Within population plastic responses to combined thermal-nutritional stress differ from those in response to single stressors, and are genetically independent across traits in both males and females.

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Key words: plasticity, temperature, nutrition, 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. 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-to-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 often < 1 for both traits, as well as changes in the expression of genetic variation across environments for egg-to-adult viability. Cross-sex genetic correlations for body size were weaker when the sexes were reared in different conditions, suggesting that the genetic basis of traits may change with the environment. Further, our results suggest that plasticity in egg-to-adult viability is genetically independent from plasticity in body size. Importantly, plasticity in response to diet and temperature individually differed from plastic shifts in response to diet and temperature in combination. By quantifying plasticity and the expression of genetic variance in response to combined stress across traits, our study reveals the complexity of animal responses to environmental change, and the need for 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 & Field, 2013; Midgley & Hannah, 2019; Wiens, 2016). 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 (Pecl et al., 2017; Van Der Putten et al., 2010), and nutritional composition of plants (Jin et al., 2019; Kreuzwieser & Gessler, 2010; Lukac et al., 2010). Specifically, plants are expected to contain less protein and varied carbohydrate content under climate change (Bowes, 1993; DaMatta et al., 2010; Dwyer et al., 2007; Richardson et al., 2002; Rosenblatt & Schmitz, 2016)**,** potentially imposing direct nutritional stress on herbivores and frugivores (Bowes, 1993; DaMatta et al., 2010; Dwyer et al., 2007; Richardson et al., 2002; Rosenblatt & Schmitz, 2016) and indirectly through changes in yeast communities. In addition, ectothermic herbivores and frugivores will be at a greater risk as they rely on ambient temperatures to maintain their metabolism (Hawlena & 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 & 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.

Phenotypic plasticity – the ability of a single genotype to express different phenotypes in different environments – is often the first response of many organisms to environmental variation (Huey et al., 2002). Plastic shifts in life-history traits in response to environmental change are common (Abram et al., 2017; Schilthuizen & Kellermann, 2014; Seebacher et al., 2015; Sgrò et al., 2016; Urban et al., 2014; Via et al., 1995). While plasticity might contribute to persistence and adaptation to environmental change, it may not always be adaptive (Ghalambor et al., 2007). In small ectotherms such as insects, many studies have revealed plastic shifts in response to either temperature or nutrition. For instance, body size (Bochdanovits & De Jong, 2003; French et al., 1998), development time (Schou et al., 2017), and reproductive success (Janowitz & Fischer, 2011; S. Wang et al., 2013) have been shown to decrease with increasing temperature. Pre-adult viability (Jang & Lee, 2018; Min et al., 2007), development time (Economos & Lints, 1984), body size (Jang & Lee, 2018; Min et al., 2007), and reproductive success (Bader & Williams, 2012) also shift plastically in response to reductions in food quality (protein and carbohydrate contents) and quantity. However wild animals are exposed to multiple stressors simultaneously (Rosenblatt & Schmitz, 2016;

Stillwell et al., 2007), which makes it important to understand plastic shifts in response to combinations of environmental stressors predicted to occur under climate change.

Recent studies focussed on understanding plastic responses to combinations of stressors have shown that trait responses to combined stress can change in unpredictable ways when compared to single stressor responses (Bubliy et al., 2012; Chakraborty et al., 2020; Kutz et al., 2019; Polak et al., 2004). Plastic responses to combined thermal-nutritional stress in insects have been explored for a range of life-history traits, including viability, developmental time, body size, and lifespan (Clissold & Simpson, 2015; Couret et al., 2014; Kutz et al., 2019; Rho & Lee, 2017; Tun-Lin et al., 2000). 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 & Lee, 2017). Similarly, in *D. melanogaster*, the negative impacts of sub-optimal diets on viability were exacerbated at higher temperatures (Kutz et al., 2019). These studies suggest that changes in temperature and nutrition may interact to pose greater levels of stress than they do individually.

Plastic responses to environmental change can vary among populations and between individuals within populations, quantified as genotype by environment interactions (G $\times$ E) (Chakraborty et al., 2023; Dreyer et al., 2016; Frankino et al., 2019; Via & Lande, 1985). Several studies have looked at among-population variation in plasticity along latitudinal gradients (Chakraborty et al., 2020; Schmidt et al., 2005; Sgrò et al., 2010; Sisodia & Singh, 2010), while fewer studies have quantified genetic variation in plasticity within a population or between individual genotypes to thermal and nutritional stressors (Chakraborty et al., 2023; Frankino et al., 2019; Lafuente et al., 2018; Mossman et al., 2016; J. B. Wang et al., 2017). Variation in plasticity within a population provides the potential for plasticity to evolve at a population level, which will determine population responses to environmental change (Frankino et al., 2019; Ghalambor et al., 2007; Lande, 2009). Greater within-population genetic variation in plasticity will therefore increase the potential for plasticity to evolve, which will allow populations to adapt to environmental change (Chevin & Hoffmann, 2017; Lande, 2009; Walter et al., 2023). Understanding the distribution of plastic responses

108 (shape of reaction norms) between individuals of a population is therefore vital for
109 determining whether populations will persist under climate change.
110
111 A powerful approach for measuring within-population variation in plasticity is to create
112 isogenic lines, which involves inbreeding lines that are collected from a natural population
113 with each resulting isogenic line representing a fixed genotype (Franěk et al., 2020; Ørsted
114 et al., 2018). Variation among isogenic lines within environments reflects snapshots of
115 standing genetic variation of the sampled population. By measuring differences in the
116 response of isogenic lines to multiple environments, we can quantify variation in plasticity
117 among genotypes (Scheiner, 1993; Via & Conner, 1995). Variation in plasticity among
118 genotypes (or significant G × E) can be caused by differences in the expression of genetic
119 variation across environments, or cross-environment genetic correlations that are less than
120 one (Via & Conner, 1995), or both. Understanding how the expression of genetic variation
121 and genetic correlations change across environment and contribute to within-population
122 variation in plasticity is essential for predicting a population's ability to adapt to long-term
123 environmental change (Ghalambor et al., 2007; Sgrò & Hoffmann, 2004; Wood & Brodie,
124 2015).
125
126 In this study, we used a set of newly derived isogenic lines of *D. melanogaster* initiated from
127 a population collected from the east coast of Australia (Chakraborty et al., 2023). These
128 lines allow us to extend upon previous studies that have examined variation in plasticity
129 between populations sampled from the latitudinal cline along the east Australian coast
130 (Chakraborty et al., 2020; Cockerell et al., 2014; Sgrò et al., 2010; van Heerwaarden et al.,
131 2016). Specifically, we used these lines to quantify sex-specific variation in plasticity in
132 response to combined thermal and nutritional stress. We subjected developing larvae to six
133 combinations of thermal and nutritional conditions that included two temperatures: 25°C
134 and 28°C, and three diets: standard, low caloric, and low protein to carbohydrate (P:C) diets.
135 These conditions were chosen to best simulate climate change, which is expected to cause a
136 3°C increase in average temperature (Screen, 2014), and to reduce protein and vary
137 carbohydrate contents in plants (DaMatta *et al.* 2010; Gowik and Westhoff 2011; IPCC
138 2018). We assessed plasticity of egg-to-adult viability and adult wing centroid size, which
139 have previously shown sensitivity to combined thermal and nutritional stress (Chakraborty

et al., 2020; Kutz et al., 2019). Given that *D. melanogaster* shows sexual size dimorphism, and that the sexes may vary in their plasticity (Hangartner et al., 2022; Pottier et al., 2021; Shingleton et al., 2017; Stillwell et al., 2010), we assessed size plasticity separately for males and females. By quantifying genetic variation in plasticity within a population in response to combinations of environmental stressors, our study sheds new insight into how animal populations may respond to ongoing climate change.

Methods

Fly stocks - isogenic lines

We used isogenic lines that were established by Chakraborty *et al.* (2023). Briefly, two hundred field-inseminated female *D. melanogaster* were collected in Duranbah, New South Wales, Australia (28.3° S, 153.5° E) in 2018. From each of these females, two independent iso-female lines were established and bred into four hundred lines. Full-sibling mating was performed in each of these four hundred lines for twenty generations following MacKay *et al.* (2012), resulting in 71 isogenic lines. Four copies of each isogenic line were reared at 25°C on standard diets (1:3 P:C ratio and 320 kcal/ L) under 12:12 light:dark cycle for twelve generations prior to the experiment, as described below. The standard diet was prepared by mixing water with potato flakes (18.18 g/ L), yeast (36.36 g/ L), agar (6.36 g/ L), and dextrose (27.27 g/ L), with addition of nipagin (6 mL/L) and propionic acid (2.5 mL/L) to prevent bacterial and fungal growth (Holleley et al., 2008). While we acknowledge the potential influence of gut microbiota on the life history traits examined, our study was designed to investigate the effects of combinations of nutrition and temperature within the constraints of our experimental design (described below); we therefore chose not to manipulate the gut microbiome so that the experimental diets (describe below) used in our study were treated the same. This meant that we could focus on the relative effects of nutrition without needing to also consider changes in the gut microbiome.

Developmental treatment conditions and choice of traits

171 We used six thermal-nutritional conditions to simulate the possible shifts in temperature
172 (Masson-Delmotte et al., 2018) and nutrition (DaMatta *et al.* 2010; Gowik and Westhoff
173 2011; IPCC 2018) under climate change. They included combinations of two temperatures:
174 25°C and 28°C, and three diets: standard diet (1:3 P:C ratio and 320 kcal/L), low caloric diet
175 (1:3 P:C and 80kcal/L, a 25% dilution of the standard diet), and low P:C diet (1:12 P:C and
176 320kcal/L). 25°C represents the average summer temperature in south-east Australia, whilst
177 28°C represents the 3°C temperature increase projected in twenty years under climate
178 change (Screen, 2014). The low caloric diet and the low P:C diets simulate a reduction of
179 protein and variation of carbohydrates in plant composition projected under climate change
180 (DaMatta *et al.* 2010). Importantly, they also reflect the nutritional range in the rotting fruit
181 upon which *Drosophila* feed in nature (Matavelli et al., 2015; Silva-Soares et al., 2017).
182 These conditions have been previously shown to successfully induce plastic responses in our
183 traits of interest, egg-to-adult viability, and wing size (Chakraborty et al., 2020; Kutz et al.,
184 2019).
185
186 Egg-to-adult viability and wing size were chosen because they are fitness-related
187 developmental traits known to be sensitive to nutrition and diet individually and in
188 combination (Chakraborty et al., 2020; Kutz et al., 2019). Egg-to-adult viability is a direct
189 indication of an individual's ability to survive to the reproductive adult stage (Kristensen et
190 al., 2015). While viability data by its nature includes missing data as we are unable to
191 measure the plasticity of traits such as body size in individuals that did not survive
192 development (Hadfield, 2008), it nonetheless represents a trait linked to fitness that has
193 previously been used in studies of plasticity (David et al., 2004; Kellermann & van
194 Heerwaarden, 2019). Larger adult body size often correlates with greater fitness in
195 ectotherms, including *Drosophila* (Kingsolver & Huey, 2008), and so plasticity in this trait will
196 likely affect fitness.
197
198 *Experimental set up - Egg lays to obtain experimental flies*
199
200 Adults from the same copy of the isogenic lines were mixed and transferred to laying plates
201 containing apple juice, agar, nipagin, and a surface layer of autoclaved yeast to encourage
202 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 vials containing 7.2 ml of their respective diets. Three replicate vials were set up 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). This resulted in a sample size of 71 isogenic lines x 2 temperatures x 3 diets x 3 replicate vials, which equals 1278 vials. Eggs were picked across 52 days, which are defined as block for the statistical analyses (described below).

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. The 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- and Y- coordinates of landmarks were determined using the software package tpsDIG (Rohlf, 2015). Using the software CoorDGen8 (Sheets, 2014), wing size was estimated using centroid size, the square root of the sum of the squared distances between each landmark (Rohlf & Slice, 1990).

Statistical analysis

Testing for overall genotype-by-environment (G × E) interactions

R was used to perform all data analysis and visualization (version 3.4.3) (R Core Team, 2017). To test for significant G × E, we used the *lme4* package (Bates et al., 2015) to fit linear mixed effects models for both traits. A binomial distribution was used to fit models for egg-

to-adult viability, while wing size was log-transformed (Labarbera, 1989), fitted with a Gaussian error distribution, and analysed separately for each sex.

Models were fitted separately for each trait. Random effects of block (defined as the days of egg picking), line, temperature-by-line, diet-by-line, and temperature-by-diet-by-line were added sequentially (see equations 1-4) and the significance of each variable was tested with log-likelihood ratio tests using the *lrtest* package (Zeileis and Hothorn, 2002).

Specifically, the first model (equation 1) included temperature and diet as the fixed effects, and block as a random effect. This model estimates the overall population response to combinations of temperature and diet while accounting for the differences in traits among blocks. Vial is not included in any of the models as a random effect because initial analyses revealed it had no significant effect.

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

In the second model (equation 2), line was added as an additional random effect to test for differences in traits across the isogenic lines.

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

In the third model (equation 3), temperature and diet were added to the random line term to test for differences across lines in their plastic response in response to temperature and diet individually (i.e. G × E), allowing random intercepts and slopes.

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

In the fourth model (equation 4), a temperature-by-diet-by-line interaction was further added as a random effect to test for differences across lines in their plastic responses to the combined effects of diet and temperature (i.e. overall G × E), allowing random intercepts.

Comparing Eqn. 4 to Eqn. 3 tests whether plasticity in response to the combined stressors differed from the plastic response to individual stressors.

$$y \sim 1 + \text{Temperature} * \text{Diet} + (1|\text{Block}) + (\text{Diet}|\text{Line}) + (\text{Temperature}|\text{Line}) + (1|\text{Temperature: Diet: Line})$$ [Eqn. 4]

In the last model (equation 5), a temperature-by-diet term was added to the random line term. Comparing the fit of Eqn. 5 to Eqn. 3 tests for significant G × E as differences among lines in both intercepts and slopes (i.e. allowing changes in expression of genetic variance and correlations as the cause of G × E).

$$y \sim 1 + \text{Temperature} * \text{Diet} + (1|\text{Block}) + (\text{Temperature: Diet}|\text{Line})$$ [Eqn. 5]

Quantifying genetic (co)variance within and across environments

Genetic (co)variances for egg-to-adult viability and wing size were estimated across treatments, and across sexes (the latter only for wing size) to illustrate the extent to which the observed G × E was due to changes in genetic variance across environments and/or genetic correlations that are less than one (Via & Lande, 1985). Because isogenic lines were used in this study, all estimates of genetic variance and genetic correlations are broad sense – they include additive and non-additive variance components.

Specifically, we used the *MCMCglmm* package (Hadfield, 2010) to fit models using Markov Chain Monte Carlo (MCMC), to estimate genetic (co)variances and genetic correlations. For each model, we used 1 million iterations with a burn-in of 100,000 iterations and a thinning interval of 500 iterations. We checked model convergence by ensuring that effective sample sizes were more than 85% of samples saved. We used weakly-informative parameter-expanded priors for the variance components, and checked their sensitivity by changing the scale of the parameters while ensuring there were no changes in the posterior distributions. A thousand iterations were kept from each model, providing the posterior distribution for all parameters estimated. In all estimations of genetic (co)variance, each combination of diet (standard, low calorie, and low P:C), temperature (25°C and 28°C), and sex (only for

298 wing size) were treated as separate traits/treatments. Models were analysed using raw
299 viability data and log-transformed wing size data.
300
301 *Quantifying genetic (co)variance in egg-to-adult viability and wing size in each sex*
302
303 To estimate genetic (co)variance in egg-to-adult viability within and across environments,
304 we fitted viability data using a binomial distribution with the generalised linear mixed
305 model.
306 $$y_{jklm} = T_j + l_{k(j)} + b_{l(jk)} + e_{m(jkl)} \text{ [Eqn. 6]}$$
307 We included viability as the response variable $y_{jklm}$and treatment T_j as the only fixed
308 effect. Random effects included isogenic line $l_{k(j)}$ and block $b_{l(j)}$ within the treatments, to
309 remove any variance due to differences between experimental blocks. The term $e_{m(jkl)}$
310 represents the residuals. For the isogenic line component, we estimated an unstructured
311 covariance matrix, which calculates the genetic variance of egg-to-adult viability within each
312 treatment, and the genetic covariance between treatments, resulting in a 6 × 6 genetic
313 (co)variance matrix. Genetic correlations between treatments were calculated from the
314 (co)variance matrix.
315
316 Genetic (co)variances and genetic correlations for wing size within and across environments
317 for each sex were estimated as described above using a Gaussian distribution.
318
319 *Quantifying cross-sex genetic correlations in wing size*
320
321 We analysed cross-sex correlations of wing size within- and between-treatments using this
322 equation,
323 $$y_{ijklm} = T_i + S_j + l_{k(ij)} + b_{l(ijk)} + e_{m(ijkl)} \text{ [Eqn. 7]}$$
324 where sex S_j was included as a fixed effect. For the isogenic line component, we estimated
325 an unstructured covariance matrix that estimates the genetic variances of wing size among
326 isogenic lines in each combination of treatment and sex, and genetic covariances between
327 combinations respectively. We subset data by treatments and combinations of treatment
328 and sex to reduce the number of (co)variance parameters estimated. Six 4 × 4 genetic

329 (co)variance matrices were generated for calculating within-treatment cross-sex genetic
330 correlations, and fifteen 2 × 2 genetic (co)variance matrices were generated for calculating
331 between-treatment cross-sex genetic correlations.
332
333 *Genetic correlations between egg-to-adult viability and wing size*
334
335 To estimate genetic correlations between egg-to-adult viability and wing size across
336 treatments, we applied equation 6 but included both egg-to-adult viability and wing size as
337 a bivariate response variable (note that wing size was averaged over both sexes and
338 estimated without sex as a fixed effect). As egg-to-adult viability and wing size were
339 measured on different scales, we standardised each trait by dividing trait values by their
340 grand mean (Houle, 1992). We applied equation 6 separately to the data from each
341 treatment, and only included isogenic line and block as random effects. An unstructured
342 covariance matrix was estimated, which calculates the genetic variance in viability and wing
343 size, and the genetic covariance between viability and wing size within each treatment. This
344 produced six 2 × 2 genetic (co)variance matrices. Genetic correlations between traits for
345 each treatment were then calculated from each (co)variance matrix.
346
347 **Results**
348
349 We characterised genetic variation in plasticity (G × E) for both egg-to-adult viability and
350 sex-specific wing size in response to combined stressors within a population. We subjected
351 *D. melanogaster* isogenic lines to six different thermal-nutritional conditions (25°C—
352 standard diet, 25°C—low caloric diet, 25°C—low P:C diet, 28°C—standard diet, 28°C—low
353 caloric diet, and 28°C—low P:C diet) and tested for significant genetic variation in plasticity
354 in egg-to-adult viability and wing size. We then calculated genetic (co)variances and genetic
355 correlations for each trait within and across environments and sexes (for wing size) to
356 understand the causes of the significant G × E. Genetic correlations between egg-to-adult
357 viability and wing size were also calculated to understand the independent evolvability
358 between traits.
359

360 *Genotype-by-environment interactions in egg-to-adult viability*

361

362 We found evidence of significant genetic variation in plasticity for viability (G × E) in
363 response to temperature and diet individually. Importantly, the plastic response to
364 combinations of temperature and nutrition varied from the plastic responses seen for either
365 temperature or nutrition individually (Table 1). When we look at the raw data, such
366 variation in plasticity is captured by non-parallel reaction norms among lines across diets
367 and temperatures (Fig. 2A).

368

369 *Genetic (co)variances in egg-to-adult viability*

370

371 We estimated genetic (co)variance matrices to determine whether significant G × E for
372 viability was caused by differences in the amount of genetic variance across environments
373 and/or cross-environment genetic correlations that are less than one (Via & Lande, 1985).
374 We found significant genetic variance for viability in all treatments (Fig. 2B), and that
375 genetic variance for viability at 28°C low P:C diet was significantly higher than the genetic
376 variance at 25°C standard diet (Fig. 2B and Fig. S1), evidenced by the non-overlapping 95%
377 highest posterior density (HPD) intervals (Fig. 2B).

378

379 Genetic correlations between treatment pairs were all positive and significantly greater
380 than zero, but mostly less than one (Fig. 2C and Fig. S2). This suggests that significant G × E
381 for viability results from both differences in the expression of genetic variance across
382 environments, and differences in how isogenic lines respond to environments (Falconer,
383 1952; Via & Lande, 1985).

384

385 *Genotype-by-environment interactions in wing size*

386

387 We found evidence for genetic variation in plasticity for wing size in males and females in
388 response to temperature and nutrition individually, and that the plastic response of size to
389 combinations of temperature and nutrition varied from the plastic responses seen for

390 temperature and nutrition individually (Table 2). Such significant within-population G × E in
391 male and female wing size is visualised as non-parallel reaction norms in Fig. 3A-B.
392
393 *Genetic (co)variances in wing size*
394
395 We found significant genetic variance in wing size for all treatments within each sex;
396 however, the distribution of genetic variance did not differ across treatments (Fig. 3C and
397 Fig. S3). Thus, significant G × E in wing size in both males and females was not caused by the
398 differences in the amount of genetic variance across the environments.
399
400 Genetic correlations in wing size across the six thermal-nutritional treatments within each
401 sex were all positive, significantly greater than zero, and mostly less than one (Fig. 3D, Fig.
402 S4 and Fig. S5). This suggests that significant G × E in wing size of both males and females
403 was caused by differences in the alleles contributing to the response across environments
404 (Falconer, 1952; Via & Lande, 1985).
405
406 *Cross-sex genetic correlations for wing size*
407
408 To determine whether wing size has a similar genetic basis in both sexes, we estimated the
409 cross-sex genetic correlations for wing size within and between all thermal-nutritional
410 treatments. This helps us to understand the potential for size to evolve independently in
411 each sex and in each environment (Falconer, 1952; Via & Lande, 1985).
412
413 Cross-sex correlations within treatments (indicated in red in Fig. 4, Fig. S6) were significantly
414 greater than zero, and large, but less than one, indicating that even though sexes share
415 some genetic basis for these traits, there is also some degree of genetic independence.
416 Some cross-sex genetic correlations were significantly smaller when males and females
417 developed in different treatments (i.e. male: 25°C low P:C – female: 28°C Standard; male:
418 28°C standard – female: 25°C low caloric, 25°C low P:C, and 28°C low caloric; male: 28°C low
419 caloric – female: 25°C standard and 28°C standard; indicated in blue in Fig. 4). This suggests
420 that the genetic basis for wing size in males and females diverges when the sexes are reared

421 in different environments, which could allow sexes to evolve independently as conditions
422 become more variable.
423
424 *Genetic correlations between egg-to-adult viability and wing size*
425
426 Finally, we estimated genetic correlations between egg-to-adult viability and wing size
427 (sexes combined) across all thermal-nutritional treatments to determine whether these
428 traits have a similar genetic basis, and whether they can evolve independently in different
429 environments (Falconer, 1952; Via & Lande, 1985). Correlations between viability and wing
430 size were not significantly different from zero in all treatments (Fig. 5 and Fig. S7), indicating
431 that these traits are genetically independent.
432
433 **Discussion**
434
435 Climate change is simultaneously imposing thermal and nutritional stress on animals (Pecl et
436 al., 2017; Van Der Putten et al., 2010), and is expected to negatively affect biodiversity in
437 the long term (Masson-Delmotte et al., 2018; Rosenblatt & Schmitz, 2016; Walters et al.,
438 2012). Phenotypic plasticity is an important means by which animals will mitigate the
439 increasing stress under climate change (Seebacher et al., 2015; Sgrò et al., 2016; Urban et
440 al., 2014), although it may not always be adaptive (Ghalambor et al., 2007; Sgrò et al.,
441 2016). Plastic responses of life-history traits can shift in response to changes in temperature
442 (French et al., 1998; Hoffmann et al., 2003; Schou et al., 2017) and nutrition individually
443 (Bader & Williams, 2012; Jang & Lee, 2018; Min et al., 2007). Temperature can also modify
444 plastic responses of life history traits to nutritional stress, causing an interactive effect
445 (Chakraborty et al., 2020; Kutz et al., 2019; Lee & Roh, 2010). While these studies improve
446 our understanding of how animals may respond to combined stress resulting from climate
447 change, they have not accounted for genetic variation in plasticity within a population.
448
449 Individuals within a population can show substantial variation in plasticity (Chakraborty et
450 al., 2023; Frankino et al., 2019; Lafuente et al., 2018; Mossman et al., 2016; J. B. Wang et al.,
451 2017). Understanding within-population variation matters because it may shape the
452 capacity for populations to adapt and persist in the face of ongoing environmental change

453 (Chevin et al., 2010; Dreyer et al., 2016; Frankino et al., 2019; Lande, 2009). Extensive
454 within-population variation in plasticity has been reported in a range of life-history traits
455 such as viability (Duun Rohde et al., 2016) and body size (Barker & Krebs, 1995; Imasheva et
456 al., 1999; Lafuente et al., 2018) in response to thermal and nutritional stress individually
457 (Frankino et al., 2019; Lafuente et al., 2018; Mossman et al., 2016; J. B. Wang et al., 2017).
458 The results of the current study are consistent with these findings. Importantly, we also
459 show that within-population variation in viability and body size plasticity in response to
460 combinations of temperature and diet differed from the plastic responses to temperature
461 and nutrition individually.
462
463 The extent to which genotypes within a population vary in their plasticity will determine
464 how the population responds to selection imposed by environmental change (Chevin et al.,
465 2010; Dreyer et al., 2016; Frankino et al., 2019). Populations with similar mean plasticity
466 (reaction norms) may vary markedly in their genotype-specific reaction norms, causing them
467 to vary in their response to selection (Dreyer et al., 2016; Frankino et al., 2019; Lande,
468 2009). Predictions of long-term evolutionary responses to environmental changes using
469 population-level assessments of plasticity, could therefore be inaccurate unless within-
470 population variation in plasticity is also considered (Dreyer et al., 2016; Frankino et al.,
471 2019; Reed et al., 2010).
472
473 We found that the significant G × E for viability was the result of both significant differences
474 in the expression of genetic variance across environments, and cross-environment genetic
475 correlations that were greater than zero but less than one. Specifically, we found that
476 genetic variance for viability was significantly higher at 28°C on a low P:C diet compared to
477 25°C on a standard diet. Bubliy *et al.* (2001) also observed that significant G × E in viability
478 was due to an increase in genetic variance for viability at elevated (32°C) compared to
479 control (25°C) temperatures. In contrast, Bubliy and Loeschcke (2002) found no differences
480 in the expression of genetic variance for viability between 13°C and 25°C. The increase in
481 genetic variance at elevated temperatures individually (Bubliy et al., 2001) or when
482 combined with nutritional stress (current study) are consistent with the idea that higher
483 levels of environmental stress can result in increases in the expression of genetic variance
484 (Hoffmann & Merilä, 1999; Imasheva et al., 1998), although the mechanisms of such

485 changes remains unknown. Our results suggest that the adaptive capacity of populations, at
486 least with respect to viability, may be enhanced by some of the changes expected to occur
487 with climate change, namely elevated temperature combined with diets low in protein but
488 high in carbohydrates (Burla & Taylor, 1982; Fowler & Whitlock, 2002).
489
490 We found that the significant G × E interactions for viability were also the result of genetic
491 correlations across environments that were above zero and often less than one. This
492 indicates that viability across some thermal-nutritional conditions is controlled by different
493 alleles or differently by the same alleles (Via & Lande, 1985), meaning that viability has
494 some capacity to evolve independently across different thermal-nutritional environments
495 (Via & Lande, 1987). While some studies have found that genetic correlations can change in
496 sign and magnitude across environments (Sgrò & Hoffmann, 2004; Wood & Brodie, 2015),
497 we did not find this to be the case for viability in the present study.
498
499 Although body size plasticity has been shown to vary within a population in response to
500 temperature (Barker & Krebs, 1995; Cavicchi et al., 1995; Lafuente et al., 2018) and nutrition
501 individually (Imasheva et al., 1999) and in combination (De Moed et al., 1997), the source of
502 this G × E has differed across studies. De Moed *et al.* (1997) found that the significant G × E
503 for body size was due to differences in the expression of genetic variance across
504 environments; namely, the expression of genetic variance for size increased only under
505 combinations of low temperature (15°C) and poor food (low protein). We found that the
506 expression of genetic variance for size did not differ across environments in either sex;
507 rather the significant G × E for body size for both sexes was due to genetic correlations
508 across treatments that were significantly more than zero but often less than one. Similarly,
509 Barker and Krebs (1995) and Imasheva *et al.* (1999) also found that genetic correlations less
510 than unity, rather than changes in the expression of genetic variance across environments,
511 were responsible for G × E for body size in response to temperature and dietary yeast
512 concentration respectively. Interestingly, De Moed *et al.* (1997) did not find an increase in
513 genetic variance for size when larvae developed at higher temperature (27.7 °C) and poor
514 food, which is consistent with the present study.
515

Differences across studies in the extent to which genetic variance and/or genetic correlations change with environment may reflect the use of different source/experimental populations, environmental conditions, and experimental approaches across studies. Yet, studies to date (reviewed in Hoffmann and Merilä (1999); Sgrò and Hoffmann (2004); Wood and Brodie (2015)) indicate that although the environmental dependency of genetic variance and correlations may be common, is not easily predicted by trait or environment.

As expected from previous studies (Cowley et al., 1986; Cowley & Atchley, 1990; Lasne et al., 2018; Reeve & Fairbairn, 1996), we found strong and positive cross-sex genetic correlations for wing size within treatments. This indicates that the genetic basis of plasticity in wing size is largely shared between males and females, but because these correlations are less than one, there is some potential for size to evolve independently between sexes (Lande, 1980; Via & Lande, 1987). More importantly, we found that some cross-sex genetic correlations were reduced when males and females developed in different environments. This suggests that there is a greater possibility for the independent evolution of wing size when the sexes experience markedly different thermal-nutritional environments.

Most studies find that cross-sex genetic correlations are large and positive (Poissant et al., 2010). However, there is some evidence that cross-sex genetic correlations can change in magnitude (Delcourt et al., 2009) and sign (Berger et al., 2014). Specifically, in the seed beetle, the cross-sex genetic correlation for fitness changed from negative under benign temperatures to positive under thermal stress (Berger et al., 2014), while in *D. serrata*, Delcourt *et al.* (2009) showed that the negative cross-sex genetic correlation for fitness became stronger on a novel diet. In the wild, individual *Drosophila* are often found in different microhabitats – different temperatures due to daily thermal fluctuations (Noer et al., 2020) and different nutritional contexts due to different niches, resources (Diepenbrock & Burrack, 2017), and nutritional requirements (Camus et al., 2018; Duxbury & Chapman, 2021; Lee et al., 2013). Thus, weaker cross-sex genetic correlations under varying environmental conditions may facilitate adaptation if changing environments favour sex-specific optima, which is often the case (Berger et al., 2014; Delcourt et al., 2009; Poissant et al., 2010).

548 Finally, genetic correlations between viability and wing size across all six thermal-nutritional
549 treatments were not different from zero. This means that these two traits do not share a
550 similar genetic basis, and are able to evolve independently (Via & Lande, 1985). Our findings
551 differ from earlier studies. For instance, Teuschl *et al.* (2007) artificially selected dung flies
552 with different body sizes – small, control, and large – and assessed their pre-adult viability in
553 combinations of high temperature and limited food. Interestingly, larger males had higher
554 pre-adult mortality, suggesting body size and viability might be negatively genetically
555 correlated. Dingle (1992) also observed a similar phenomenon in milkweed bugs under food
556 shortage, such that higher pre-adult survival was traded off with smaller body size, which
557 again indicates a negative genetic correlation between these two traits (Via & Lande, 1985).
558 The fact that we found no correlation between viability and wing size suggests that the
559 genetic correlation between viability and body size may be species- and condition-specific.
560
561 While this study shows that plastic responses to temperature and diet individually vary
562 markedly from plastic shifts in response to temperature and diet in combination, we
563 acknowledge that our experimental conditions are not a direct reflection of conditions
564 experienced in nature. In particular, we used constant temperatures, when in nature
565 temperatures fluctuate. Future studies should incorporate thermal variation and
566 fluctuations to better reflect daily temperature fluctuations, since plastic responses of traits
567 such as body size traits (Czarnoleski et al., 2013), fertility and productivity traits (Rodrigues
568 et al., 2022) to varying temperature regimes can differ from those in response to constant
569 temperatures. That said, our study nonetheless illustrates for the first time how plasticity in
570 response to single stressors does not predict plastic shifts in response to the same stressors
571 individually.
572
573 Finally, our estimates of genetic (co)variance include non-additive components of variation.
574 In particular, epistatic variance can be significantly increased in highly inbred isogenic lines
575 (Huang et al., 2020; Mackay, 2010). While positive and non-directional epistasis generally
576 align with predictions from additive genetic variance, negative epistasis may lead to
577 canalisation (Carter et al., 2005). Due to the potential contribution of epistatic variance to
578 our estimates of genetic (co)variance, direct predictions of evolutionary responses to
579 combinations of stressors from our data are not possible. Nonetheless, our data do allow us

580 to quantify genetic variation for plasticity within a population across traits and sexes, and
581 explore how the expression of genetic variation and covariation changes with the
582 environment and sex.
583
584 In summary, we explicitly assessed the genetic basis of within-population variation in
585 plasticity of egg-to-adult viability and wing size in *D. melanogaster* in response to combined
586 thermal-nutritional conditions. We demonstrated that individual genotypes varied in their
587 plastic responses, and that the plastic response to temperature and diet individually differed
588 from the plastic response to both temperature and nutrition in combination. We also found
589 that the source of G $\times$ E resulted from changes in the expression of genetic variance
590 (viability only), and genetic correlations less than unity across environments (both traits).
591 Additionally, our results suggest that the independent evolution of wing size between the
592 sexes may be possible when the sexes experience different environmental conditions, and
593 that viability and wing size are genetically independent, at least under the conditions
594 assessed here. Our research sheds light on the complex genetic basis of life-history traits in
595 response to combined stressors, and the potential for the genetic basis of traits to change
596 across environments. Accurate predictions of how species may respond to climate change
597 will require studies of plasticity that examine the longer-term consequences of G $\times$ E and
598 sex-specific adaptation on evolutionary trajectories (Wood & Brodie, 2015).
599

600 **Tables and graphs**

601

602 **Table 1**. Linear models testing for genotype (Line) by environment interactions in egg-to-
603 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) + (Temperature|Line) + (Diet|Line)	1.3 to 1.2	<0.001
1.4	y ~ 1 + Temperature * Diet + (1|Block) + (Temperature|Line) + (Diet|Line) + (1|Temperature:Diet:Line)	1.4 to 1.3	<0.001
1.5	y ~ 1 + Temperature * Diet + (1|Block) + (Temperature:Diet|Line)	1.5 to 1.3	<0.001

604

605 **Table 2**. Linear models testing for genotype (Line) by environment interactions in wing size
606 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	log(y) ~ 1 + Temperature * Diet + (1|Block)	-	-
2.2	log(y) ~ 1 + Temperature * Diet + (1|Block) + (1|Line)	2.2 to 2.1	<0.001
2.3	log(y) ~ 1 + Temperature * Diet + (1|Block) + (Temperature|Line) + (Diet|Line)	2.3 to 2.2	<0.001
2.4	log(y) ~ 1 + Temperature * Diet + (1|Block) + (Temperature|Line) + (Diet|Line) + (1|Temperature:Diet:Line)	2.4 to 2.3	<0.001
2.5	log(y) ~ 1 + Temperature * Diet + (1|Block) + (Temperature:Diet|Line)	2.5 to 2.3	0.007
	Female wing size (y) model		

3.1	log(y) ~ 1 + Temperature * Diet + (1|Block)	-	-
3.2	log(y) ~ 1 + Temperature * Diet + (1|Block) + (1|Line)	3.2 to 3.1	<0.001
3.3	log(y) ~ 1 + Temperature * Diet + (1|Block) + (Temperature|Line) + (Diet|Line)	3.3 to 3.2	<0.001
3.4	log(y) ~ 1 + Temperature * Diet + (1|Block) + (Temperature|Line) + (Diet|Line) + (1|Temperature:Diet:Line)	3.4 to 3.3	<0.001
3.5	log(y) ~ 1 + Temperature * Diet + (1|Block) + (Temperature:Diet|Line)	3.5 to 3.3	<0.001

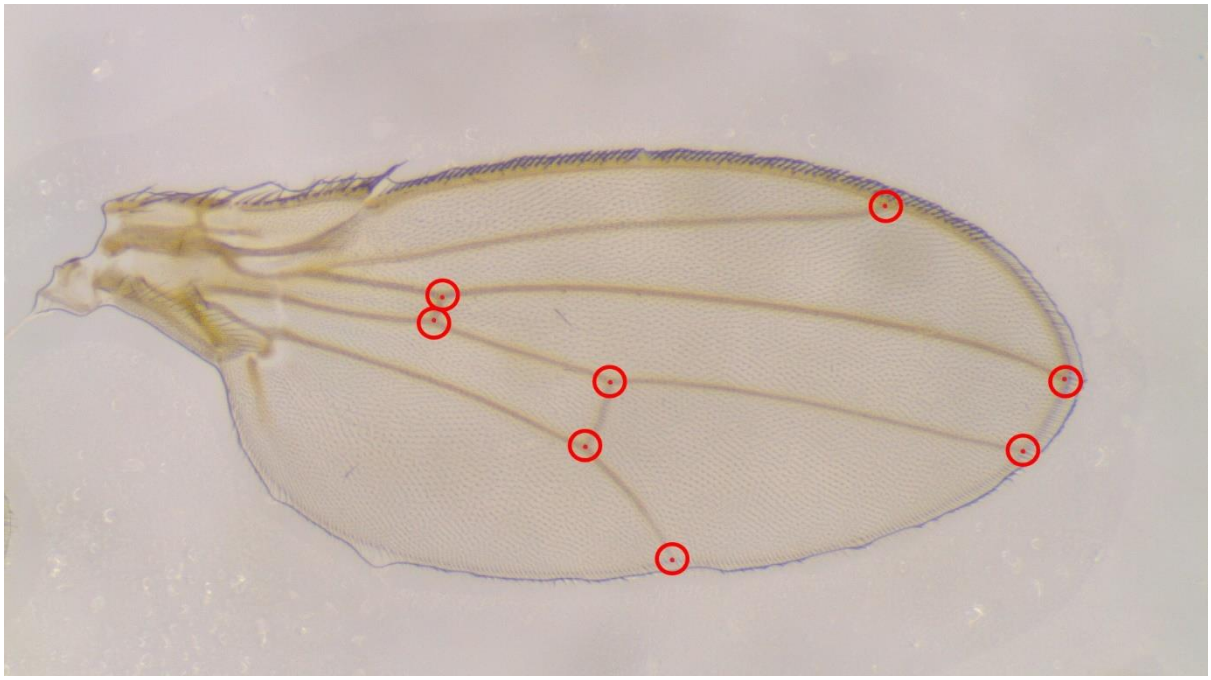
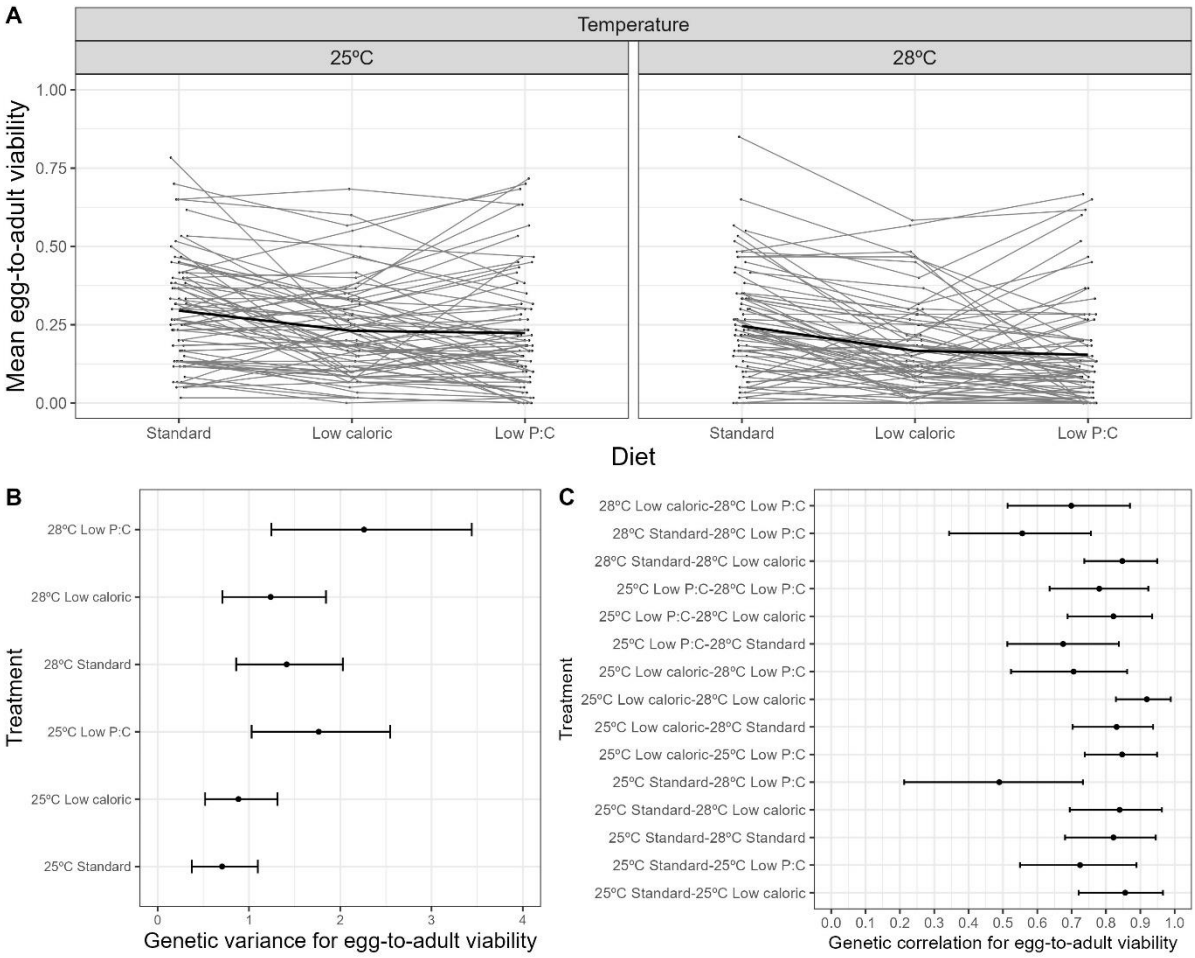


Fig. 1. Landmarks used for calculating wing centroid size, indicted by red dots.

612



613

614 **Fig. 2.** (**A**) Reaction norms of mean egg-to-adult viability of 71 isogenic lines across six
615 thermal-nutritional treatments. Solid black line in both panels represents the overall
616 population mean reaction norm, and the light grey lines the reaction norms for each
617 isogenic line (genotype) across the three diets. For ease of visualisation we have separated
618 the panels by temperature. (**B**) Estimates of genetic variance (which includes non-additive
619 genetic variance) for egg-to-adult viability in response to six combined thermal-nutritional
620 treatments, and (**C**) genetic correlation (broad sense) for egg-to-adult viability across
621 treatments. Error bars show 95% upper and lower highest posterior density (HPD) intervals
622 in panels B and C.

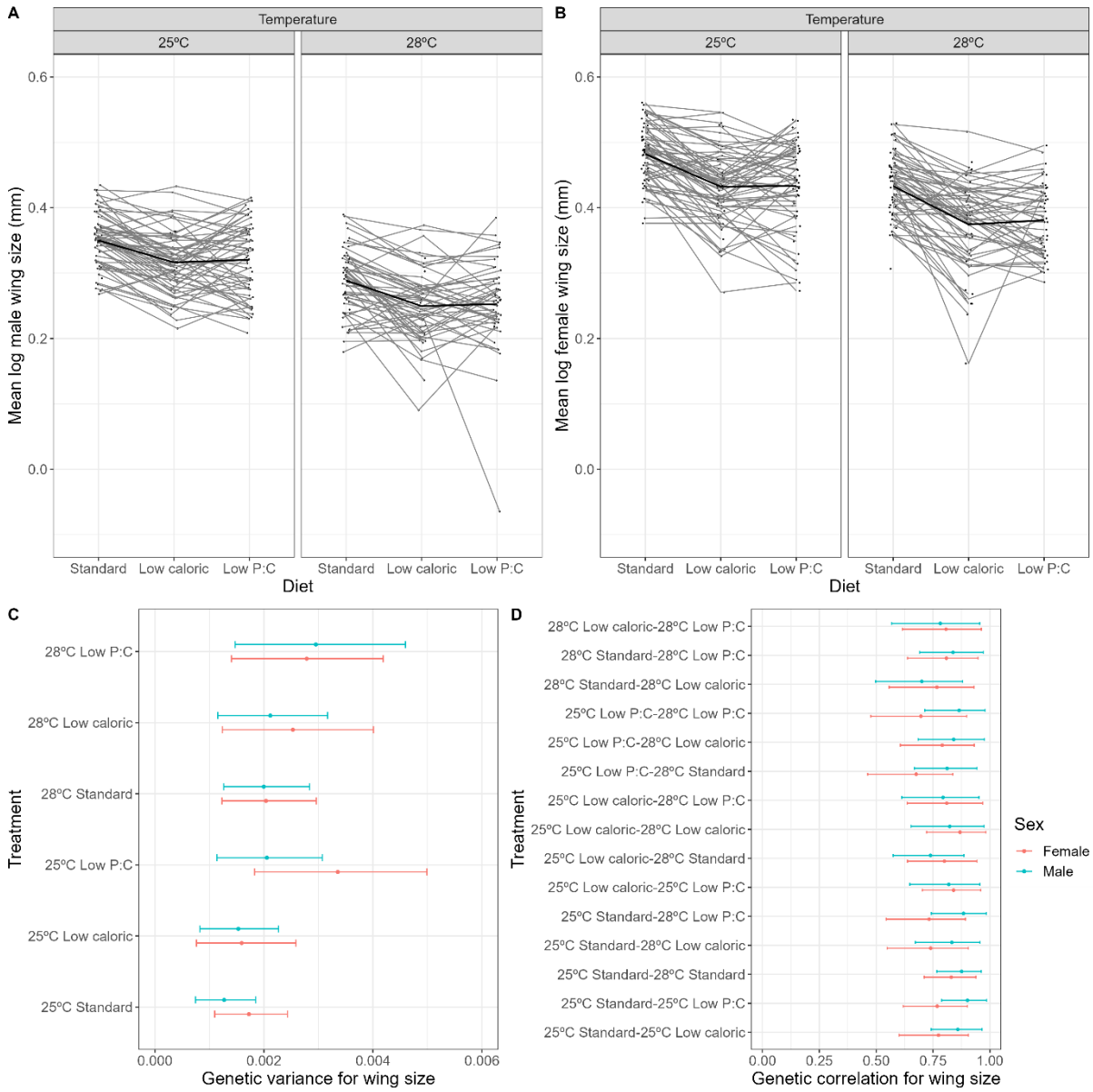


Fig. 3. Reaction norms of mean log wing size of (**A**) males and (**B**) females of 71 isogenic lines across six combined thermal-nutritional treatments. Solid black line in each panel represents the overall population mean reaction norm, and the light grey lines the reaction norms for each isogenic line (genotype) across the three diets. For ease of visualisation we have separated the panels by temperature. (**C**) Estimates of genetic variance (which includes non-additive genetic variance) for males and female wing size in response to six combine thermal-nutritional treatments, and (**D**) Genetic correlation (broad sense) for wing size across treatments for males and females. Error bars show 95% upper and lower HPD intervals in panels C and D.

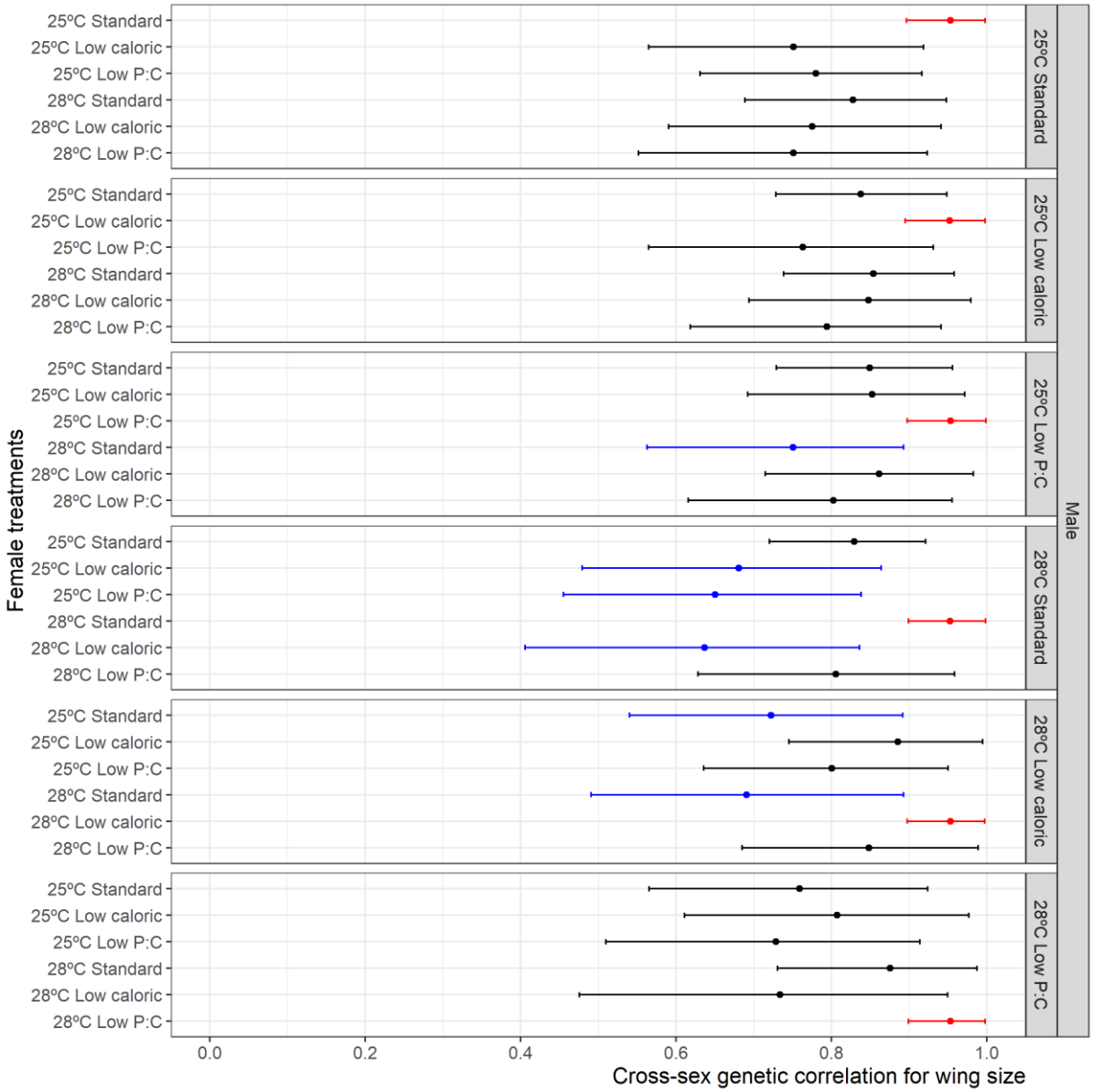


Fig. 4. Cross-sex genetic correlation (broad sense) for wing size across six combined thermal-nutritional treatments. Error bars show 95% upper and lower HPD intervals. Red error bars indicate the cross-sex genetic correlation within treatments. The blue error bars show significantly smaller cross-sex genetic correlations across certain treatment combinations compared to the cross-sex genetic correlations within treatments (red error bars).

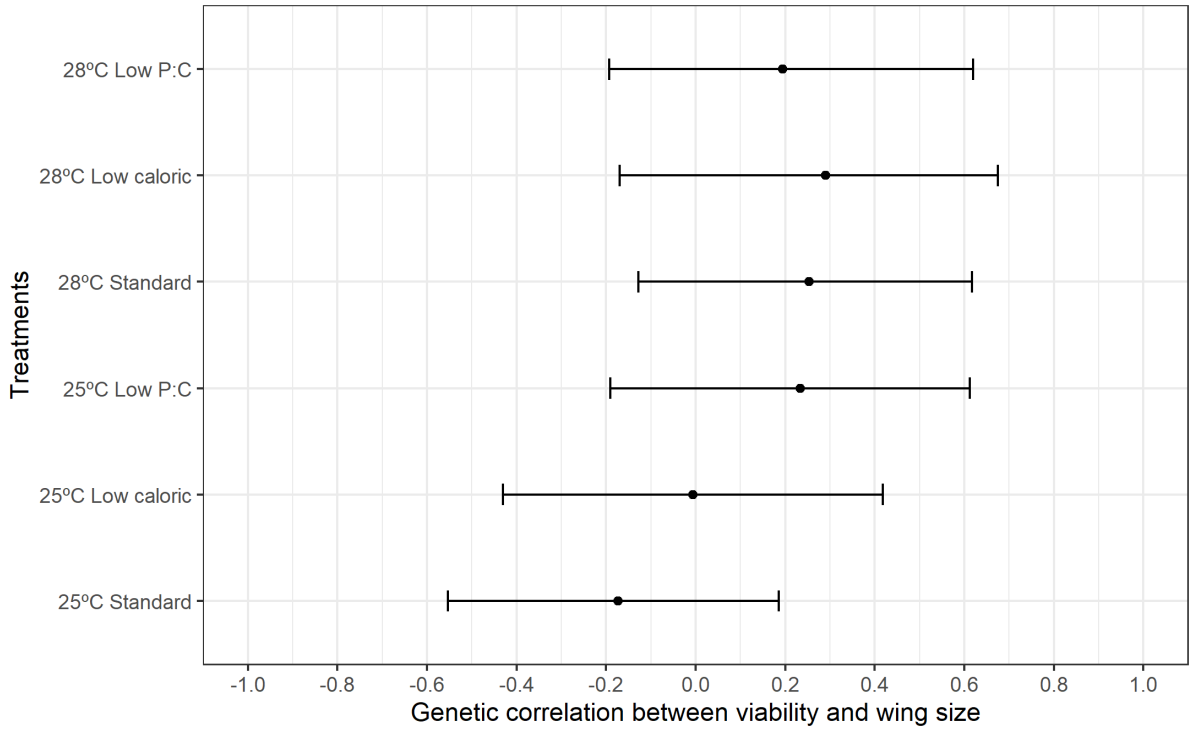


Fig. 5. Genetic correlation (broad sense) between egg-to-adult viability and wing size within each of six combined thermal-nutritional treatments. Wing size of both sexes was combined for estimating the genetic correlation between egg-to-adult viability and wing size. Error bars shows 95% upper and lower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

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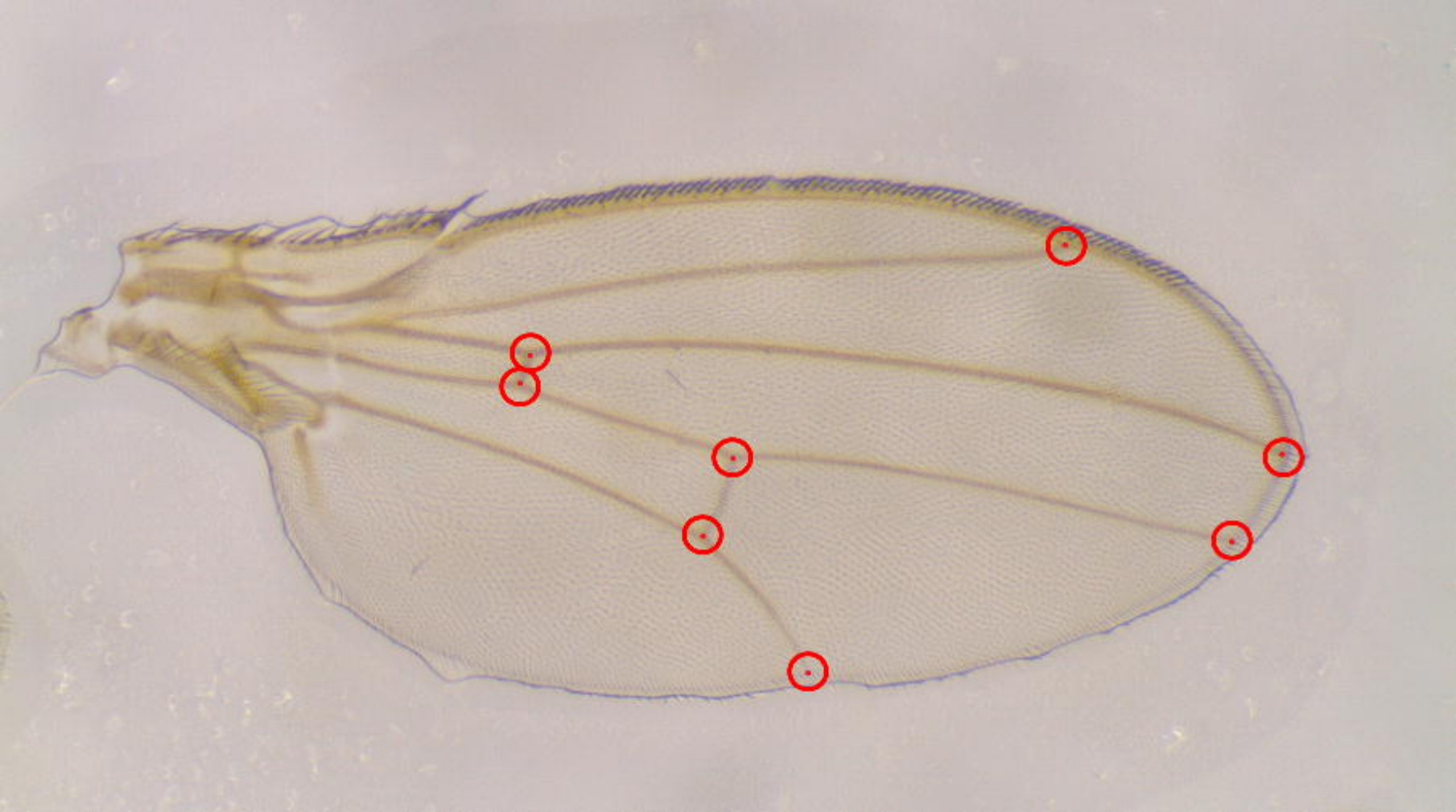

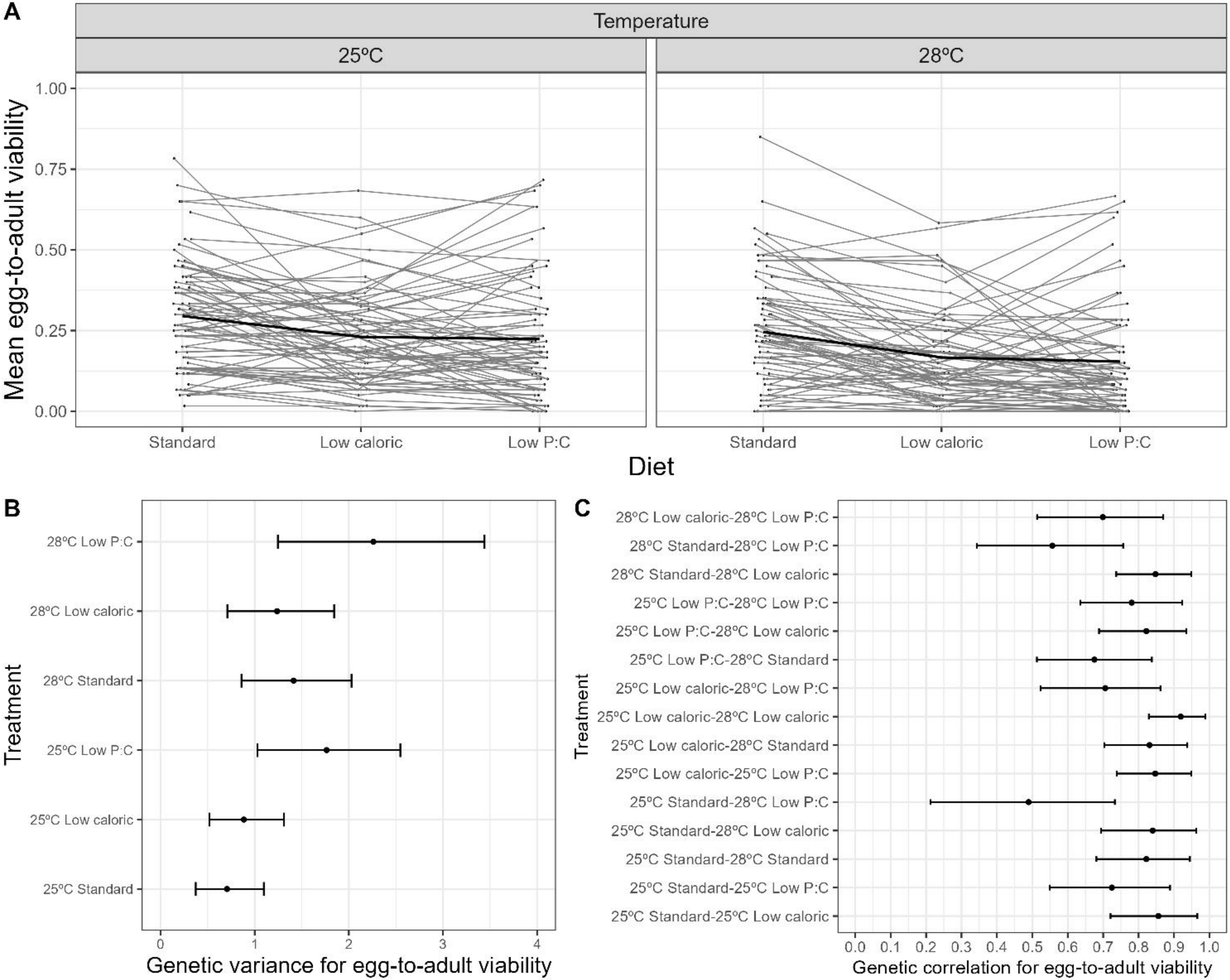
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Temperature
25ºC
28ºC
Mean egg-to-adult viability
1.00
0.75
0.50
0.25
0.00
Standard
Low caloric
Low P:C
Diet
B
Treatment
28ºC Low P:C
28ºC Low caloric
28ºC Standard
25ºC Low P:C
25ºC Low caloric
25ºC Standard
0
1
2
3
4
Genetic variance for egg-to-adult viability
C
Treatment
28ºC Low caloric-28ºC Low P:C
28ºC Standard-28ºC Low P:C
28ºC Standard-28ºC Low caloric
25ºC Low P:C-28ºC Low P:C
25ºC Low P:C-28ºC Low caloric
25ºC Low P:C-28ºC Standard
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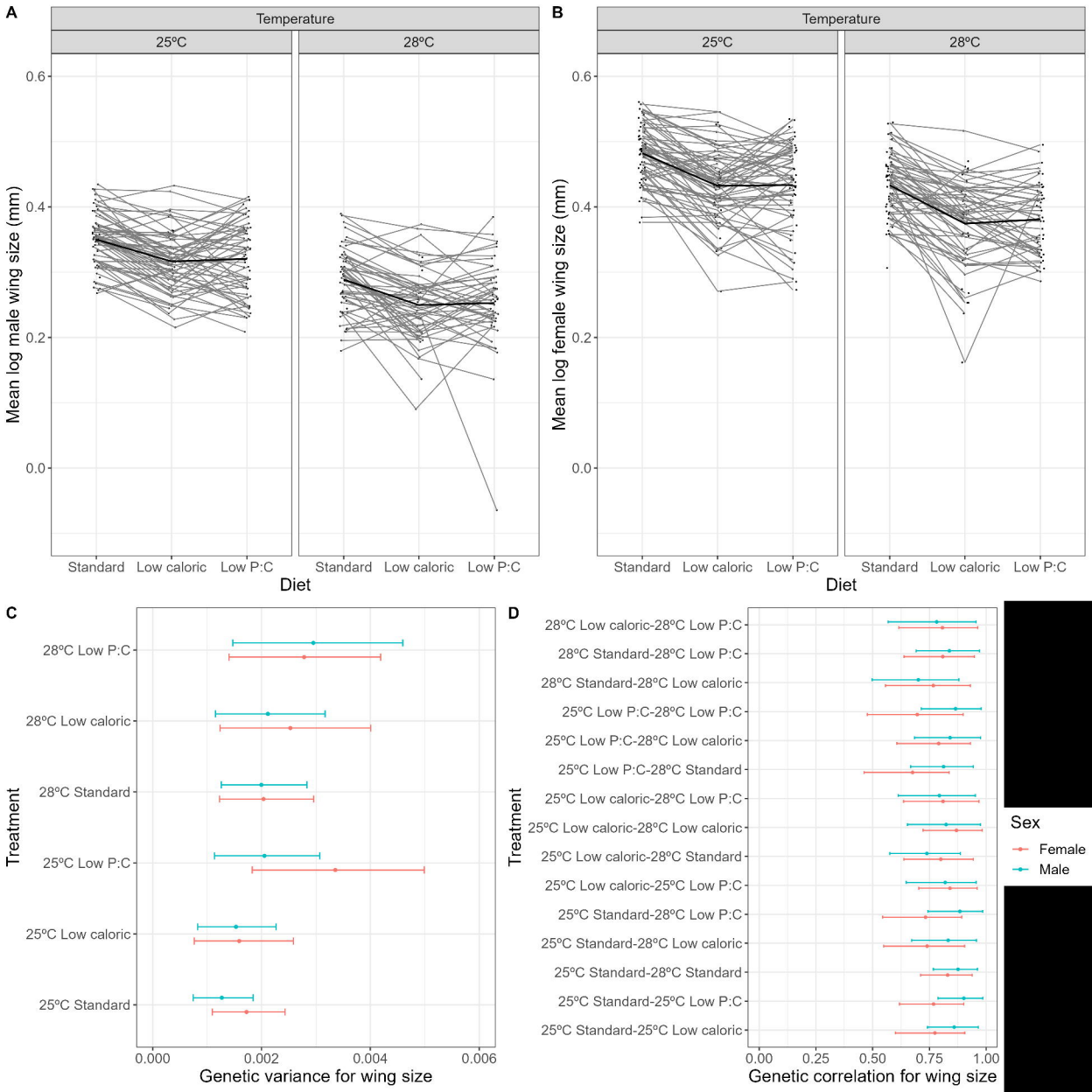
25ºC Standard-28ºC Standard
25ºC Standard-25ºC Low P:C
25ºC Standard-25ºC Low caloric
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Genetic correlation for egg-to-adult viability


A
Temperature
25ºC
28ºC
Mean log male wing size (mm)
0.6
0.4
0.2
0.0
Standard
Low caloric
Low P:C
Diet
B
Mean log female wing size (mm)
C
Treatment
28ºC Low P:C
28ºC Low caloric
28ºC Standard
25ºC Low P:C
25ºC Low caloric
25ºC Standard
0.000
0.002
0.004
0.006
Genetic variance for wing size
D
28ºC Low caloric-28ºC Low P:C
28ºC Standard-28ºC Low P:C
28ºC Standard-28ºC Low caloric
25ºC Low P:C-28ºC Low P:C
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25ºC Standard-28ºC Low caloric
25ºC Standard-28ºC Standard
25ºC Standard-25ºC Low P:C
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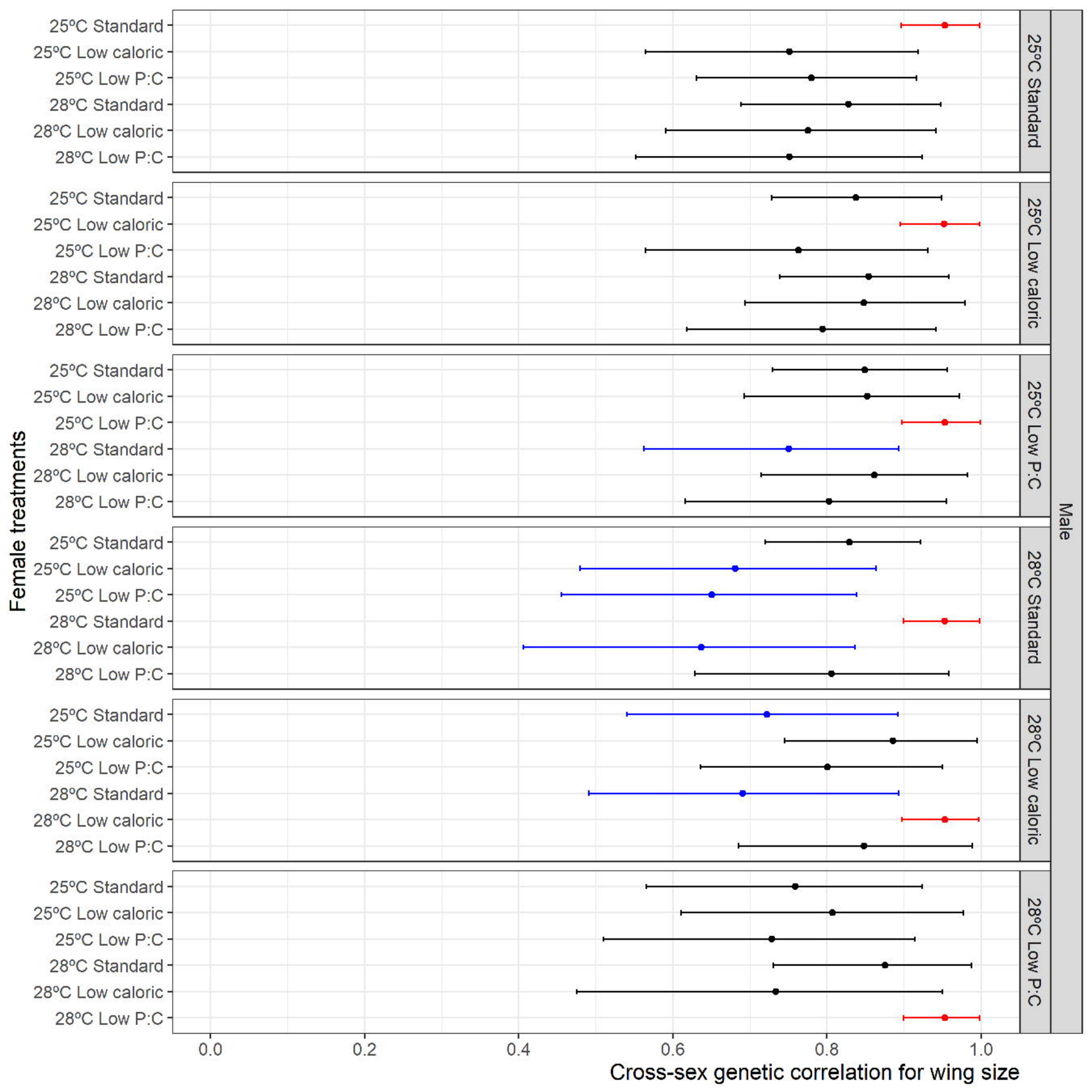
0.00
0.25
0.50
0.75
1.00
Genetic correlation for wing size
Sex
Female
Male


Female treatments
25ºC Standard
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25ºC Standard
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25ºC Low P:C
28ºC Standard
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Male
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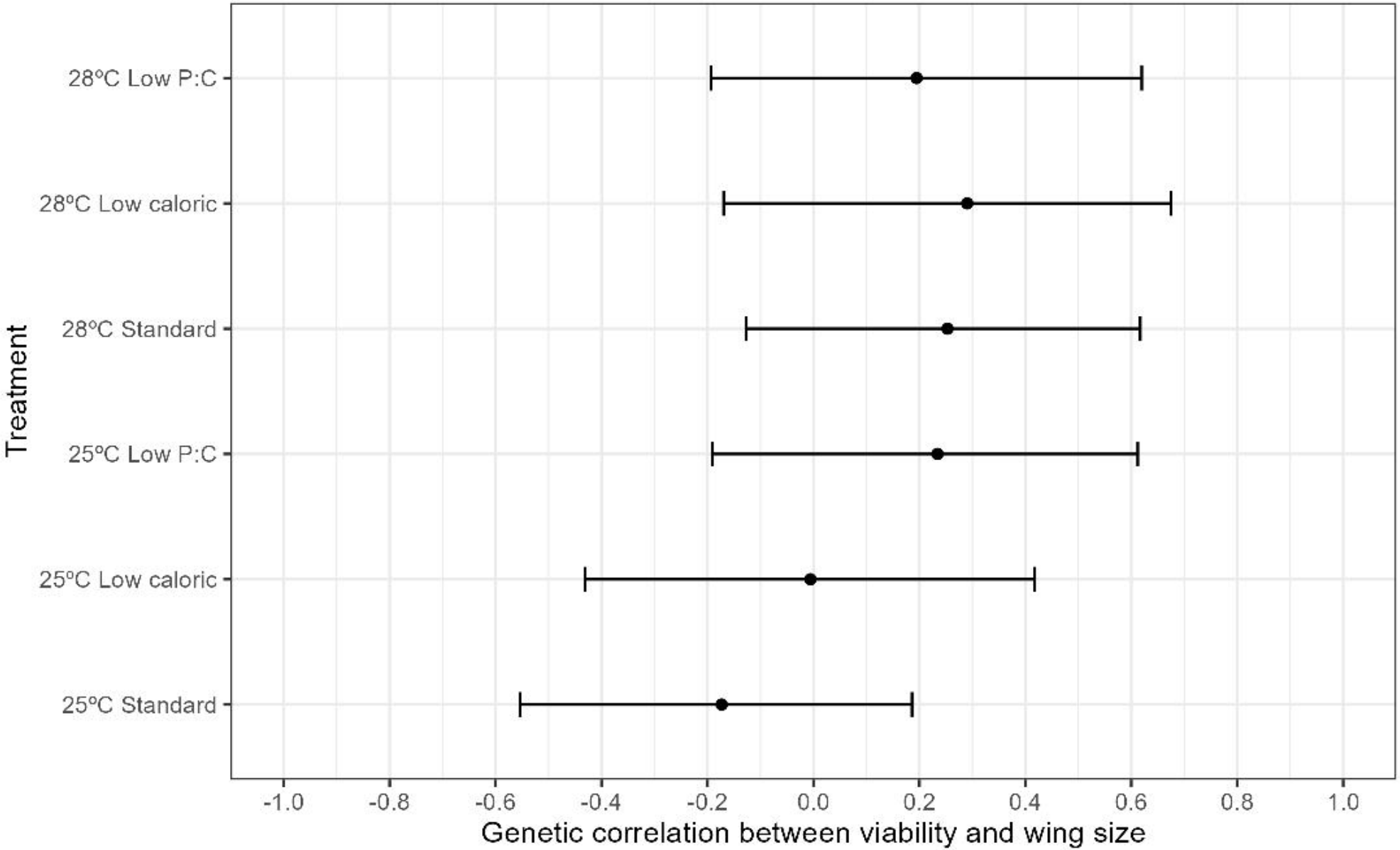
0.2
0.4
0.6
0.8
1.0
Cross-sex genetic correlation for wing size


28ºC Low P:C
28ºC Low caloric
28ºC Standard
25ºC Low P:C
25ºC Low caloric
25ºC Standard
Treatment
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
Genetic correlation between viability and wing size