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Genetic covariances promote climatic adaptation in Australian *Drosophila*

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Evolutionary potential for adaptation hinges upon the orientation of genetic variation for traits under selection, captured by the additive genetic variance-covariance matrix (G), as well as the evolutionary stability of G. Yet studies that assess both the stability of G and its alignment with selection are extraordinarily rare. We evaluated the stability of G in three *Drosophila melanogaster* populations that have adapted to local climatic conditions along a latitudinal cline. We estimated population- and sex-specific G matrices for wing size and three climatic stress-resistance traits that diverge adaptively along the cline. To determine how G affects evolutionary potential within these populations, we used simulations to quantify how well G aligns with the direction of trait divergence along the cline (as a proxy for the direction of local selection) and how genetic covariances between traits and sexes influence this alignment. We found that G was stable across the cline, showing no significant divergence overall, or in sex-specific subcomponents, among populations. G also aligned well with the direction of clinal divergence, with genetic covariances strongly elevating evolutionary potential for adaptation to climatic extremes. These results suggest that genetic covariances between both traits and sexes should significantly boost evolutionary responses to environmental change.

KEY WORDS: **B matrix, G matrix, local adaptation, sexual dimorphism, selection.**

Climatic conditions are currently changing around the globe, elevating the risk of extinction for many organisms (Collins et al. 2013). Populations in changing environments can potentially persist by adapting to new conditions (Hoffmann and Sgrò 2011), yet the potential for rapid adaptation depends upon the pattern of genetic variation and covariation within and among traits targeted by selection (Fisher 1930; Lande 1979; Lande and Arnold 1983). The additive genetic variance-covariance matrix (G) summarizes the distribution of genetic variation among multiple traits, and plays a central role in predicting short-term evolutionary responses to selection (Lande 1979).

How genetic covariation between traits impacts adaptation ultimately depends on the alignment between G and the direction of selection on the same traits (Lande 1979; Lande and Arnold 1983). On the one hand, adaptive potential is highest when selection aligns closely with the genetic axis showing greatest variability (Walsh and Blows 2009). On the other hand, misalignment between selection and genetic variation restricts adaptation, and in extreme cases may generate absolute genetic constraints, despite abundant genetic variation for individual traits (Schluter 1996; Hansen and Houle 2008; Agrawal and Stinchcombe 2009; Kirkpatrick 2009; Walsh and Blows 2009). Genetic covariances between the sexes can similarly influence the evolutionary trajectories of female and male phenotypes (Bonduriansky and Chenoweth 2009; Cox and Calsbeek 2009). For traits expressed by both sexes, G is partitioned into submatrices that describe trait variances and covariances within each sex (G_m for males and G_f for females), and a submatrix (B) describing genetic covariances between sexes (Lande 1980). Positive and strong between-sex genetic covariances can promote adaptation when the direction of selection on males and females is the same, or constrain adaptation when the direction of selection differs between the sexes (Lande 1980; Bonduriansky and Chenoweth 2009; Connallon and Hall 2016).

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Predicting the impacts of genetic variances and covariances on adaptation is further complicated by the potential for evolution of G itself. Theoretical models suggest that selection, mutation, genetic drift, and migration are all likely to affect G to some extent (Lande 1979; Jones et al. 2004; Guillaume and Whitlock 2007). Empirical evidence suggests that G matrices are often evolutionarily stable (Arnold et al. 2008), although several studies have documented changes in G due to selection (e.g., Shaw et al. 1995; Blows et al. 2004; Careau et al. 2015; Uesugi et al. 2017), drift (Phillips et al. 2001), migration (Nosil et al. 2006), or interactions with the environment (Wood and Brodie 2015). Finally, some parts of G may be more susceptible to change than others. For example, theoretical models of within-generation change in G predict that stabilizing selection more strongly constrains sex-specific genetic variances (G_f and G_m) than between-sex genetic covariances (B) (Barker et al. 2010; Gosden and Chenoweth 2014), whereas directional selection can elevate or reduce genetic covariances between the sexes (McGlothlin et al. 2019). Nevertheless, the long-run evolutionary stability of G and its submatrices remains an open question, with important implications for adaptation and the evolution of sexual dimorphism.

Meaningful predictions about a population's capacity for rapid adaptation require knowledge of both selection and the G matrix, posing a major empirical challenge for studies of adaptation in the wild. Unsurprisingly, given the difficulty of directly estimating selection and G, few studies measure both in the same population, and even fewer measure them within each sex (Cox and Calsbeek 2009; Poissant et al. 2010; Wyman and Rowe 2014; Cox et al. 2017). One approach for addressing this challenge is to complement replicated estimates of the G matrix with indirect estimates of selection through the study of adaptive trait divergence along environmental clines. Cline studies provide a powerful approach to infer adaptive divergence among populations that are connected by gene flow (Haldane 1948; Endler 1977). Establishment and maintenance of repeatable and genetically based clinal trait divergence requires sustained selection for local adaptation, particularly when gene flow among populations is high (Lenormand 2002). Trait clines can therefore indicate directions (and in ideal cases, strengths) of selection along environmental gradients (Hoekstra et al. 2004; Mullen and Hoekstra 2008). Classical cline theory predicts that directional selection near range limits will align with the direction of phenotypic divergence along the cline (García-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997). As such, species with repeatable and genetically based trait clines are tractable systems for assessing the alignment between G and selection, as inferred from divergence in targeted traits near cline ends.

Here, we examine phenotypic divergence and the stability of G (including its sex-specific submatrices, G_m, G_f, and B) for wing size and climatic stress-resistance traits (desiccation resistance, cold recovery time, and heat knockdown time) in *Drosophila melanogaster* populations that have locally adapted to climatic selection along a latitudinal cline. Australian *Drosophila* populations have played a key role in studies of climatic adaptation, repeatedly exhibiting genetically based clinal divergence in body size and thermal stress traits, despite strong gene flow across the cline (Kennington et al. 2003; Hoffmann and Weeks 2007; Sgrò et al. 2010; Hangartner et al. 2015; Lasne et al. 2018). Although desiccation resistance has shown less consistent clinal divergence than our other focal traits (Hoffmann et al. 2001; Lasne et al. 2018), studies conducted since The Millenial Drought (1996–2010) have reported elevated desiccation resistance in temperate populations from southeastern Australia, where desiccation stresses have been particularly intense (Lasne et al., 2018, 2019; the current study). In light of these observations, our four focal traits are among the best candidates for adaptive divergence by latitude across eastern Australia.

Using a quantitative genetic breeding design, we estimated population- and sex-specific G matrices for three populations spanning the full gradient of climatic conditions along Australia's east coast (tropical Cairns, subtropical Brisbane, and temperate Melbourne). Using simulations, we then quantified the degree of alignment between population- and sex-specific G matrices and the direction of climatic selection inferred from clinal patterns of trait divergence (Hoffmann and Weeks 2007; Sgrò et al. 2010; Hangartner et al. 2015). Our analyses addressed three questions. First, how stable is the G matrix, and its sex-specific submatrices, across the geographic range of Australian *Drosophila melanogaster*? Second, how well does G align with the inferred direction of selection at the cline ends? Finally, what is the quantitative impact of between-trait and between-sex genetic covariances on the capacity for climatic adaptation? Our analyses reveal that G (including its sex-specific components) is stable across the range, and aligns well with the direction of climatic selection at the cline ends. Importantly, our results suggest that genetic covariances between traits and sexes may promote adaptation to climatic extremes.

Methods

FIELD COLLECTION AND LABORATORY MAINTENANCE

Flies were collected in April–May 2015 from three sites on the east coast of Australia: tropical Cairns (–17.523°S/146.09°E), subtropical Brisbane (–26.857°S/152.955°E), and temperate Melbourne (–37.734°S/145.450°E). Flies from these sites therefore inhabit warm, high-humidity conditions year-round in northern Cairns, shifting toward slightly lower humidity and milder winters in Brisbane, and increasingly colder, drier conditions southward to Melbourne. Two generations after collection,

mass-bred populations were founded for each location using 10 males and 10 females from each of 65 iso-female lines. Each population was maintained at discrete generations in 6 × 250 mL bottles containing 60 mL of potato, yeast, and sucrose medium. Densities were approximately 300–400 flies per bottle to ensure a census population size of 2000+ individuals. Populations were treated with an antibiotic at generation three to cure them of *Wolbachia* and kept at a constant temperature of 25°C, with a 12:12 h light:dark cycle, for at least 10 more generations. Such conditions are roughly native for the Brisbane population. While estimates of genetic variation are potentially affected by exposing field-fresh stocks to native versus nonnative conditions in the laboratory (e.g., Sniegula et al. 2016), our maintenance of all populations under identical conditions for multiple generations prior to use is expected to minimize this issue here (Hoffmann and Sgrò 2018).

QUANTITATIVE GENETIC BREEDING DESIGN

A paternal half-sibling full-sibling breeding design (Lynch and Walsh 1998) was used to estimate additive genetic variances and covariances among sexes and traits (desiccation resistance, cold recovery time, heat knockdown time, and wing size) for each of the three populations. We performed the experiments in four blocks: Block 1 after 13 (Brisbane), 15 (Cairns), or 16 (Melbourne) generations of mass breeding, Block 2 in the following generation and Blocks 3 and 4 after another nine and 10 generations, respectively. Blocks 1 and 2 were performed in November 2015, and Blocks 3 and 4 in February–March 2016.

Parents of focal animals were reared under controlled densities of approximately 40 eggs per vial and collected as virgins within 6 h of emergence over two days. Females and males were separated using light CO_2 anesthesia and held in separate vials by sex, at densities of approximately 20–30 individuals per vial, until two days old. For each block, 50 virgin males (sires) per population (150 in total) were randomly selected from holding vials. Each sire was placed in a vial containing 6 mL of food media, ad libitum live yeast, and five virgin females (dams), and left to mate for three days. Each dam was then placed individually in a separate vial and allowed to lay eggs for 24 h, then moved to a fresh vial and allowed to lay eggs for another 24 h. Focal animals were collected within one day of emerging. Females and males were separated using light CO_2 anesthesia, held in separate vials by sex and family, and left to recover for another 48 h. At five to seven days old, two females and two males per dam were tested for each stress assay. In summary, we mated at least 100 sires to each of five dams per population. We then scored focal traits on two females and males per dam, measuring 1019–1062 females and males per trait and population, and 25,068 flies in total.

TRAIT ASSAYS

Desiccation resistance

Flies were desiccated individually in 5 mL glass vials topped with gauze in five sealed glass tanks containing silica desiccant (10% relative humidity) at 25°C. Flies were scored as dead when no movement was detected and flies were scored at hourly intervals until all the flies had died (van Heerwaarden and Sgrò 2014). Flies were scored by the same person, with populations and sexes randomized between tanks. The time interval when 90% mortality was reached was used for the statistical analyses (Griffin et al. 2016).

Cold recovery time

Individual flies were placed into 5 mL Eppendorf tubes and submerged for 5 h in a 10% glycol solution maintained at 0°C (Hoffmann et al. 2002). Flies were then removed from the bath and placed at 25°C. Cold recovery time was measured as the time taken for flies to stand upright after removing them from the bath. Flies were scored by the same three observers over four runs, with populations and sexes randomized between observers.

Heat knockdown time

Flies were placed individually into 5 mL vials with plastic caps and immersed in a preheated recirculating water bath at 39°C. Each fly was scored for heat knockdown time, defined as the time taken for each fly to be knocked down and become immobile (Sgrò et al. 2010). Heat knockdown time was scored over two days per block, with four runs performed each day, and with populations and sexes randomized between runs. The same two people scored all assays.

Wing size

Individual flies were put in 5 mL Eppendorf tubes and frozen at –20°C for subsequent wing size measurements. The right wing (or the left if the right wing was damaged) was removed from each fly with fine forceps, mounted on a glass slide with double-sided tape, and protected with a cover slip. Wing images were captured with a Wild M3 dissector microscope (Leica, Heerbrugg, Switzerland) attached to a digital camera, and landmarked for the eight junctions of longitudinal veins with the wing margins or cross-veins (Liefting et al. 2009). Their x and y coordinates were recorded using the program TPSDIG version 1.31, written by F. J. Rohlf. Wing size was calculated as centroid size (square root of the sum of the squared inter-landmark distances) (Hoffmann and Shirriffs 2002). To check for measurement error, we measured repeatability for wing centroid-size estimates. Repeat measures were highly correlated ($r > 0.99$, $n = 100$).

ANALYSES OF POPULATION DIVERGENCE

Divergence in trait means

We fitted linear models using maximum likelihood in the MIXED procedure of SAS (SAS Institute, Cary, NC) to test for population divergence in trait means. These models were run separately per trait, with sex, population, their interaction, and block included as fixed factors. Scorer and run, nested within block, were included as fixed factors for cold recovery time and heat knockdown time. Tank was included as a fixed factor for desiccation resistance. Post hoc pairwise comparisons were constructed in these models using Tukey tests, comparing least square means and adjusting for multiple comparisons.

Estimation of G matrices

Next, we estimated population-specific G matrices using restricted maximum likelihood (REML) in ASReml-R 3.0 (while matrices could also have been estimated in MIXED, we turned to ASReml-R because its routines do so more efficiently; we did not use ASReml-R to test for population divergence in trait means because it does not implement maximum likelihood, which is preferable for testing fixed effects). For each population, we fitted a linear mixed model to the traits measured in each sex (giving eight sex-specific traits in total), estimating G as an unstructured covariance matrix (Lynch and Walsh 1998):

$$\mathbf{G} = \begin{pmatrix} \mathbf{G}_m & \mathbf{B} \\ \mathbf{B}^T & \mathbf{G}_f \end{pmatrix}$$

where G_m and G_f are the male and female G matrices, B is the cross-sex covariance matrix, and T denotes matrix transposition. Each model included traits and blocks as fixed effects, and sire and dam (nested within sire) as random effects. Sire effects were multiplied by 4 to estimate G (Lynch and Walsh 1998). To determine whether these effects were significantly different from zero, we used log-likelihood ratio tests that compared full models to reduced ones with the relevant (co)variance components set to zero (van Heerwaarden and Sgrò 2014). Each model also included sex-specific residual variances and covariances for cold recovery time and wing size (which were measured on the same individual in each sex), and sex-specific residual variances for heat and desiccation resistance (which were measured on different individuals in each sex). Because traits were measured on different scales, each trait was standardized to unit variance by population prior to analyses (to show the effects of trait standardization, we also report G matrices based on unstandardized data in Table S1).

We then refitted the models in a Bayesian framework using the *MCMCglmm* R package (Hadfield 2010) to sample the marginal posterior distribution of each G. Equivalent likelihood-based distributions can be obtained using an REML-multivariate normal sampling approach (REML-MVN; Houle and Meyer 2015), but are not yet widely available for complex models like ours. We fitted models using weakly informative inverse-Wishart priors, with parameters for the distribution set to 0.001 for the degrees of freedom and a diagonal matrix containing values of one-third of the phenotypic variance for the scale. Distributions were estimated from 110,000 MCMC iterations sampled every 100 iterations following an initial burn-in period of 10,000 iterations. Autocorrelations between the 1000 samples were below the recommended level of 0.1, yielding effective samples sizes of several hundred for all estimates. We inspected plots of traces and posterior distributions to ensure that models converged. We further explored the use of parameter expanded priors, which gave similar results to those presented here but made models take longer to converge. Bayesian estimates of G (Table S2) were also similar to REML estimates (Table 1), with 95% confidence intervals for the former consistently including the latter.

Population differences in G and its submatrices

To test for population differences in G and its submatrices (B, G_f, and G_m), we used a fourth-order genetic covariance tensor. Such tensors provide a general framework for characterizing differences among G matrices, and for identifying trait combinations that differ most in genetic variation among them (Hine et al. 2009). Tensors can be decomposed into a set of eigentensors (E_i), each representing an independent component of variation among the original matrices (in our case, the maximum number of nonzero components was two, one less than the number of matrices compared). Once an E_i capturing variation among matrices is identified, it can be decomposed into eigenvectors (e_{i1}, e_{i2}, etc.) describing the trait combinations that differ most among matrices, and eigenvalues describing the amount of variance for each combination. Additional details of tensor-based comparisons of G can be found in Hine et al. (2009), Aguirre et al. (2014), and Gosden and Chenoweth (2014).

We applied the tensor to the 1000 MCMC samples of each matrix using a modified version of the *R* routine in Aguirre et al. (2014), which compares the observed divergence of matrices to a null distribution where divergence is driven by random sampling variation. Since a feature of the original routine was identified as likely to make this null distribution too narrow, we used an improved method described in detail in Morrissey et al. (submitted). Briefly, we constructed our null distribution from the posterior means of 1000 models, each re-fitted to a single randomization of the data. Each randomization took one MCMC sample, shuffled sire values among populations, and re-combined shuffled values with non-shuffled dam and residual effects to simulate a new set of phenotypes that were randomized at the level of interest. Models were then re-fitted to randomized data using the same burn-in and thinning interval as the original models. To reduce computational burden, however, we estimated each posterior mean from 200 MCMC samples, which was the smallest number required to

Table 1. G matrices of genetic variance and covariance components (±SE) for cold recovery time (Cold), desiccation resistance (Des), heat knockdown time (Heat), and wing size (Size) in females (F) and males (M) from (a) Cairns (tropical northern Australia), (b) Brisbane (subtropical central Australia), and (c) Melbourne (temperate southern Australia). Female submatrices (G_f) are on the top left, male submatrices (G_m) are on the bottom right, and submatrices of cross-sex covariances (B) are shaded grey in between. Estimates are based on REML using variance-standardized data, and significant values ($P < 0.05$) are marked in bold.

a) Cairns

	Cold_F	Des_F	Heat_F	Size_F	Cold_M	Des_M	Heat_M	Size_M
Cold_F	0.21 ± 0.09	−0.05 ± 0.08	0.00 ± 0.08	**−0.25 ± 0.08**	**0.19 ± 0.07**	0.04 ± 0.07	−0.01 ± 0.07	**−0.19 ± 0.07**
Des_F		**0.43 ± 0.13**	**−0.22 ± 0.10**	0.04 ± 0.09	0.02 ± 0.07	**0.23 ± 0.09**	−0.10 ± 0.08	−0.01 ± 0.08
Heat_F			**0.54 ± 0.14**	−0.12 ± 0.10	0.08 ± 0.07	−0.06 ± 0.09	**0.28 ± 0.09**	0.04 ± 0.09
Size_F				**0.54 ± 0.14**	0.02 ± 0.08	0.10 ± 0.09	−0.12 ± 0.08	**0.47 ± 0.11**
Cold_M					**0.13 ± 0.08**	**−0.13 ± 0.07**	−0.01 ± 0.06	−0.01 ± 0.07
Des_M						**0.24 ± 0.11**	−0.05 ± 0.07	−0.01 ± 0.08
Heat_M							**0.24 ± 0.10**	−0.01 ± 0.07
Size_M								**0.32 ± 0.11**

b) Brisbane

	Cold_F	Des_F	Heat_F	Size_F	Cold_M	Des_M	Heat_M	Size_M
Cold_F	**0.21 ± 0.10**	0.06 ± 0.08	−0.02 ± 0.07	0.04 ± 0.09	**0.24 ± 0.08**	−0.09 ± 0.08	−0.04 ± 0.07	0.07 ± 0.07
Des_F		**0.42 ± 0.12**	−0.16 ± 0.08	**0.23 ± 0.09**	−0.10 ± 0.08	**0.29 ± 0.10**	−0.06 ± 0.08	**0.20 ± 0.08**
Heat_F			**0.33 ± 0.11**	−0.01 ± 0.08	−0.04 ± 0.08	−0.04 ± 0.09	**0.24 ± 0.08**	−0.03 ± 0.07
Size_F				**0.36 ± 0.12**	−0.03 ± 0.08	0.14 ± 0.09	−0.02 ± 0.07	**0.33 ± 0.09**
Cold_M					**0.26 ± 0.11**	0.01 ± 0.08	−0.06 ± 0.07	−0.01 ± 0.07
Des_M						**0.45 ± 0.13**	0.00 ± 0.08	0.02 ± 0.08
Heat_M							**0.20 ± 0.09**	−0.04 ± 0.07
Size_M								**0.24 ± 0.10**

c) Melbourne

	Cold_F	Des_F	Heat_F	Size_F	Cold_M	Des_M	Heat_M	Size_M
Cold_F	**0.26 ± 0.09**	0.12 ± 0.08	0.00 ± 0.07	−0.05 ± 0.07	**0.22 ± 0.07**	0.01 ± 0.08	−0.03 ± 0.07	−0.02 ± 0.07
Des_F		**0.40 ± 0.12**	−0.01 ± 0.08	0.09 ± 0.08	−0.01 ± 0.07	**0.18 ± 0.09**	0.01 ± 0.08	0.14 ± 0.09
Heat_F			**0.35 ± 0.11**	−0.01 ± 0.08	0.04 ± 0.07	**−0.18 ± 0.09**	**0.21 ± 0.09**	0.00 ± 0.08
Size_F				**0.29 ± 0.11**	0.00 ± 0.07	0.04 ± 0.08	−0.06 ± 0.08	**0.31 ± 0.09**
Cold_M					**0.23 ± 0.09**	−0.05 ± 0.08	−0.05 ± 0.07	−0.06 ± 0.07
Des_M						**0.45 ± 0.13**	−0.07 ± 0.09	0.06 ± 0.09
Heat_M							**0.36 ± 0.11**	0.03 ± 0.08
Size_M								**0.39 ± 0.12**

obtain a stable estimate (Walter et al. 2018). Based on the overlaps of posterior means and 95% HPD intervals calculated from the observed and null distributions, we inferred whether population differences in G were greater than expected by chance alone (for further details, see Aguirre et al. 2014 and Morrissey et al. submitted).

We also characterized population differences in the overall amount of genetic variation in each G matrix. To do so, we calculated the total size of each matrix as its trace (the sum of its diagonal elements), and compared traces between populations based on overlaps of posterior means and 95% HPD intervals calculated from the 1000 MCMC samples. As G matrices did not differ in size, their submatrices were not further analyzed.

QUANTIFYING THE IMPACT OF GENETIC COVARIANCES ON EVOLVABILITY

To test whether genetic covariances promote or constrain local adaptation in our three populations sampled along the East coast of Australia, we calculated "evolvability"—the predicted evolutionary trajectory of a population relative to the direction favored by selection (Hansen and Houle 2008)—using the posterior MCMC samples for each G matrix (Cairns, Brisbane, and Melbourne), coupled with simulated directional selection vectors that were either (1) random and unbiased in direction (hereafter "random selection"), or (2) in the same direction as clinal divergence for the four traits (hereafter "local selection"). Thus, local selection in each cline-end population was assumed to occur in the same direction as the change in trait mean in cline-end populations relative to the range center (for detailed methods see the Supplementary Information). As mentioned above, the clinal divergence patterns of our traits—as documented here and in several previous studies (see above)—imply that each plays a role in adaptation to local climatic conditions, although we acknowledge the absence of direct estimates of local selection on these traits.

We tested whether or not G aligned with patterns of clinal divergence by contrasting mean evolvability under random selection versus evolvability in response to the implied direction of local selection at the cline ends. A stronger response to local relative to random selection implies an alignment between G and the direction of clinal divergence; a weaker response implies misalignment. To examine effects of between-trait and between-sex genetic covariances on evolvability, we performed simulations using: (1) the full G matrix for each population, (2) a reduced G matrix with between-sex covariances set to zero (B = 0), and (3) a minimal G matrix with all covariances set to zero (B and off-diagonal elements of G_f and G_m set to zero). Finally, to evaluate the plausible range of sex-specific selection scenarios, we performed simulations assuming that: (1) magnitudes of selection per trait were identical between the sexes, and (2) magnitudes of selection per trait were uncorrelated between the sexes.

Results

POPULATION-SPECIFIC TRAIT MEANS AND G MATRICES

For all four traits, we detected significant population divergence across the cline from its warmer, more humid end in tropical Cairns in the north to its cooler, drier end in temperate Melbourne in the south (Table S3; Fig. 1). Flies from Cairns and Brisbane had similar cold resistance, but were significantly less cold resistant than flies from Melbourne. Females were significantly less cold-resistant than males. There was also a significant population-by-sex interaction for this trait (Table S3A; Fig. 1A), with sexual dimorphism in cold resistance being roughly twice as large in Melbourne than in other populations (Fig. S1A). Heat resistance differed significantly among all three populations, decreasing from Cairns to Brisbane, and from Brisbane to Melbourne (Table S3B; Fig. 1B). Females were less heat resistant than males, with no significant population-by-sex interaction for this trait (Table S3B, Fig. S1B; Fig. 1B). Likewise, desiccation resistance differed among all three populations, increasing from Cairns to Brisbane, and from Brisbane to Melbourne (Table S3C; Fig. 1C). Females were more desiccation resistant than males, and there was a significant population-by-sex interaction for this trait (Table S3C; Fig. 1C), with sexual dimorphism in desiccation resistance increasing from Cairns to Brisbane, and from Brisbane to Melbourne (Fig. S1C). Lastly, wing size also differed among all three populations, increasing from Cairns to Brisbane, and from Brisbane to Melbourne (Table S3D; Fig. 1D). Females were larger than males, and there was no significant population-by-sex interaction in size (Table S3D, Fig. S1D; Fig. 1D).

Log likelihood tests revealed significant additive genetic variance for all traits and populations (sire variances significant at $p < 0.05$; Table 1). In each population, genetic covariances between the sexes were often significant and mostly positive (Table 1). In contrast, genetic covariances between traits (within sexes) were generally weak, with only three being significant in Cairns (two in females and one in males), one in Brisbane (in females), and none in Melbourne (Table 1). G matrices using unstandardized data were qualitatively similar (Table S1).

TESTS OF POPULATION DIVERGENCE IN G MATRICES AND SEX-SPECIFIC SUBMATRICES

Our tensor comparison did not find significant population divergence in G or its sex-specific submatrices G_m, G_f, or B (Fig 1E–H), based on the overlapping 95% HPD intervals of observed and random eigentensors (E_1 and E_2). Nor did populations differ in the total amount of genetic variation in each G, based on overlapping traces of 0.71 (95% HPD: 0.50–0.91) for Cairns, 0.68 (95% HPD: 0.50–0.88) for Brisbane, and 0.64 (95% HPD: 0.46–0.82) for Melbourne.

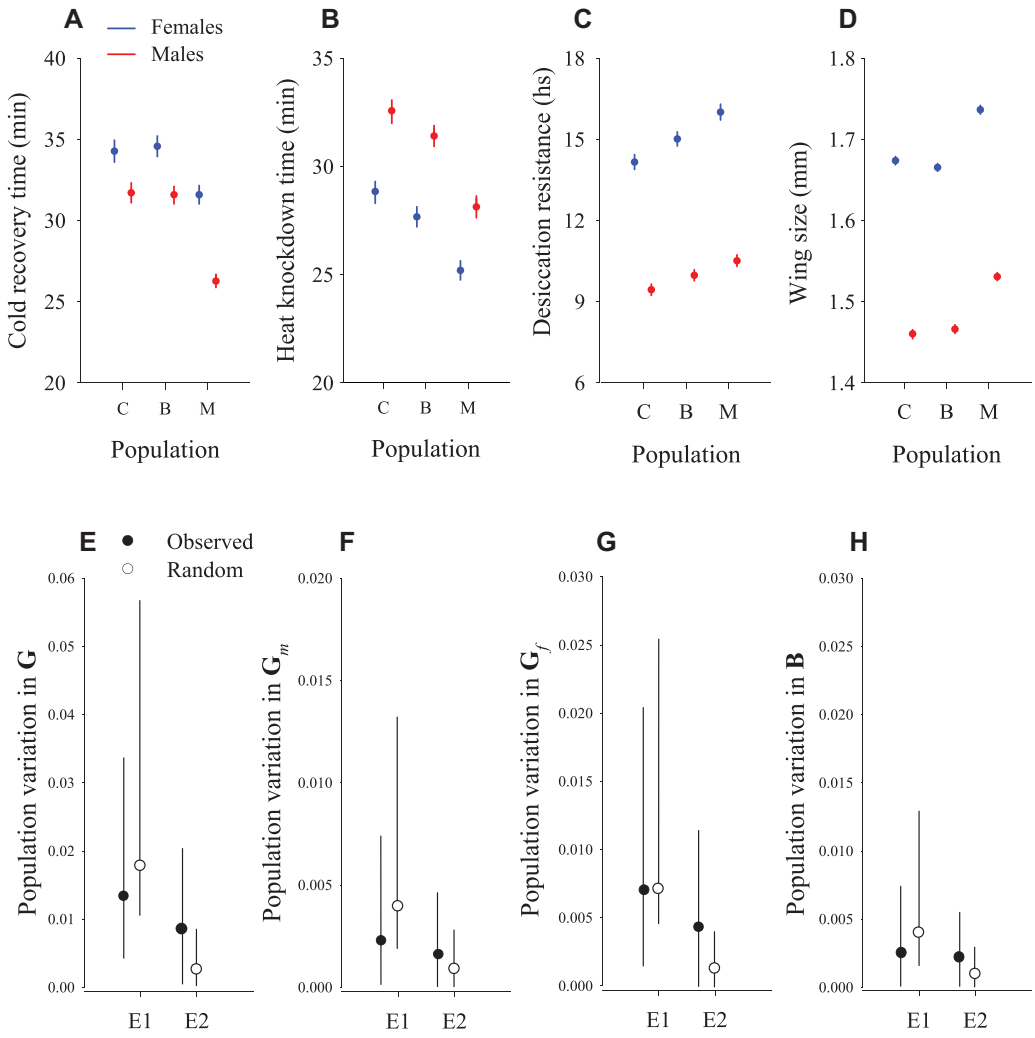

Figure 1. Panels (A)–(D) show mean trait values (±SE) for female and male *D. melanogaster* from Cairns (C, tropical northern Australia), Brisbane (B, subtropical central Australia), and Melbourne (M, temperate southern Australia). (A) Cold recovery time (min), (B) heat knockdown time (min), (C) desiccation resistance (hs), and (D) wing size (mm). Panels (E)–(H) show the results of the genetic covariance tensor analyses, where E_1 and E_2 are eigentensors capturing divergence among populations for observed and randomized (E) G matrices, (F) within-male submatrices (G_m), (G) within-female submatrices (G_f), and (H) between-sex submatrices (B). Non-overlapping 95% HPD intervals indicate significant divergence among populations.

GENETIC COVARIANCES AND EVOLVABILITY

Evolvability simulations showed that both forms of genetic covariance—covariances between traits (within sexes), and covariances between sexes—promote local adaptation to conditions at cline ends (i.e., directions of local selection at cline ends were assumed to follow the direction of clinal divergence for each trait; see Methods). Three specific results point to positive effects of genetic covariances on evolvability. First, mean evolvability in response to local selection was higher than evolvability in response to random selection (Fig. 2), which indicates an alignment between G and the direction of local selection. Second, the inclusion of each set of genetic covariance terms (between-trait and between-sex covariances) increased evolvability to cline-end conditions (Fig. 3). We estimate that while between-trait covariances improve evolvability by up to 1.5-fold relative to the hypothetical scenario where all genetic covariances are zero (e.g., the blue curve in Fig. 3, bottom left), effects of between-trait covariances on evolvability were largely small and nonsignificant (see Fig. 3, bottom). In contrast, between-sex covariances significantly increased evolvability in both sexes, with B consistently boosting evolvability by roughly 1.5- to twofold (see Fig. 3, top). These results are robust to sex differences in selection; genetic covariances had similar quantitative effects on evolvability when the relative magnitudes of selection per trait were identical between the sexes or uncorrelated between them (Fig. S2).

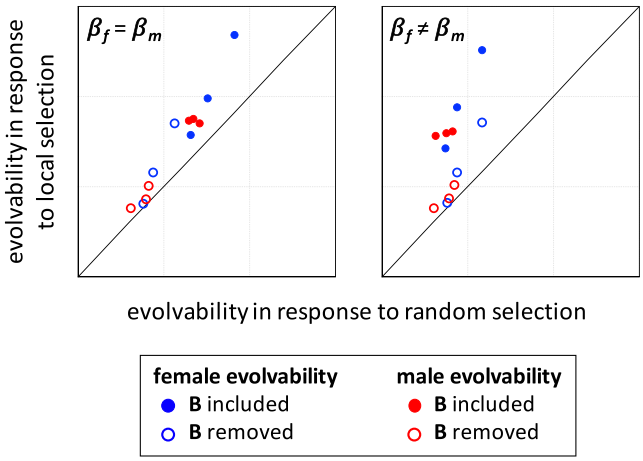

Figure 2. Alignment between G and the direction of clinal divergence. Shown are simulation results for mean evolvability in response to: (1) local selection (*y*-axis), where simulated vectors of selection favor the directions of observed clinal divergence in *D. melanogaster*; and (2) random selection (*x*-axis), where simulated vectors of selection are randomly distributed in the four trait dimensions. Evolvability results for each sex are shown using the full G matrix (solid circles), and a reduced G matrix with cross-sex covariances set to zero (B = 0). Results in the left-hand panel assume that selection gradients for each trait are identical between the sexes ($\beta_f = \beta_m$, where β_i is the vector of selection gradients for the *i*th sex). Simulations in the right-hand panel assume that magnitudes of selection on each trait are uncorrelated between the sexes ($\beta_f \neq \beta_m$). Evolvability was significantly greater in response to local selection than random selection ($P < 0.01$ for all contrasts; Mann–Whitney *U* tests of evolvability under local versus random selection; evolvability projections based on simulations of selection gradients for each of 1000 MCMC samples of G, per population). Additional details of the simulations can be found in the Supplementary information S1.

Discussion

Evolutionary trajectories of populations, including capacity to adapt to environmental change, hinge upon the orientation and stability of the G matrix for traits that mediate fitness in changing environments (Lande 1979; Schluter 1996; Kopp and Matuszewski 2014). Within this context, our study addresses three important, yet unresolved, questions. How stable is G among populations of a broad-ranging species? How well does G align with the direction of clinal divergence, inferring the direction of selection at cline ends? What is the quantitative impact of genetic covariances on adaptive evolutionary potential (i.e., "evolvability"; Hansen and Houle 2008) in contexts of environmental change? Here, we show that for three geographically distinct populations of *Drosophila melanogaster* sampled from its east Australian range, G and its sex-specific submatrices for ecologically important traits are stable in magnitude and orientation (we detected no significant population differences in G). We further showed that genetic covariances between traits have modest effects on the adaptive potential of populations at cline ends, whereas covariances between sexes increase this potential by strongly aligning G with the direction of selection for all traits examined.

STABILITY OF THE G MATRIX AND ITS SEX-SPECIFIC SUBMATRICES

Whether or not G matrices are geographically and temporally stable has important implications for the long-run predictability of evolution, the emergence and resolution of genetic constraints, population persistence, and the geographic breadth and limits to species' ranges (Arnold et al. 2008; Duputié et al. 2012; Chevin 2013; Polechová and Barton 2015; Polechová 2018). Stability in G, or lack thereof, also reflects the evolutionary forces that maintain genetic variation in quantitative traits, with mutational constraints expected to impose some degree of constancy in G, and variability in environment, selection, and drift allowing for divergence in G (Lande 1979; Jones et al. 2004; Guillaume and Whitlock 2007). G matrices from natural and experimental populations often show conserved aspects of structure, yet several studies also document evolutionary change in G over time or space (Arnold et al. 2008; Wood and Brodie 2015; Puentes et al. 2016). We observed no significant divergence in G or its submatrices across our three geographically distinct populations of *D. melanogaster*. The stability of G across the range is striking, given the wide geographic distances between study populations, and the disparate environmental contexts in which they evolved.

Lack of divergence in any of the sex-specific components of G is notable, given prior theoretical and empirical studies of divergence in G_f, G_m, or B. Most theory for the evolution of sex differences focuses on changes in sex-specific trait means, rather than changes in variances and covariances (Lande 1980; Wyman et al. 2013; Connallon 2015; Connallon and Hall 2016), and there are currently few clear predictions regarding divergence of G_f, G_m, and B. Nevertheless, two studies have explored within-generation changes in the sex-specific elements of G as a means of generating hypotheses about their evolutionary dynamics. Barker et al. (2010) showed that multivariate stabilizing selection reduced G_f and G_m more than it reduced B, from which they hypothesized that G_f and G_m should be more evolutionarily stable than B (e.g., the former are less prone to divergence by genetic drift). McGlothlin et al. (2019) extended Barker's model by incorporating effects of sex-differences in directional selection on within-generation changes in G. They showed that directional selection can either elevate or decrease cross-sex genetic covariances, although only sexually antagonistic selection was likely to maintain positive between-sex covariances. Previous empirical tests for divergence in G_f, G_m, and B, while rare, seem to agree that G_f and G_m are more evolutionarily stable that B (e.g., in garter snake populations: Barker et al 2010; populations of *Drosophila serrata*: Gosden and

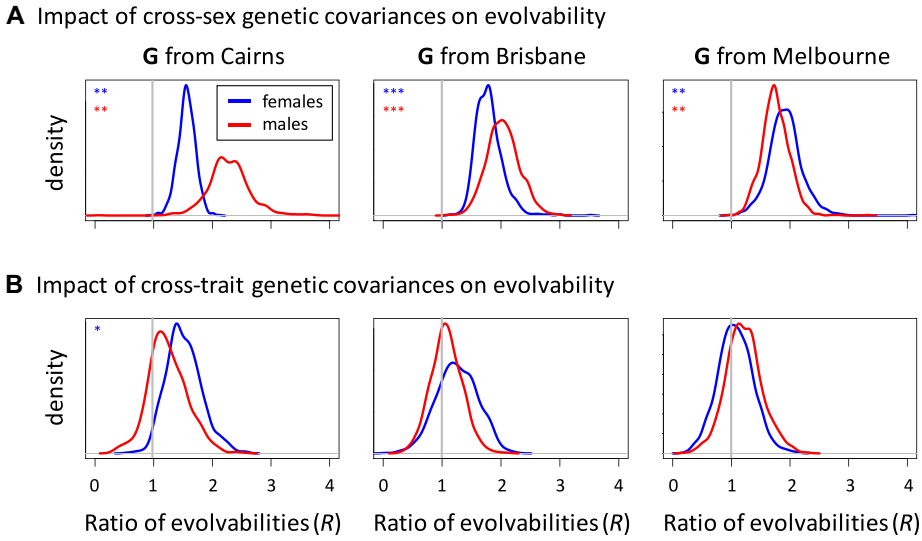

Figure 3. **Genetic covariances elevate evolutionary potential for adaptation to climatic conditions at the cline ends. Top Panels: distributions of the ratio of evolvability with all genetic covariances included versus evolvability with cross-sex covariances excluded. *R* depicts the relative increase (when $R > 1$) or decrease (when $R < 1$) in evolvability due to cross-sex genetic covariances; the distribution of *R* for each population is based on evolvability projections using 1000 MCMC samples of G for the population. Bottom Panels: distributions of the ratio of evolvability with cross-trait covariances included (but B = 0) versus evolvability with all genetic covariances excluded. *R* depicts the relative increase (when $R > 1$) or decrease (when $R < 1$) in evolvability due to cross-trait genetic covariances. Results assume that selection gradients are identical between the sexes ($\beta_f = \beta_m$, where β_i is the vector of selection gradients for the *i*th sex). Similar results were obtained under the assumption that the magnitudes of selection gradients, per trait, are uncorrelated between the sexes ($\beta_f \neq \beta_m$; see Fig. S2). Asterisks denote cases where 95% of the distribution of *R* did overlap with $R = 1$: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.0001$, where *P* is twice the proportion of the distribution of *R* that fell below 1. Additional details of the simulations can be found in the Supplementary information S1.**

Chenoweth 2014), whereas our findings imply that all elements of G are stabile. Moving forward, we need genetically explicit models of evolutionary change in G_f, G_m, and B to predict how different forms of sex-specific selection influence evolutionary divergence of the elements of G (see McGlothlin et al. 2019 for discussion). On the empirical front, three studies are clearly insufficient to make generalizations about the relative stability of the components of G matrix, and as such, further empirical work is clearly warranted.

THE IMPACT OF GENETIC COVARIANCES ON ADAPTIVE POTENTIAL

By combining empirical estimates of G with simulated responses to selection, we quantified the impact of between-trait and between-sex genetic covariances on evolvability under climatic conditions at the cline ends. Genetic covariances strongly elevated the adaptive potential, with G in all three populations aligning with the inferred direction of selection in cline-end environments. Although genetic covariances between traits had a modest impact on evolvability, covariances between sexes consistently elevated evolvability within each sex by a factor of roughly 1.5- to twofold. The greater impact of between-sex covariances on evolvability (relative to within-sex covariances between traits) arises from the fact that between-sex covariances are consistently larger than between-trait covariances (as documented here and in many other studies: Table 1; Poissant et al. 2010). When selection aligns between the sexes—as appears likely for the four traits considered here (Lasne et al. 2018)—strong and positive between-sex genetic covariances greatly facilitate adaptation (Lande 1980; Connallon 2015; Connallon and Hall 2016).

To our knowledge, our study is the first to quantify the impact of both genetic covariances between traits and between sexes on the evolvability of local adaptation. A literature survey by Agrawal and Stinchcombe (2009) found that genetic covariances among traits seem to have little effect, on average, on rates of multivariate adaptation. Far fewer studies have quantified the extent to which multivariate evolution is facilitated or constrained by between-sex genetic covariances (Lewis et al. 2011; Gosden et al. 2012; Stearns et al. 2012; Poissant et al. 2016; Cox et al. 2017). These studies report evidence that B constrains (Lewis et al. 2011; Gosden et al. 2012; Stearns et al. 2012; Poissant et al. 2016), or marginally impacts (Cox et al. 2017) sex-specific adaptation. The different results of these studies, relative to ours, could reflect differences in the traits under consideration, or the specific environmental contexts in which sex-specific selection was evaluated. For example, sexual antagonism appears to be particularly common among

sexually selected traits and phenotypes mediating adult fitness components, compared to traits affecting viability (Chippindale et al. 2001; Lewis et al. 2011; Gosden et al. 2012; Connallon and Matthews 2019). Theory also predicts that sexually antagonistic selection is more likely to emerge in relatively stable or benign environments compared to stressful ones (reviewed in Connallon and Hall 2018), which has garnered some empirical support (Long et al. 2012; Berger et al. 2014; De Lisle et al. 2018; but see Holman L and Jacomb 2017; Martinossi-Allibert et al. 2018). Our focus on environmental stress resistance traits that show parallel clinal divergence in the sexes provides context for sexually concordant local selection along the cline (Connallon 2015; Lasne et al. 2018), under which cross-sex genetic covariances should facilitate adaptation to extremes of the environmental gradient.

Whether G facilitates or constrains adaptation ultimately depends on whether genetic variation is available in the direction of local selection. Given the logistical challenges of measuring fitness and genetic variances in wild populations, few studies directly estimate both selection and G. Our indirect approach for inferring (locally) directional selection allowed us to evaluate the orientation of G relative to selection that is reflected in clinal divergence, yielding clear evidence that G matrices align well with evolutionary divergence toward cline ends. These results suggest that selection for clinal divergence has not eroded genetic variation in the direction of local selection. The pattern is consistent with theory predicting an increase in genetic variation along lines of directional selection (Barton 2001; Jones et al. 2004; Jones et al. 2012), providing scope for rapid divergence in peripheral populations that are newly founded or fragmented from the rest of the species range.

AUTHOR CONTRIBUTIONS

SH, TC, and CMS designed the experiments. SH and CL collected the data and SH, KM, and TC analyzed the data. SH wrote the manuscript and CMS, CL, KM, and TC revised earlier drafts. All authors read and approved the final draft of the manuscript.

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DATA ARCHIVING

Data deposited at Dryad: The doi for our data is https://doi.org/10.5061/dryad.hk29749.

LITERATURE CITED

Agrawal, A. F. and J. R. Stinchcombe. 2009. How much do genetic covariances alter the rate of adaptation? Proc. R. Soc. B 276:1183–1191.

Aguirre, J., E. Hine, K. McGuigan, and M. Blows. 2014. Comparing **G**: multivariate analysis of genetic variation in multiple populations. Heredity 112:21–29.

Arnold, S. J., R. Bürger, P. A. Hohenlohe, B. C. Ajie, and A. G. Jones. 2008. Understanding the evolution and stability of the **G**-Matrix. Evolution 62:2451–2461.

Barker, B. S., P. C. Phillips, and S. J. Arnold. 2010. A test of the conjecture that **G**-matrices are more stable than **B**-matrices. Evolution 64:2601–2613.

Barton, N. H. 2001. Adaptation at the edge of a species' range. Pp. 365–392 i*n* J. Silvertown and J. Antonovics eds. Integrating ecology and evolution in a spatial context. vol. 14. Blackwell Scientific, Oxford, U.K.

Berger, D., E. C. Berg, W. Widegren, G. Arnqvist, and A. A. Maklakovl. 2014. Multivariate intralocus sexual conflict in seed beetles. Evolution 68:3457–3469.

Blows, M. W., S. F. Chenoweth, and E. Hine. 2004. Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. Am. Nat. 163:329–340.

Bonduriansky, R., and S. F. Chenoweth. 2009. Intralocus sexual conflict. Trends Ecol. Evol. 24:280–288.

Careau, V., M. E. Wolak, P. A. Carter, and T. Garland. 2015. Evolution of the additive genetic variance–covariance matrix under continuous directional selection on a complex behavioural phenotype. Proc. R. Soc. B. 20151119.

Chevin, L. M. 2013. Genetic constraints on adaptation to a changing environment. Evolution 67:708–721.

Chippindale, A. K., J. R. Gibson, and W. R. Rice. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in Drosophila. Proc. Natl. Acad. Sci. USA 98:1671–1675.

Collins, M., R. Knutti, J. Arblaster, J.-L. Dufresne, T. Fichefet, P. Friedlingstein et al. 2013. Long-term climate change: projections, commitments and irreversibility. *in* T. F. Stocker, D. Qin, G-KP, M. Tignor, J. Ska, A. N. Boschung, Y. Xia, V. Bex et al., eds. Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, U.K.

Connallon, T. 2015. The geography of sex-specific selection, local adaptation, and sexual dimorphism. Evolution 69:2333–2344.

Connallon, T., and M. D. Hall. 2016. Genetic correlations and sex-specific adaptation in changing environments. Evolution 70:2186–2198.

Connallon, T., and M. D. Hall. 2018. Environmental changes and sexually antagonistic selection. Encyclopedia of Life Sciences. John Wiley & Sons, Chichester, U.K.

Connallon, T., and G. Matthews. 2019. Cross-sex genetic correlations for fitness and fitness components: connecting theoretical predictions to empirical patterns. Evol. Lett. 3:254–262.

Cox, R., R. Costello, B. Camber, and J. McGlothlin. 2017. Multivariate genetic architecture of the *Anolis* dewlap reveals both shared and sex-specific features of a sexually dimorphic ornament. J. Evol. Biol. 30:1262–1275.

Cox, R. M., and R. Calsbeek. 2009. Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. Am. Nat. 173:176–187.

De Lisle, S. P., D. Goedert, A. M. Reedy, and E. I. Svensson. 2018. Climatic factors and species range position predict sexually antagonistic selection across taxa. Proc. R. Soc. B. 373:20170415.

Duputié, A., F. Massol, I. Chuine, M. Kirkpatrick, and O. Ronce. 2012. How do genetic correlations affect species range shifts in a changing environment? Ecol. Lett. 15:251–259.

Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton, NJ.

Fisher, R. A. 1930. The genetic theory of natural selection. Clarendon Press, Oxford, U.K.

García-Ramos, G., and M. Kirkpatrick. 1997. Genetic models of adaptation and gene flow in peripheral populations. Evolution 51:21–28.

Gosden, T. P., and S. F. Chenoweth. 2014. The evolutionary stability of cross-sex, cross-trait genetic covariances. Evolution 68:1687–1697.

Gosden, T. P., K. L. Shastri, P. Innocenti, and S. F. Chenoweth. 2012. The **B**-matrix habors significant and sex-specific constraints on the evolution of multicharacter sexual dimorphism. Evolution 66:2106–2116.

Griffin, P. C., S. B. Hangartner, A. Fournier-Level, and A. A. Hoffmann. 2016. Genomic trajectories to desiccation resistance: convergence and divergence among replicate selected *Drosophila* lines. Genetics 116:187104.

Guillaume, F., and M. C. Whitlock. 2007. Effects of migration on the genetic covariance matrix. Evolution 61:2398–2409.

Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J. Stat. Softw. 33:1–22.

Haldane, J. 1948. The theory of a cline. J. Genet. 48:277–284.

Hangartner, S. B., A. A. Hoffmann, A. Smith, and P. C. Griffin. 2015. A collection of Australian *Drosophila* datasets on climate adaptation and species distributions. Sci. Data 2:150067.

Hansen, T. F., and D. Houle. 2008. Measuring and comparing evolvability and constraint in multivariate characters. J. Evol. Biol. 21:1201–1219.

Hine, E., S. F. Chenoweth, H. D. Rundle, and M. W. Blows. 2009. Characterizing the evolution of genetic variance using genetic covariance tensors. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364:1567–1578.

Hoekstra, H. E., K. E. Drumm, and M. W. Nachman. 2004. Ecological genetics of adaptive color polymorphism in pocket mice: geographic variation in selected and neutral genes. Evolution 58:1329–1341.

Hoffmann, A. A., A. Anderson, and R. Hallas. 2002. Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. Ecol. Lett. 5:614–618.

Hoffmann, A. A., R. Hallas, C. Sinclair, and P. Mitrovski. 2001. Levels of variation in stress resistance in Drosophila among strains, local populations and geographic regions: patterns for desiccation, starvation, cold resistance and associated traits. Evolution 55:1621–1630.

Hoffmann, A. A., and C. M. Sgrò. 2018. Comparative studies of critical physiological limits and vulnerability to environmental extremes in small ectotherms: how much environmental control is needed? Integr. Zool. 13:355–371.

Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. Nature 470:479–485.

Hoffmann, A. A., and J. Shirriffs. 2002. Geographic variation for wing shape in *Drosophila serrata*. Evolution 56:1068–1073.

Hoffmann, A. A., and A. R. Weeks. 2007. Climatic selection on genes and traits after a 100 year-old invasion: a critical look at the temperate-tropical clines in *Drosophila melanogaster* from eastern Australia. Genetica 129:133–147.

Holman, L., and F. Jacomb. 2017. The effects of stress and sex on selection, genetic covariance, and the evolutionary response. J. Evol. Biol. 30:1898–1909.

Houle, D., and K. Meyer. 2015. Estimating sampling error of evolutionary statistics based on genetic covariance matrices using maximum likelihood. J. Evol. Biol. 28:1542–1549.

Jones, A. G., S. J. Arnold, and R. Burger. 2004. Evolution and stability of the **G**-matrix on a landscape with a moving optimum. Evolution 58:1639–1654.

Jones, A. G., R. Bürger, S. J. Arnold, P. A. Hohenlohe, and J. C. Uyeda. 2012. The effects of stochastic and episodic movement of the optimum on the evolution of the **G**-matrix and the response of the trait mean to selection. J. Evol. Biol. 25:2210–2231.

Kennington, W. J., J. Gockel, and L. Partridge. 2003. Testing for asymmetrical gene flow in a *Drosophila melanogaster* body-size cline. Genetics 165:667–673.

Kirkpatrick, M., and N. H. Barton. 1997. Evolution of a species' range. Am. Nat. 150:1–23.

Kirkpatrick, M. 2009. Patterns of quantitative genetic variation in multiple dimensions. Genetica 136:271–284.

Kopp, M., and S. Matuszewski. 2014. Rapid evolution of quantitative traits: theoretical perspectives. Evol. Appl. 7:169–191.

Lande, R. 1979. Quantitative genetic-analysis of multivariate evolution, applied to brain–body size allometry. Evolution 33:402–416.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Lasne, C., S. B. Hangartner, T. Connallon, and C. M. Sgrò. 2018. Cross-sex genetic correlations and the evolution of sex-specific local adaptation: insights from classical trait clines in *Drosophila melanogaster*. Evolution 72:1317–1327.

Lasne, C., B. Van Heerwaarden, C. M. Sgrò, and T. Connallon. 2019. Quantifying the relative contributions of the X chromosome, autosomes, and mitochondrial genome to local adaptation. Evolution 73:262–277.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends Ecol. Evol. 17:183–189.

Lewis, Z., N. Wedell, and J. Hunt. 2011. Evidence for strong intralocus sexual conflict in the Indian meal moth, *Plodia interpunctella*. Evolution 65:2085–2097.

Liefting, M., A. A. Hoffmann, and J. Ellers. 2009. Plasticity versus environmental canalization: Population differences in thermal responses along a latitudinal gradient in *Drosophila serrata*. Evolution 63:1954–1963.

Long, T. A. F., A. F. Agrawal, and L. Rowe. 2012. The effect of sexual selection on offspring fitness depends on the nature of genetic variation. Curr. Biol. 22:204–208.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Inc., Sunderland, USA.

Martinossi-Allibert, I., U. Savković, M. Đorđević, et al. 2018. The consequences of sexual selection in well-adapted and maladapted populations of bean beetles. Evolution 72:518–530.

McGlothlin, J. W., R. M. Cox, and E. D. Brodie. 2019. Sex-specific selection and the evolution of between-sex genetic covariance. J. Heredity 110:422–432.

Morrissey, M. B., S. Hangartner, and K. Monro. 2019. A note on simulating null distributions for G matrix comparisons. https://doi.org/10.1111/evo.13842

Mullen, L. M., and H. E. Hoekstra. 2008. Natural selection along an environmental gradient: a classic cline in mouse pigmentation. Evolution 62:1555–1570.

Nosil, P., B. Crespi, C. Sandoval, and M. Kirkpatrick. 2006. Migration and the genetic covariance between habitat preference and performance. Am. Nat. 167:E66-E78.

Phillips, P. C., M. C. Whitlock, and K. Fowler. 2001. Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. Genetics 158:1137–1145.

Poissant, J., M. B. Morrissey, A. G. Gosler, J. Slate, and B. C. Sheldon. 2016. Multivariate seletion and intersexual genetic constraints in a wild bird population. J. Evol. Biol. 29:2022–2035.

Poissant, J., A. J. Wilson, and D. W. Coltman. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. Evolution 64:97–107.

Polechová, J., and N. H. Barton. 2015. Limits to adaptation along environmental gradients. Proc. Natl. Acad. Sci. USA 112:6401–6406.

Polechová, J. 2018. Is the sky the limit? On the expansion threshold of a species' range. PLoS Biol. 15:e2005372.

Puentes, A., G. Granath, and J. Ågren. 2016. Similarity in **G** matrix structure among natural populations of *Arabidopsis lyrata*. Evolution 70:2370–2386.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

Sgrò, C. M., J. Overgaard, T. N. Kristensen, K. A. Mitchell, F. E. Cockerell, and A. A. Hoffmann. 2010. A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia. J. Evol. Biol. 23:2484–2493.

Shaw, F. H., R. G. Shaw, G. S. Wilkinson, and M. Turelli. 1995. Changes in genetic variances and covariances: G whiz! Evolution 49:1260–1267.

Sniegula, S., M. J. Golab, S. M. Drobniak, and F. Johansson 2016. Seasonal time constraints reduce genetic variation in life-history traits along a latitudinal gradient. J. Anim. Ecol. 85:187–198.

Stearns, S. C., D. R. Govindaraju, D. Ewbank, and S. G. Byars. 2012. Constraints on the coevolution of contemporary human males and females. Proc. R. Soc. B. 279:4836–4844.

Uesugi, A., T. Connallon, A. Kessler, and K. Monro. 2017. Relaxation of herbivore-mediated selection drives the evolution of genetic covariances between plant competitive and defense traits. Evolution 71:1700–1709.

van Heerwaarden, B., and C. M. Sgrò. 2014. Is adaptation to climate change really constrained in niche specialists? Proc. R. Soc. B. 281: 20140396.

Walsh, B., and M. W. Blows. 2009. Abundant genetic variation+ strong selection = multivariate genetic constraints: a geometric view of adaptation. Annu. Rev. Ecol. Evol. Syst. 40:41–59.

Walter, G. M., J. D. Aguirre, M. W. Blows, and D. Ortiz-Barrientos. 2018. Evolution of genetic variance during adaptive radiation. Am. Nat. 191:E108–128.

Wood, C. W., and E. D. Brodie. 2015. Environmental effects on the structure of the **G**-matrix. Evolution 69:2927–2940.

Wyman, M., J. Stinchcombe, and L. Rowe. 2013. A multivariate view of the evolution of sexual dimorphism. J. Evol. Biol. 26:2070–2080.

Wyman, M. J., and L. Rowe. 2014. Male bias in distributions of additive genetic, residual, and phenotypic variances of shared traits. Am. Nat. 184:326–337.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. **G** matrices of genetic variance and covariance components (±SE) for cold recovery time (Cold), desiccation resistance (Des), heat knockdown time (Heat), and wing size (Size) in females (F) and males (M) from (A) Cairns (tropical northern Australia), (B) Brisbane (subtropical central Australia), and (C) Melbourne (temperate southern Australia).

Table S2. Bayesian-estimated **G** matrices of genetic variance and covariance components (with 95% CI) for cold recovery time (Cold), desiccation resistance (Des), heat knockdown time (Heat), and wing size (Size) in females (F) and males (M) from (A) Cairns (tropical northern Australia), (B) Brisbane (subtropical central Australia), and (C) Melbourne (temperate southern Australia).

Table S3. Linear models of (A) cold recovery time (min), (B) heat knockdown time (min) (C) desiccation resistance (hs) and (D) wing size (mm) of female and male *D. melanogaster* originating from three populations.