doi:10.1111/evo.13647

Quantifying the relative contributions of the X chromosome, autosomes, and mitochondrial genome to local adaptation*

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Received August 17, 2018
Accepted November 1, 2018

During local adaptation with gene flow, some regions of the genome are inherently more responsive to selection than others. Recent theory predicts that X-linked genes should disproportionately contribute to local adaptation relative to other genomic regions, yet this prediction remains to be tested. We carried out a multigeneration crossing scheme, using two cline-end populations of *Drosophila melanogaster,* to estimate the relative contributions of the X chromosome, autosomes, and mitochondrial genome to divergence in four traits involved in local adaptation (wing size, resistance to heat, desiccation, and starvation stresses). We found that the mitochondrial genome and autosomes contributed significantly to clinal divergence in three of the four traits. In contrast, the X made no significant contribution to divergence in these traits. Given the small size of the mitochondrial genome, our results indicate that it plays a surprisingly large role in clinal adaptation. In contrast, the X, which represents roughly 20% of the *Drosophila* genome, contributes negligibly—a pattern that conflicts with theoretical predictions. These patterns reinforce recent work implying a central role of mitochondria in climatic adaptation, and suggest that different genomic regions may play fundamentally different roles in processes of divergence with gene flow.

KEY WORDS: **Adaptive divergence, *Drosophila*, mtDNA, stress resistance, X chromosome.**

Environmental conditions vary across species' ranges, leading to selection for local adaptation (Haldane 1948; Endler 1977). Nevertheless, the evolution of local adaptation is compromised by gene flow across the species' range and genetic drift in peripheral populations (Yeaman and Otto 2011; Blanquart et al. 2012). Strong gene flow can offset selection for genetic divergence between populations, leading to sustained migration loads and limited adaptation to local conditions (García-Ramos and Kirkpatrick 1997; Lenormand 2002; Kirkpatrick and Barton 2006). The harmful effects of migration may be compounded by genetic drift in small populations within the range, including those at range margins. In extreme cases, gene flow and drift can lead to the collapse of peripheral populations and restricted species' ranges (Polechová and Barton 2015; Connallon and Sgrò 2018; Polechová 2018).

The scope of adaptive genetic differentiation between populations depends on the balance between spatially varying selection, gene flow, and drift—a balance that is likely to vary across the genome. Some regions of the genome are inherently more responsive to selection than others. For example, loci that are the strongest determinants of local fitness may exhibit pronounced population divergence, despite strong gene flow limiting divergence through most of the genome (Hoban et al. 2016). Tight physical linkage between locally adaptive alleles can also facilitate genetic divergence between populations, and favor inversions that suppress recombination within locally adapted haplotypes (Dobzhansky 1970; Kirkpatrick and Barton 2006; Via and West 2008; Nosil et al. 2009; Yeaman and Whitlock 2011). These predictions can account for geographically variable inversion polymorphisms that appear to play important roles in climatic adaptation (e.g., Knibb et al. 1981; Krimbas and Powell 1992;

*This article corresponds to Richards, T. J. 2019. Digest: All genomes are not equal: How variation in autosomal, sex chromosome, and mitochondrial genomes contributes to the process of local adaptation. Evolution. https://doi.org/10.1111/evo.13667.

Carla M. Sgrò and Tim Connallon equally co-supervised the research; author order was decided by a coin toss.

Weeks et al. 2002; Anderson et al. 2003; Stefansson et al. 2005; Kirkpatrick 2010; Lowry and Willis 2010; Kapun et al. 2016).

Chromosome-specific features of ploidy and sex-biased inheritance can also affect the balance between local selection and gene flow, leading to different opportunities for local adaptation across the genome. For example, haploid expression of X-linked genes in males (or of Z-linked genes in females) enhances the expression of genetic variation for fitness and facilitates a stronger response to selection at X-linked compared to autosomal genes (Avery 1984). This effect can theoretically lead to faster rates of X-linked than autosomal divergence between species (Faster-X theory: Charlesworth et al. 1987; Orr 2010; Meisel and Connallon 2013), and to disproportionate contributions of X-linked genes to local adaptation with gene flow (Lasne et al. 2017; Connallon et al. 2018). Homoplasmic expression of mitochondrial-encoded (mtDNA) genes may similarly enhance the responsiveness of the mitochondrial genome to selection (e.g., Bergstrom and Pritchard 1998), and thereby elevate its contribution to local adaptation (see later). X-linked and mitochondrial genes also exhibit sex-biased inheritance, with the X transmitted twice as often through females as males, and mitochondria exhibiting strict maternal inheritance. Because the dispersal rates of females and males are often sexually dimorphic (Trochet et al. 2016), sex-biased genetic transmission influences the *effective* strength of gene flow for autosomal, X-linked, and mitochondrial genes, leading to chromosomal differences in evolutionary constraint due to gene flow (Hedrick 2007).

Few empirical studies to date have explicitly tested for differential contributions of sex chromosomes, mitochondria, and autosomes to local adaptation. Although several studies show that X-linked genes often diverge more rapidly than autosomes between species (see Meisel and Connallon 2013; Charlesworth et al. 2018), there are currently little data on the contributions of the X and autosomes to adaptive diversification within species (but see Heyer and Segurel 2010; Lambert et al. 2010; Allen et al. 2017). Likewise, although mitochondrial genomes are known to contribute substantially to local adaptation in some instances (e.g., Camus et al. 2017), the overall contribution of mitochondrial genes to local adaptation remains an open question.

Here, we used a multigeneration crossing design to partition the relative contributions of the autosomes, X chromosome, and mitochondrial genome to adaptive differentiation between cline-end populations of *Drosophila melanogaster* from eastern Australia (one tropical and the other temperate). We first developed simple population genetic models for local adaptation of X-linked, autosomal, mitochondrial, and Y-linked genes, which illustrate the heightened potential for genetic divergence of X-linked and uniparentally inherited genes relative to those on autosomes. We then tested these predictions by quantifying the contributions of these different chromosomal classes to divergence in four ecologically important traits: heat resistance, starvation resistance, desiccation resistance, and wing size. These traits display parallel patterns of clinal divergence across the latitudinal gradients of eastern Australia (James et al. 1995; Hoffmann et al. 2002; Hoffmann and Weeks 2007; Sgrò et al. 2010; Lasne et al. 2018), North America (Schmidt et al. 2005), and India (Parkash and Munjal 1999; Parkash et al. 2012; Rajpurohit et al. 2013), highlighting their likely importance in climatic adaptation in *D. melanogaster*. The maintenance of these trait clines through time (James et al. 1995; Hoffmann et al. 2002; Hoffmann and Weeks 2007; Sgrò et al. 2010; Lasne et al. 2018) and in spite of strong gene flow across the latitudinal gradient in eastern Australia (Kennington et al. 2003; Turner et al. 2008) further implies that their patterns of divergence reflect responses to local selection. By combining chromosome-specific estimates of trait divergence with line-cross analysis of the dataset (e.g., Carson and Lande 1984; Reinhold 1998; Blackmon and Demuth 2016), we tested for elevated contributions of each chromosome type to clinal divergence relative to their baseline proportions in the *Drosophila* genome (i.e., ~80% of genes are autosomal, ~20% are X-linked, and less than 1% are mitochondrial-encoded; Adams et al. 2000; Wolff et al. 2016). Our results suggest that genetic divergence between these clinally adapted *Drosophila* populations is dominated by autosomal and mitochondrial genes. We discuss these results, including the unexpectedly low contribution of the X to trait divergence, in light of theoretical predictions for the genetic architecture of local adaptation.

Methods

THEORETICAL MODELS

To clarify the predicted contributions of autosomal, X-linked, and uniparentally inherited genes to local adaptation, we applied Moran's classical model of divergence with gene flow between a pair of populations (see Moran, 1959, 1962; Charlesworth and Charlesworth 2010, ch. 4; Lasne et al. 2017; Charlesworth and Barton 2018). Our application of Moran's model accommodates different modes of transmission and sex differences in selection and migration. Results comparing the autosomes and X were previously developed by Lasne et al. (2017), and are summarized below. We are unaware of similar applications of Moran's model to genes with uniparental inheritance, although such applications of Moran's model are straightforward.

Following Moran (1959, 1962), we assume that there are two equal-sized populations under selection for local adaptation to a pair of habitats (one population in habitat 1, and the other in habitat 2). The populations exchange migrants at equal rates. For each model of inheritance, local adaptation is mediated by a single locus with two alleles, A and a; allele A is favored in

habitat 1 and allele a is favored in habitat 2. We assume that the strength of selection is symmetrical between the habitats, and that selection and dispersal parameters are small: s and $d << 1$, where s is the selection coefficient against a locally maladaptive allele, and d is the proportion of individuals that disperse in each generation. For simplicity, we assume that alleles have additive effects on local fitness, and selection is equal between the sexes ($s_m = s_f = s$, although eqs. (1)–(4) allow for exploration of sex differences in selection, i.e., $s_m \neq s_f$).

At equilibrium, the difference in allele frequency between the two populations depends on the mode of linkage, the strength of selection, and the pattern of sex-specific dispersal (denoted d_f and d_m for females and males, respectively). The equilibrium allele frequency difference between populations at an mtDNA locus is:

$$\delta_{mito} = \sqrt{1 + \left(\frac{2d_f}{s_f}\right)^2} - \frac{2d_f}{s_f}. \quad (1)$$

Equilibrium frequency difference for a Y-linked locus is:

$$\delta_Y = \sqrt{1 + \left(\frac{2d_m}{s_m}\right)^2} - \frac{2d_m}{s_m}. \quad (2)$$

Derivations of equations (1) and (2) are provided in Appendix 1. Following Lasne et al. (2017), allele frequency divergence equilibria for autosomal and X-linked loci, respectively, are:

$$\delta_A = \sqrt{1 + \left[\frac{4\left(d_f + d_m\right)}{s_f + s_m}\right]^2} - \frac{4\left(d_f + d_m\right)}{s_f + s_m}, \quad (3)$$

and

$$\delta_X = \sqrt{1 + \left[\frac{2\left(2d_f + d_m\right)}{s_f + s_m}\right]^2} - \frac{2\left(2d_f + d_m\right)}{s_f + s_m}. \quad (4)$$

Note that these results can also be expressed in terms of standard F_{ST} statistics. For the jth mode of inheritance, and given the symmetry of Moran's model, $F_{ST} = (\delta_j)^2$.

To validate the analytical approximations, we carried out Wright-Fisher simulations of the model, using exact deterministic recursions to predict changes in genotype frequencies in each generation, and multinomial sampling of genotype frequencies to incorporate genetic drift. The life cycle of the simulations includes the following series of events:

1. Within each habitat, random mating among N_m breeding males and N_f breeding females yields offspring for the next generation, with adults dying after reproduction,
2. Offspring disperse after birth, with each male dispersing with probability d_m, and each female dispersing with probability d_f,
3. Local selection occurs within each habitat prior to reproduction,
4. In each habitat, multinomial sampling of the predicted adult genotype frequencies generates the breeding adults, completing the generational cycle.

Each simulation run was initiated with equal frequencies of the two alleles within each of the two habitats. To eliminate the effects of the initial conditions on the results, each simulation run lasted $4N_e$ generations, where the effective population size, N_e, is defined following standard theory (Hartl and Clark 2007, pp. 123–125):

$$N_e = \frac{4N_m N_f}{N_f + N_m}. \quad (5)$$

Effective population size for autosomal genes is $2N_e$, and the effective size for X-linked genes is adjusted by a factor of:

$$\frac{9\left(N_f + N_m\right)}{8\left(N_f + 2N_m\right)}, \quad (6)$$

which reduces to the familiar ¾ when $N_f = N_m$, and otherwise ranges between 9/16 and 9/8. In the absence of genetic hitchhiking, the effective population sizes for mitochondrial and Y-linked genes are N_f and N_m, respectively. However, because Y chromosomes are nonrecombining and show markedly reduced levels of diversity due to hitchhiking (Wilson-Sayres et al. 2014), Y-linked genes are likely to be far more susceptible to genetic drift compared to other regions of the genome. We therefore define the Y-linked effective population size as $N_Y = f N_m$, where f, a constant between 0 and 1, adjusts for elevated effects of hitchhiking on the Y. All simulations were coded and run in R (R Core Team 2017).

POPULATION COLLECTION AND MAINTENANCE

Cline-end populations of *D. melanogaster*, from Melbourne (temperate climate; latitude/longitude: −37.733/145.433) and Innisfail (tropical climate; −17.517/146.017), were collected in April 2016. For each population, 50 field-caught females were used to initiate isofemale lines. In the second generation following field collection, all lines were treated with tetracycline [0.3 mg·mL^{-1}] to eliminate the possible presence of *Wolbachia*. Mass-bred populations were initiated in generation three by randomly sampling 10 virgin males and females from each of the 50 isofemale lines and mixing them together. In generation four, the two mass-bred populations were treated with chloramphenicol [25 mg·L^{-1}] and ampicillin [6 mg·mL^{-1}] to further eliminate the presence of microbial entities transmitted through the maternal lines and that could have been responsible for some of the genetic divergence between populations. As a consequence of these antibiotic treatments, effects of the cytoplasmic environment are limited to the mitochondrial genome. Subsequent generations

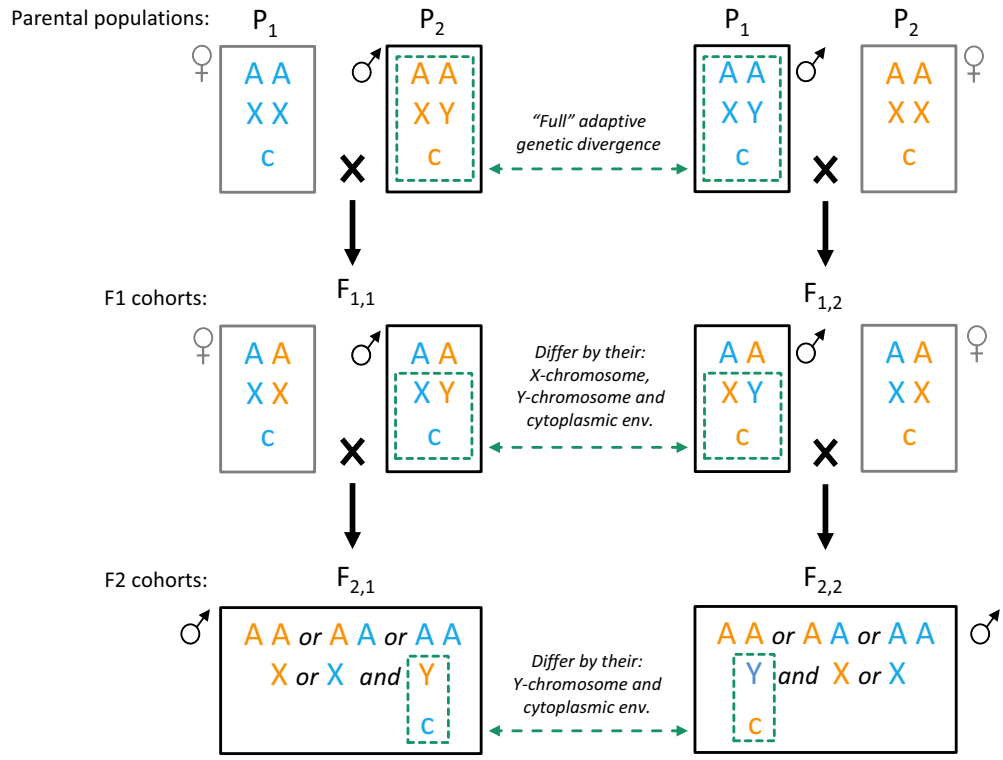

Figure 1. Three-generation crossing scheme to isolate the influence of the autosomes (A), X-chromosome (X), Y-chromosome (Y), and cytoplasmic environment (c, including mitochondria), on phenotypic divergence between two populations. Females from the P_1 population (Melbourne; blue genetic background) are crossed with males from the P_2 population (Innisfail; orange genetic background), and reciprocally. Resulting $F_{1,1}$ and $F_{1,2}$ cohorts mate within their respective cohorts and result in $F_{2,1}$ and $F_{2,2}$ cohorts. Dotted green boxes encompass genetic elements expected to be divergent between male populations/cohorts of same generation.

were propagated by randomly distributing emerging adult flies from each population across five 250-mL bottles, each containing 60 mL of agar-dextrose-potato-yeast medium. Populations were maintained at constant 25°C, 12:12 light:dark cycle, at approximately 300 flies per bottle, ensuring a census population size of about 1500 individuals.

POPULATION CROSSING SCHEME

To quantify the relative contributions of different elements of the genome to population divergence in our focal traits, we carried out a three-generation crossing scheme initiated by reciprocal crosses between the two parental populations, Melbourne and Innisfail, hereafter referred to as P_1 and P_2, respectively (Fig. 1).

Two generations prior to phenotyping, approximately 500 virgin P_1 females and 500 P_2 males were mass-crossed. We used the same number of flies from each parental population for reciprocal crosses (Fig. 1). The crosses resulted in two F_1 cohorts, labeled $F_{1,1}$ and $F_{1,2}$, which are derived from P_1 mothers and P_2 mothers, respectively. We subsequently carried out crosses using flies from within single F_1 cohorts: the resulting F_2 cohorts were labeled $F_{2,1}$ (produced from mothers and fathers from the $F_{1,1}$ cohort) and $F_{2,2}$ (produced from mothers and fathers from the $F_{1,2}$ cohort). Under this crossing scheme: (1) males from P_1 and P_2 differ by population of origin for their entire genomes; (2) $F_{1,1}$ and $F_{1,2}$ males differ for their X, Y and mitochondrial genomes, and (3) $F_{2,1}$ and $F_{2,2}$ males systematically differ for only their Y and mitochondrial genomes (Fig. 1). The comparison of male trait values across all three experimental generations provides information about the contributions of the autosomes, X chromosome, Y chromosome, and mitochondrial genome, to population divergence (see next and Fig. 1).

To phenotype all six experimental populations/cohorts together in the same generation (i.e., P_1, P_2, $F_{1,1}$, $F_{1,2}$, $F_{2,1}$, and $F_{2,2}$), we repeated the reciprocal crosses between parental populations one generation prior to experiments. Four days after all crosses were initiated, parental populations ($P_1 \times P_1$ and $P_2 \times P_2$) and the four remaining crosses ($P_1 \times P_2$; $P_2 \times P_1$; $F_{1,1} \times F_{1,1}$; $F_{1,2} \times F_{1,2}$) were transferred into 250-mL specimen containers containing blue-dyed media covered with live yeast to stimulate oviposition. Adult flies were left to lay eggs overnight (for about 12 h) at 25°C and eggs were picked and transferred into vials containing 7 mL of media the next morning. Egg density was controlled at exactly 20 eggs per vial for wing size, and approximately 50 eggs per vial for the stress resistance traits. Twenty-four hours

posteclosion, males were separated from females under light CO_2 anesthesia and allowed to recover for 48 h prior to phenotyping.

Our phenotyping experiments focus exclusively on males, enabling us to obtain large sample sizes for precision estimates of trait means in each experimental population/cohort. These estimates are essential for quantifying the contributions of the autosomes, X chromosome, mitochondrial genome, and Y chromosome to phenotypic divergence between Melbourne and Innisfail populations (see next). Our focus on males also reflects the nature of the crossing design and inference approach that we adopt. The design exploits the fact that males only inherit one X chromosome—a feature of X-linked inheritance that makes the crossing approach feasible for isolating effects of the X on divergence relative to other regions of the genome (see Carson and Lande 1984; Reinhold 1998; Mittleman et al. 2017).

TRAIT ASSAYS

All experiments were performed at a constant temperature, 25°C, between March and August 2017, spanning generations 26 and 36 of laboratory culture. For logistical reasons, starvation, desiccation, and heat resistance assays were performed in two experimental blocks in which all six populations/cohorts were equally represented.

Wing size

For each experimental population/cohort, the right wing of about 470 males was removed with forceps and mounted onto glass slides previously covered with double-sided tape. Each slide was then covered with a glass cover slip to protect the wing from damage during size measurements. A total of 2840 wings were photographed using a Leica M80 stereomicroscope (Leica Heerbugg, Switzerland) mounted with a digital camera. Using the software tpsDIG2 (version 2.17 from Rohlf 2006), eight vein landmarks were obtained for each wing. Landmark x and y coordinates were then processed in CoordGen8 software (Sheets 2003) to estimate centroid size (the square root of the sum of the squared distance between each landmark and the centroid), which is a good proxy for wing size (Lasne et al. 2018).

Starvation resistance

Four days posteclosion, 625 males per population/cohort were individually transferred from normal food medium into 40-mL vials, each containing 7 mL of non-nutritive agar medium (Lasne et al. 2018). For the entire duration of the experiment, vials were covered with a wet towel to avoid desiccation. A total of 3738 individuals were tested for resistance to starvation stress at 25°C, with mortality scored at eight-hour intervals until all flies died (Lasne et al. 2018).

Heat resistance

Over 910 five-day-old males per population/cohort were phenotyped for heat resistance. Each fly was individually placed in a 5-mL glass vial and subsequently submerged into a water bath, maintained at a constant temperature of 39°C. Heat resistance was measured as heat knockdown time, the time taken for a fly to become immobilized (Hoffmann et al. 2002; Lasne et al. 2018). The heat resistance assays were performed over two blocks of 15 runs, each with equal representation from the six populations/cohorts. A total of 5480 flies were scored for their resistance to heat stress.

Desiccation resistance

A total of 315 five-day-old males of each population/cohort were individually transferred into 5-mL glass vials, each covered with fine gauze. Vials were subsequently distributed across three sealed glass tanks (each population/cohort was equally represented within each tank), containing silica gel to maintain relative humidity between 1% and 5% at 25°C. A total of 1890 flies were checked every hour until all flies died, with resistance to desiccation stress measured as time until death (Hallas et al. 2002; Lasne et al. 2018).

DATA ANALYSIS

Population trait means

Levene and Shapiro-Wilk tests were used prior to statistical analyses to confirm assumptions of homogeneity of variance and normality in all four traits. Significant differences between populations/cohorts were tested using linear models for all stress resistance traits and wing size. Population, run (for heat resistance), tank (for desiccation resistance), and block were treated as fixed effects, with run and tank effects nested within blocks. All statistical analyses were performed in R (R Core Team 2017). For each population and trait, least square means and standard errors of the means were extracted from the model and used for subsequent index estimation analysis.

Estimates of chromosomal contributions to population differentiation

Estimates of the relative contributions of the X, autosomes, and mitochondrial genome to trait divergence between Melbourne and Innisfail, can be obtained from our data under two simplifying assumptions: that (1) chromosomes contribute additively to population divergence for each trait (e.g., there is no dominance or epistatic interactions between chromosomes from different populations), and (2) Y-linked effects are negligible on those same traits. We later validate these assumptions by performing line cross analyses (see below). Moreover, assumption 2 is likely to be true for our purposes: we focus on body size and physiological traits that are relevant to survival under stress, whereas the *Drosophila* Y chromosome primarily affects male fertility (Charlesworth 2001; Chippindale and Rice 2001).

Our general approach is similar to that of several previous studies (see Carson and Lande 1984; Reinhold 1998; Mittleman et al. 2017), but with modifications tailored to our crossing scheme. Under the assumption stated above, we can calculate the proportions of total trait divergence between populations that are attributable to the X, autosomes, and mitochondrial genome (see Appendix 2). The contribution of the X chromosome to trait divergence is:

$$I_X = \frac{\left(\bar{F}_{1,1} - \bar{F}_{1,2}\right) - \left(\bar{F}_{2,1} - \bar{F}_{2,2}\right)}{\bar{P}_1 - \bar{P}_2}, \quad (7)$$

where terms with overbars denote the true mean breeding values for a given trait in the parental populations and F_1 and F_2 cohorts. The contribution of the autosomes to divergence is:

$$I_A = 1 - \frac{\bar{F}_{1,1} - \bar{F}_{1,2}}{\bar{P}_1 - \bar{P}_2}. \quad (8)$$

The contribution of the mitochondrial genome to divergence is:

$$I_C = \frac{\bar{F}_{2,1} - \bar{F}_{2,2}}{\bar{P}_1 - \bar{P}_2}. \quad (9)$$

Note that the Y-linked effects, should they exist, are expected to inflate I_A and dampen I_C; I_X will not be affected (see Appendix 2 for details).

Our experiments provide point estimates of the population means for each experimental population, which we used to evaluate equations (5)–(7). The error in each estimate of the population trait mean contributes cumulatively to error in the overall estimate of the divergence indices (eqs. (5)–(7)). To calculate confidence intervals (CIs) for the index estimates, we modeled the distribution of the indices, given our data, by carrying out simulations in R (R Core Team 2017). We modeled population means in equations (5)–(7) as a series of independent random draws, each from a normal distribution with mean and standard deviation equal to the estimated mean and standard error for the given trait and population (i.e., based on the population least square means and standard errors of the means obtained from ANOVA; see above). Each set of random draws for a trait yielded six simulated values for population trait means (i.e., from populations P_1, P_2, and cohorts $F_{1,1}$, $F_{1,2}$, $F_{2,1}$, and $F_{2,2}$), which were then used to calculate I_X, I_A, and I_C. This procedure was carried out 10 million times, resulting in 10 million simulated indices, which we used to approximate the distributions of I_X, I_A, and I_C, given our data. In our results, we present the median and 95% CI of these distributions.

LINE-CROSS ANALYSIS

The above indices can partition the contributions of the autosomes, the X, and the mitochondrial genome to trait divergence, provided these genomic regions contribute additively to phenotypic differences between cline-end populations. To validate this assumption, and test for nonadditive effects (i.e., dominance and epistasis), we carried out a line-cross analysis using the same dataset. We used the R software package, SAGA (Blackmon and Demuth 2016), to estimate the composite genetic effects (hereafter CGEs) contributing to population divergence in each trait. The approach uses corrected Akaike's information criterion to explore all possible models of dominance and epistasis between chromosomes and estimate the contribution of each CGE using model Akaike weights. For each CGE, variable importance to population phenotypic divergence (v_i) is calculated by summing Akaike weights of all models in which the CGE occurs. CGEs with v_i scores greater than 0.5 are the most likely contributors to trait divergence between populations (for further details of the methodology, see Blackmon and Demuth 2016; van Heerwaarden and Sgrò 2017).

Results

CHROMOSOME-SPECIFIC PREDICTIONS FOR ADAPTIVE DIVERGENCE WITH GENE FLOW

Assuming equally strong selection at loci with different modes of inheritance, theory predicts that autosomal genes will show lower divergence between populations than genes on the X chromosome, Y chromosome, and mitochondrial genome (Fig. 2). When males and females disperse at the same rate, adaptive divergence is greatest for uniparentally transmitted loci (Y-linked and mitochondrial genes), followed by X-linked loci (Fig. 2, middle panel). Male-limited dispersal enhances population divergence of mitochondrial and X-linked loci relative to those on the Y and autosomes (Fig. 2, left panel). Female-limited dispersal enhances Y-linked divergence, and leads to similarly low divergence for X-linked, mitochondrial and autosomal genes (Fig. 2, right panel).

Stochastic simulations show that the predictions of our analytical models (eqs. (1)–(4)) provide excellent approximations of chromosome-specific patterns of local adaptation as long as the product of the effective population size and the strength of selection is large (see Supporting Information Figs. S1–S8). In contrast, when effective population size is small and selection is weak relative to gene flow, drift dampens divergence between populations (compare Supporting Information Figs. S1–S4, where selection dominates in population divergence, with Supporting Information Figs. S5–S8, where drift has a more pronounced impact). Drift is expected to play a particularly pronounced role in the evolution of Y-linked genes, due to both the reduced effective population size of males compared to females and the heightened impact of genetic hitchhiking on the Y compared to other regions of a genome (Wilson-Sayres et al. 2014). Simulations that take both of these issues into account show that the Y is less likely to maintain polymorphism involved in local adaptation compared to the autosomes, X chromosome, and mitochondrial genome (Supporting Information Figs. S5–S8). Drift should,

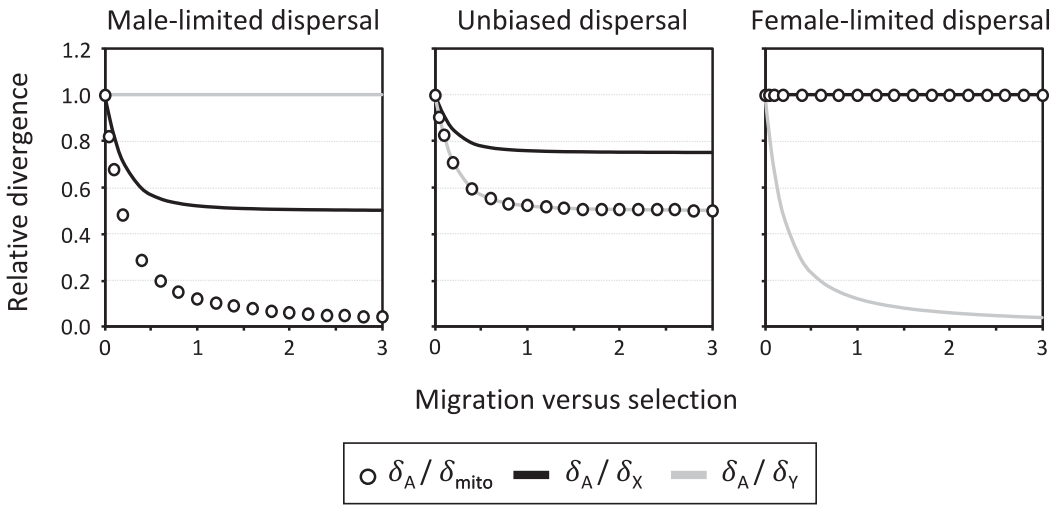

Figure 2. Adaptive divergence under migration-selection balance in population genetic models of local adaptation. Results are based on equations (1) and (2) of the present study, and 5a and 5b of Lasne et al. (2017). Each panel shows how the relative strength of migration and selection influences allele frequency differences between the two populations at an X-linked, Y-linked, and mitochondrial locus relative to an autosomal locus. Migration is male-limited (left panel), equal between the sexes (middle panel), or female-limited (right panel). The x-axis shows the sex-averaged migration rate relative the strength of selection (assumed equal between the sexes): $d/s = [(d_m + d_f)/2]/s$. Additional simulation results can be found in the Supporting Information (Figs. S1–S8).

therefore, dampen the predicted contribution of the Y to local adaptation.

Estimates of sex-specific dispersal in *Drosophila* suggest that species vary in their relative dispersal rates of females vs. males, with similar rates of sex-specific dispersal in *D. melanogaster* (reviewed in Markow and Castrezana 2000). In the absence of chromosomal biases in the genetic basis of our four focal traits, we therefore expect the mitochondrial genome and sex chromosomes to disproportionately contribute to adaptive divergence between our cline-end populations relative to their sizes within the *Drosophila* genome. It is worth noting, however, that chromosomal biases in gene content will modify these theoretical expectations, perhaps in a trait-by-trait manner. We return to this point in the Discussion section.

CHROMOSOMAL INDICES OF GENETIC DIFFERENTIATION

We found significant population differences in trait means for all four traits (Table 1, Fig. 3A–D), with block, run, and tank identity also having significant effects on the three stress resistance traits (Table 1). The cline-end populations, Melbourne (P_1) and Innisfail (P_2), were the most phenotypically divergent for all four traits, with Melbourne males being larger and more resistant to starvation and desiccation stress than Innisfail males. Males from Innisfail were significantly more resistant to heat stress than those from Melbourne. F_1 and F_2 cohorts were (phenotypically) intermediate between the parental populations for wing size, desiccation resistance, and starvation resistance (Fig. 3B–D), whereas F_1 cohorts were more resistant to heat stress than both parental populations and F_2 cohorts. It is worth noting that, for each trait, levels of divergence between Melbourne and Innisfail parental male populations are very similar to those described in Lasne et al. (2018), suggesting that adaptive genetic differentiation between populations is maintained despite being in the laboratory for a number of generations.

The three divergence indices, I_X, I_A, and I_C, are each expected to have values between 0 and 1 as long as the X-linked, autosomal, and mitochondrial genes all have concordant effects on trait divergence between the cline-end populations. Such concordance would occur, for example, if both autosomal and X-linked genes sampled from Melbourne tend to increase wing size. Negative indices or indices in excess of 1 indicate conflicting effects of different chromosomes on trait divergence between populations (e.g., Melbourne-derived autosomal genes tend to increase, whereas X-linked genes tend to decrease, wing size). With this in mind, Figure 3 shows that the relative contributions of the X, autosomes, and mitochondrial genome to clinal divergence vary among the four traits.

The autosomes contribute significantly to divergence between cline-end populations for heat resistance, starvation resistance, and wing size (i.e., the 95% CI for I_A is greater than 0 for these three traits; Fig 3E,G,H). Autosomes are also estimated to account for roughly 50% of population divergence in desiccation resistance (median $I_A = 0.51$), although the CI are too broad to reject a hypothesis of no autosomal contribution to divergence (Fig. 3F). Finally, autosomes contribute disproportionately to

Table 1. Linear model testing for difference in wing size, starvation, heat, and desiccation resistance between populations/cohorts (i.e., P_1, P_2, $F_{1,1}$, $F_{1,2}$, $F_{2,1}$, $F_{2,2}$).

Trait	Source	*df*	SS	*F*	*P* Value
Wing size (mm)	Population	5	1.545	210.26	<0.001
	Error	2834	4.165		
Starvation resistance (hours)	Population	5	41,504	23.354	<0.001
	Block	1	3542	9.966	0.002
	Error	3731	1,326,104		
Heat knockdown time (minutes)	Population	5	6084	14.71	<0.001
	Block	1	8236	99.566	<0.001
	Run (Block)	48	13,022	3.28	<0.001
	Error	5425	448,726		
Desiccation resistance (hours)	Population	5	134.3	6.127	<0.001
	Block	1	67.5	15.396	<0.001
	Tank (Block)	4	552	31.48	<0.001
	Error	1879	8237.2		

population divergence in two traits: heat resistance and wing size (additive autosomal effects dominate in wing size divergence, whereas autosomal dominance-by-dominance epistasis dominates in heat resistance evolution; see below). For these traits, autosomes account for more than 80% of the divergence between Melbourne and Innisfail (whereas, only 80% of *D. melanogaster* genes are autosomal).

The X chromosome does not significantly contribute to divergence between the cline-end populations in any of the stress resistance traits (i.e., the 95% CI for I_X overlapped with 0 and I_X median ≤ 0 in all three traits; Fig. 3E–G). Although we estimated that the X chromosome accounts for about 8.5% of wing size divergence between Melbourne and Innisfail, the 95% CI for this trait overlaps with 0 (Fig. 3H) and the X contributes less to divergence than expected given that roughly 20% of *Drosophila* genes are X-linked (i.e., the upper 95% CI for I_X is less than 0.2).

Mitochondrial genes significantly contributed to divergence between the cline-end populations in all three stress-resistance traits but not in wing size. Their effects account for about 77%, 67%, and 30% of the divergence in desiccation, heat, and starvation resistance, respectively (Fig 3E–G). These results strongly contrast with the small proportion of the *Drosophila* genome comprised by the mitochondrial genome.

For point of contrast, we also estimated the relative contributions of the X chromosomes and autosomes to divergence using trait means from parental populations and the reciprocal F_1 cohorts. This approach, which neglects potential effects of the Y chromosome and mitochondrial genome, has been widely used in previous studies (see Reinhold 1998), yet it can yield upwardly biased estimates of the contribution of the X to trait divergence (Mittleman et al. 2017). Indeed, in our dataset, neglecting the F_2 generation overestimates the contribution of the X chromosome to divergence along the cline. Rather, analyses using the F_1 approach (sensu Reinhold 1998) suggest that X-linked genes may disproportionately contribute to divergence between Melbourne and Innisfail in desiccation and starvation resistance, whereas the full dataset implies the opposite (Fig. 3F,G: point estimates, shown as open diamonds, are greater than the fraction of the *Drosophila* genome that is X-linked).

ESTIMATES OF TRAIT GENETIC ARCHITECTURE THROUGH LINE-CROSS ANALYSIS

To test for nonadditive effects between chromosomes, we performed line-cross analyses using the full dataset. Divergence in wing size and starvation resistance was predominantly attributable to autosomal additive effects (Aa), with v_i scores of 0.92 and 0.68, respectively (Fig. 4; Supporting Information Table S1), and no significant composite (epistatic) genetic effects contributing to trait divergence (Fig. 4). In contrast to our initial analysis based on chromosome indices, line-cross analysis can isolate the additive effects of Y-linked and mitochondrial genes on divergence (Ya and Ca in Fig. 4, respectively; the latter refers to cytoplasmic-inherited maternal effects—the mitochondria in our case; see Mather and Jinks 1982; Kearsey and Pooni 1996; Fox et al. 2004). Additive mitochondrial genetic effects predominate in the genetic basis of divergence in desiccation resistance (v_i score = 0.97, Fig. 4). In addition, mitochondrial additive effects were near-significant for starvation resistance (v_i score = 0.5, Fig. 4), although error bars barely overlapped with 0. Finally, the genetic architecture of divergence in heat resistance was, if anything, dominated by nonadditive genetic interactions

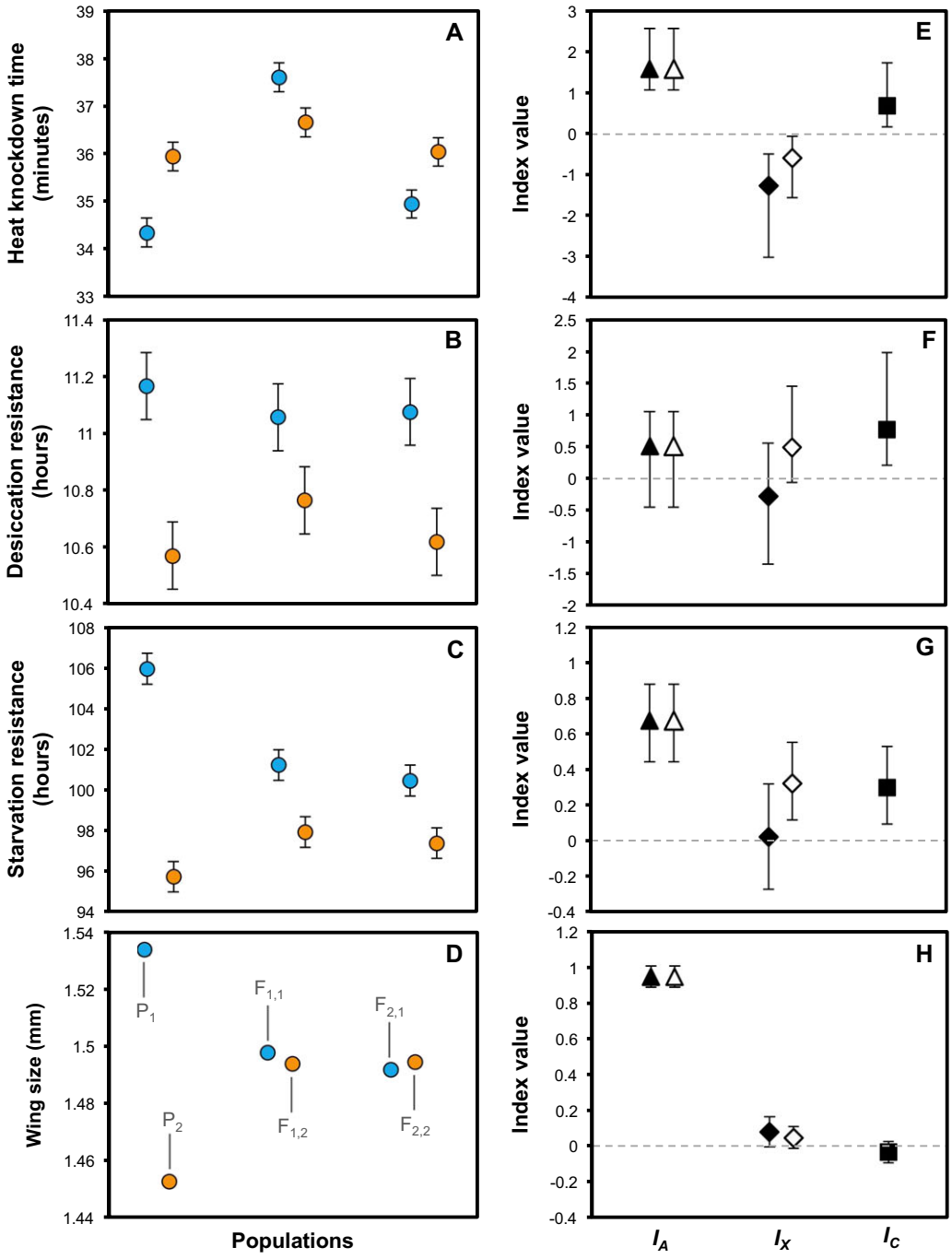

Figure 3. Population trait means and corresponding divergence indices, I_X, I_A, and I_C, for heat resistance, desiccation resistance, starvation resistance, and wing size. Left column: populations/cohorts (P_1, P_2, $F_{1,1}$, $F_{1,2}$, $F_{2,1}$, and $F_{2,}$) trait means (+/− 1 SE) for (A) heat resistance measured as heat knockdown time, (B) desiccation resistance measured as survival time, (C) starvation resistance measured as survival time, and (D) wing size. The two parental populations, Melbourne (P_1) and Innisfail (P_2), are presented in blue and orange, respectively. This color coding is propagated in F_1 and F_2 cohorts to signify the original maternal population (Melbourne or Innisfail). Right column: black closed symbols represent I_X, I_A, and I_C index medians (+/− 95% CI) for (E) heat resistance, (F) desiccation resistance, (G) starvation resistance, and (H) wing size. For point of contrast, I_X and I_A values estimated using the approach of Reinhold (1998) are represented by open symbols for all four traits (see discussion).

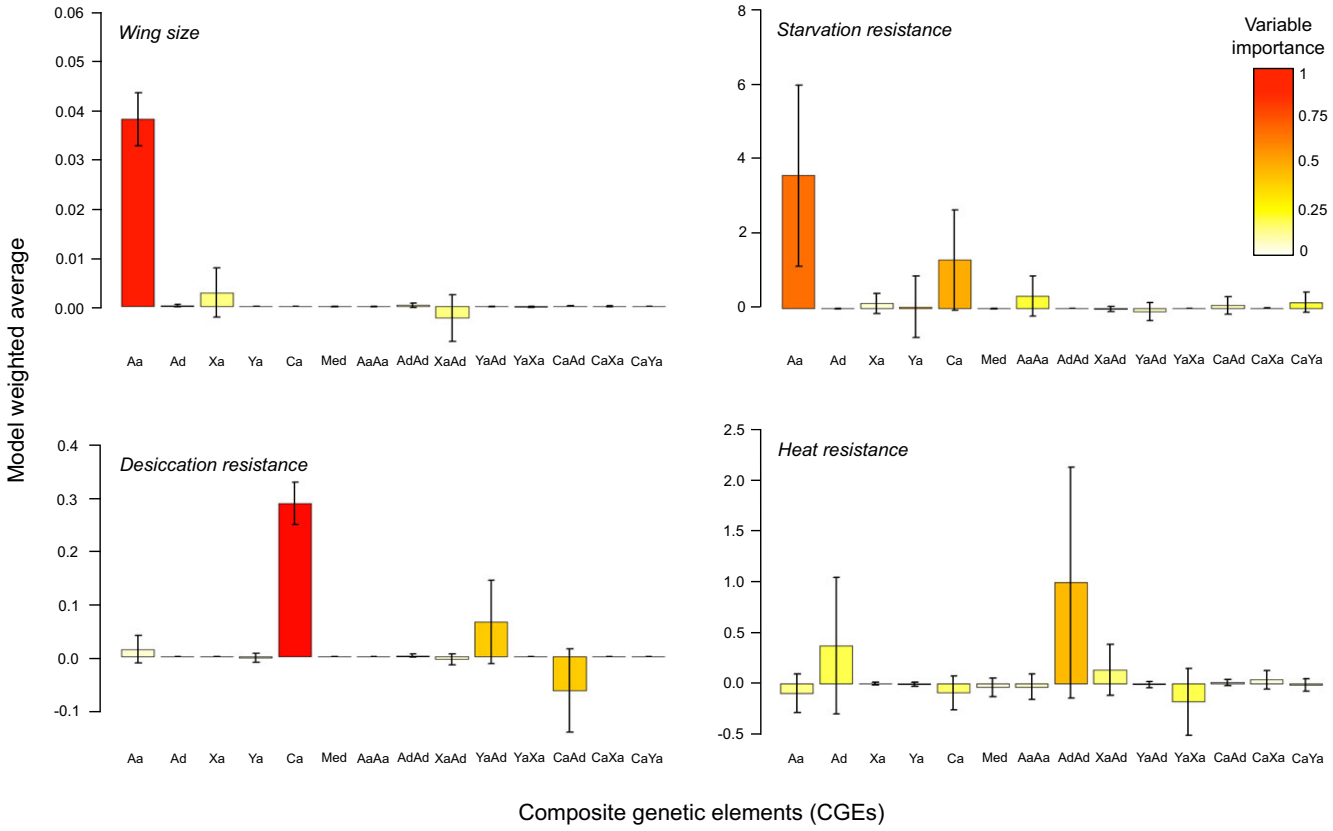

Figure 4. **Model weighted parameter estimates for wing size, starvation resistance, desiccation resistance, and heat resistance in *Drosophila melanogaster*. The color of the bar indicates the variable importance (v_i) score, from not important in light yellow to very important in red. The height of each bar reflects the magnitude of each CGE and their error bars indicate unconditional standard errors (see Blackmon and Demuth 2016). Additive effects: Aa, autosomal additive; Xa, X-linked additive; Ya, Y-linked additive; and Ca, additive cytotype (mitochondria only in the present study). Nonadditive effects: Ad, autosomal dominance; Med, Dominance maternal; AaAa, autosomal additive by additive epistasis; AdAd, autosomal dominance by dominance epistasis; XaAd, X-linked additive by autosomal dominance epistasis; YaAd, Y-linked additive by autosomal dominance epistasis; YaXa, Y-linked additive by X-linked additive epistasis; CaAd, additive cytotype by autosomal dominance epistasis; XaAd, X-linked additive by autosomal dominance epistasis; YaAd, Y-linked additive by autosomal dominance epistasis.**

(Fig. 4), with the highest v_i score ($v_i = 0.46$) for autosomal dominance by dominance epistasis (AdAd). We note that there was a high degree of model uncertainty regarding the genetic basis of divergence in heat resistance. In none of the four traits were X-linked or Y-linked genes found to importantly contribute to population divergence.

Discussion

The different modes of inheritance and expression of autosomal, sex-linked, and mitochondrial genes can potentially lead to chromosomal disparities in the genomic basis of local adaptation (Lasne et al. 2017; see above). Using simple population genetic models of adaptation with gene flow, we predicted that chromosomes with sex-biased inheritance-–including the X chromosome, Y chromosome, and mitochondrial genome-–should contribute disproportionately to local adaptation, relative to their sizes within the genome. We tested these predictions on four classic, clinally diverged traits from east Australian *D. melanogaster* populations.

We found that autosomal and mitochondrial genes contribute substantially to adaptive divergence between cline-end populations, whereas the X chromosome has, at most, a minor influence on divergence in these traits. Our results, therefore, add to accumulating evidence for the importance of the mitochondrial genome in adaptation (e.g., Morales et al. 2015; James et al. 2016; Camus et al. 2017), while also raising questions about the scope of "faster-X effects" in adaptive evolution (i.e., the extent to which X-linked genes diverge more rapidly than autosomal genes during adaptation; Presgraves, 2008, 2018; Meisel and Connallon 2013; Lasne et al. 2017; Charlesworth et al. 2018; Connallon et al. 2018). Below, we discuss potential causes of misalignment between some of our theoretical predictions and empirical results, including potential effects of trait genetic architecture

on chromosome-specific patterns of evolutionary divergence. We also outline advantages of our empirical methodology for quantifying the contributions of different chromosomal classes within a genome to adaptive divergence, and provide suggestions for future research on the genomic basis of local adaptation.

(MIS)ALIGNMENT BETWEEN THEORETICAL PREDICTIONS AND EMPIRICAL PATTERNS

Population genetics theory predicts that X-linked genes will disproportionately contribute to local adaptation, relative to autosomal genes, under most conditions of sex-specific selection and migration (see Fig. 2; Lasne et al. 2017; Connallon et al. 2018). Likewise, Y and mitochondrial linkages are expected to facilitate adaptive divergence between populations, relative to autosomal genes, as long as sex differences in migration are not extreme (see Fig. 2). From this theory, and assuming that loci potentially responding to local selection are randomly distributed across the genome, then the X chromosome and mitochondrial genome should contribute more to adaptive divergence between populations than predicted from their relative sizes within the *Drosophila* genome.

Although the CIs are too broad to reject a hypothesis of no contribution of X-linked genes to divergence in three out of four traits, the lack of evidence for a significant contribution of the X chromosome to clinal divergence (median $I_X < 0.2$ in all four traits, and no significant X-linked effects in the line-cross analysis) implies that genes affecting variation in these traits may in fact be nonrandomly distributed across the genome, and in particular, depauperate on the X. Indeed, previous studies suggest an important role for autosomal inversion polymorphisms in adaptive trait divergence along clines in *D. melanogaster* in North America (Durmaz et al. 2018) and Australia (Weeks et al. 2002; Rako et al. 2006). Our field sampling strategy is expected to capture a representative collection of genotypes from each of the cline-end populations; based on previous studies of Australian *D. melanogaster* populations, our samples are likely to include the inversions that segregate across the cline (Anderson et al. 2005; Kolaczkowski et al. 2011). Genomic differentiation between tropical and temperate populations of Australian *D. melanogaster* has been linked to these inversion polymorphisms (Turner et al. 2008; Kolaczkowski et al. 2011; Fabian et al. 2012; Kapun et al. 2016). Inversion polymorphisms in *D. melanogaster* appear to be less common on the X-chromosomes compared to the autosomes (Charlesworth et al. 1987; Kolaczkowski et al. 2011; Connallon et al. 2018; but see Chakraborty et al. 2018), which may lead to fewer opportunities for local adaptation by way of major segregating inversions.

Interestingly, we found that the mitochondrial genome disproportionately contributed to adaptive divergence between populations in the three stress resistance traits. Several other lines of empirical evidence have emerged during the last 20 years to suggest that mitochondria may play an important role in climatic adaptation (see Mishmar et al. 2003; Balloux et al. 2009; Morales et al. 2015; Camus et al. 2017). For example, Camus et al. (2017) recently linked geographic variation in mitochondrial haplogroups to clinal divergence in thermal performance traits between tropical and temperate Australian populations of *D. melanogaster*. Our study suggests that the mitochondrial genome's contribution to local adaptation may not be limited to thermotolerance traits, but rather extends to starvation resistance, desiccation resistance, and potentially other physiological performance traits. These patterns are consistent with theory, although the large effects of the mitochondrial genome could imply that genes involved in climatic adaptation are enriched in mitochondrial genomes.

TESTING FOR EFFECTS OF X-LINKAGE ON ADAPTIVE PHENOTYPIC DIVERGENCE

Studies seeking to quantify the contribution of the X chromosome to adaptive trait divergence between populations have traditionally used reciprocal crossing designs, where the trait differences between the F_1 hybrid male cohorts are compared to differences between males from parental populations (Reinhold 1998). This inference approach rests upon two important assumptions. First, the approach assumes that Y-linked and cytoplasmic effects (including mitochondria) negligibly affect trait divergence. However, neglecting the F_2 generation leads to spurious attribution of mitochondrial and Y-linked effects to the X chromosome, creating a bias toward detecting large-X effects in trait divergence (Mittleman et al. 2017; contrast open and filled diamonds in Fig. 3E–H). A second key assumption is that the chromosomes contribute additively to trait divergence between populations. This assumption can be empirically validated using line-cross analysis methods (an approach that we advocate), although in practice, such validation is rare.

The line-cross analysis carried out here confirms that Y-linked genes contribute negligibly to clinal divergence in body size and stress resistance traits (as suggested by previous work in *Drosophila*; see Chippindale and Rice 2001). The analysis also validates the additivity assumption for three of the four traits that we examined. Consistent with previous studies using Australian cline-end populations of *D. melanogaster*, we found that a simple autosomal additive model explained phenotypic divergence for wing size (Gilchrist and Partridge 1999; van Heerwaarden and Sgrò 2011). Autosomal and mitochondrial additive effects also contributed to divergence for starvation resistance, whereas mitochondrial additive effects predominated in divergence for desiccation resistance. These results contrast with Kennington et al. (2001), who found that epistatic effects better explained population divergence for both traits (although these differences could be due to the use of different cline-end populations and

analytical approaches, that is, Joint-scaling in Kennington et al. 2001 vs. full information theory in the present study). Finally, in agreement with van Heerwaarden and Sgrò (2011), autosomal epistatic effects impacted population divergence in heat resistance.

By focusing on traits that consistently display clinal divergence by latitude in spite of strong gene flow (Kennington et al. 2003; Lasne et al. 2018), we sought to quantify the role of the X, autosomes, and mitochondrial genome to *adaptive* phenotypic divergence. Because direct evidence of adaptive divergence can be difficult to acquire in other contexts of population differentiation, many previous studies of X-linked divergence are based on contexts of trait evolution where strong evidence for adaptation is lacking (e.g., Reinhold 1998; for discussion, see Mittleman et al. 2017). Given the centrality of selection in maintaining stable trait divergence with gene flow, clinal-diverged populations provide exceptionally useful systems for testing theoretical predictions about the role of X-linkage in facilitating adaptation (e.g., Lasne et al. 2017; Connallon et al. 2018). Nevertheless, such experiments are rare. Thus, further empirical work is needed before we can draw any general conclusions regarding faster-X effects in local adaptation. Indeed, conclusions of the current study, although useful, are limited to our four focal traits, and to patterns of divergence in males. Thankfully, the empirical approach that we take, and which we advocate, is applicable to any species where common-garden breeding experiments are feasible. The general tractability of the approach opens the door to experiments in a wide range of species that harbor sex chromosomes (e.g., Bachtrog et al. 2014).

APPENDIX 1

Contributions of Mitochondrial, Y, X-Linked and Autosomal Genes to Local Adaptation

Predictions for the Y and mitochondria can be obtained by modeling local adaptation in a haploid population and then taking sex-specific migration and selection parameters into account.

Following Charlesworth and Charlesworth (2010, ch. 4), we adapt the two-patch haploid model (based on Moran 1962), assuming that strengths of selection and population sizes are symmetrical between two subpopulations of the species, and that selection and migration are weak (we can therefore ignore terms of order O(s^2, d^2, sd) in the models, where s is the selection coefficient and d is the dispersal rate).

The change in frequency due to migration in population 1 is given by:

$$\Delta q_{1(\mathrm{mig})} = q_1\,(1-d) + dq_2 - q_1 = d\,(q_2 - q_1)\,.$$

The change due to selection is:

$$\Delta q_{1(\mathrm{sel})} = sq_1\,(1-q_1)\,.$$

Because the model is symmetrical, $q_1 = 1 - q_2$, and the total change in frequency across a generation is:

$$\Delta q_1 = \Delta q_{1(\mathrm{sel})} + \Delta q_{1(\mathrm{mig})} = sq_1\,(1-q_1) + d\,(1-2q_1)\,.$$

At equilibrium ($\Delta q_1 = 0$), the frequency of the locally adapted allele within population 1 is:

$$\hat{q}_1 = \frac{1 - \frac{2d}{s} + \sqrt{1 + \left(\frac{2d}{s}\right)^2}}{2}\,.$$

The allele frequency difference between population is:

$$\delta_{hap} = \hat{q}_1 - \hat{q}_2 = 2\hat{q}_1 - 1 = \sqrt{1 + \left(\frac{2d}{s}\right)^2} - \frac{2d}{s}.$$

When the locus is encoded in the mitochondria, we have:

$$\delta_{mito} = \sqrt{1 + \left(\frac{2d_f}{s_f}\right)^2} - \frac{2d_f}{s_f}.$$

And when the gene is Y-linked, we have:

$$\delta_Y = \sqrt{1 + \left(\frac{2d_m}{s_m}\right)^2} - \frac{2d_m}{s_m}.$$

Autosomal and X-linked models are obtained from Lasne et al. (2017):

$$\delta_A = \sqrt{1 + \left[\frac{4\left(d_f + d_m\right)}{s_f + s_m}\right]^2} - \frac{4\left(d_f + d_m\right)}{s_f + s_m},$$

$$\delta_X = \sqrt{1 + \left[\frac{2\left(2d_f + d_m\right)}{s_f + s_m}\right]^2} - \frac{2\left(2d_f + d_m\right)}{s_f + s_m}.$$

APPENDIX 2

Estimating the Contribution of the X (and Other Chromosomes) to Divergence

The true population means for autosomal (A), X-linked (X), cytoplasm (C), and Y-linked (Y) genes in Southern and Northern populations (1 and 2) are given by: $\bar{A}_1$, $\bar{A}_2$, $\bar{X}_1$, $\bar{X}_2$, $\bar{C}_1$, $\bar{C}_2$, $\bar{Y}_1$, and $\bar{Y}_1$. We define the population-averaged effects of each chromosome as:

$$\bar{A} = \frac{\bar{A}_1 + \bar{A}_2}{2}\,,$$

$$\bar{X} = \frac{\bar{X}_1 + \bar{X}_2}{2}\,,$$

$$\bar{C} = \frac{\bar{C}_1 + \bar{C}_2}{2}\,,$$

$$\bar{Y} = \frac{\bar{Y}_1 + \bar{Y}_2}{2}\,.$$

The population differences are defined as:

$$\delta_A = \bar{A}_2 - \bar{A}_1,$$

$$\delta_X = \bar{X}_2 - \bar{X}_1,$$

$$\delta_C = \bar{C}_2 - \bar{C}_1,$$

$$\delta_Y = \bar{Y}_2 - \bar{Y}_1.$$

Pure population phenotypes are given by:

$$\begin{aligned}\bar{P}_1 &= \bar{A}_1 + \bar{X}_1 + \bar{C}_1 + \bar{Y}_1 \\ &= \bar{A} + \bar{X} + \bar{C} + \bar{Y} - \frac{\delta_A}{2} - \frac{\delta_X}{2} - \frac{\delta_C}{2} - \frac{\delta_Y}{2},\end{aligned}$$

$$\begin{aligned}\bar{P}_2 &= \bar{A}_2 + \bar{X}_2 + \bar{C}_2 + \bar{Y}_2 \\ &= \bar{A} + \bar{X} + \bar{C} + \bar{Y} + \frac{\delta_A}{2} + \frac{\delta_X}{2} + \frac{\delta_C}{2} + \frac{\delta_Y}{2}.\end{aligned}$$

Assuming strictly additive effects across all chromosome combinations, we can define the mean trait values among F_1 males as a function of the maternal and paternal populations of origin. F_1 males with a mother from population i have mean phenotype $F_{1,i}$. Using the expressions from above, we have:

$$\begin{aligned}\bar{F}_{1,1} &= \frac{\bar{A}_1 + \bar{A}_2}{2} + \bar{X}_1 + \bar{C}_1 + \bar{Y}_2 \\ &= \bar{A} + \bar{X} + \bar{C} + \bar{Y} - \frac{\delta_X}{2} - \frac{\delta_C}{2} + \frac{\delta_Y}{2},\end{aligned}$$

$$\begin{aligned}\bar{F}_{1,2} &= \frac{\bar{A}_1 + \bar{A}_2}{2} + \bar{X}_2 + \bar{C}_2 + \bar{Y}_1 \\ &= \bar{A} + \bar{X} + \bar{C} + \bar{Y} + \frac{\delta_X}{2} + \frac{\delta_C}{2} - \frac{\delta_Y}{2}.\end{aligned}$$

F_2 individuals are produced by crossing F_1s within maternal lines (i.e., $F_{1,2}$ males are crossed with $F_{1,2}$ females, and likewise for $F_{1,1}$ individuals). The average phenotype for F_2 males with population i grandmothers is $F_{2,i}$. Using the expressions above, we have:

$$\begin{aligned}\bar{F}_{2,1} &= \frac{1}{4}\bar{A}_1 + \frac{1}{2}\left(\frac{\bar{A}_1 + \bar{A}_2}{2}\right) + \frac{1}{4}\bar{A}_2 + \frac{1}{2}\bar{X}_1 + \frac{1}{2}\bar{X}_2 + \bar{C}_1 + \bar{Y}_2 \\ &= \bar{A} + \bar{X} + \bar{C} + \bar{Y} - \frac{\delta_C}{2} + \frac{\delta_Y}{2},\end{aligned}$$

$$\begin{aligned}\bar{F}_{2,2} &= \frac{1}{4}\bar{A}_1 + \frac{1}{2}\left(\frac{\bar{A}_1 + \bar{A}_2}{2}\right) + \frac{1}{4}\bar{A}_2 + \frac{1}{2}\bar{X}_1 + \frac{1}{2}\bar{X}_2 + \bar{C}_2 + \bar{Y}_1 \\ &= \bar{A} + \bar{X} + \bar{C} + \bar{Y} + \frac{\delta_C}{2} - \frac{\delta_Y}{2}.\end{aligned}$$

Autosomal, X-linked, and cytoplasmic and Y-linked contributions to population divergence in males can be partitioned as follows:

$$I_A = 1 - \frac{\bar{F}_{1,1} - \bar{F}_{1,2}}{\bar{P}_1 - \bar{P}_2} = \frac{\delta_A}{\delta_A + \delta_X + \delta_C + \delta_Y} + 2\varepsilon_Y,$$

$$I_X = \frac{\left(\bar{F}_{1,1} - \bar{F}_{1,2}\right) - \left(\bar{F}_{2,1} - \bar{F}_{2,2}\right)}{\bar{P}_1 - \bar{P}_2} = \frac{\delta_X}{\delta_A + \delta_X + \delta_C + \delta_Y},$$

$$I_C = \frac{\bar{F}_{2,1} - \bar{F}_{2,2}}{\bar{P}_1 - \bar{P}_2} = \frac{\delta_C}{\delta_A + \delta_X + \delta_C + \delta_Y} - \varepsilon_Y,$$

where:

$$\varepsilon_Y = \frac{\delta_Y}{\delta_A + \delta_X + \delta_C + \delta_Y}.$$

I_X provides a clear estimate of the contribution of the X chromosome to population divergence. I_A and I_C do cleanly exclude effects of Y-linked genes on divergence, yet they will capture unbiased estimates of autosomal and cytoplasmic effects when the Y contributes negligibly to divergence (e.g., $\varepsilon_Y = 0$). Because the *Drosophila* Y primarily affects traits directly linked to male fertility (Charlesworth 2001), the assumption that the Y does not contribute to divergence in body size, thermal performance, desiccation resistance, and starvation resistance in this species is probably reasonable.

Following the logic of Orr (1998), we expect the set of deltas to all have the same sign as long as divergence of the trait is driven by natural selection. In this case, any Y chromosome effects will inflate the inferred contribution of the autosomes to divergence, and dampen the inferred contribution of the cytoplasm.

AUTHOR CONTRIBUTIONS

C.L., T.C., and C.M.S. designed the study, analyzed the data, and wrote the manuscript. C.L. performed laboratory experiments. B.V.H. contributed to data analysis and editing the manuscript.

ACKNOWLEDGMENTS

We are grateful to Fiona Cockerell, Teresa Kutz, Chris Allan, Sonia Sanchez, Michael Roast, Felix Zajitschek, Hariz Ahmad, Taylor Graham, and Oscar Formoso for assistance with fly maintenance and phenotyping scoring, and to Damian Dowling, the AE, and two anonymous reviewers for valuable comments and suggestions on an earlier version of the manuscript. Support for this research was provided by the School of Biological Sciences at Monash University and the Australian Research Council.

DATA ARCHIVING

Data have been uploaded to Dryad: https://doi.org/10.5061/dryad.t732dt8

LITERATURE CITED

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne, P. G. Amanatides, S. E. Scherer, P. W. Li, R. A. Hoskins, R. F. Galle et al. 2000. The genome sequence of *Drosophila melanogaster*. Science 287:2185–2195.

Allen, S. C., R. Bonduriansky, C. M. Sgrò, and S. F. Chenoweth. 2017. Sex-biased transcriptome divergence along a latitudinal gradient. Mol. Ecol. 26:1256–1272.

Anderson, A. R., J. E. Collinge, A. A. Hoffmann, M. Kellett, and S. W. McKechnie. 2003. Thermal tolerance trade-offs associated with the right arm of chromosome 3 and marked by the *hsr-omega* gene in *Drosophila melanogaster*. Heredity 90:195–202.

Anderson, A. R., A. A. Hoffmann, S. W. McKechnie, P. A. Umina, and A. R. Weeks. 2005. The latitudinal cline in the *In(3R)Payne* inversion polymorphism has shifted in the last 20 years in Australian *Drosophila melanogaster* populations. Mol. Ecol. 14:851–858

Avery, P. 1984. The population genetics of haplo-diploids and X-linked genes. Genet. Res. 44:321–341.

Bachtrog, D., J. E. Mank, C. L. Peichel, M. Kirkpatrick, S. P. Otto, T. L. Ashman, M. W. Hahn, J. Kitano, I. Mayrose, R. Ming et al. 2014. Sex determination: why so many ways of doing it? PLoS Biol. 12:e1001899.

Balloux, F., L. J. Handley, T. Jombart, H. Liu, and A. Manica. 2009. Climate shaped the worldwide distribution of human mitochondrial DNA sequence variation. Proc. Biol. Sci. 276:3447–3455.

Bergstrom C. T., and J. Pritchard. 1998. Germline bottlenecks and the evolutionary maintenance of mitochondrial genomes. Genetics 149:2135–2146

Blackmon, H., and J. P. Demuth. 2016. An information-theoretic approach to estimating the composite genetic effects contributing to variation among generation means: moving beyond the join-scaling test for line cross analysis. Evolution 70:420–432.

Blanquart, F., S. Gandon, and S. L. Nuismer. 2012. The effects of migration and drift on local adaptation to heterogeneous environment. J. Evol. Biol. 25:1351–1363.

Camus, M. F., J. N. Wolff, C. M. Sgrò, and D. K. Dowling. 2017. Experimental support that natural selection has shaped the latitudinal distribution of mitochondrial haplotypes in Australian *Drosophila melanogaster*. Mol. Biol. Evol. 34:2600–2612.

Carson, H. L., and R. Lande. 1984. Inheritance of a secondary sexual character in *Drosophila silvestris*. P. Natl. Acad. Sci. USA 81:6904–6907.

Chakraborty, M., N. W. VanKuren, R. Zhao, X. Zhang, S. Kalsow, and J. J. Emerson. 2018. Hidden genetic variation shapes the structure of functional elements in *Drosophila*. Nat. Genet. 50:20–25.

Charlesworth, B. 2001. Genome analysis: more *Drosophila* Y chromosome genes. Curr. Biol. 11:R182–R184.

Charlesworth, B., and N. H. Barton. 2018. The spread of an inversion with migration and selection. Genetics. 208:377–382.

Charlesworth, B., J. L. Campos, and B. C. Jackson. 2018. Faster-X evolution: theory and evidence from *Drosophila*. Mol. Ecol. 27:3753–3771.

Charlesworth, B., and D. Charlesworth. 2010. Elements of evolutionary genetics. Roberts and Company Publishers, Greenwood Village, CO.

Charlesworth, B., J. A. Coyne, and N. H. Barton. 1987. The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. 130:113–146.

Chippindale, A. K., and W. R. Rice. 2001. Y chromosome polymorphism is a strong determinant of male fitness in *Drosophila melanogaster*. PNAS. 98:5677–5682.

Connallon, T., C. Olito, L. Dutoit, H. Papoli, F. Ruzicka, and L. Yong. 2018. Local adaptation and the evolution of inversions on sex chromosomes and autosomes. Phil. Trans. Roy. Soc. B. 373:20170423. 10.1098/rstb.2017.0423

Connallon, T., and C. M. Sgrò. 2018. In search of a general theory of species' range evolution. PLoS Biol. 16:e2006735. https://doi.org/10.1371/journal.pbio.2006735

Dobzhansky, T. 1970. Genetics of the evolutionary process. Columbia Univ. Press, New York.

Durmaz, E., C. Benson, M. Kapun, P. Schmidt, and T. Flatt. 2018. An inversion supergene in *Drosophila* underpins latitudinal clines in survival traits. J. Evol. Biol. 31:1354–1364.

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

Fabian, D. K., M. Kapun, V. Nolte, R. Kofler, P. S. Schmidt, C. Schlotterer, and T. Flatt. 2012. Genome-wide patterns of latitudinal differentiation among populations of *Drosophila melanogaster* from North America. Mol. Ecol. 21:4748–4769.

Fox, C. W., M. E. Czesak, and W. G. Wallin. 2004. Complex genetic architecture of population differences in adult lifespan of a beetle: nonadditive inheritance, gender differences, body size and a large maternal effect. J. Evol. Biol. 17:1007–1017.

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

Gilchrist, A. S., and L. Partridge. 1999. A comparison of the genetic basis of wing size divergence in three parallel body size clines of *Drosophila melanogaster*. Genetics 153:1775–1787.

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

Hallas, R., M. Schiffer, and A. A. Hoffmann. 2002. Clinal variation in *Drosophila serrata* for stress resistance and body size. Genet. Res. 79:141–148.

Hartl, D. L., and A. G. Clark. 2007. Principles of population genetics. Sinauer Associates, Inc. Publishers, Sunderland, MA.

Hedrick, P. W. 2007. Sex: differences in mutation, recombination, selection, gene flow, and genetic drift. Evolution 61:2750–2771.

Heyer, E., and L. Segurel. 2010. Looking for signatures of sex-specific demography and local adaptation on the X-chromosome. Genome Biol. 11:203. https://doi.org/10.1186/gb-2010-11-1-203

Hoban, S., J. L. Kelly, M. F. Antolin, G. Bradburd, D. B. Lowry, M. L. Poss, L. K. Reed, A. Storfer, and M. C. Whitlock. 2016. Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. Am. Nat. 188:379–397.

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., 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.

James, A. C., R. B. Azevedo, and L. Partridge. 1995. Cellular basis and developmental timing in a size cline of *Drosophila melanogaster*. Genetics 140:659–666.

James, J E., G. Piganeau, and A. Eyre-Walker. 2016. The rate of adaptive evolution in animal mitochondria. Mol. Ecol. 25:67–78.

Kapun, M., D. K. Fabian, J. Goudet, and T. Flatt. 2016. Genomic evidence for adaptive inversion clines in *Drosophila melanogaster*. Mol. Biol. Evol. 63:1317–1336.

Kearsey, M. J., and H. S. Pooni. 1996. The genetical analysis of quantitative traits. Chapman and Hall, London, U.K.

Kennington, W. J., A. S. Gilchrist, D. B. Goldstein, and L. Partridge. 2001. The genetic bases of divergence in desiccation and starvation resistance among tropical and temperate populations of *Drosophila melanogaster*. Heredity 87:363–372.

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. 2010. How and why chromosome inversion evolve. PLoS Biol. 8:e1000501. https://doi.org/10.1371/journal.pbio.1000501

Kirkpatrick, M., and N. Barton. 2006. Chromosome inversions, local adaptation and speciation. Genetics 173:419–434.

Knibb, W. R., J. G. Oakeshott, and J. B. Gibson. 1981. Chromosome inversion polymorphism in *Drosophila melanogaster*. II. Geographic clines and climatic associations in Australia, North America and Asia. Genetica 58:213–221.

Kolaczkowski, B., A. D. Kern, A. K. Holloway, and D. J. Begun. 2011. Genomic differentiation between temperate and tropical Australian populations of *Drosophila melanogaster*. Genetics 187:245–260.

Krimbas, C. B., and J. R. Powell. 1992. Drosophila inversion polymorphism. CRC Press, Boca Raton, FL.

Lambert, C. A., C. F. Connelly, J. Madeoy, R. Qiu, M. V. Olson, and J. M. Akey. 2010. Highly punctuated patterns of population structure on the X chromosome and implications for African evolutionary history. Am. J. Hum. Genet. 86:34–44.

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., C. M. Sgrò, and T. Connallon. 2017. The relative contributions of the X chromosome and autosomes to local adaptation. Genetics 205:1285–1304.

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

Lowry, D. B., and J. H. Willis. 2010. A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. PLoS Biol. 8. https://doi.org/10.1371/journal.pbio.1000500

Markow T. A., and S. Castrezana. 2000. Dispersal in cactophilic *Drosophila*. Oikos 89:378–386.

Mather, K., and J. L. Jinks. 1982. Biometrical genetics: the study of continuous variation. Cambridge Univ. Press, Cambridge, U.K.

Meisel, R. P., and T. Connallon. 2013. The faster-X effect: integrating theory and data. Trends Genet. 29:537–544.

Mishmar, D., E. Ruiz-Pesini, P. Golik, V. Macaulay, A. G. Clark, S. Hosseini, M. Brandon, K. Easley, E. Chen, M. D. Brown et al. 2003. Natural selection shaped regional mtDNA variation in humans. PNAS 100:171–176.

Mittleman, B. E., B. Manzano-Winkler, J. B. Hall, K. L. Korunes, and M. A. F. Noor. 2017. The large X-effect on secondary sexual characters and the genetics of variation in sex comb tooth number in *Drosophila subobscura*. Ecol. Evol. 7:533–540.

Morales, H. E., A. Pavlova, L. Joseph, and P. Sunnucks. 2015. Positive and purifying selection in mitochondrial genome of a bird with mitonuclear discordance. Mol. Ecol. 24:2820–2837.

Moran, P. A. P. 1959. The theory of some genetical effects of population subdivision. Aust. J. Biol. Sci. 12:109–116.

———. 1962. The statistical processes of evolutionary theory. Clarendon Press, Oxford, U.K.

Nosil, P., D. J. Funk, and D. Ortiz-Barrrientos. 2009. Divergent selection and heterogeneous genomic divergence. Mol. Ecol. 18:375–402.

Orr, H. A. 1998. Testing natural selection vs. genetic drift in phenotypic evolution using quantitative trait locus data. Genetics 149: 2099–2104.

———. 2010. The population genetics of beneficial mutations. Phil. Trans. R. Soc. B 365:1195–1201.

Parkash, R., D. D. Aggarwal, and B. Kalra. 2012. Coadapted changes in energy metabolites and body color phenotypes for resistance to starvation and desiccation in latitudinal populations of *D. melanogaster*. Evol. Ecol. 26:149–169.

Parkash, R., and A. K. Munjal. 1999. Climatic selection of starvation and desiccation resistance in populations of some tropical drosophilids. J. Zool. Syst. Evol. Res. 37:195–202.

Polechová, J. 2018. Is the sky the limit? On the expansion threshold of a species' range. PLoS Biol 16:e2005372. https://doi.org/10.1371/journal.pbio.2005372

Polechová, J., and N. H. Barton. 2015. Limits to adaptation along environmental gradients. PNAS 112:6401–6406.

Presgraves, D. C. 2008. Sex chromosomes and speciation in *Drosophila*. Trends Genet. 24:336–343.

———. 2018. Evaluating genomic signatures of "the large X-effect" during complex speciation. Mol. Ecol. 27:3822–3830. https://doi.org/10.1111/mec.14777

R Core Team. 2017. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rajpurohit, S., O. Nedved, and A. G. Gibbs. 2013. Desiccation tolerance of Indian drosophilids. Comp. Biochem. Physiol. A Physiol. 164:391–398.

Rako, L., A. R. Anderson, C. M. Sgrò, A. J. Stocker, and A. A. Hoffmann. 2006. The association between inversion *In(3R)Payne* and clinally varying traits in *Drosophila melanogaster*. Genetica 128: 373–384.

Reinhold, K. 1998. Sex linkage among genes controlling sexually selected traits. Behav. Ecol. Sociobiol. 44:1–7.

Rohlf, F. J. 2006. *tpsDig*, version 2.10. Department of Ecology and Evolution, State University of New York, Stony Brook, New York.

Schmidt, P. S., L. Matzkin, M. Ippolito, and W. F. Eanes. 2005. Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. Evolution 59:1721–1732.

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.

Sheets, H. D. 2003. IMP—integrated morphometrics package. Department of Physics, Canisius College, Buffalo, New York.

Stefansson, H., A. Helgason, G. Thorleifsson, V. Steinthorsdottir, G. Masson, J. Barnard, A. Baker, A. Jonasdottir, A. Ingason, V. G. Gudnadottir et al. 2005. A common inversion under selection in Europeans. Nat. Genet. 37:129–137.

Trochet, A., E. A. Courtois, V. M. Stevens, M. Baguette, A. Chaine, D. S. Schmeller, and J. Clobert. 2016. Evolution of sex-biased dispersal. Q. Rev. Biol. 91:297–320.

Turner, T. L., M. T. Levine, M. L. Eckert, and D. J. Begun. 2008. Genomic analysis of adaptive differentiation in *Drosophila melanogaster*. Genetics 179:455–473.

van Heerwaarden, B., and C. M. Sgrò. 2011. The effect of developmental temperature on the genetic architecture underlying size and thermal clines in *Drosophila melanogaster* and *D. simulans* from the east coast of Australia. Evolution 65:1048–1067.

———.2017. The quantitative genetic basis of clinal divergence in phenotypic plasticity. Evolution 71:2618–2633.

Via, S., and J. West. 2008. The genetic mosaic suggests a new role for hitchhiking in ecological speciation. Mol. Ecol. 17:4334–4345.

Weeks A. R., S. W. McKechnie, and A. A. Hoffmann. 2002. Dissecting adaptive clinal variation: markers, inversions and size/stress associations in *Drosophila melanogaster* from a central field population. Ecol. Lett. 5:756–763.

Wilson-Sayres M. A., K. E. Lohmueller, and R. Nielsen. 2014. Natural selection reduced diversity on human Y chromosomes. PLoS Genet. 10:e1004064.

Wolff, J. N., M. F. Camus, D. J. Clancy, and D. K. Dowling. 2016. Complete mitochondrial genome sequences of thirteen globally sourced strains of fruit fly (*Drosophila melanogaster*) from a

powerful model for mitochondrial research. Mitochondr. DNA 27: 4672–4674.

Yeaman, S., and S. P. Otto. 2011. Establishment and maintenance of adaptive genetic divergence under migration, selection and drift. Evolution 65:2123–2129.

Yeaman, S., and M. C. Whitlock. 2011. The genetic architecture of adaptation under migration-selection balance. Evolution 65:1897–1911.

Associate Editor: J. Jensen
Handling Editor: M. R. Servedio

Supporting Information

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

Figure S1. Comparisons between FST predictions from the analytical models provided in the main text and stochastic Wright-Fisher simulations.
Figure S2. Comparisons between FST predictions from the analytical models provided in the main text and stochastic Wright-Fisher simulations.
Figure S3. Comparisons between FST predictions from the analytical models provided in the main text and stochastic Wright-Fisher simulations.
Figure S4. Comparisons between FST predictions from the analytical models provided in the main text and stochastic Wright-Fisher simulations.
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Figure S7. Comparisons between FST predictions from the analytical models provided in the main text and stochastic Wright-Fisher simulations.
Figure S8. Comparisons between FST predictions from the analytical models provided in the main text and stochastic Wright-Fisher simulations.
Table S1. Model weighted parameter estimates, unconditional standard errors, and variable importance (v_i) scores for each of the different composite genetic effects (CGEs) contributing to the divergence between a tropical and temperate population of *Drosophila melanogaster* for wing size, starvation, desiccation, and heat resistance.