Hierarchical Assessment of Mutation Properties in Daphnia magna

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ABSTRACT Understanding the context-dependence of spontaneous mutations is crucial to predicting evolutionary trajectories. In this experiment, the impact of genetic background and trait-type on mutational susceptibility was investigated. Mutant and non-mutant lines of six unique genotypes from two populations of Daphnia magna were phenotypically assayed using a common-garden experiment. Morphological, life-history, and behavioral traits were measured and estimates of the mutation parameters were generated. The mutation parameters varied between the populations and among genotypes, suggesting differential susceptibility to mutation depending upon genomic background. Traits also varied in their susceptibility to mutation with behavioral traits evolving more rapidly than life-history and morphological traits. These results may reflect the unique selection histories of these populations.

Spontaneous mutations, uncorrected errors that occur during DNA replication and repair, are the ultimate source of genetic variation. Small fractions of incoming mutations are beneficial, provide the fuel for adaptive evolution (Batallion 2000; Eyre-Walker and Keightley 2007; Perfeito et al. 2007). However, a majority of spontaneous mutations are either neutral or deleterious with respect to fitness (Eyre-Walker and Keightley 2007; Halligan and Keightley 2009). Normally, purifying selection eliminates a majority of the deleterious variants from a population, leaving behind the beneficial and neutral mutations. However, in populations where selection is relaxed or eliminated genetic drift governs the fate of incoming mutations. Unlike selection, which fixes beneficial mutations in a population, genetic drift results in the stochastic fixation of mutations, regardless of the effect of those mutations (erratum: Nature 453: 128) on fitness. Given that the deleterious mutation rate is much higher than the beneficial mutation rate (Kibota and Lynch 1996; Eyre-Walker and Keightley 1999; Denver et al. 2004; Haag-Liautard et al. 2007), populations that evolve under genetic drift accumulate deleterious mutations, and experience a gradual erosion of fitness. In populations where conditions of relaxed selection persist for extended periods, the interaction between genetic drift and deleterious mutations can ultimately result in the extinction of the population through mutational meltdown (Lynch et al. 1993; Lande 1994; Lynch et al. 1995; Zeyl et al. 2001).

Despite the importance of mutation in shaping the attributes of natural populations, our understanding of the context-dependence of mutation parameters, particularly the phenotypic effects of mutations, is still limited. Current evidence indicates that mutational effects vary in different environments. For example, mutational effects can be exacerbated under stressful environments (e.g., Kondrashov and Houle 1994; Latta et al. 2015), particularly when the environmental stressor is high population density (Agrawal and Whitlock 2010). However, non-density forms of environmental stress have varying influence on mutational effects, including reducing and even eliminating the effects of mutation on fitness (Agrawal and Whitlock 2010). Additionally, there appears to be genotypic variation in the spontaneous mutation parameters. For example, some genotypes within a species have high mutation rates, while other genotypes have low mutation rates (Demerec 1937; Haag-Liautard et al. 2007; Latta et al. 2013). Finally, traits that differ in their genetic architecture are differentially susceptible to the effects of mutation accumulation (MA) (Houle 1992; Houle et al. 1996; Rowe and Houle 1996; Lynch et al. 1999; Halligan and Keightley 2009; Latta et al. 2015). Specifically, traits with complex genetic architectures controlled by numerous genetic loci, such as life history traits, appear to be more prone to MA than...
traits with simple genetic architectures, such as morphological traits (Houle 1992; Latta et al. 2015). Additionally, evidence suggests that quantitative traits have higher mutational heritability than gene expression traits, which may be due to the large number of genes controlling quantitative traits, or because gene expression traits are more sensitive to environmental variation (Rifkin et al. 2005; Huang et al. 2016).

Genotypic variation in the mutation parameters has been demonstrated in several organisms including Drosophila melanogaster (Haag-Liautard et al. 2007), Chlamydomonas reinhardtii (Kraemer et al. 2017), Daphnia pulex (Latta et al. 2013), and various species of rhabditid nematodes (Baer et al. 2005). The patterns that have emerged from these studies suggest that differences in the underlying mutation rates among genotypes, and/or differences in the epistatic interactions between new mutations and the genomic background in which they arise, may contribute to variation in estimates of mutational effects (Remold and Lenski 2004; Weinreich et al. 2005; Phillips 2008; Le Gac and Doebeli 2010; Ness et al. 2015; Kronholm et al. 2017). One genomic feature that predicts genotypic variability in the mutation parameters is the pre-existing load of deleterious mutations the genotype harbors. Several studies report increases in mutation rate estimates in genotypes with high initial mutation loads (Ávila et al. 2006; Agrawal and Wang 2008; Sharp and Agrawal 2012), which may contribute to rapid changes in fitness in these loaded genotypes.

Trait variation in mutation rates and effects has also been demonstrated in numerous systems. The working hypothesis is that physiologically complex traits controlled by numerous genetic loci present large mutational targets because the number of genetic loci controlling a trait is directly proportional to the mutation rate for that trait (Houle 1992; Houle et al. 1996; Rowe and Houle 1996; Latta et al. 2015). For example, fitness traits display greater mutational susceptibility than morphological traits, putatively due to the complex genetic architecture of life-history traits relative to morphological traits. One set of traits that may be especially susceptible to mutation due to its complex genetic architecture are those under the control of the nervous system, such as behavioral traits. The effect of spontaneous mutation on neural function, and on behavior specifically, has largely been neglected with most phenotypic studies focusing on life-history and morphological traits (e.g., Azevedo et al. 2002; Estes et al. 2004; Latta et al. 2015). However, the effect of mutation on behavioral traits has been investigated in a few systems. In Caenorhabditis elegans, behavioral performance declines at a rate similar to fitness traits (Ajie et al. 2005; Estes et al. 2005). Similarly, MA lines of Drosophila are less motile than associated control populations (Lattimer et al. 2014), and mutations have a stronger effect on male reproductive performance than female reproductive performance (Mallet and Chippindale 2011; Mallet et al. 2012; Sharpe and Agrawal 2013; Almbror and Simmons 2014), both of which suggest these observations may be explained by differences in the mutational target size of the behavioral traits investigated.

To examine genotypic and trait-specific variation in susceptibility to the effects of mutation, we conducted an MA experiment on six unique genotypes of Daphnia magna isolated from a broad latitudinal gradient. Daphnia are an ideal system for MA experiments as they can be maintained clonally in the lab, which allows the accumulation of mutations in a heterozygous state in naturally occurring genomes (Keith et al. 2016). Following MA, a phenotypic assay in which life-history, morphological, and behavioral traits were measured was used to compare the performance of the mutation lines and controls. This experimental design allows us to use a hierarchical approach to simultaneously examine both genotypic and trait-specific variability in mutational effects, and test the nature of the context-dependence of spontaneous mutation.

**MATERIALS AND METHODS**

**Study System**

The aquatic microcrustacean Daphnia is a model organism for ecological and evolutionary biology studies (e.g., Miner et al. 2012). The cyclical parthenogenetic nature of Daphnia makes them an ideal organism to use in MA experiments because clonal reproduction can be maintained in the lab using specific environmental conditions. In these experiments, D. magna were reared under a 16L:8D photoperiod at a constant temperature of 18°C.

The D. magna genotypes used in this experiment were collected along a latitudinal gradient that captures a range of environmental variation including temperature and photoperiod (Table S1). Three unique genotypes from each of two populations (Germany and Israel) were used to initiate the control and mutant lines. These populations capture variation in local selective pressures that may influence the phenotypic effects of mutation. Importantly, this sampling design permits an assessment of mutation parameter variability both among genotypes within a population, and among populations.

The stock cultures for each genotype were maintained in 250 mL beakers containing 175–200 mL of Aechener Daphnien Medium (ADaM; Klüttgen et al. 1994) under a constant photoperiod (16L:8D) and temperature (18°C), and fed the unicellular green alga Scenedesmus obliquus ad libitum (2-3 times per week). One concern in MA experiments is the maintenance of a stable control during the MA phase of the experiment. In Daphnia, control lines are maintained by establishing populations of large size, which minimizes the number of incoming deleterious mutations (Flynn et al. 2016). However, this approach also generates opportunity for control lines to adapt to the lab environment during the MA phase. In order to minimize the possibility of adaptation in the control populations during the MA phase we maintained stock cultures of each genotype for 6 months under these lab conditions prior to initiation of the MA experiment.

**Mutation Accumulation Experiment**

Control and mutant lines were initiated from clonally produced offspring of a single asexual female isolated from the stock cultures of each genotype (Table S1). Control lines were maintained in two replicate 3 L jars containing 2 L of ADaM, under constant temperature (18°C) and photoperiod (16L:8D), and fed the unicellular green alga Scenedesmus obliquus ad libitum. The media in the jars was replaced every 2-3 weeks, and individuals in the two replicate jars were mixed to maintain as much genetic variation including temperature and photoperiod (Table S1). Three unique genotypes from each of two populations (Germany and Israel) were used to initiate the control and mutant lines. These populations capture variation in local selective pressures that may influence the phenotypic effects of mutation. Importantly, this sampling design permits an assessment of mutation parameter variability both among genotypes within a population, and among populations.

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Mutant lines for each genotype were established by generating five replicate clonal lineages from each of six genotypes (30 lineages total). These lineages (MA lines) were initiated by placing a single clonally produced female in a 250 mL beaker containing 100 mL of ADaM supplemented with Scenedesmus obliquus at a concentration of 600,000 cells/mL. All MA lines were maintained in conditions identical to the control lines (16L:8D, 18°C). The food/media mixture in each beaker was replaced once per week, and each line was fed a prescribed volume of concentrated Scenedesmus three days after the media replacement to reset the algal cell concentration in the beaker to 600,000 cells/mL.
Each MA line was propagated from generation to generation over the course of the experiment via single offspring descent by taking a single juvenile from the second clutch of the mother. A series of backups were maintained in parallel with the focal lineages in the event that the single individual intended to be used to establish the next generation died before reproduction, or was a male. In the event that the focal lineage and all backups either died before reproduction, or were all males, the lineage was declared extinct and a new replicate lineage was established from the control lines. In this experiment there were two mutant lines, both descended from a single German genotype, which were restarted from the control population. For these lines, the number of generations of divergence from the control population was calculated from the time of line re-initiation. Thus, our estimates of the number of generations of divergence presented here represent the number of generations in which lines evolved under genetic drift after isolation from the control population. The two lines that were restarted from the control populations undoubtedly experienced mutation accumulation while in the control populations. However, individuals in control populations are under strong purifying selection (Flynn et al. 2016), so the mutations that these lines accrued while in the control population should be predominantly neutral with respect to fitness. In total, the MA phase of the experiment was conducted for approximately 2.5 years and resulted in an experiment-wide average of 22 generations of divergence between MA lines (Table S2).

**Phenotypic Assay**

Life-history, morphological, and behavioral traits for mutant and control individuals were assayed simultaneously using a common-garden experiment. Single juvenile females (five for each MA line, and 15 for each control line) were isolated from the MA lines or control populations and placed in 150 mL beakers containing 100 mL of ADaM supplemented with *S. obliquus* to yield a concentration of 600,000 cells/mL. Beakers were randomized on trays and placed in an environmental chamber under standard laboratory conditions (16L:8D, 18°C). Over the course of the assay the food/media mixture was replaced every other day to ensure individuals had sufficient food, and the trays containing beakers were rotated in the environmental chamber every day to minimize the effect of micro-environmental differences within the chamber. The single females were reared under these conditions until release of their second clutch. This one-generation acclimation period serves to minimize maternal effects. Two to four individuals were then isolated from the second clutch and the mother was removed from the beaker. The date of birth of the second clutch individuals was recorded, and then the individuals were reared until maturity (first deposition of eggs in the carapace) and the date recorded to allow an estimate of age at maturity.

Upon reaching maturity, individuals were placed under a Leica M1G5C microscope and the number of eggs in the carapace were counted to obtain an estimate of fecundity. Body size at maturity and behavioral data were determined using a Leica microscope and camera to obtain video recordings of mature individuals. Body size was measured from still frames isolated from the video. Behavior of individuals was assessed by placing them in a 16 mm diameter by 3 mm depth arena containing 700 µL of ADaM and recording movement within the arena for 20 sec. Individual trajectories were then tracked with ImageJ software (Schneider et al. 2012) using the MTrack plugin (Meijering 2008). Three aspects of behavior were quantified using the tracking data: 1) maximum velocity, 2) mean velocity, and 3) the standard deviation of velocity (which provides an estimate of the erratic nature of movement).

**Data Analysis**

Estimates of the mutational bias (ΔM), a mutation parameter that describes the sign and magnitude of phenotypic change resulting from mutation, were obtained for each trait for each of the six genotypes by determining the mutation-line mean phenotype (zm) for each of the five MA lines, and the mean phenotype (z0) from control lines. The per-generation change in mean phenotype (Rm) was estimated as the slope of the weighted least-squares regression of zm on the line-specific number of generations of divergence where estimates of zm were used to set the y-intercept and estimates of z0 were weighted by the inverse of their sampling variance. Estimates of ΔM were generated by scaling Rm by the control mean phenotype (z0). Because ΔM is a scaled metric, it facilitates comparisons between traits and genotypes that may have differed in initial mean phenotype.

In order to compare estimates of ΔM among traits, genotypes, and populations we used Kruskal-Wallis tests with our estimates of ΔM. Specifically, to assess variation in trait susceptibility to mutation we used estimates of the absolute value of ΔM, which represents the magnitude of phenotypic change in response to mutation. To assess variation in ΔM at the level of genotype and population we used the original estimates of ΔM, which represents both the magnitude and direction of phenotypic change in response to mutation.

Estimates of evolvability (CVm2), which describes the per-generation rate of input of new mutational variance, were generated by first scaling the raw data by the corresponding MA line mean. The scaled data for a genotype-specific trait was then subjected to variance partitioning using a random effects model under restricted maximum likelihood (REML) as implemented by the lme4 package (Bates et al. 2015) in Program R (R Core Team 2016). This procedure yielded estimates of the within- and among-line components of variance, which correspond to the environmental variance (Ve) and genetic variance among MA lines (Vg), respectively. Estimates of CVm2 for each genotype-specific trait were calculated by dividing Vg by the number of generations of mutational divergence averaged across all MA lines for a genotype. Because the data were scaled prior to analysis, the estimates of CVm2 we obtain are dimensionless and allow comparisons among traits and genotypes.

**Data Availability**

Data are provided with this article as a supporting file (File S1). Supplemental material available at Figshare: https://doi.org/10.25387/g3.6799034.

**RESULTS**

**Mutational Bias and Evolvability**

The majority of ΔM estimates for each genotype-specific trait were significant (Table 1; Table S2). The individual estimates varied by two orders of magnitude and varied in sign, with some traits increasing in response to mutation while other traits decreased. In contrast, the majority of CVm2 estimates measured after an average of 22 generations of divergence were not significantly different from zero (Table 1; Table S2). Significant estimates of CVm2 occurred solely within genotype GC. Specifically, age at maturity, egg number, max velocity, and mean velocity showed significant estimates of CVm2 within genotype GC (Table 1; Table S2).

**Mutational Bias Among Traits**

Estimates of the absolute value of ΔM, which represents the magnitude of phenotypic change, was high for maximum velocity, mean velocity, and standard deviation of velocity relative to age at maturity, body size, and egg number (Figure 1A). However, the estimates of ΔM did not
significantly differ among individual traits (Figure 1A; Table 1; Table S3). When the individual traits were pooled into behavioral traits (max velocity, mean velocity, and standard deviation of velocity) and non-behavioral traits (age at maturity, body size, and egg number), behavioral traits displayed significantly larger estimates of the absolute value of $\Delta M$ than non-behavioral traits (Figure 1B; Table S3).

**Mutational Bias Among Genotypes**

Estimates of $\Delta M$ varied significantly among individual genotypes comprising the German and Israel populations (Figure 1C; Table 1; Table S3). Overall, the three genotypes within the German population were characterized by negative estimates of $\Delta M$ for a majority of the traits examined (Table 1). Averaged across traits, two of the three genotypes (GA and GB) had overall negative estimates of $\Delta M$ (Figure 1C; Table 1; Table S3). In contrast, the trait-specific estimates of $\Delta M$ for the Israel genotypes were predominantly positive, resulting in genotypic estimates of $\Delta M$ that are positive for two of the three genotypes (IA and IB; Figure 1C; Table 1; Table S3).

**Mutational Bias Among Populations**

Estimates of $\Delta M$ for the German and Israel populations, obtained by pooling the trait-specific estimates across all genotypes derived from each population, were significantly different (Figure 1C; Figure 1D; Table 1; Table S3). Specifically, the average estimate of $\Delta M$ for the German population was negative, indicating that trait values tend to decrease relative to the control following MA. Alternatively, the average estimate of $\Delta M$ for the Israel population was positive, suggesting trait values tend to increase relative to the control following MA.

**DISCUSSION**

Spontaneous mutations are the ultimate source of genetic variation, but our understanding of the context-dependence of mutation parameters is still limited. Environmental differences, genotypic variation and genetic architecture are all factors that influence estimates of spontaneous mutation rates and effects. For example, stressful environments can intensify or weaken mutational effects (Agrawal and Whitlock 2010). Additionally, traits that have complex genetic architectures are especially prone to spontaneous mutations because of their larger mutational target size (Houle 1992; Landry et al. 2007). Investigating both genotypic and trait-specific variability in mutational effects provides a means to understand the various context-dependencies of spontaneous mutations.

| Population | Genotype | Trait  | GOD  | $z_0$ | $\Delta M$ | CVm² |
|------------|----------|-------|------|-------|------------|------|
| G          | A        | AM    | 19.2 | 14.2  | -0.0038    | 0.0000|
| G          | A        | Egg   | 19.2 | 6.2   | -0.0037    | 0.0000|
| G          | A        | Size  | 19.2 | 3.1   | -0.0001    | 0.0000|
| G          | A        | MaxV  | 19.2 | 49.8  | -0.0438    | 0.0000|
| G          | A        | MeanV | 19.2 | 10.3  | -0.0400    | 0.0000|
| G          | A        | SDV   | 19.2 | 9.2   | -0.0424    | 0.0000|
| G          | B        | AM    | 21.4 | 13.4  | -0.0038    | 0.0000|
| G          | B        | Egg   | 21.4 | 4.9   | 0.0022     | 0.0000|
| G          | B        | Size  | 21.4 | 2.9   | 0.0005     | 0.0000|
| G          | B        | MaxV  | 21.4 | 25.4  | -0.0275    | 0.0000|
| G          | B        | MeanV | 21.4 | 4.7   | -0.0257    | 0.0000|
| G          | B        | SDV   | 21.4 | 4.2   | -0.0277    | 0.0000|
| G          | C        | AM    | 22.8 | 14.2  | 0.0003     | 0.0000|
| G          | C        | Egg   | 22.8 | 4.3   | -0.0028    | 0.0053|
| G          | C        | Size  | 22.8 | 3.0   | -0.0005    | 0.0000|
| G          | C        | MaxV  | 22.8 | 73.4  | 0.0006     | 4.9231|
| G          | C        | MeanV | 22.8 | 16.7  | 0.0003     | 0.0048|
| G          | C        | SDV   | 22.8 | 13.7  | -0.0015    | 0.0002|
| I          | A        | AM    | 24.8 | 14.8  | -0.0013    | 0.0000|
| I          | A        | Egg   | 24.8 | 6.0   | 0.0017     | 0.0000|
| I          | A        | Size  | 24.8 | 2.9   | 0.0021     | 0.0000|
| I          | A        | MaxV  | 24.8 | 49.9  | 0.0085     | 0.0000|
| I          | A        | MeanV | 24.8 | 7.8   | 0.0125     | 0.0000|
| I          | A        | SDV   | 24.8 | 9.2   | 0.0083     | 0.0000|
| I          | B        | AM    | 23.0 | 14.0  | 0.0007     | 0.0000|
| I          | B        | Egg   | 23.0 | 6.6   | 0.0033     | 0.0000|
| I          | B        | Size  | 23.0 | 2.9   | 0.0010     | 0.0000|
| I          | B        | MaxV  | 23.0 | 69.5  | 0.0048     | 0.0000|
| I          | B        | MeanV | 23.0 | 14.9  | 0.0001     | 0.0000|
| I          | B        | SDV   | 23.0 | 14.3  | 0.0005     | 0.0000|
| I          | C        | AM    | 23.4 | 14.2  | -0.0001    | 0.0000|
| I          | C        | Egg   | 23.4 | 6.5   | 0.0016     | 0.0000|
| I          | C        | Size  | 23.4 | 3.1   | -0.0001    | 0.0000|
| I          | C        | MaxV  | 23.4 | 66.5  | -0.0032    | 0.0000|
| I          | C        | MeanV | 23.4 | 10.8  | -0.0020    | 0.0000|
| I          | C        | SDV   | 23.4 | 11.7  | -0.0003    | 0.0000|
In the context of trait evolution by mutation, the magnitude of $\Delta M$ estimates is hypothesized to be directly proportional to the mutational target size for a trait. In our examination of trait evolution within *D. magna*, the absolute value of $\Delta M$ was higher for behavioral traits than other phenotypic traits. The greater sensitivity of behavioral traits (mean velocity, max velocity, and standard deviation of velocity) to spontaneous mutations suggests that the genetic architecture underlying these traits is more complex than the genetic architecture of life-history traits (age at maturity and egg number), and morphological traits (body size). Given that previous studies indicate life-history traits have a high susceptibility to mutation relative to morphological traits due to the complex genetic architecture associated with life-history traits (Houle et al. 1996; Latta et al. 2015), results from our phenotypic assay suggest behavioral traits may be more complex than life-history traits. These results agree with previous MA experiments that found behavioral traits are large mutational targets (Ajie et al. 2005; Estes et al. 2005). There was little evidence to suggest that significant mutational variability for the traits arose during the experiment, with only age at maturity, egg number, max velocity, and mean velocity in genotype GC producing significant estimates of $CV_m^2$, (Table S2). The limited divergence among MA lines, reflected by the large proportion of non-significant estimates of $CV_m^2$, is likely due to a combination of the low number of generations of divergence among MA lines for each genotype (five lines per genotype) and the limited number of mutant lines associated with each genotype (five lines per genotype).

In general, variation in $\Delta M$ among genotypes indicates that genomic background may influence the rate at which phenotypic evolution occurs in the absence of selection. Specifically, the magnitude of $\Delta M$ may be an indicator of the underlying mutation rate (Remold and Lenski 2004; Weinreich et al. 2005; Phillips 2008; Le Gac and Doebeli 2010; Ness et al. 2015; Kronholm et al. 2017), while the sign of $\Delta M$ may indicate the context of the ancestral selective regimes. Estimates of $\Delta M$ also varied significantly among individual genotypes within *D. magna*. Two genotypes that originated from the German population (GA and GB) exhibited large negative estimates of $\Delta M$, while genotype GC had a small negative estimate of $\Delta M$. In contrast, genotypes IA and IB from the Israel population exhibited intermediate positive estimates of $\Delta M$, while genotype IC was slightly negative. The variation in $\Delta M$ among genotypes observed here may reflect differences in the underlying mutation rate and/or type of selection acting on specific clonal genotypes. Genotypes that evolved quicker, genotypes GA, GB and IA, may have elevated mutation rates. These genotypes may have also experienced strong directional selection due to the local predation regime and may be optimally adapted to high predator densities. In contrast, genotypes that evolved slowly (GC, IB, and IC), may have lower underlying mutation rates, and also may be a result of stabilizing selection resulting from fluctuating selection that arises due to seasonal changes in predator density. Thus, these genotypes may be optimally adapted to intermediate or low predator densities. While these suppositions require further experimentation, they may help demonstrate the influence of fluctuating environments on genetic diversity within populations, and its influence on genotypic susceptibility to mutation.

A comparison of mutation parameter estimates from two ecologically divergent populations of *D. magna* indicated that the magnitude of $\Delta M$ in the German population was greater than the estimate from the Israel population, suggesting traits in the German population evolve under relaxed selection faster than traits in the Israel population. Additionally, the sign of $\Delta M$ for the German population is negative while the estimate from the Israel population is positive. Mutated lines among the Israel population displayed increased first egg number, increased body size, and higher velocities relative to non-mutated controls. A possible explanation for this seemingly adaptive trend requires an understanding of the ancestral environment of the *D. magna* used in the experiment. *Daphnia* that originate in environments containing visually-feeding predators experience selection that drives trait evolution to minimize detectability, resulting in small body size and small egg number (e.g., Fisk et al. 2007), and minimal movement. When these *Daphnia* are released from selection, new mutations generate phenotypes that would be deleterious in the context of the ancestral environment, such as those observed in this study. Additionally, the increased velocities may have resulted from the release of selection from a warm thermal environment. Given that the Israel population originated from a warmer environment, individuals in this population likely maintain physiological function without the requirement of expending excess energy through behavior to generate metabolic heat. In the absence of selection, new mutations may result in unnecessary energy expended.

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**Figure 1** Mutational bias ($\Delta M$) estimates among traits, trait type, genotypes, and populations. (A) Trait specific estimates of the absolute value of $\Delta M$; (B) Trait type estimates of the absolute value of $\Delta M$; (C) Genotypic estimates of $\Delta M$; (D) Population estimates of $\Delta M$. Error bars are ± SE. Size = Body length at maturity; AM = Age at maturity; Egg = Egg number at maturity; MeanV = Maximum velocity; MeanV = Mean velocity; SDV = Standard deviation of velocity. Non-behavioral = Size, AM, Egg; Behavioral = MaxV, MeanV, SDV. A, B, C = Individual genotypes comprising each population. G = German population; I = Israel population.
as movement. In contrast, the German population evolved smaller body size, smaller egg number and slower movement in the absence of selection. These results suggest the ancestral phenotypes in this population include large body size, large egg number, and faster movement. These ancestral phenotypes are characteristic of Daphnia populations that originate from an environment free of visual predators, but in which gape-limited ambush invertebrate predators predominate.

In summary, susceptibility to deleterious mutation varies at the trait, genotypic, and population levels. Variation at the level of traits is directly proportional to the putative mutational target size of the trait, with traits under the control of numerous genetic loci, such as behavior, displaying more susceptibility to mutation than traits under the control of few genetic loci. Additionally, genotypes and populations vary in their susceptibility to mutation, and this variation likely reflects a combination of variation in the underlying mutation rates and the unique selection histories among genotypes.

ACKNOWLEDGMENTS
We thank Dr. Charles F. Baer and an anonymous reviewer for their comments that improved the quality of this paper. We thank Dieter Ebert for providing the D. magna genotypes used in this project. The project described was supported by an Institutional Development Award (IDeA) to LCL from the National Institute of General Medical Sciences of the National Institutes of Health under Grant #P20GM103408, and grants from the M. J. Murdock Charitable Trust, Reed College start-up funds, and National Science Foundation (MCB-1150213) to SS. The experiment was conceived by LL, SS, and SE. The experiment was designed by LL, SS, and SE. The experiment was conducted by LL, SS, SE, DD, RM, SK, and WR. The data were analyzed by LL and SE. The manuscript was written by LL and SE. The manuscript was reviewed prior to submission by LL, SS, SE, DD, RM, SK, and WR.

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