OXFORD

Environmental dependence of mutational (co)variances of adaptive traits

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Abstract

Standing genetic variation, and capacity to adapt to environment change, will ultimately depend on the fitness effects of mutations across the range of environments experienced by contemporary, panmictic, populations. We investigated how mild perturbations in diet and temperature affect mutational (co)variances of traits that evolve under climatic adaptation, and contribute to individual fitness in *Drosophila serrata*. We assessed egg-to-adult viability, development time and wing size of 64 lines that had diverged from one another via spontaneous mutation over 30 generations of brother–sister mating. Our results suggested most mutations have directionally concordant (i.e., synergistic) effects in all environments and both sexes. However, elevated mutational variance under reduced macronutrient conditions suggested environment-dependent variation in mutational effect sizes for development time. We also observed evidence for antagonistic effects under standard versus reduced macronutrient conditions, where these effects were further contingent on temperature (for development time) or sex (for size). Diet also influenced the magnitude and sign of mutational correlations between traits, although this result was largely due to a single genotype (line), which may reflect a rare, large effect mutation. Overall, our results suggest environmental heterogeneity and environment-dependency of mutational effects could contribute to the maintenance of genetic variance.

Keywords: *Drosophila serrata*, mutation accumulation, diet, development time, size

Introduction

Mutation is the ultimate source of all genetic variation, and the frequency distribution of mutational effects plays a key role in evolutionary phenomena including the maintenance of quantitative genetic variance (Johnson & Barton, 2005; Walsh & Lynch, 2018) and the extinction risk of small populations (Lande, 1995; Lynch & Gabriel, 1990; Lynch et al., 1995). Evidence is markedly consistent in suggesting that new mutations typically have moderately deleterious fitness effects (Halligan & Keightley, 2009; Keightley & Lynch, 2003), contributing to standing genetic variation for ~50–100 generations before being eliminated by selection (Houle et al., 1996; McGuigan et al., 2015).

In contrast to this evidence of deleterious mutation, evidence of pervasive local adaptation (Hereford, 2009; Leimu & Fischer, 2008) suggests frequent mutations with beneficial fitness effects, where these mutations may have antagonistic or conditionally neutral fitness effects in other environments. Evidence has emerged that phenotypic effects of mutations can differ between environments. These investigations have typically contrasted benign with stressful levels of an environmental factor (e.g., addition of a chemical toxin, or large change in temperature) (reviewed in Agrawal & Whitlock, 2010; Berger et al., 2021; Martin & Lenormand, 2006), or natural environments that are highly divergent along multiple environmental axes (e.g., Roles et al., 2016; Weng et al., 2020). However, it is the distribution of fitness effects under the range of environmental conditions that individual's experience over their immediate spatial range and lifecycle that will determine whether mutations are rapidly eliminated by selection, or persist for longer, contributing to standing genetic variation and therefore, potentially, to adaptation to altered environmental conditions in the future. How mutational (co) variances vary across the relatively limited range of within-population environmental experiences is largely unknown (Conradsen et al., 2022; Garcia-Dorado et al., 2000).

For sexually reproducing taxa with separate sexes, sex differences in fitness effects of mutations can also influence their contribution to standing genetic variation. Evidence suggests mutations typically affect fitness in the same direction in both sexes, but heterogeneity in size and direction of phenotypic effects have been reported (Allen et al., 2017; Connallon & Clark 2011; Mallet et al., 2011; McGuigan et al., 2011; Sharp & Agrawal, 2013). Sex-specific environmental effects on mutational (co)variances have rarely been investigated (Latimer et al., 2014), and thus their potential influence on standing genetic variation is unknown.

Fitness is complex, determined by multiple traits, (Arnold, 2003; Shaw, 2019), where the specific combination of trait values associated with high fitness differs among environments (Svensson et al., 2021). Evolution toward a multivariate fitness optimum will be determined by the genetic correlations

among the fitness-determining traits (Lande, 1979; Schluter, 1996; Walsh & Blows, 2009). Environment-dependent allelic effects can cause genetic correlations to change markedly when the same population of genotypes encounters different environmental conditions (Sgrò & Hoffmann, 2004; Wood & Brodie, 2015). While mutation has been shown to cause correlations among diverse traits (e.g., Dugand et al., 2021; Estes & Phillips, 2006; McGuigan et al., 2014), very little is known of the environmental sensitivity of such mutational correlations, and thus how mutation versus historical selection contribute to heterogeneity in multivariate adaptive potential under different environmental conditions.

Here, we investigate how mild perturbations to nutrition and temperature during larval development in the vinegar fly *Drosophila serrata* influence phenotypic (co)variance contributed by spontaneous mutations. Climate is a major driver of diversity from global to local scales (Addo-Bediako et al., 2000; Andrewartha & Birch, 1954). Distributions of phenotypes and taxa depend on taxon-specific climatic tolerances and indirect responses to abiotic effects, particularly changes in quantity or nutritional quality of food (Abarca & Spahn, 2021; Huxley et al., 2021; Kellermann & van Heerwaarden, 2019; Thomas et al., 2017). In taxa such as *Drosophila serrata*, larval dependence on rotting fruits exposes them to heterogeneity in food quantity and quality, as well as temperature, where the extent of shade introduces heterogeneity among colocated fruits.

In this study, we aimed to infer the potential for heterogeneous selection to influence the contribution of mutations to standing genetic variation. To do so, we estimate mutational variance within and covariance among environments and sexes to infer if and how mutational effects change in these different contexts. We focused on three traits implicated in climatic adaptation: survival (egg-to-adult viability), development time, and size. Development time and size have diverged along latitudinal clines in *Drosophila* (e.g., Huey et al., 2000; James et al., 1995) including *D. serrata* (Hallas et al., 2002; Sgrò & Blows, 2003), with larger size and longer development time at higher latitudes. Although responses vary (i.e., there is evidence of genotype by environment variation), both development time and size also exhibit plastic responses to developmental temperature and larval nutrition in *Drosophila* (e.g., Bitner-Mathe & Klaczko, 1999; Chakraborty et al., 2021; Liefting et al., 2009). We estimated the mutational (co)variance in these key life-history traits in males and females from a panel of mutation accumulation lines in *D. serrata*, finding evidence of both environment independent and dependent mutational effects.

Methods

Population history

A panel of mutation accumulation (MA) lines were founded by one of the highly homozygous *D. serrata* genomic reference panel (DsGRP) lines (Reddiex et al., 2018), DsGRP-226. Each MA line, established by a single breeding pair, was maintained by brother–sister mating for 30 generations followed protocols described in McGuigan et al. (2011). The low effective population size in each line minimizes the opportunity for selection, with random sampling (drift) predominantly determining if mutations are fixed or lost (Lynch et al., 1999). Each MA line was maintained by up to 10 replicate brother–sister matings (all derived from a single mating

pair each generation), mitigating extinction risk (by avoiding chance events, and preventing fixation of lethal mutations). Each generation, one of these vials was chosen randomly to provide parents for the next generation, ensuring that unintentional selection on productivity traits (e.g., female fecundity) was avoided. After this mutation accumulation phase, the census population size was increased to ~90 (three vials of ~30 adults, admixed each generation).

MA studies typically estimate mutational variance, the per generation rate of increase in phenotypic variation due to new mutations; here, the unknown effective population size after expansion precludes accurate estimation of that parameter (Lynch & Hill, 1986). However, assaying MA lines simultaneously in multiple environments allows us to interpret differences in the magnitude of among-line (co)variance as changes in the phenotypic effects of the sampled mutations. Evidence suggests selection effectively prevents deleterious mutation accumulation in populations as small as $N = 10$ (Estes et al., 2004; Katju et al., 2015; Luijckx et al., 2018), while beneficial mutations arise and fix rarely, even in large populations ($N > 1,000$) (e.g., Denver et al., 2010). We therefore expect among-line variation in these *D. serrata* MA lines to predominantly reflect drift-driven fixation of mutations during the 30 generations of brother–sister mating, while mutations arising after expansion will contribute to within-line variance.

Experimental treatments

Several replicate vials of four virgin four-day-old males and females from each MA line were placed on a treacle-enriched diet, with a live yeast paste, to encourage egg laying. Forty eggs were randomly allocated to each of 16 vials (640 eggs per line), with four vials assigned to each of four environmental treatments. Treatments corresponded to a fully factorial design with two levels of diet and of temperature. Eggs were introduced to 100 mm × 25 mm vials containing 10 ml of either the standard laboratory diet (per 1 L water: 37 g inactivated torula yeast [protein source], 54 g raw cane sugar [carbohydrate source] and 18 g agar with [antimicrobials] 6 ml propionic acid and 12 ml nipagin, a 10% w/v solution of methyl-4-hydrobenzoate in methanol) or a reduced macro-nutrient diet (two-thirds of the yeast [24.7 g] and sugar [36 g] of the standard diet, with all other ingredients unchanged). Larvae experienced their assigned diet throughout development to pupation. Developing larvae (on both diets) were exposed to either standard rearing conditions of constant 25 °C, or a 3-hr heat shock (30 °C) per day for the first three days of larval development, and thereafter held at 25 °C. The variability of nutritional quality of natural food sources for the *D. serrata* are not known, and the difference to the well-tolerated laboratory diet make it difficult to clearly extrapolate from the imposed dietary manipulation to natural heterogeneity in resources. We imposed differences in overall macronutrient availability consistent with mild increases in resource competition (e.g., McGuigan, 2009). The temperature differences however were chosen to capture some part of the natural thermal variability the ancestral population experienced. Inseminated female *D. serrata* were caught in Brisbane, Australia in October 2011 to establish the DsGRP panel (Reddiex et al., 2018). In the decade 2002–2011, Brisbane's average daily maximum temperatures exceeded 30 °C in the hottest months (30.4 and 30.3 °C in January and February, respectively), while the October and annual average

daily maximums over that period were 26.8 and 26.4 °C, respectively (Australian Bureau of Meteorology, 2023).

Consistent with our goal of applying mild environmental perturbations, there was no difference in mean egg-to-adult viability (measured as detailed below) among the four environments (Figure 1A; Supplementary Table S1; Supplementary Figure S1). However, as anticipated, we found evidence of sexual dimorphism and plastic responses for the other traits (for full details see Supplementary Materials). Development time was delayed by 15 hr under reduced macronutrient conditions, while the heat shock treatment accelerated development by 2 hr (Figure 1B; Supplementary Table S1; Supplementary Figure S1). Females developed ~5 hr faster than males, but there was no difference in how the sexes responded to the environments (Figure 1B; Supplementary Table S1; Supplementary Figure S1). The effect of environment on size was sex-specific, with males, but not females, decreasing size in response to heat shock, and while both sexes decreased size on the reduced macronutrient diet, there was some evidence that females responded more strongly than males (Figure 1C; Supplementary Table S1; Supplementary Figure S1).

Flies were reared together in one temperature controlled room (12:12 light:dark cycle), with heat-shocked vials transferred to an incubator chamber (Rcom Maru D H&B 380, Autoelex Co. Ltd) for the 3 hr 30 °C treatment. Positions of the rearing vials within the constant temperature room were randomized across all levels of the experiment. For logistical reasons, the experiment was conducted in a complete block design, with two blocks, initiated one week apart, each consisting of 32 MA lines (64 MA lines in total).

Phenotype assays

Egg-to-adult viability and development time were recorded for each vial, separately for males and females. Eclosed flies were removed twice daily (morning and afternoon, ~ 6 hr apart) over the duration of adult eclosion (day 10 through 16 post laying), with time recorded in 15 min intervals. Eclosion in *Drosophila* occurs within a relatively narrow (~5 hr) window during 24 hr, with individuals missing a window then delaying eclosion to the following window (Mark et al., 2021; Skopik & Pittendrigh, 1967). Development time (in hours) of each vial was then determined as the average eclosion time for males, females or for both sexes pooled. Egg-to-adult viability (survival) was the total number of males, females or flies (both sexes) eclosing per vial. Eggs were randomly allocated to the four treatments, and we therefore expect that the average sex ratio per environment was constant, but heterogeneous sex-specific mortality rates during development may cause divergence in adult sex ratio among environments. We estimated the sex ratio of emergent adults as: (1 + female)/(1 + males), where the addition of 1 ensured there were no undefined values.

Of 1,024 vials initiated with 40 eggs, 60 had no eclosion, and were treated as missing data and excluded from analyses. A further 23 vials had no male eclosion and 15 vials no female eclosion, and were excluded from their respective sex-specific analyses. Exclusion of zero eclosion vials will upwardly bias estimates of mean viability across the population. These vials were relatively evenly distributed among environments (13–18 of the 256 vials per treatment had no eclosion), but not genotypes: 20 of the 64 MA lines had ≥1 zero eclosion vial; five lines had four to eight (25%–50%) zero eclosion vials. This suggests both stochastic microenvironmental

variation and mutation influenced viability, and exclusion of zero emergence vials will downwardly bias estimates of mutational variance. Viability data (with zeros excluded) and development time were normally distributed, while sex ratio was log-normal distributed and transformed to a log scale for
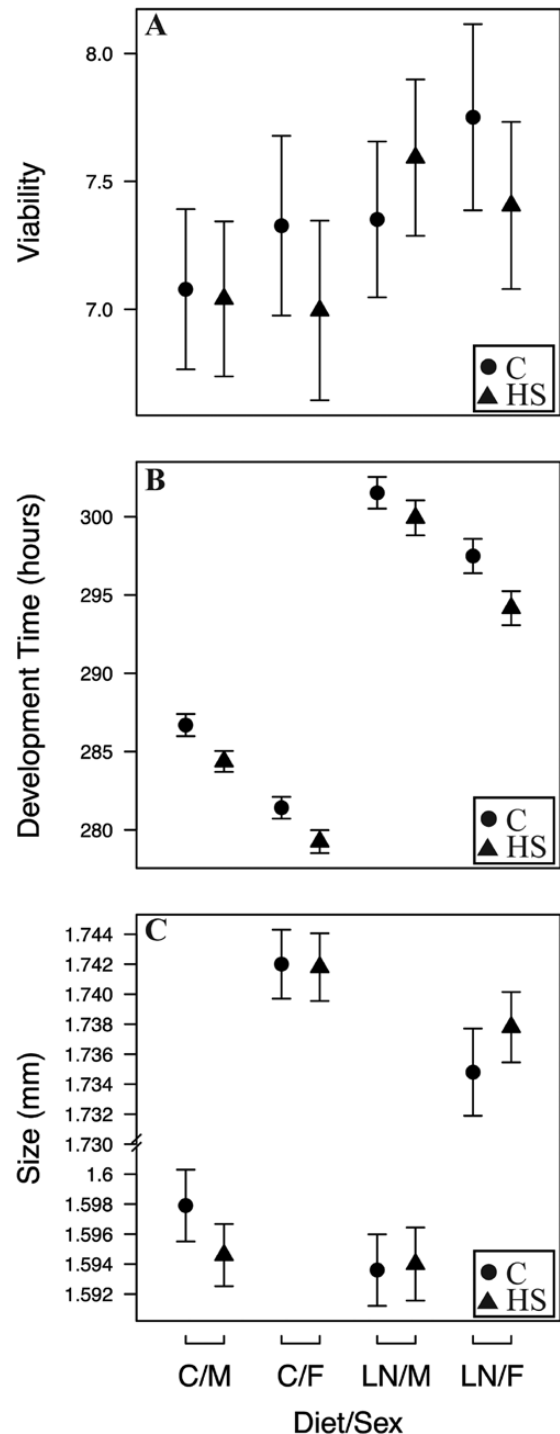
Figure 1. Effects of environment and sex on trait means of the mutation accumulation (MA) lines. Environment and sex-specific means (±*SE*) are plotted for A) viability; B) development time; and C) wing size. Diet (C = control, normal nutrition; LN = low nutrition) and sex (male = M; female = F) are indicated on the x-axis, while symbols indicate estimates from standard rearing temperature (circles) versus the heat-shock treatment (triangles). For further details see Supplementary Materials.

analysis. Four outliers (>3.5 *SD* of the mean) were detected and excluded from all analyses.

Up to five males, and five females per vial were assayed for wing size (7,735 wings in total), a proxy of body size in *Drosophila* (Robertson & Reeve 1952). Flies collected from the first experimental block (32 lines) were processed at The University of Queensland, while those from the second experimental block (32 lines) were processed at Monash University. Due to differences in available equipment, the image collection protocol differed between blocks. For block 1, all wings per vial (up to five males and five females) were photographed simultaneously (as described in Dugand et al., 2021), while for block 2 all wings were photographed individually. Within each block, images were randomly ordered, and the positions of nine landmarks recorded per wing (as described in Dugand et al., 2021) by a single researcher per block. For block 1, common environment effects on wings are confounded with measurement error at the vial level, but image randomization ensured systematic changes in landmarking through time were not confounded with MA line or environment.

Landmark coordinates were aligned using a full Procrustes fit in MorphoJ (Klingenberg, 2008). Centroid size (the square root of the sum of the squared distances between each landmark and their centroid), was recorded as a measure of total wing size. Centroid size data were normally distributed, with values rescaled (×100) prior to analysis to increase resolution of estimates. To obtain vial-mean values of size (consistent with the vial-level values for the other traits), we first assessed the presence of outliers (>3.5 *SD*) within each sex and treatment for each line, which might affect the estimates of vial mean. We excluded 15 observations (0.19% of the data), distributed across 14 lines. We then calculated vial means (per sex, and in total), and again assessed the presence of extreme values (>3.5 *SD* from the mean) within each experimental block, treatment and sex; a further 13 observations (from 12 MA lines) were excluded as outliers. Additionally, one MA line had 10 observations > 3.5 *SD* (and a further seven observations > 3.0 *SD*) from their respective mean; we retained all these observations and implemented analyses both including and excluding this extreme line, as detailed further in the Results.

Data analysis

Mutational (co)variances across environments and sexes for each trait

We first tested the null hypothesis that our MA lines had acquired mutations with effects on the measured traits. Separately for each trait and sex in each environment (28 models in total), we used PROC MIXED in SAS (SAS Institute Inc., 2012) to implement a restricted maximum likelihood (REML) fit of the mixed model:

$$y = \mu + \mathbf{X}\mathbf{B} + \mathbf{Z}\mathbf{M} + \epsilon \quad (1)$$

where y was the vector of observations, and μ was the mean trait value. The design matrix $\mathbf{X}$ associated each observation (vial) with its fixed effects (B); here, experimental block, a categorical variable with two levels, was the only fixed effect. The design matrix $\mathbf{Z}$ associated vials with their level of the random effect of genotype (MA line, M) to estimate the among-line (mutational) variance. The residual variance, ϵ, was modeled separately for each experimental block to accommodate any (uncontrollable) temporal heterogeneity

in laboratory conditions. Log-likelihood ratio tests (LRT), comparing a model in which the among-line variance was estimated to one in which it was constrained to zero, were used to determine statistical support for mutational variance (Supplementary Table S2).

Having confirmed that all traits (except sex ratio) were affected by mutations in each environment and sex (Supplementary Table S2), we investigated further how mutational (among-line) variance varied. For each trait, we modified model (1) to include diet, temperature, sex and their interactions as fixed effects and, at the among-line variance level, to estimate the unconstrained 8 × 8 matrix of among-line (co)variance among each level of diet, temperature and sex. To account for the nonindependence of male and female observations from the same vial, we modeled the residual variance as an unstructured covariance matrix between the sexes; we allowed for heterogeneity in the magnitude of this residual variance by estimating block, diet, and temperature specific residuals.

To determine whether mutational effects differed with sex or environment, we used factor analytic modeling (Hine & Blows, 2006; Meyer & Kirkpatrick, 2005). This application of factor analytic modeling is conceptually equivalent to testing the null hypothesis that a cross-environment (or cross-sex) mutational correlation is 1.0 (given sampling error), but allows us to test all pairwise combinations simultaneously, and to also consider heterogeneity in the magnitude of among-line variance. Where mutations influence the trait identically (within sampling error) in both sexes and all environments, only one dimension of among-line variance will be supported (i.e., only one eigenvalue of the among-line covariance matrix is above zero). Heterogeneity in either magnitude or direction of mutational effect across environments and/or sexes will result in more dimensions of variation. Thus, we tested the hypothesis that mutational effects were environment and/ or sex-dependent by fitting a series of nested models, constrained to have zero, one, two or three dimensions of variation (implemented using TYPE = FA0(*n*) in PROC MIXED), and comparing the fit of these nested models using likelihood ratio tests, where the difference in the number of parameters between models defines the degrees of freedom for the test.

To visualize all parameter estimates, we placed robust confidence intervals around variance component estimates (including eigenvalues) using the REML-MVN sampling approach (Houle & Meyer, 2015; Meyer & Houle, 2013; Sztepanacz & Blows, 2017). We obtained the unconstrained among-MA line covariance matrix from the fitted model, and used the mvrnorm function of the MASS package (Venables & Ripley, 2002) in R to draw 10,000 random samples from the distribution $N \sim (\hat{\theta}, \mathbf{V})$ where $\hat{\theta}$ was the vector of REML covariance parameter estimates, and $\mathbf{V}$ was the asymptotic variance–covariance matrix. While the REML variance estimates were constrained to be positive, the REML-MVN samples were not (i.e., were on the G-scale: Houle & Meyer, 2015).

To aid interpretation of the magnitude of among-line (mutational) variance in the different environments and sexes, where trait mean and variance also differed (Figure 1; Supplementary Table S2), we followed Hansen and Houle (2008) to estimate mean-standardized (i.e., I, opportunity for selection) and variance-standardized (i.e., H², heritability) among-line variance–covariance matrices: $\mathbf{L}_\mu = \mathbf{L} \oslash (\bar{z}\bar{z}')$ and $\mathbf{L}_\sigma = \mathbf{L} \oslash (\sigma\sigma')$ where $\mathbf{L}$ was the 8 × 8 among-line variance covariance matrix, $\bar{z}$ and σ the 8-element vectors of trait

mean and standard deviation, respectively, (and $\bar{z}'$ and σ' their transpose), while $\oslash$ indicates element-wise division. Estimates of $\bar{z}$ and σ were obtained from a modified model, fit separately to each trait in each sex in each environment:

$$y = \mu + \epsilon \qquad (2)$$

Where μ, the intercept, provided the estimate of $\bar{z}$ and ϵ an estimate of the phenotypic variance (σ^2). The rnorm function in R was used to draw 10,000 estimates of $\bar{z}$ from $N \sim (\mu, SD)$, where SD was the standard deviation of μ, and 10,000 estimates of σ from $N \sim (\sqrt{\sigma^2}, \sqrt{asy})$, where asy was the asymptotic variance of ϵ. These samples were randomly paired with the 10,000 REML-MVN estimates of among-line variance to provide confidence estimates for scaled mutational variance estimates.

Mutational correlations among traits and their response to environment

Finally, we investigated whether the environment influenced mutational correlation among traits. With 64 MA lines, we did not have sufficient power to estimate all $n(n + 1)/2$ unique covariance parameters of the full multivariate model for all traits in all environments and sexes (210 parameters). Our primary focus in this study was the effect of the environment, and in general we observed a greater effect on mutational variance of environment than sex (see Results). We therefore fit a modified version of model (1), omitting the effect of sex, and modeling trait-specific responses to all other fixed effects. For the random effects (among-line and residual), we modeled unconstrained among-trait covariance matrices separately for each diet and temperature (and for the residual, in each experimental block). We used LRT to test the hypothesis that among-line pairwise trait correlations differed between diets by comparing the unconstrained model fit to a model in which, for each of the three trait pairs, the correlations were constrained to have the same value on both diets. We implemented this LRT using the "covtest" function in PROC GLIMMIX in SAS (SAS Institute Inc., 2012). We again extracted the Fisher Inverse information matrix and estimated REML-MVN CI per parameter, as detailed above.

Results

Mutational (co)variances between environments and sexes for each trait

Egg-to-adult viability

There was strong statistical support for among-line (mutational) variance in viability (within each environment and sex: Supplementary Table S2; multivariate analysis, 0 to 1 dimension contrast: $\chi^2 = 113.8$, df = 8, $p < .0001$). Observations indicate that most of this mutational variance was due to mutations with similar effects in all environments and sexes (i.e., context-independent). Specifically, all cross-environment and cross-sex correlations were positive and strong (Supplementary Table S3), and the loadings on the first eigenvector (Viab1) were in the same direction and of similar magnitude for all environments and sexes (Table 1).

There was weak statistical support for context-dependent mutational effects (1 to 2 dimensions contrast: $\chi^2 = 13.9$, df = 7, $p = .0530$). This second eigenvector (Viab2) was characterized by opposing loadings of the two diets (Table 1), suggesting the presence of mutations that increased viability on one experimental diet, while decreasing it on the other

(Figure 2A). Exclusion of MA-190, with extreme values of wing size (but not viability: Figure 2A and C), had little effect on the magnitude of among-line variance (overlapping 95% CI of eigenvalues, Supplementary Table S4), or on the pattern of among environment covariation (eigenvector loadings: Supplementary Tables S5 and S6). Similarly, there was no evidence of differences in mean viability among environments (Figure 1; Supplementary Table S1; Supplementary Figure S1), and interpretation of the cross-environment and cross-sex patterns of mutational covariance were the same when considering them on mean or variance standardized scales (Supplementary Tables S5 and S6).

Estimates of among-line variance in viability were typically higher for females than males in the same environment (Supplementary Table S2). This was reflected in slightly higher loadings of females for both eigenvectors (Viab1 and Viab2; Table 1). However, CI of among-line variances substantially overlapped between males and females, and the between-sex correlations within each environment were strong (≥ 0.89) with CI including 1.0 (Supplementary Table S3). We further note that there was no statistical support for among-line (mutational) (co)variance in adult sex ratio, irrespective of the environment (within each environment: Supplementary Table S2; multivariate analysis, 0 to 1 dimension: $\chi^2 = 0.32$, df = 4, $p = .9885$). This suggests mutations had consistent effects on male and female viability, but must be interpreted cautiously given the missing information on sex ratio of embryos.

Egg-to-adult development time

For development time, there was strong statistical support for two dimensions of mutational covariance (1 to 2 dimensions contrast: $\chi^2 = 26.9$, df = 7, $p = .0003$; 2 to 3 dimensions contrast: $\chi^2 = 4.3$, df = 6, $p = .6361$; Table 1). All pairwise development time correlations were positive and strong (Supplementary Table S3), and all environments and both sexes loaded strongly, in the same direction, on the first eigenvector (DevT1: Table 1). Thus, similar to viability, mutational effects on development time were predominantly context-independent. However, two patterns consistent with context-dependent mutational effects were also apparent.

First, the among-line variance in development time was 2.0–3.8 times higher under reduced macronutrients than under standard diet conditions (Figure 2B; Supplementary Tables S2 and S3). This is reflected in the larger loadings on DevT1 for reduced macronutrient environments (Table 1). Mean development time (Figure 1; Supplementary Table S1) and total (phenotypic) variance (Supplementary Table S2) in development time were also higher on the reduced macronutrient than standard diet. Accounting for these differences in scale, there was 1.8–3.4 (I) or 1.2–2.1 (H^2) times more among-line variance when macronutrients were reduced (Supplementary Table S2). Reflecting this persistent, scale-independent, diet effect on the magnitude of mutational variance, the 1st eigenvector, with larger loadings for low macronutrient environments, was coincident on all three scales (Supplementary Table S5; dot products > 0.99: Supplementary Table S6). Overall, while the shared direction of loadings on the major axis of among-line variance (DevT1) indicated that mutations affected development time in a consistent direction (increasing or decreasing it) under all environments and both sexes, the larger contribution from reduced macronutrient conditions suggests that the phenotypic effects of the mutations are larger under those conditions.

Table 1. Results of eigenanalyses of the unconstrained among-line covariance matrices estimated for each trait. The characteristics of the first two dimensions of among-line variance are reported for viability (Viab), development time (DevT), and wing size (Size). The eigenvalues are presented in the first row (90% CI from REML-MVN sampling shown in the row below, in italics). The normalized eigenvector loadings are reported below their respective eigenvalue, showing the loading of each diet (C = control, standard; LN = low macronutrient)/temperature (C = control, standard; HS = heatshock)/sex (M = male; F = female).

Diet/temperature/sex	Viab1	Viab2	DevT1	DevT2	Size1	Size2
	22.15 *13.59–30.54*	**4.05** *1.36–6.73*	**240.08** *148.37–330.11*	**41.40** *18.44–64.65*	**20.88** *14.40–27.47*	**2.09** *1.09–3.09*
C/C/M	0.327	0.245	0.223	0.422	0.365	0.342
C/C/F	0.412	0.338	0.239	0.409	0.316	0.405
C/HS/M	0.334	0.042	0.210	0.413	0.307	0.269
C/HS/F	0.404	0.566	0.247	0.340	0.326	0.297
LN/C/M	0.287	−0.231	0.424	0.043	0.378	−0.172
LN/C/F	0.382	−0.500	0.470	−0.009	0.416	−0.543
LN/HS/M	0.312	−0.361	0.449	−0.427	0.396	0.019
LN/HS/F	0.350	−0.265	0.431	−0.429	0.306	−0.484

The second pattern in mutational contributions to development time variation was apparent in the opposing sign but similar magnitude loadings on DevT2 under standard diet conditions (with or without heat shock) versus under reduced macronutrients and thermal shock (Table 1). This suggests that mutations increasing development time under one set of environmental conditions had antagonistic effects, decreasing it in the alternative environments (and vice versa). This pattern was not affected by differences in scale (phenotypic mean or variance), with eigenvectors being coincident (DevT2 dot products > 0.99) on all three scales (Supplementary Table S6).

MA-190, a line with extremely large wing size (Figure 2C), was also notable for having a short development time on the reduced macro-nutrient conditions relative to all other MA lines (Figure 2B). Exclusion of MA-190 reduced the among-line variance by ~13% (Supplementary Table S4). However, there remained strong statistical support for two dimensions of among-line variance (i.e., for mutational effects to depend on environment: 1 to 2 dimensions contrast: $\chi^2 = 21.1$, df = 7, $p = .0036$), and patterns of covariance remained the same (eigenvector loadings were very similar: Supplementary Table S5; dot products > 0.99: Supplementary Table S6).

Adult wing size

There was statistical support for two dimensions of among-line variance in wing size (1 to 2 dimensions contrast: $\chi^2 = 42.4$, df = 7, $p < .0001$; 2 to 3 dimensions contrast: $\chi^2 = 10.2$, df = 6, $p = .1165$) (Table 1, Supplementary Tables S2 and S3). Again, most mutational variance was context-independent (Size1 was characterized by similar sized loadings all in the same direction; Table 1). The loadings on Size1 were highly similar (dot product > 0.99) on raw, mean standardized and variance standardized scales (Supplementary Tables S5 and S6). As expected, given its extreme value in both sexes and all environments (Figure 2C), MA-190 contributed very strongly to the first eigenvector; excluding MA-190 from the estimation of among-line covariances resulted in the first eigenvalue decreasing by 50% (measurement or mean-standardized scales) or 34% (variance-standardized scale) (Supplementary Table S4). However, MA-190 did not affect the orientation of the major axis of mutational variance (dot products > 0.98 between 1st eigenvectors when MA-190 was included versus excluded: Supplementary Table S6).

The statistically supported second eigenvector (Size2) showed sex-limited antagonistic effects across diet: mutations had opposing effects on size under control diet conditions (irrespective of sex) and on female size under low macronutrient conditions (Table 1). This axis of mutational variation was not influenced by MA-190 (Supplementary Tables S4–S6; factor analytic modeling when MA-190 excluded, 1 to 2 dimensions contrast: $\chi^2 = 42.04$, df = 7, $p < .0001$). Further, the very strong similarity of the Size2 vector on raw, mean and variance standardized scales (dot products > 0.98: Supplementary Table S6) suggests that the inference of mutations with sex-limited effects was not a reflection of the strong sexual dimorphism in size (Figure 1C; Supplementary Table S2), any sex differences in plastic responses to the environments (Figure 1C), or sex differences in the magnitude of phenotypic variance (Supplementary Table S2).

Mutational correlations among traits and their response to environment

Consistent with the per-trait observation that nutrition had the greatest effect on among-line variance, the pairwise trait correlations were also notably similar between the two thermal environments within each diet, but differed between the two diets (Table 2; contrast of unconstrained model estimates versus constraining, for each of the three pairs of traits, the correlations to be the same for each diet: $\chi^2 = 10.60$, df = 3, $p = .0141$). Diet particularly affected the correlation between development time and size, which was very weak on the control diet (0.05 or –0.15, without or with heat shock, respectively), but moderately negative under reduced macro-nutrient conditions across both thermal regimes (–0.43 or –0.54) (Table 2). MA-190, which had extremely large wing size and for which development time was less sensitive to diet (Figure 2B and C), contributed strongly to the pattern of diet-specific trait correlations (Table 2); when MA-190 was excluded from the analyses, the null hypothesis that the three pairwise correlations were not influenced by diet was accepted ($\chi^2 = 5.04$, df = 3, $p = .1692$).

Discussion

The phenomenon of environment-dependency in the effects of alleles, which leads to changes in quantitative genetic
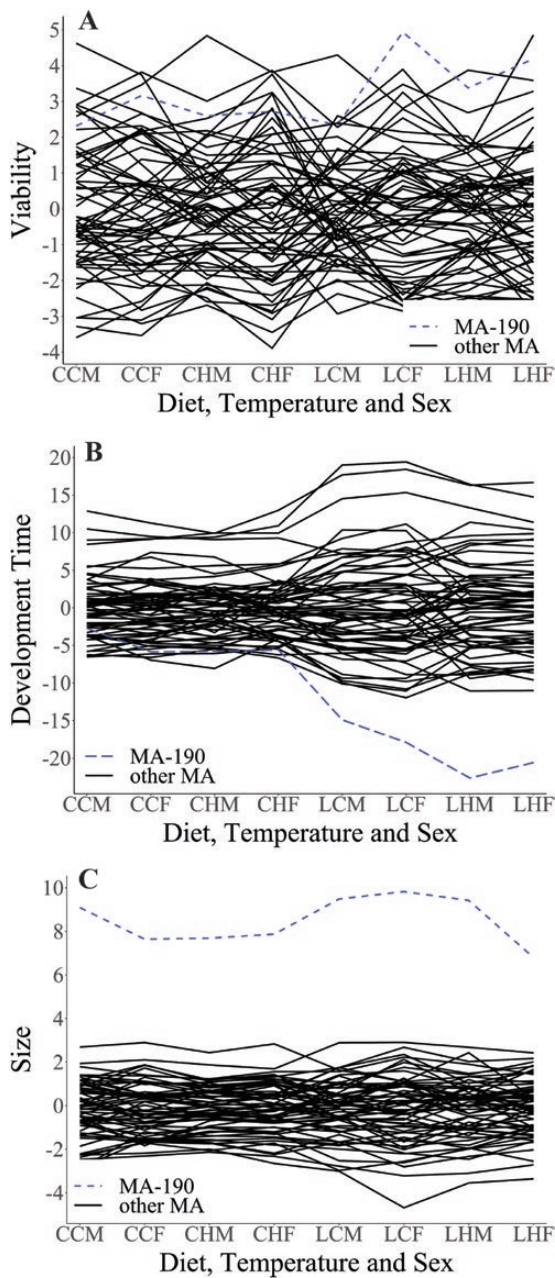
Figure 2. Among-line (co)variance across environments and sexes. For A) viability (count of eclosed adults); B) development time (in hours); and C) wing size (mm), the breeding value (BLUPs) deviations from their respective diet (C or L for control or low nutrition), temperature (C or H for control or heat shock), and sex (M or F for male or female) specific trait mean are plotted. MA-190, with extreme large wing size, is highlighted (dashed line) in each panel.

variances and covariances across different environments, is well established (Sgrò & Hoffmann, 2004; Wood & Brodie, 2015). However, the frequency distribution of mutations that must ultimately cause these conditional (co)variances has received limited attention. To address this knowledge gap, we assessed whether mutational effects on phenotypes were heterogenous under mild environmental perturbations. Our findings suggested mutation predominantly introduced variance with directionally concordant (synergistic) effects on a trait in all considered environments and both sexes. However, we also found evidence for a smaller contribution to phenotypic

variation from mutations with environment-dependent effects.

Several studies, contrasting more extreme environmental differences, in taxa ranging from unicellular microbes to fish, have likewise concluded that mutational effects are predominantly positively correlated among environments (Baer et al., 2006; Fry & Heinsohn, 2002; Latimer et al., 2014; Miller et al., 2022; Ostrowski et al., 2005; Sane et al., 2018). Similarly, available evidence suggests the average mutational effect may be synergistic between the sexes (Connallon & Clark, 2011; Mallet et al., 2011; Sharp & Agrawal, 2013). The prevalence of directionally concordant mutational effects, coupled with pervasive evidence that mutations are typically deleterious (Halligan & Keightley, 2009), suggests that the duration of transient contributions of segregating mutations to standing genetic variation will be environment independent. However, further information is required on how, for traits such as those considered here, the relationship to total fitness (and thus the strength and direction of selection) itself varies with environmental context.

Our analyses also provided supported for mutations with context-dependent phenotypic effects, implicating both mutations with heterogenous magnitude of synergistic effects (potentially including conditionally neutral mutations) and mutations with antagonistic effects. Specifically, nutrition appeared to have a greater effect than temperature (or their interaction) on cross-environment mutational (co)variance. We note that a greater effect of temperature may have been observed if we had considered more extreme temperatures, and/or longer duration of exposure to hot temperatures. However, it is intriguing that our manipulative restriction of macronutrients, slowing development and weakly decreasing size, but not affecting preadult viability, revealed patterns consistent with other studies in which more extreme manipulations of environment (including temperature) have been applied. A review of 18 studies of mutational effects concluded that increased population density (but not other environmental factors such as temperature or chemical food additives) consistently increased the magnitude of mutational effects on fitness traits (viability, survival and productivity) (Agrawal & Whitlock, 2010).

The heightened influence of nutrition may reflect the hypothesized significance of the genetic basis of nutritional resource acquisition (and efficient utilization) and allocation (Parsons, 2005). This genetic architecture not only determines the nature of life-history trait covariances (Houle, 1991) but also strongly influences the maintenance of genetic variance in traits under persistent directional selection (Rowe & Houle, 1996). Nutritional density and energetic demands will vary over large and small temporal and spatial scales (Parsons, 2005; Rosenblatt & Schmitz, 2016). Thus, natural heterogeneity in nutritional environment may broadly impact on how mutations contribute to standing genetic variance. In particular, smaller phenotypic effects of mutations under nutritionally permissive conditions can weaken selection, allowing mutations to reach higher frequency in the population; coupled with larger phenotypic effects under reduced nutrition density (as seen here for development time), higher allele frequencies would increase genetic variance and the capacity of the population to respond to selection.

Studies of mutational variance under natural (field) conditions capture the total influence of complex, multivariate, environments (e.g., Roles et al., 2016; Rutter et al., 2018),

Table 2. The among-line correlations between traits within each environmental context. The pairwise correlations (95% REML-MVN CI, in italics)[a] between viability, development time (Dev. Time) and size are presented for larvae reared on each diet (C = control, standard; LN = low macronutrient), temperature (Temp: C = control, standard; HS = heatshock) for analyses including all MA lines (All) or excluding MA-190 (no 190).

Lines	Diet	Temp	Viability: Dev. Time	Viability: Size	Dev. Time: Size
All	C	C	0.26 *−0.28, 0.70*	−0.31 *−0.80, 0.21*	0.05 *−0.38, 0.54*
	C	HS	0.30 *−0.42, 0.81*	−0.18 *−0.91, 0.52*	−0.15 *−0.79, 0.46*
	LN	C	−0.12 *−0.88, 0.36*	0.19 *−0.32, 0.77*	−0.43 *−0.82, −0.04*
	LN	HS	−0.12 *−0.73, 0.40*	0.33 *−0.21, 0.92*	−0.54 *−0.95, −0.13*
No 190	C	C	0.29 *−0.21, 0.72*	−0.50 *−1.11, 0.05*	0.16 *−0.34, 0.83*
	C	HS	0.34 *−0.37, 0.83*	−0.54 *−1.68, 0.20*	−0.09 *−0.91, 0.72*
	LN	C	0.02 *−0.81, 0.55*	−0.06 *−0.71, 0.64*	−0.22 *−0.78, 0.33*
	LN	HS	0.08 *−0.66, 0.68*	−0.17 *−1.55, 0.85*	−0.37 *−1.31, 0.41*

[a] CI are based on a minimum of 9,268 MVN samples. This is fewer than 10,000 due to negative variance estimates in some MVN samples.

but the relative contribution of different environmental factors, and their biologically relevant interactions with one another, have rarely been dissected. Here, the effect of diet on mutational (co)variances depended on temperature or sex for development time and size, respectively (Table 1). We focused explicitly on nutrition and temperature because growth rates of food sources (bacteria, fungi, plants etc) are thermally dependent (Rosenblatt & Schmitz, 2016), and studies of phenotypic responses to the environment (i.e., plasticity) have demonstrated temperature dependent nutritional effects (e.g., Chakraborty et al., 2020; Lee et al., 2015). The results here, with robust statistical support for mutational variation that was dependent on the interaction of environmental factors and sex, strongly suggests that greater empirical attention should be paid to assessing mutational effects under more complex contexts.

The three traits considered here, viability, development time and size, have been extensively studied in several species of *Drosophila*. Estimates of phenotypic and genetic correlations among the traits (within and among populations) are highly variable in both sign and magnitude among studies, and among treatments within studies (e.g., Chakraborty et al., 2022; Chippindale et al., 2003; Horvath & Kalinka, 2016; James et al., 1995; Sgrò & Blows, 2004; Willi & Hoffmann, 2012). This lability of association is consistent with evidence from the current study, where the mutational correlations between the traits were typically weak, suggesting that mutations predominantly affect each trait independently. Keightley and Ohnishi (1998) chemically induced mutations in *D. melanogaster* and reported correlations between these three traits (viability, development time, and size) were <|0.19| (average |0.11|); these correlations were weaker than observed among the other six life-history traits analyzed (<|0.49|, average |0.18|). Other estimates of mutational correlation between pairs of life-history traits also tend to be stronger than observed here (Estes et al., 2005; Houle et al., 1994; Keightley et al., 2000; McGuigan & Blows, 2013).

Our data suggest pleiotropic effects among traits are conditional on the environment (diet). Size and development time exhibited a weak correlation under control diet

conditions (Table 2, 0.05 or −0.15 with or without heat shock, respectively), but are significantly negatively correlation under reduced macronutrient conditions (Table 2, −0.43 or −0.54). The stronger correlation under reduced macronutrients was mainly driven by MA-190 (Table 2), which was characterized by extremely large size, and which extended development by considerably less than the population mean under reduced macronutrient conditions (Figure 2B and C). It is not possible, from the available information, to determine whether the observed multivariate phenotype (and its response to diet) was due to a single large effect mutation, or to coincident mutations with independent effects on size and development time under reduced macronutrient conditions. The potential for mutation to conditionally couple or decouple development time and adult size may be particularly important for understanding climate adaptation in holometabolous insects, and these mutational effects warrant greater scrutiny.

Both development time and size are sexually dimorphic in *Drosophila*, as observed in this study (Figure 1, Supplementary Table S1). For both traits, we found overwhelming evidence that most mutations had sexually concordant effects. However, we found some evidence of sex-limited, environment-dependent, effects on size, where mutations that increased (decreased) size on standard food decreased (increased) female, but not male, size on a reduced macronutrient diet. Two previous studies of size (thorax length, or total length) under standard culture conditions in *D. melanogaster* found mutations with sexually concordant effects accounted for all (Wayne & Mackay, 1998) or the vast majority (Keightley & Ohnishi, 1998) of mutational variance. Further studies are necessary to determine whether size (or growth) is typically influenced by sex-dependent environmental dependence, and whether factors such as the direction and magnitude of sexual dimorphism in size predict heterogeneity in mutational effects.

While mutational variance was robustly supported for all other traits, we found no evidence that mutation affected the adult sex ratio in this population of *D. serrata*. The sex of developing embryos in each of the 40 eggs placed in a vial

were not determined, and so it is not possible to infer whether sex ratio was maintained constantly from fertilization to sexual maturity, nor whether there was mutational variance in sex ratio later in adult life due to differential survival. In contrast to our observation of mutationally invariant adult sex ratio, Pannebakker et al. (2008) found mutational variance for sex ratio was of a similar magnitude to that typical of life-history traits in the parasitoid wasp *Nasonia vitripennis*.

Conclusions

Our findings, and those of previous studies of more extreme environments, suggest that most mutational variance has environmentally unconditional effects. Nonetheless, a substantial portion of the phenotypic variation introduced by mutation had environment and/or sex-specific effects. Extending our understanding of how these environment-dependent effects influence how segregating mutations contribute to adaptive potential will depend on coupling information on mutational effects with frequency distribution of the selection environments a population encounters (e.g., Stinchcombe et al., 2010). Approaches combining phenotypic and genomic data from mutation accumulation populations with the historical records of selection captured by the within and among population genomic diversity may also lend insight to how environmental heterogeneity in mutational effects and selection collectively influence standing genetic variation.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Data analyzed in this manuscript is available from https://doi.org/10.48610/01993ca.

Author contributions

C.M.S. and K.M. conceived the study. All authors contributed to experimental design, N.C.A., A.K., and R.J.D. conducted the experiments and collected the data. A.K. and K.M. analyzed the data. All contributed to writing the manuscript.

Conflict of interest:

The authors declare no conflict of interest.

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