The Phenotypic Effects of Spontaneous Mutations in Different Environments

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ABSTRACT: Understanding the context dependence of mutation represents the current frontier of mutation research. In particular, understanding how traits vary in their abilities to accrue mutational variation and how the environment influences expression of mutant phenotypes yields insight into evolutionary processes. We conducted phenotypic assays in four environments using a set of *Daphnia pulex* mutation accumulation lines to examine the context dependence of mutation. Life-history traits accrued mutational variance faster than morphological traits when considered in individual environments. Across environments, the mutational variance in plasticity was also greater for life-history traits than for morphological traits, although this pattern was less robust. In addition, the expression of mutational variance depended on the environment, which resulted in changes in the rank order of genotype performance across environments in some cases. Such cryptic genetic variation resulting from mutation may maintain genetic diversity and allow for rapid adaptation in spatially or temporally variable environments.

Keywords: mutation accumulation, phenotypic plasticity, life history, morphology.

Introduction

Mutation is the ultimate source of all genetic variation, but very few estimates of the most important parameters related to mutation are available. For example, direct estimates of the rate of spontaneous mutation exist for only a few organisms (e.g., Denver et al. 2004; Haag-Liautard et al. 2007; Marriage et al. 2009). In addition, the phenotypic effects of spontaneous mutations (on which natural selection acts) have only been measured in a few species and environments (e.g., Kondrashov and Houle 1994; Baer et al. 2006; but see, e.g., Matsuba et al. 2013). Even when measured, the distributions of the size or the directions of the phenotypic effects of mutation are typically only assayed for a single ancestral genotype in a single environment (for reviews, see Baer et al. 2007; Halligan and Keightley 2009). Quantifying the phenotypic effects of mutation in multiple environments, however, is critical for achieving a deeper understanding of the role of mutation in evolution, including the genetic architecture of complex traits, the rate at which traits can change, and the ecological and evolutionary consequences of phenotypic plasticity.

Here we quantify changes in the phenotypic effects of spontaneous mutation on a wide variety of traits in different environments using a mutation-accumulation (MA) approach. MA experiments (reviewed in Halligan and Keightley 2009) involve propagating multiple parallel lineages initiated from a single ancestor. Each MA lineage is maintained separately, with individual lines propagated through single-progeny descent in order to form a maximal bottleneck each generation. This bottleneck minimizes the impact of selection relative to drift and ensures that spontaneous mutations, even when deleterious, can accrue in descendent genomes at the rate at which they occur. Mutations resulting in sterility or juvenile mortality prevent propagation of the line and thus will not be detected using an MA approach. Descendants of each line can be sequenced to estimate the rate of mutation (e.g., Xu et al. 2012) or can be assayed phenotypically to determine the effects of spontaneous mutation on a variety of traits (e.g., Latta et al. 2013; Schaack et al. 2013).

Phenotypic assays of descendant MA lines in a single environment typically reveal an increase in phenotypic variance (V_p) among lines over time for any given trait. This can be attributed to the unique set of mutations that accumulate in each of the separate descendent lineages, which result in an increased level of genotypic variance among lines due to mutation (mutational variance, V_m).

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Estimates of V_m are frequently scaled to either the environmental variance (V_e) or the mean trait value ($\bar{z}$) to generate dimensionless measures of the variance due to mutation (the mutational heritability [h^2_m] or coefficient of mutational variability [CV_m]) that facilitate comparisons among traits. Variation in the level of standardized V_m among the traits measured is thought to relate to either (*a*) the genetic architecture of the trait or (*b*) the historical selection regime favoring canalization or plasticity of the trait (Stearns et al. 1995; Baer 2008).

Most MA experiments have shown that, on average, mutations are deleterious. However, some studies suggest that the distribution of fitness effects includes a greater fraction of beneficial mutations than previously thought (Bataillon 2000; Chang and Shaw 2003; Joseph and Hall 2004; Hall and Joseph 2010). In these cases, a shift in the mean for fitness traits may not be observed, but an increase in the among-line variance is detected (e.g., Rutter et al. 2010). One way to assess potentially beneficial mutations is to assay MA lines in multiple environments. Such assays can reveal whether the phenotypic effects of mutation differ among environments because they are either (1) unconditionally deleterious (but differ in magnitude of effects), (2) conditionally neutral, or (3) conditionally beneficial. The evolutionary implications of these different scenarios are significant, as the release of cryptic genetic variation—genetic variation that is only visible to selection under certain environmental conditions—provides fodder for natural selection to act rapidly rather than waiting for new mutations to arise, accumulate, and facilitate adaptation to new conditions (McGuigan and Srgo 2009). Even in the case of conditionally neutral or deleterious mutations, an increase in genetic variance can have significant evolutionary consequences in natural populations in the form of increased genetic loads or higher potential for inbreeding depression.

It has been proposed that harsh environments, in particular, may be more likely to reveal cryptic genetic variation, but few studies have assayed MA lines in multiple environments (Kondrashov and Houle 1994; Martin and Lenormand 2006; but see Garcia-Dorado et al. 1999). In addition to environment-specific effects, certain revealing traits are also expected to demonstrate distinctive patterns based on the genetic architecture of the trait, the number of underlying mutational targets, and the type of selection acting on the trait (reviewed in Scheiner 1993). For example, life-history traits will show more mutational variance than morphological traits because they are more complex (i.e., influenced by a greater number of loci). Further, if the plasticity of a given trait is determined by the same number of loci underlying the trait or a proportional number of other loci, we expect mutational variance for plasticity of life-history traits to also be greater than that for morphological traits. Finally, if a particular mutation exhibits different effects in different environments (e.g., antagonistic pleiotropy), then we also predict rank-order changes among lineages across environments. Quantifying the context-dependent effects of mutation on traits in multiple environments represents the first step toward understanding both the evolutionary history and evolutionary potential of mutation to shape phenotypes in stressful, fluctuating, or complex environments.

Methods

Mutation Accumulation and Phenotypic Assays

MA Experiment with Daphnia pulex. *Daphnia* are aquatic microcrustaceans (order: Cladocera) that have long served as model organisms for population and community ecology studies and, more recently, for ecological, evolutionary, and ecotoxicological genomics (Seyfert et al. 2008; Miner et al. 2012). We initiated *Daphnia pulex* MA lines in 2004 from progeny of a single female originating from Slimy Log Pond, Oregon. The isolate used was the same as that used for the whole-genome-sequencing project for *D. pulex* ("the chosen one" [TCO]; Colbourne et al. 2011).

We maintained each MA line by single-progeny descent under standard lab conditions (see below). Specifically, each generation, we chose one female offspring to propagate each line. We maintained backup individuals to restore lines if focal individuals failed to reproduce. Although lines were typically propagated with a single offspring, backup individuals (two) were isolated into plastic tubes containing 50 mL of water and otherwise maintained under similar conditions to focal individuals as replicates. This was to ensure that if the focal individual used to propagate the line died, it would be possible to continue advancing the line without going back to the previous generation. Thus, in this experiment, the number of bottlenecks and the number of generations are equivalent; although when backups were used, the bottleneck was two individuals instead of one. Occasionally, when the focal individual and the replicate backups were dead, it was necessary to go back to the previous generation (maintained at 10°C). In those cases, a new focal individual and new backups were isolated, rendering that lineage a generation behind others in the experiment.

Single-progeny descent creates a bottleneck leading to an effective population size of ~1 for each lineage during the experiment. This experimental design minimizes the effects of selection relative to genetic drift and allows the accumulation of nonlethal, nonsterilizing mutations. Over time, unique mutations accumulate in each lineage. Individuals from 10 MA lines were isolated (after 76–125 generations [avg. = 93] of mutation accumulation), and their offspring were raised for two generations under stan-

dard lab conditions (replicates) to acclimate individuals and minimize maternal effects prior to performing phenotypic assays.

Assaying Traits across Environments. We performed phenotypic assays on those replicates from each line that survived the two generations of acclimation ($n = 3$–12) under standard laboratory conditions and in three additional environmental conditions (hereafter referred to as environment: standard, copper, low food, and high temperature). Conditions were based on estimates of stressful but nonlethal conditions determined during pilot experiments. For standard conditions, we cultured individual animals in 100 mL of filtered lake water (collected from Lake Lemon, Bloomington, IN) in 250-mL beakers. We provided each *Daphnia* with ~1.5×10^6 cells of live *Scenedesmus obliquus* algae daily. We placed these beakers in environmental chambers (Percival Scientific, Perry, IA) under uniform light (12L : 12D photoperiod) and temperature (20°–21°C) conditions.

The remaining environments differed from standard conditions in the following ways: copper (+20 μg Cu^{2+}/L, added as anhydrous copper sulfate, $CuSO_4$); low food (individuals received 0.9×10^6 cells of live *S. obliquus* daily); and high temperature (beakers maintained at 22°–23°C). Table A1 (tables A1–A5 available online) provides a summary of conditions for each environment. We randomly arranged all beakers, except for the high-temperature environment, which was housed in a separate chamber, and low-food animals, which were grouped by environment but still randomized with respect to the other variables.

We recorded birth date, time to and body length at first reproduction (maturity), timing of second and third reproductions, the number of live offspring for clutches 1–3, and date of death for each individual. We measured morphological traits from digital photographs taken on the day of their first and second clutches using a SPOT camera and Nikon SMZ1000 and E800 microscopes (analyzed with ImageJ, version 1.48, accurate to 0.01 mm; Abramoff et al. 2004; Schneider et al. 2012). We measured body length (base of spine to crown of head), width (at widest point, taken perpendicular to body-length line), and spine length. We also calculated three composite measures: total length (body length + spine length), juvenile growth rate (mm/day change in body length from birth to first clutch date), and adult growth rate (mm/day change in body length from first clutch date to second clutch date). All data collected and used in analyses are deposited in the Dryad Digital Repository: http://doi.org/10.5061/dryad.mj5m8 (Latta et al. 2013).

Statistical Analysis

The Impact of Mutation on Different Traits and in Different Environments. To examine the impact of mutation on different traits and in different environments, we used the nlme package (Pinheiro et al. 2014) in the R program, version 3.0.3, to extract estimates of the among-line component of variance (ΔV) for each trait in each of the four environments using a random-effects ANOVA model under restricted maximum likelihood, with line treated as a random effect. To ensure all comparisons involved the same underlying sets of spontaneous mutations, we included only MA lines for which there was sufficient replication in all four environments (any environment $n > 3$ replicates) in this analysis. To generate an estimate of the mutational heritability (h^2_m), we subjected the raw data to ANOVA and extracted estimates of ΔV and the residual variance from the model, which provides an estimate of the environmental variance (V_e). The mutational heritability (h^2_m) was then calculated as $\Delta V/V_e$. Note that estimates of ΔV are often scaled by the number of generations of divergence prior to calculation of h^2_m; however, since we used the same MA lines for all calculations, the generations of divergence for these lines (96 generations) represented a constant in the calculation of h^2_m that we ignored.

We also generated estimates of the mutational coefficient of variability (CV_m) using two methods. First, we used the estimates of ΔV extracted from the raw data and then divided these values by the treatment-specific mean trait value. Second, we scaled the raw data to the treatment-specific mean trait value and then applied ANOVA to the scaled data. Although this second method ignores the sampling variance of the group mean, by using the scaled data, the estimate of ΔV we obtain following ANOVA provides an estimate of CV^2_m, so we calculated the CV_m as $(\Delta V)^{1/2}$. As above, we did not scale estimates of ΔV by the number of generations of divergence; nor did we multiply by 100 in order to express the CV_m as a percentage, as is common. We obtained 95% confidence intervals for the estimates of ΔV and V_e that were used to calculate h^2_m and for the ΔV used to calculate CV_m, using the intervals function in nlme.

To test for differences in h^2_m and CV_m between types of traits, we classified traits as either life history or morphological (L or M in table S1; tables S1, S2 available online). We then used Kruskal-Wallis one-way ANOVA by ranks to test for significant differences between types of traits in R. To test for differences in h^2_m and CV_m among environments, we pooled all traits measured in a single environment, regardless of the type of trait, and used the Kruskal-Wallis test. We then used the Wilcoxon rank sum test to make pairwise comparisons between each of the environ-

ments and a sequential Bonferroni to account for multiple comparisons.

The Impact of Mutation on Plasticity. To test the extent of variability in phenotypic plasticity (genotype × environment [G × E] effects), we used three approaches. First, we applied a two-factor mixed effects ANOVA under restricted maximum likelihood on either the raw data (for estimates of the interaction mutational heritability [h^2_mI]) or the treatment mean-scaled data (for estimates of the interaction mutational coefficient of variability [CV_mI]), with environment treated as a fixed effect, and line and line × environment interaction modeled as random effects in the lme4 package (Bates et al. 2014) in R for the same data set described above ($n = 5$ MA lines). In these models, we also included an observation-level factor to account for heterogeneity in the residual variance among treatments (for sample code and examples from B. Bolker, see http://rpubs.com/bbolker/varfac). From models applied to the raw data, we then extracted estimates of the line × environment interaction variance (which describes the amount of variation in reaction norms among lines) and the residual variance to serve as an estimate of V_e. From these, we calculated an estimate of h^2_mI by treating the interaction variance similarly to estimates of ΔV described above and then dividing this estimate by V_e. For models applied to the treatment mean-scaled data, we took the square root of estimates of the interaction variance to obtain an estimate of CV_mI. We obtained 95% confidence intervals for the variance components using the confint function.

We used Kruskal-Wallis tests in program R to compare the h^2_mI and CV_mI among trait types. To test the extent to which different environments elicit plasticity among MA lines, we pooled all trait-specific estimates of the interaction h^2_mI and CV_mI for each environment and then performed pairwise comparisons among the four environments. For example, an estimate of the amount of plasticity that the standard environment induces was obtained by averaging across all traits in the pairwise comparisons of standard versus copper, standard versus high temperature, and standard versus low food. We used Kruskal-Wallis and Wilcoxon tests in R to examine significant differences among environments.

For the second approach, we used all available data (that is, data for any trait in any pair of environments for which it was possible to measure the variance) instead of limiting our analyses to lines in which the same mutations would be present in all four environments as above. We used two-factor ANOVA to examine the mutational generation of G × E interactions. Specifically, we applied two-factor linear models with line and environment as main effects to test for a significant G × E interaction across all possible pairs of environments. For discrete traits (e.g., timing events and clutch sizes), we fit a generalized linear model with line and environment as main effects, assuming a Poisson distribution, and then obtained *P* values using a χ^2 test on the analysis of deviance table. For continuously distributed traits (e.g., sizes and growth rates), we fit a general linear model with line and environment as main effects and estimated *P* values with *F*-tests. In contrast to the previously described analyses in which we treated line and line × environment interaction as random effects in order to extract model variance components, in these tests line, environment, and interaction were all treated as fixed effects. As a consequence, the results we obtain from these analyses are specific to the lines and environments included in the ANOVA and are not necessarily generalizable to MA lines and environments not included in the analysis. We compared the number of significant environment, line, or interaction effects between trait types or pairs of environments using a χ^2 test or a *z*-test for proportions, respectively.

A significant interaction term in this model can occur either because the rank order of trait means among MA lines changes across environments or because of variance inflation in one of the environments relative to the other. To distinguish between these two possibilities, we inferred that any models that revealed a significant interaction term only (i.e., no significant main effects) indicated a change in rank order among the MA lines. Alternatively, any models that had at least one significant main effect (environment, line, or both) were interpreted as evidence of variance inflation in one of the two environments and verified by visual inspection of the reaction norm plot. To perform these analyses, we used all available MA lines (5–10 lines) with sufficient within-line replication in a pair of environments ($n > 2$) and performed all possible pairwise environment comparisons for each trait, resulting in 96 total ANOVAs.

Finally, in addition to the pairwise environmental comparisons described above, we used two-factor linear modeling to test for G × E effects across all environments simultaneously. We used the same approach as described above—fixed-effects generalized linear models assuming a Poisson distribution for discrete traits and a normal distribution for continuously distributed traits. In this analysis, we used only the five MA lines with sufficient replication ($n > 3$) for each trait in all environments.

Results

The Impact of Mutation on Different Traits and in Different Environments

Life-history traits had significantly higher levels of mutational variation than morphological traits based on es-

timates of h^2_m ($\chi^2_1 = 8.06$; $P = .005$; fig. 1; tables A2, S1). The two methods we used to estimate CV_m were statistically equivalent (fig. A1; table A2), and therefore we present only the results for the CV_m estimates obtained from the data that was scaled to the mean prior to ANOVA in the main text; results for both methods of CV_m estimation can be found in the appendix. The scaled data indicated that estimates of CV_m were also higher for life-history traits than for morphological traits ($\chi^2_1 = 13.27$; $P = .000$; figs. 1, A1; tables A2, S1). Kruskal-Wallis tests also indicated a significant effect of the environment on the amount of expressed mutational variation (figs. 1, A1; tables A2, S1) for both estimates of h^2_m ($\chi^2_3 = 15.90$; $P = .001$) and CV_m ($\chi^2_3 = 11.37$; $P = .010$). In particular, Wilcoxon tests indicated that high temperature resulted in significantly higher estimates of h^2_m than the other three environments and that the standard conditions had higher estimates of h^2_m than the copper environment (table A2). The standard and high-temperature environments also had higher estimates of CV_m than the copper environment (table A2). However, after Bonferroni correction, high temperature resulted in higher estimates of h^2_m and CV_m only when compared to the environment with added copper (table A2). The mean trait values and associated standardized mutational variances (CV_m) for each trait in each environment are presented in figure A2.

The Impact of Mutation on Plasticity

Estimates of h^2_mI and CV_mI reflect the influence of mutation on phenotypic plasticity among MA lines. While estimates of h^2_mI did not differ between life-history and morphological traits, estimates of CV_mI were significantly higher for life-history traits than for morphological traits (fig. 2; tables A2, S2). Estimates of both h^2_mI and CV_mI did vary according to environment, (fig. 2; tables A2, S2). Specifically, pairwise comparisons in which high temperature was one of the two environments resulted in higher estimates of h^2_mI and CV_mI than pairwise comparisons that included copper exposure or low food (tables A2, S2). After Bonferroni correction, none of the comparisons between the high-temperature and copper environments were significant.

The 96 two-factor ANOVAs employed to test for significant effects of environment, line, and/or line × environment interactions across pairs of environments are presented in table A5 (with illustrations of each predicted effect in fig. 3*A*–3*D*). In 40 cases, there was at least a significant effect of environment (e.g., fig. 3*E*), 16 cases showed at least a significant effect of line (e.g., fig. 3*F*), and 17 cases indicated at least a significant interaction (e.g., fig. 3*G*, 3*H*). In 10 of the 17 cases, where there was a significant interaction detected, there were no main effects of environment or line (table A3), which means there were changes in the rank order of MA line trait values across environments. The remaining 7 ANOVAs were accompanied by a significant effect of environment, line, or both (table A3). In these cases, visual inspection of the reaction norm plots indicated that the interaction terms were due to an increase in variance in one of the two environments compared.

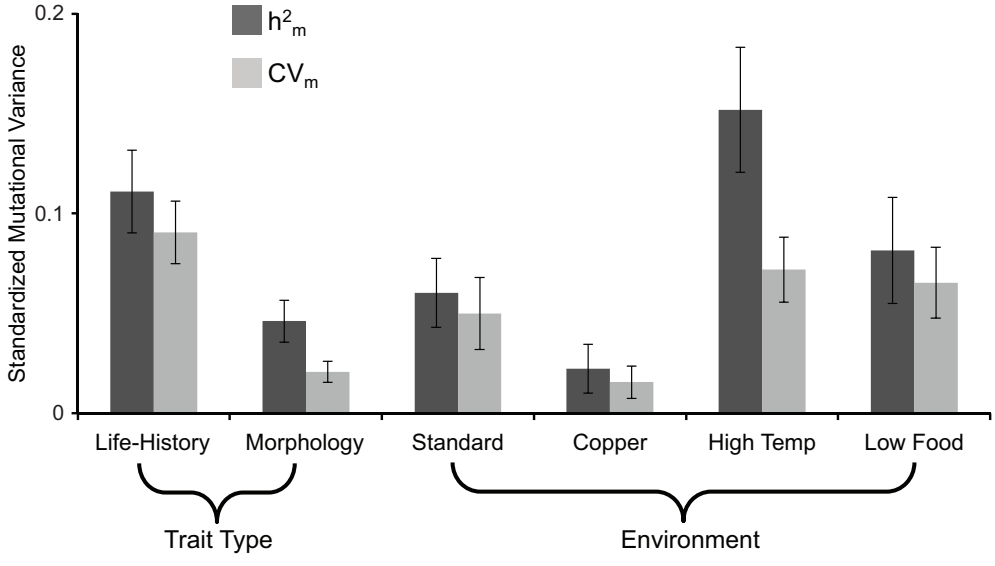

Figure 1: Average estimates of standardized metrics (mutational heritability [h^2_m] and coefficient of mutational variability [CV_m]) that describe the amount of expressed mutational variance for each of the trait types (life history or morphological) and for each environment. Error bars are ±SE.

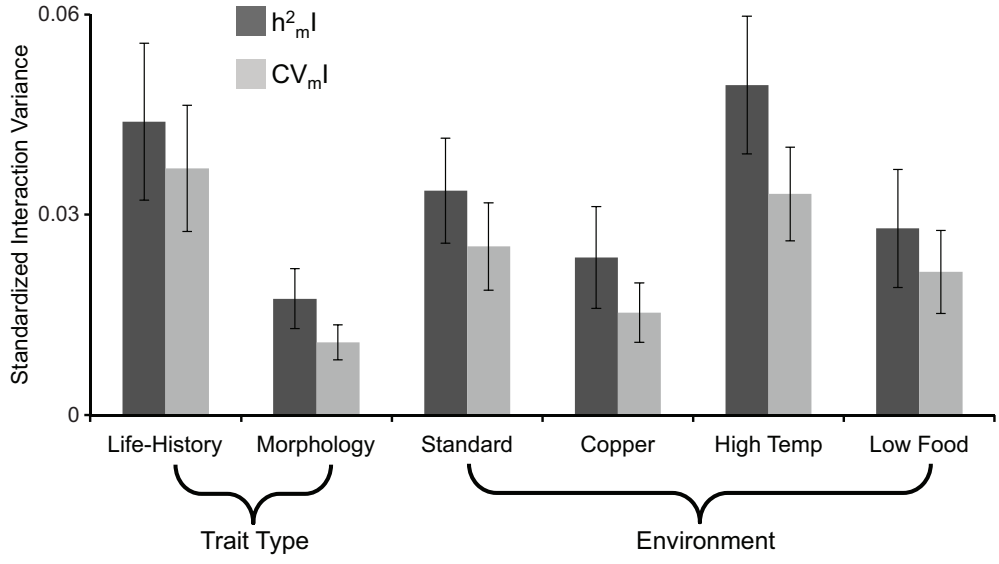

Figure 2: Average estimates of the interaction variance (interaction heritability [h^2_mI] and interaction mutational coefficient of variability [CV_mI]) for the two types of traits and four different environments. Estimates for the environments were generated by including all pairwise environmental comparisons in which the focal environment was one of the two environments compared. Error bars are ±SE.

A detailed examination shows that life-history and morphological traits do not differ, in terms of the proportions of these trait types exhibiting environment, line, or interaction effects (table A4). The low-food environment accounts for the largest proportion (34%) of environment effects and is significantly higher than the standard and high-temperature treatments, although neither is significant after Bonferroni correction (fig. 4; table A4). The high-temperature environment accounts for the highest proportion of line (38%) and interaction (47%) effects (fig. 4; table A4). The proportion of line effects for high temperature is significantly higher than the proportion in the copper environment only, but not after Bonferroni correction (table A4). The proportion of interaction effects for high temperature is significantly higher than all of the other environments; however, after Bonferroni correction, the proportion of significant high-temperature interaction effects is only significantly higher than the low-food treatment.

Finally, we used a set of separate two-factor ANOVAs to test for main effects and interactions for each of the 16 traits across all four of the environments simultaneously. Significant line × environment effects were observed for 3 traits (second clutch size, second adult instar body size, and juvenile growth rate [fig. 5]), and main effects of line and environment were also observed for 3 and 10 traits, respectively (full results in table A5).

Discussion

Mutation is the ultimate source of new genetic variation, but how genetic variation manifests as phenotypic variation can depend on the genetic architecture of a trait and the influence of the environment. Specifically, the genetic architecture of a trait determines how much variation can be generated for that trait by mutation (Lynch et al. 1999). For example, traits with complex genetic architectures have more mutational targets and may display either more variability because of mutation (Houle et al. 1996) or greater robustness to mutations (de Visser et al. 2003) than do traits with simple genetic architectures. The environment in which a mutation occurs can also influence how strong an effect that mutation has on a phenotype (e.g., Fry et al. 1996; Vassilieva et al. 2000; Baer 2008). Characterizing the effects of mutation across trait types and environments can deepen our understanding of the evolutionary process.

In our study, life-history traits exhibited higher levels of mutational variability than morphological traits, supporting our prediction that life-history traits likely have more mutational targets than morphological traits. These results were consistent between the two dimensionless metrics (h^2_m and CV_m; fig. 1) and were consistent in each environment (figs. A1, A2). Specifically, nonparametric comparisons of h^2_m and CV_m, estimated from five MA lines, indicate significantly higher estimates of standardized mutational variance in life-history traits than in morpholog-

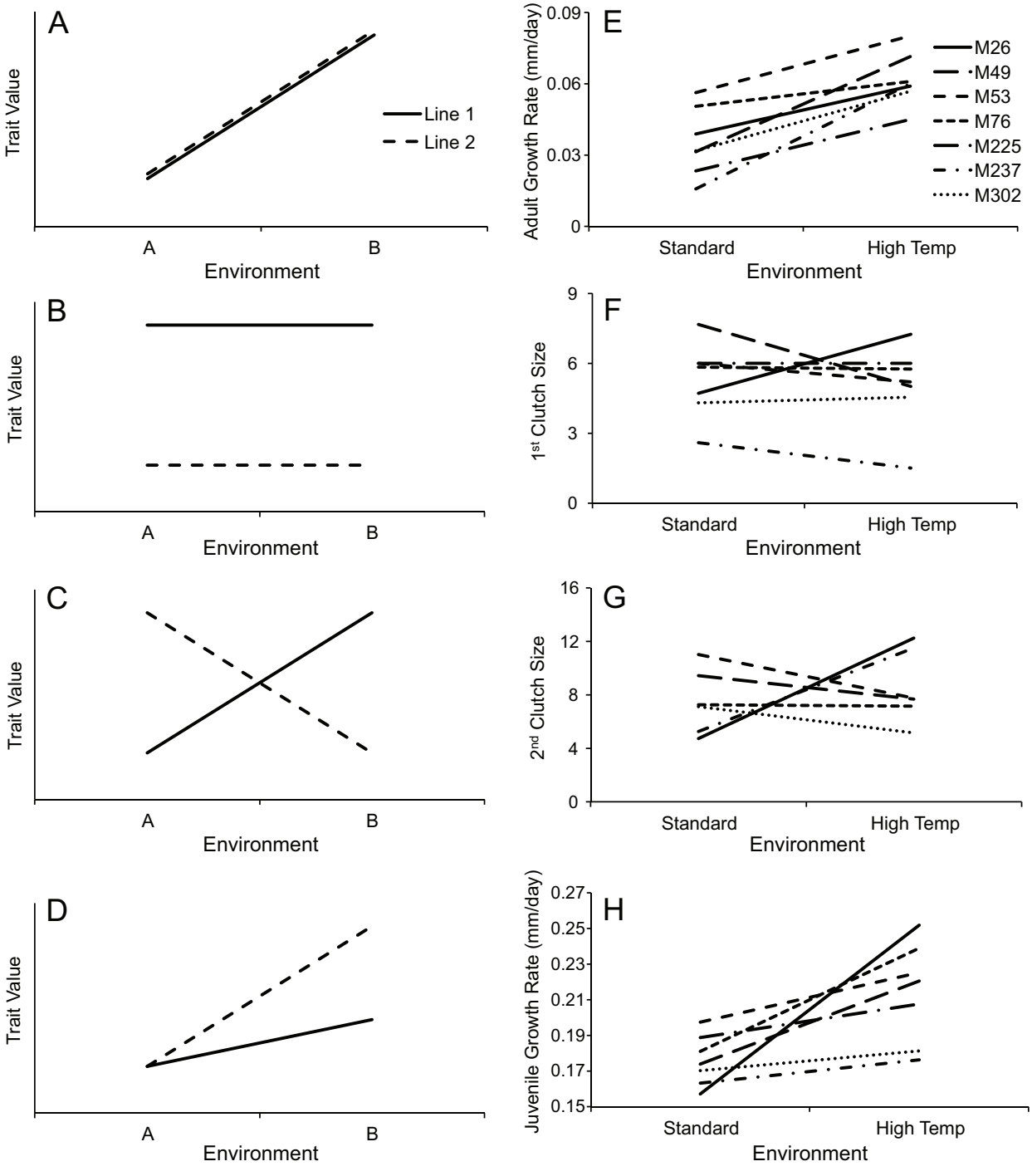

Figure 3: Examples of predicted (*left column*) and observed (*right column*) trait values for mutation-accumulation (MA) lines assayed in two environments when there are significant effects of environment (*A*, *E*), MA line (*B*, *F*), and environment × line interactions (*C*, *D*, *G*, *H*). Predicted patterns are illustrated for only two MA lines, whereas observed trait values are shown for all MA lines with sufficient replication.

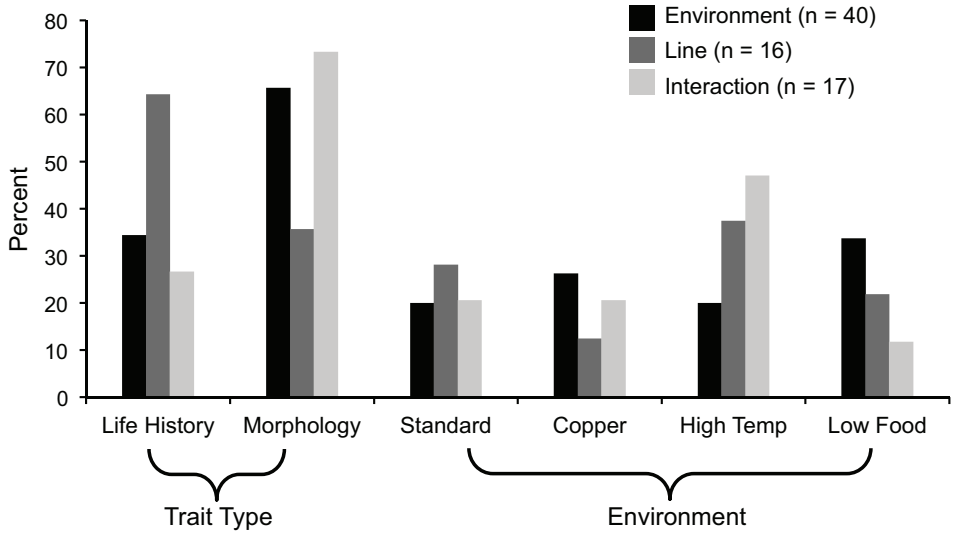

Figure 4: Percentage of environment, line, and environment × line effects obtained based on two-factor ANOVA for either trait type or environment.

ical traits (fig. 1). This result is corroborated by ANOVAs in which there was a nonsignificant trend showing a higher proportion of significant main effects of line in life-history traits than in morphological traits (fig. 4; table A4). Previous studies using an MA framework have also suggested that life-history traits diverge faster and have more complex genetic architectures than morphological traits (Houle et al. 1996; Lynch et al. 1999).

Just as life-history traits accrue mutational variance faster than morphological traits, our estimates of h^2_mI and CV_mI, which describe the amount of plasticity in a trait, also accrue mutational variance faster for life-history traits than for morphological traits. These results suggest that plasticity in life-history traits may have more mutational targets than plasticity in morphology and, perhaps more generally, that the complexity of the genetic architecture for a trait is correlated with the architecture for plasticity in that trait as well. This difference was observed only in our estimates of CV_mI, however, limiting our confidence in this scenario versus the alternative (loci controlling life-history and morphological traits are either a subset of, or different from, the loci influencing plasticity of these traits). Additional empirical work could distinguish between these two models in order to understand the architecture of plasticity further.

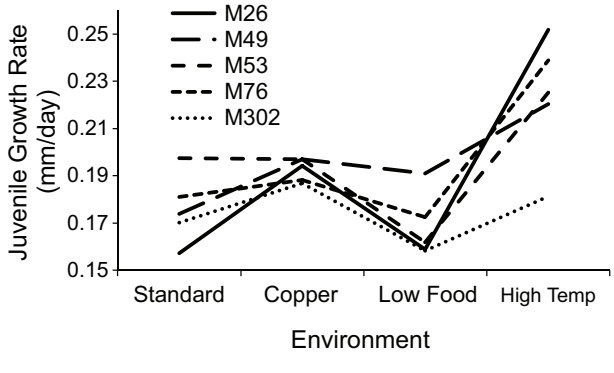

Figure 5: Rank-order changes of five mutation-accumulation lines (M26, M49, M53, M76, and M302) across four environments for a composite trait, juvenile growth rate.

Environment-dependent expression of mutational variance (fig. 1) drives variation in plasticity among these lineages. Specifically, it changes (*a*) the rank order of MA line-specific phenotypic means across environments (e.g., fig. 5) and (*b*) the variance among MA lines across environments (figs. 3*E*–3*H*). Both of these outcomes are important for evolution. First, changes in rank order provide a mechanism for the maintenance of genetic diversity in natural populations that occupy spatially or temporally variable environments (Gillespie and Turelli 1989). Additionally, changes in rank order across environments result in trade-offs between genotypes and provide a mechanism for the evolution of ecological specialization (Futuyma and Moreno 1988).

The second outcome, differences in variance across environments, means that the efficacy of selection can vary across environments, which can effect the rate at which populations evolve. For example, populations in environments with greater levels of expressed mutational variance could evolve faster than populations in environments that

mask mutational variation (Goddard et al. 2005). While levels of mutational variance were lowest in the copper exposure, they were highest in the high-temperature environment (fig. A2). This finding is corroborated by studies looking at mutational variance in flies, where both high (Fernandez and Lopez-Fanjul 1997) and low (Fry et al. 1996) temperatures have been shown to increase variance relative to standard laboratory temperatures. In light of current concerns about global climate change, this observation poses an interesting question—to what extent can the release of expressed mutational variance by changing temperature mitigate the negative biological consequences of global climate change?

Understanding the context dependence of spontaneous mutations is a major challenge for understanding the evolutionary process. Life-history and morphological traits vary in the rate at which they accrue mutational variation reflecting significant differences in their underlying genetic architecture. Further, the impact of mutation on plasticity varies among trait types and environments. Finally, for some traits, the mean values for each lineage change rank order, and the levels of observable mutational variance change across environments. Together, such variable levels of expression of mutational variance across traits and environments have major consequences, especially in habitats that are complex or in which things have changed rapidly. These findings, while illuminating, underscore the need for a deeper understanding of how the mutational process shapes the relationship between genotype and phenotype and, thereby, evolutionary change.

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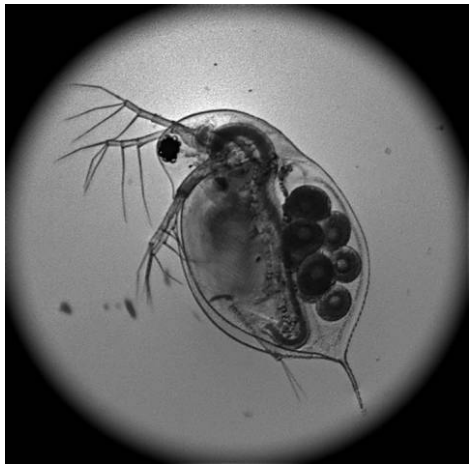

A female *Daphnia pulex*. This aquatic microcrustacean serves as an important model system in ecology and evolution. Photo credit: Sarah Schaack.