietary yeast concentration respectively. These
468 differences across studies are likely to arise due to differences in the source populations and
469 experimental conditions used.
470
471 As expected from previous studies, we found that wing size exhibited sexual dimorphism
472 (females were larger than males), and there were strong and positive cross-sex genetic
473 correlations within treatments (Cowley *et al.* 1986; Cowley and Atchley 1990; Reeve and
474 Fairbairn 1996; Lasne *et al.* 2018). Strong and positive cross-sex genetic correlations indicate

475 that the genetic basis of plasticity in wing size is largely shared in males and females, though
476 because these correlations are less than one there is some potential for independent
477 evolution of size plasticity between the sexes (Lande 1980; Via and Lande 1987). Cross-sex
478 genetic correlations were reduced when males and females developed in different
479 environments. Thus, there is a greater possibility of independent evolution in wing size
480 plasticity when the sexes experience markedly different nutritional-thermal environments.
481 Delcourt *et al.* (2009) and Berger *et al.* (2014) have also reported changes in magnitude of
482 cross-sex genetic correlation of fitness when rearing males and females in different
483 environments. This matters because, in the wild, individual *Drosophila* are often found in
484 different microhabitats – different temperatures due to daily thermal fluctuations (Noer *et*
485 *al.* 2020) and different nutrients due to different niches, resources (Diepenbrock and
486 Burrack 2017), and nutritional requirements (Lee *et al.* 2013; Camus *et al.* 2018; Duxbury
487 and Chapman 2021). Estimating cross-sex genetic correlation between males and females
488 that developed in different environments may help improve the accuracy when estimating
489 the individual evolvability between sexes.
490
491 Finally, genetic correlations between viability and wing size across all six thermal-nutritional
492 treatments were not different from zero. This means that these two traits do not share a
493 similar genetic basis, and are able to evolve independently (Via and Lande 1985). Our
494 findings differ from earlier studies. For instance, Teuschl *et al.* (2007) artificially selected
495 dung flies with different body sizes – small, control, and large – and assessed their pre-adult
496 viability in combinations of high temperature and limited food. Interestingly, larger males
497 had higher pre-adult mortality, suggesting body size and viability might be negatively
498 genetically correlated. Dingle (1992) also observed a similar phenomenon in milkweed bugs
499 under food shortage, such that higher pre-adult survival was traded off with smaller body
500 size, which again indicates a negative genetic correlation between these two traits (Via and
501 Lande 1985). The fact that we found no correlation between viability and wing size suggests
502 that the genetic correlation between viability and body size may be species- and condition-
503 specific.
504
505 In summary, we explicitly assessed within-population variation in plasticity of egg-to-adult
506 viability and sex-specific wing size in *D. melanogaster*, in response to combined thermal-

507 nutritional conditions. We demonstrated that, even though combined thermal-nutritional
508 stress reduced viability and wing size (i.e., reduced performance) on average, individual
509 genotypes still varied in their plastic responses, such that some genotypes had increased
510 viability/wing size under increasingly stressful conditions. Additionally, our results suggest
511 that the independent evolution of wing size between the sexes may be possible when the
512 sexes experience different environmental conditions, and that viability and wing size are
513 also genetically independent. Our research sheds light on the importance of measuring the
514 extent of plasticity of fitness-related life-history traits in response to combined stressors, as
515 well as measuring cross-sex genetic correlation between treatments. This will be crucial for
516 making accurate predictions of how species may respond to climate change.
517
518 **Results – Tables and graphs**
519
520 **Table 1**. Linear models testing for genotype (Line) by environment interactions in egg-to-
521 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

522
523 **Table 2**. Linear models testing for genotype (Line) by environment interactions in wing size
524 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

525

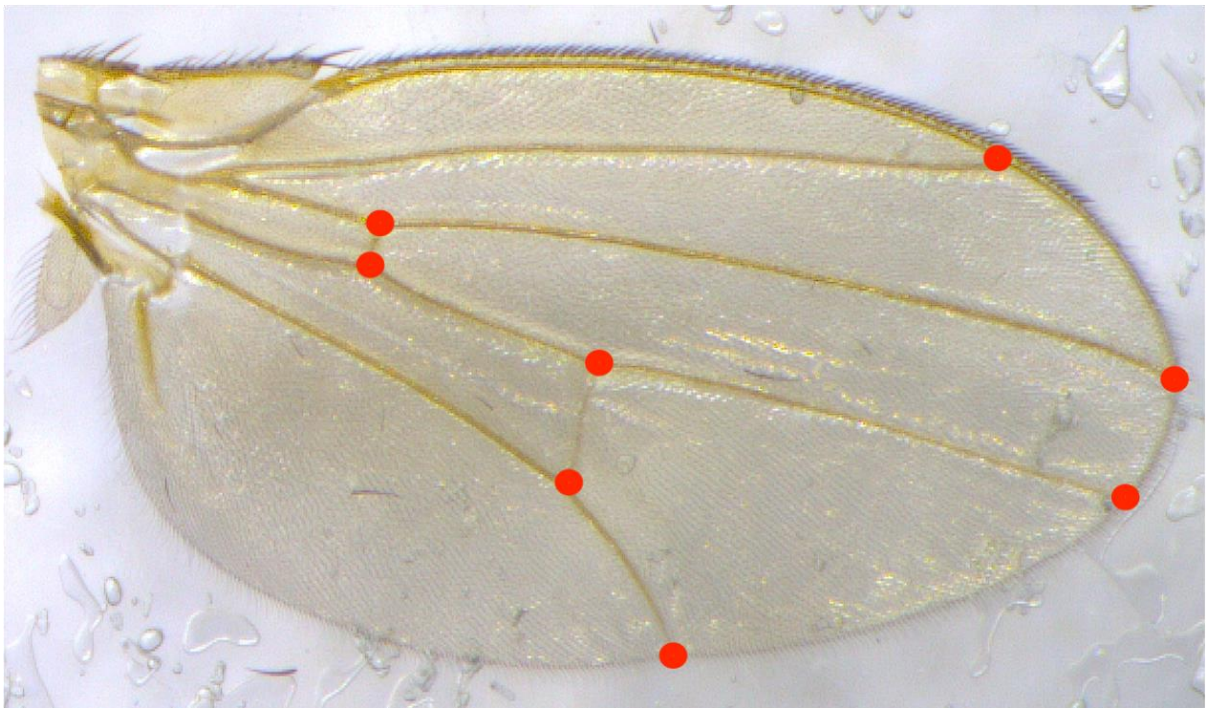


Fig. 1. Landmakrs used for calculating wing centroid size, indicted by red dots.

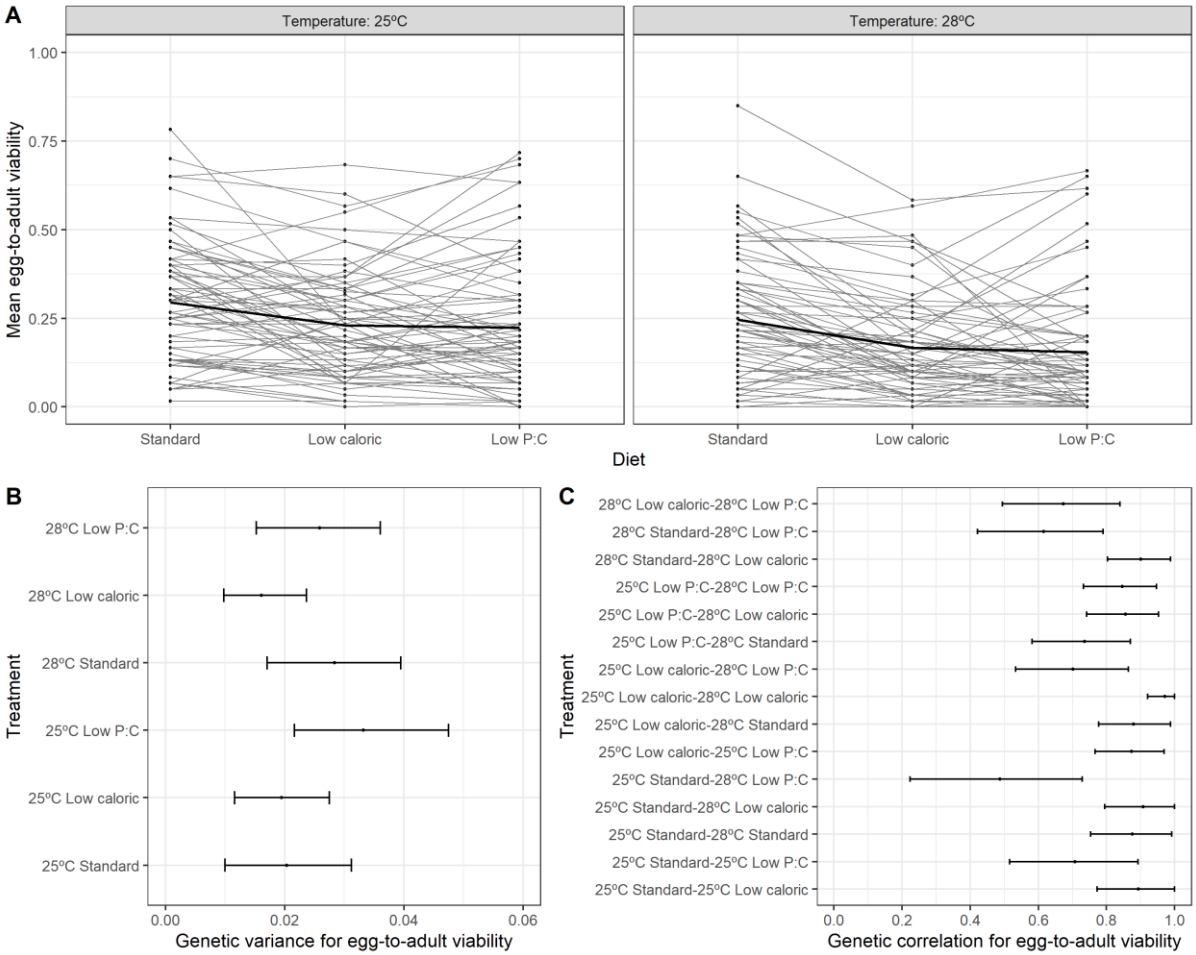
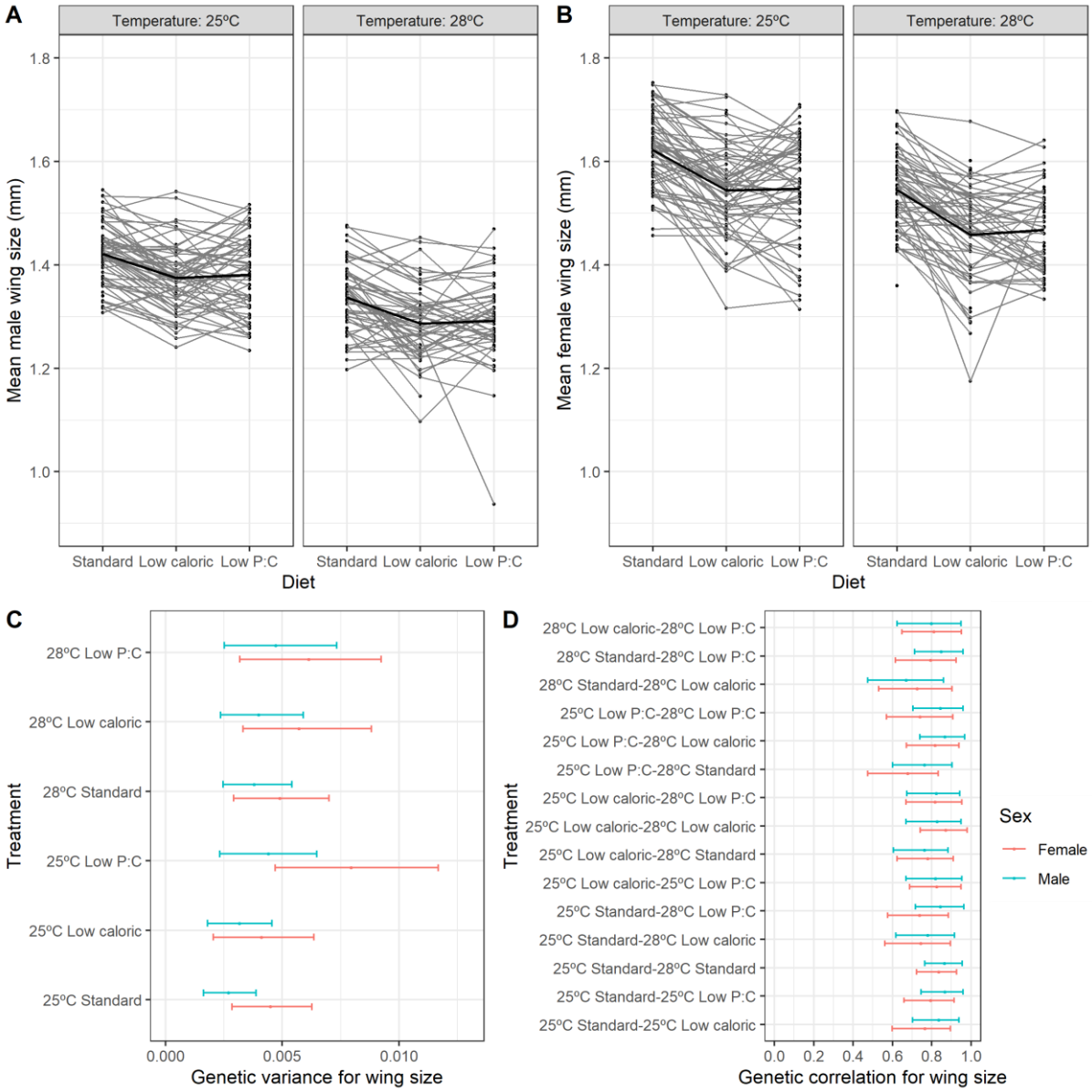


Fig. 2. Mean egg-to-adult viability of each isogenic lines (**A**), estimates of its genetic variance in response to six combined thermal-nutritional treatments, (**B**) and the genetic correlation within and across environments for viability (**C**). Black line presents the population mean in

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



534
535 **Fig. 3.** Mean wing size of (**A**) males and (**B**) females of isogenic lines, estimates of their (**C**)
536 genetic variance, and (**D**) the genetic correlation within and across six combined thermal-
537 nutritional treatments. Black line presents the population mean in panels A and B. Error bars
538 shows 95% upper and lower HPD intervals in panels C and D.

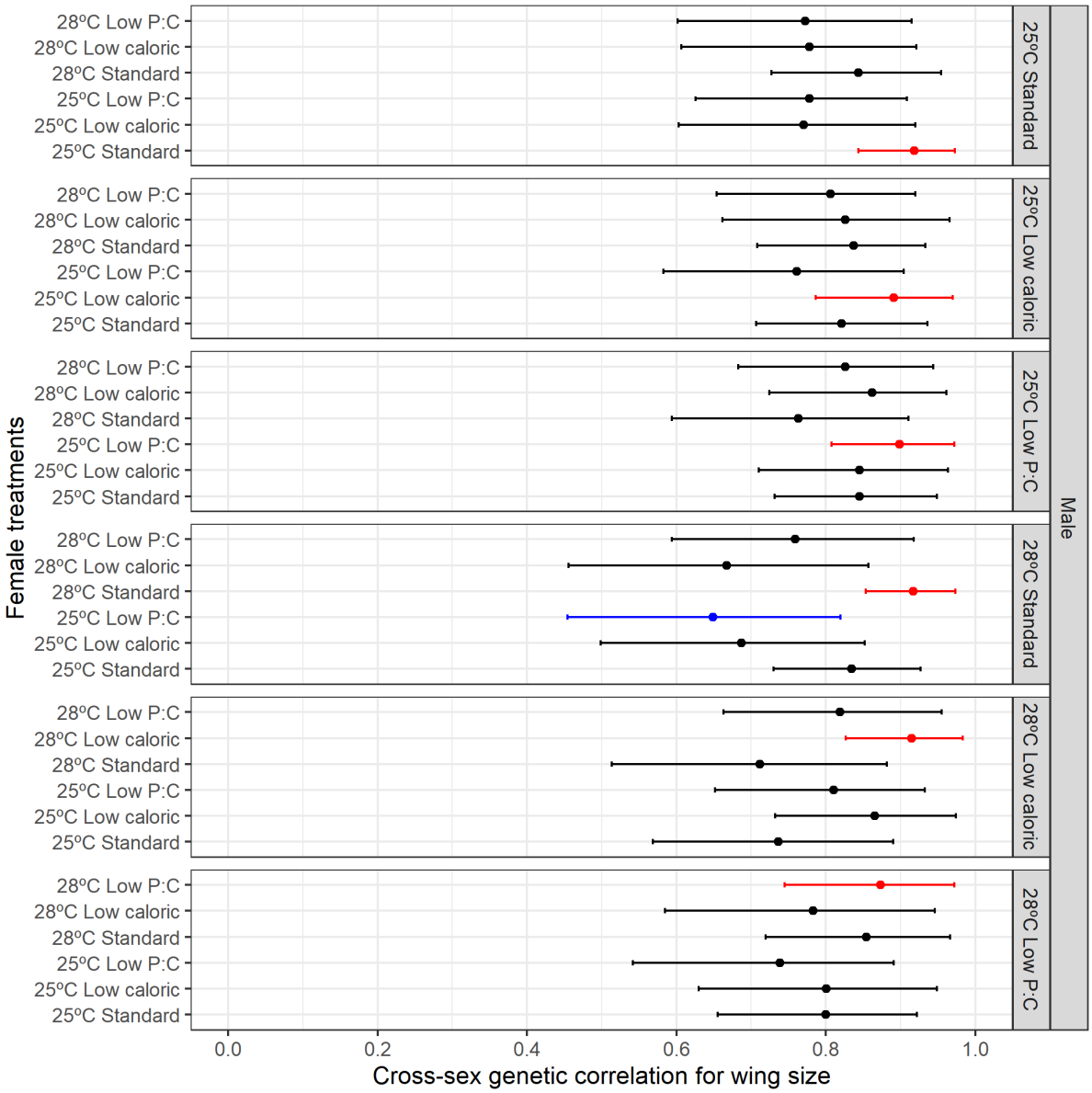


Fig. 4. Cross-sex genetic correlation for wing size between six combined thermal-nutritional treatments. Error bars shows 95% upper and lower HPD intervals. Red error bars indicate cross-sex genetic correlation within treatments. The blue error bar shows a significantly smaller cross-sex genetic correlation 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.

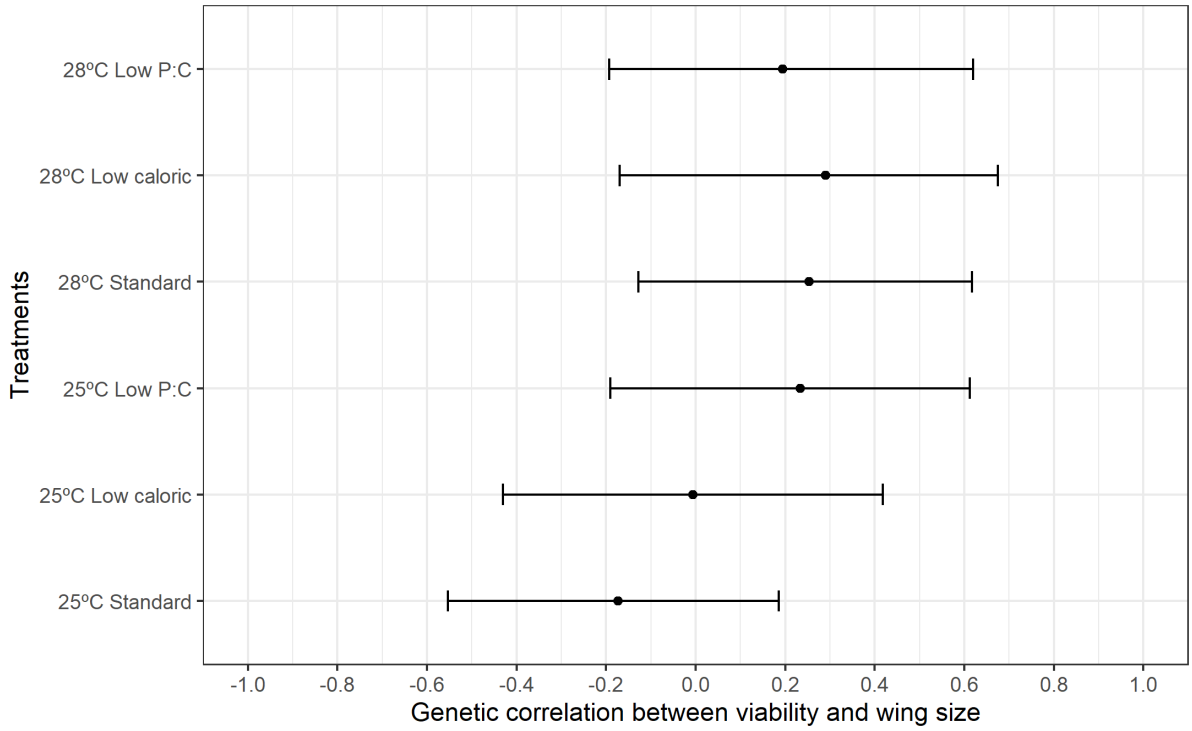


Fig. 5. Genetic correlation between egg-to-adult viability and wing size within each of six combined thermal-nutritional treatments. Wing size of both sexes was combined for estimating the genetic correlation between egg-to-adult viability and wing size. Error bars shows 95% upper and lower HPD intervals.

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553

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