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## MUTATION, SELECTION, AND THE MAINTENANCE OF LIFE-HISTORY VARIATION IN A NATURAL POPULATION

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**Abstract.**—In an effort to provide insight into the role of mutation in the maintenance of genetic variance for life-history traits, we accumulated spontaneous mutations in 10 sets of clonal replicates of *Daphnia pulex* for approximately 30 generations and compared the variance generated by mutation with the standing level of variation in the wild population. Mutations for quantitative traits appear to arise at a fairly high rate in this species, on the order of at least 0.6 per character per generation, but have relatively small heterozygous effects, changing the phenotype by less than 2.5% of the mean. The mean persistence time of a new mutation affecting life-history/body-size traits is approximately 40 generations in the natural population, which requires an average selection coefficient against new mutations of approximately 3% in the heterozygous state. These data are consistent with the idea that the vast majority of standing genetic variance for life-history characters may be largely a consequence of the recurrent introduction of transient cohorts of mutations that are at least conditionally deleterious and raise issues about the meaning of conventional measures of standing levels of variation for fitness-related traits.

**Key words.**—*Daphnia pulex*, deleterious mutation, life-history variation, mutation accumulation, mutation-selection balance.

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The standing pool of adaptive genetic variation determines a population's ability to respond evolutionarily to short-term selective challenges in a changing environment. Based on this general principle, hundreds of studies have estimated heritabilities and genetic covariance matrices in natural populations in an effort to reveal the evolutionary potential of quantitative characters (see reviews in Falconer and Mackay 1996; Roff 1997; Lynch and Walsh 1998). All such studies implicitly assume that the genetic components of variance extracted by conventional methods (e.g., covariances between relatives) are reasonable measures of the genetic variation that is actually utilizable in adaptive evolution. That this basic premise is unlikely to be strictly true is suggested by what we know about the ultimate source of genetic variation—mutation.

Numerous studies in a wide array of species have revealed that the mutational rate of production of new variation for morphological, behavioral, and life-history traits is sufficient to increase heritabilities by approximately 0.1 to 1.0 percent per generation (see reviews in Lande 1976; Lynch 1988; Houle et al. 1996; Lynch and Walsh 1998). However, mutation-accumulation experiments have also consistently shown significant declines in fitness-related traits in lines taken through repeated population bottlenecks (Crow and Simmons 1983; Kibota and Lynch 1996; Keightley and Caballero 1997; Lynch and Walsh 1998), which supports the common belief that the vast majority of mutations have deleterious effects on fitness. Most spontaneous mutations are slightly recessive and only decrease fitness by one or two percent in the heterozygous state (Crow and Simmons 1983; Houle et al. 1997). On average, such mutations will typically segregate in populations for several tens of generations before being eliminated by natural selection. This is because the average persistence time of a deleterious mutation is approximately equal to the reciprocal of the selection coefficient against heterozygotes (Barton 1990; Crow 1993; Houle et al. 1996). Thus,

it is conceivable that a large fraction of the genetic variation revealed in quantitative-genetic assays of natural populations is simply a reflection of the recurrent introduction of transient cohorts of deleterious alleles by mutation. It is still an open question as to whether most mutations are detrimental in all ecological settings. If this is the case, then the sea of deleterious mutations, individually kept at low frequencies by selection-mutation balance, may typically obscure the much smaller pool of potentially adaptive variation.

Support for the selection-mutation balance hypothesis is provided by the observation that the average persistence times for mutations affecting life-history and morphological characters are, respectively, on the order of 50 and 100 generations in natural populations of a diversity of species (Houle et al. 1996). Such estimates, derived as ratios of standing genetic variance to the rate of mutational variance, are quite consistent with the results from laboratory mutation-accumulation experiments referred to above, as they imply average selection coefficients of heterozygous mutations of one to two percent. Because of limitations in the existing data, most of the analyses presented in Houle et al. (1996) were rather crude, relying on estimates of genetic variances and mutational variances derived from different populations, often raised under rather different conditions. Nevertheless, there is no obvious reason why the average result should be greatly biased.

In an effort to achieve a clearer understanding of the role of mutation in the maintenance of genetic variance in natural populations, we have performed a long-term mutation-accumulation experiment on several clones derived from a natural population of the freshwater planktonic cladoceran *Daphnia pulex*. By performing this work with multiple clones, we avoided the possibility that our results are a peculiar feature of any single genotype and also eliminated the possibility of line divergence resulting from the segregation of variation in the base population. The field population has

been the previous subject of extensive quantitative-genetic analyses for life-history traits (Lynch and Deng 1994), allowing us to compute the average persistence times of new mutations.

## METHODS

### *Experimental Protocol*

The progenitors of the mutation-accumulation experiment were 10 distinct genetic clones of *D. pulex* taken from Amazon Pond in Eugene, Oregon. From a single clutch from each of these 10 stem mothers, 10 individuals were isolated singly into 250-mL beakers containing approximately 100 mL of a food and pond water mixture. These 100 individuals comprised the initial (time zero) experimental lines. In the laboratory, *D. pulex* reproduces entirely by ameiotic parthenogenesis, so that all lines were maintained as clones. Each generation, each of the 100 lines was propagated by taking a single offspring from the second clutch. Additional offspring from this clutch (no more than five, to minimize the opportunity for selection on new mutations) were placed in a backup beaker to be used only for the case in which the single individual died before reproduction. The 100 experimental lines were maintained in this manner for 2.5 years in a walk-in environmental chamber maintained at 12°C. Throughout the period of line maintenance and in the assays, the food consisted of a suspension of the green alga *Scenedesmus*, maintained in 100 mL of filtered pond water at a constant spectrophotometric level of 95% light transmittance (650 nm). The water used in the study was originally collected from the study site and was reused throughout the experiment by recycling it through a Vortex diatomaceous earth filter.

The lines were assayed for life-history characters on three occasions, equivalent to an average of 7, 16, and 32 generations of mutation accumulation. When lines are maintained as single individuals that reproduce only every few days, it is essentially impossible to keep them completely synchronous. Nevertheless, the degree of asynchrony in the experimental lines was small, ranging from 30 to 35 generations in the final assay. At the time of each of the three assays, 81, 80, and 30 of the original 100 lines remained. Most of the losses prior to the first two assays were a consequence of obvious laboratory accidents, but during the last 15 or so generations of mutation-accumulation, the lines became progressively more difficult to maintain and some of the extinctions may have been a direct consequence of deleterious mutation accumulation.

The general assay design followed the protocol outlined in Lynch (1985). Prior to analysis, each of the surviving lines was replicated into five sublines by taking progeny from the second clutch (first clutch progeny are sometimes reduced in size relative to those from later clutches). These sublines were then maintained in a randomized design under controlled food and temperature conditions for two generations, again propagating the lines from generation to generation with single offspring derived from the second clutch. The third generation of such subdivision represents the measurement generation, at which time a single random offspring was taken from each replicate (yielding 405, 400, and 150 individuals in the three assays). This procedure of subdivision ensures

that any variance resulting from genetic and/or environmental maternal and grandmaternal effects will contribute to the within-line component of variance, rather than to the among-line components (Lynch 1985). This is critical because it is from the latter that the mutational variances are derived.

Size at maturity was measured to the nearest 0.01 mm using a Wild M8 dissecting microscope fitted with an ocular micrometer. For the first two assays, for each of the first five clutches, the date of birth, total number of live progeny, and average size of offspring were also measured, whereas the final assay only acquired data through the second clutch. As in our previous work (e.g., Lynch and Deng 1994), we are able to obtain fairly accurate estimates of ages at reproduction by recording the ages of embryos carried by the mothers and back-extrapolating to the age of release of the preceding clutch. Average offspring sizes were calculated by measuring five randomly chosen progeny, except in the rare event that less than five progeny were born, in which case all offspring were measured.

As a control for this experiment, we employed an obligately parthenogenetic lineage of *D. pulex*. At the outset of the experiment, we accumulated a large supply of resting eggs produced by this clone and kept them frozen until newborns were needed for an experimental assay. Such progeny were acquired by taking a portion of the resting eggs through repeated cycles of cold/dark and warm/light conditions. Descendants of 100 control hatchlings were employed in the first two assays, but for unknown reasons the control performed very poorly in the third assay (despite the normal behavior of our experimental animals), and it is excluded from this final analysis.

### *Analysis of Data*

For each of the 10 sets of lines in each of the three assays, the within- and among-line components of variance were obtained by one-way ANOVA. The overall estimate of environmental variance ( $V_e$ ) for each trait was then obtained as the average of the 30 estimates (three for each of the 10 sets of lines) of this parameter. For sizes at birth and maturity, the estimates of  $V_e$  were corrected for measurement error (by subtracting estimates of the variance of repeated measures on the same individuals, averaged over many individuals). All other traits were assumed to be measured without error.

Because of the clonal nature (and permanent heterozygosity) of our lines, the rate of increase in variance among lines provides an estimate of the rate of production of mutational variance ( $V_m$ ). This quantity was estimated for each of the 10 lines by weighted least-squares regression of the among-line components of variance for the three line-specific assays on generation number. To account for the heteroscedasticity of sampling error among assays, the weights for these regressions were taken to be the inverse of the sampling variance for the individual variance component estimates, and the regressions were fitted through the origin. The 10 line-specific estimates were then averaged to obtain an overall estimate of  $V_m$  for each trait, with the standard errors of the estimates being obtained by simply treating the ten estimates as independent variables. The standard errors for the mutational heritabilities,  $V_m/V_e$  ( $V_m$  is traditionally excluded from the

TABLE 1. Estimates of the initial mean ( $\bar{z}_0$ ), rate of input of mutational variance ( $V_m$ ), environmental component of variance ( $V_e$ ), rate of change of the mean ( $R_m$ ), and mutational heritability ( $h_m^2$ ). Standard errors are given in parentheses.

Character	$\bar{z}_0$	$V_m$	$V_e$	$R_m$	$h_m^2$
Size at maturity (mm)	1.87 (0.03)	0.00016 (0.00006)	0.01628 (0.00187)	-0.0108 (0.0046)	0.0097 (0.0036)
Age at maturity (days)	23.15 (0.58)	0.05502 (0.01683)	15.08640 (2.09403)	-0.1160 (0.0425)	0.0046 (0.0014)
Instar duration (days)	6.68 (0.06)	0.00277 (0.00035)	0.59887 (0.05355)	-0.0192 (0.0011)	0.0057 (0.0010)
Size at birth (mm)	0.62 (0.01)	0.00002 (0.00000)	0.00017 (0.00001)	-0.0055 (0.0010)	0.0048 (0.0005)
Clutch size:					
1st	9.00 (0.86)	0.21481 (0.10874)	24.16294 (1.81957)	0.0360 (0.0652)	0.0102 (0.0052)
2nd	16.46 (2.36)	0.60183 (0.21708)	43.43631 (6.35424)	0.2421 (0.1774)	0.0175 (0.0072)
3rd	18.20 (1.62)	0.20339 (0.16299)	70.36116 (6.75070)	0.5293 (0.1173)	0.0031 (0.0023)
4th	21.69 (0.57)	0.76428 (0.22589)	95.87365 (9.92599)	0.5154 (0.0663)	0.0096 (0.0031)
5th	24.64 (1.31)	0.37244 (0.22310)	114.04067 (10.40918)	0.3386 (0.0889)	0.0038 (0.0021)
Survival to maturity	1.00 (0.01)	0.00018 (0.00007)	0.14682 (0.00443)	-0.0206 (0.0021)	0.0012 (0.0005)

denominator because of its very small value relative to  $V_e$ , were obtained from the standard formula for the variance of a ratio (eq. A1.19b, Lynch and Walsh 1998), taking the sampling variance of the numerator and the denominator into consideration.

Estimates of the rate of change in the means of characters ( $R_m$ ) over the course of the mutation-accumulation experiment were also obtained by applying weighted least-squares regression to the assay-specific means for each of the 10 sets of lines. Overall estimates of  $R_m$  (and their standard errors) were then obtained by treating the 10 estimates as independent. Our estimates of  $V_m$  and  $R_m$  will be biased to the extent that selection against deleterious mutations was responsible for the line extinctions.

Lower-bound estimates of the zygotic mutation rates for characters  $U_{min}$  and upper-bound estimates for the average heterozygous effects of mutations ( $\bar{a}_{max}$ ) were obtained by applying the estimates of  $R_m$  and  $V_m$  to the techniques of Bateman (1959) and Mukai et al. (1972). The estimates were obtained by use of equations (5.23) and (5.24a) provided in Lynch (1994), which account for bias caused by the sampling error of  $R_m$  and  $V_m$ . (Note that there is a typographical error in eq. 5.24a;  $R_m^2$  should be replaced by  $R_m$ .) Standard errors for  $U_{min}$  and  $\bar{a}_{max}$  were obtained by applying the formula for the variance of a ratio (eq. A1.19b, Lynch and Walsh 1998) under the assumption that the estimates for the changes in means and variances are statistically independent.

The mean persistence times of mutations in the natural population were obtained by dividing the standing levels of total genetic variance for the traits by their mutational variances,  $\bar{t}_p = V_G/V_m$ , and correcting for the bias caused by the sampling variance of the denominator (eq. A1.19a, Lynch and Walsh 1998). Several (up to 24) independent estimates of  $V_G$  are available for many of the traits observed in this study for the same population from which the mutation-accumulation lines were extracted (Lynch and Deng 1994; M. Pfrender, unpubl.) Most of these estimates were obtained under environmental conditions similar or identical to those employed in the mutation-accumulation assays. For each character/assay, the original data were converted to coefficients of genetic variation ( $CV_G = \sqrt{V_G/\bar{z}}$ ), where  $\bar{z}$  is the mean phenotype for the trait, and then a mean value of this parameter was obtained for each character by averaging over all available estimates for that character. The genetic variance estimates employed in the following analyses were obtained

as  $[(CV_G \cdot \bar{z}_0)^2]$ , where  $\bar{z}_0$  is the time-zero estimate of the mean phenotype for a character derived as the average intercept of the regressions that estimated  $R_m$ .

## RESULTS

Based on the properties of our control line, the experimental conditions imposed on our mutation-accumulation lines appear to have been quite constant throughout the course of the experiment. The only differences observed between the controls in the first and second assays were slight increases in the sizes at birth and maturity, amounting to 0.009 (0.004) and 0.006 (0.001) mm/generation, respectively. For these two traits, we corrected the overall estimates of  $R_m$  by subtracting the control rates of change from the rates observed for the mutation-accumulation lines. Such treatment assumes that this trend held throughout the course of the experiment, because as noted above estimates were not acquired for the control in the final assay.

The estimates of the mutational variance for the majority of the characters are statistically significant, yielding mutational heritabilities in the range of 0.001–0.018, with an average value of 0.0066 (Table 1). In addition, the average effects of mutations are directional—decreasing size at birth and maturity, age at maturity, instar duration, and survival from birth to maturity, and increasing clutch sizes.

The observed temporal changes in the means and among-line variances are consistent with a fairly high mutation pressure to new alleles with fairly small effects. Application of the Bateman-Mukai technique yielded lower-bound estimates of the zygotic mutation rate (per character per generation) ranging from 0.02 to 1.95 (Table 2). The average estimate of  $U_{min}$  is 0.57 per character per generation. The lower-bound estimates of the average mutational effects, taken as a proportion of the initial means of the traits, are all less than 0.06 in absolute value, with an average absolute value of 0.024. The estimated persistence times of new mutations in the natural population fall in the range of 18–72 generations, with an average value of 41 (6) generations (Table 3).

## DISCUSSION

The estimates of mutational heritability obtained in this study are, on average, four times greater than those obtained in an earlier experiment with another clone of the same species (Lynch 1985). However, these differences may simply

TABLE 2. Estimates of the genomic mutation rate ( $U_{min}$ ), the average heterozygous effect ( $\bar{a}_{max}$ ), and the average effect as a fraction of the initial mean phenotype ( $\bar{a}'_{max}$ ). The estimates for the size of the first clutch are undefined because the standard error of the change in the mean exceeds the mean.

Character	$U_{min}$	$\bar{a}_{max}$	$\bar{a}'_{max}$
Size at maturity (mm)	0.729 (0.627)	-0.015 (0.007)	-0.008 (0.004)
Age at maturity (days)	0.101 (0.075)	-0.687 (0.269)	-0.030 (0.012)
Adult instar duration (days)	0.046 (0.007)	-0.068 (0.007)	-0.010 (0.001)
Size at birth (mm)	1.513 (1.345)	-0.003 (0.002)	-0.005 (0.005)
Clutch size:			
2nd	0.021 (0.077)	0.907 (1.706)	0.055 (0.104)
3rd	0.496 (0.850)	0.215 (0.229)	0.012 (0.013)
4th	0.164 (0.064)	1.304 (0.420)	0.060 (0.019)
5th	0.122 (0.145)	0.702 (0.553)	0.029 (0.023)
Survival to maturity	1.946 (0.854)	-0.008 (0.003)	-0.008 (0.003)

be due to greater selective mortality in the previous study, where after 75 generations of mutation accumulation only eight of 50 original lines remained at the time of the single assay. Although substantial line loss also occurred in the current experiment, most of this arose following the second of the three assays, so the overall effect on the estimates of  $h_m^2$  (which utilized data from all three assays) should have been reduced. The average value of  $h_m^2$  that we obtained in this study, approximately 0.007, is in fact quite similar to what has been found in studies with other species. For example, a summary of the existing data for well-studied characters, provided in Lynch and Walsh (1998), indicates that  $h_m^2$  is approximately 0.003–0.005 for bristle numbers and body weight in *Drosophila*, 0.003–0.017 for body weight and bone lengths in mice, and 0.009 and 0.003 for size and reproductive traits in maize and rice, respectively. The average  $h_m^2$  for various life-history characters in *Drosophila* (Houle et al. 1994; Fernandez and Lopez-Fanjul 1997; Pletcher et al. 1998) is 0.004 (0.001).

Our estimates of zygotic mutation rates for quantitative characters, with an average lower bound of approximately 0.6 (per character per generation), are also consistent with previous estimates in the literature. For example, the average estimate of  $U_{min}$  for egg-to-adult viability derived from several mutation-accumulation experiments with *Drosophila* is approximately 0.6 (Crow and Simmons 1983; Lynch and Walsh 1998), whereas  $U_{min}$  for total fitness in *Arabidopsis*, again estimated with a mutation-accumulation experiment, is approximately 0.1 (Schultz et al., unpubl.) Indirect estimates of the genomic mutation rate to deleterious alleles in plants,

obtained by comparing the fitness of selfed and outcrossed progeny, range from approximately 0.2 to 1.5 (B. Charlesworth et al. 1990; D. Charlesworth et al. 1994; Johnston and Schoen 1995), and other indirect lines of evidence suggest that zygotic mutation rates for quantitative traits are commonly at least 0.2 per character per generation in eukaryotic species (ch. 12, Lynch and Walsh 1998). Even the very low genomic rate of mutations deleterious to fitness observed in the bacterium *E. coli* appears to be consistent with those for higher organisms, once one accounts for differences in genome size and numbers of cell divisions per generation (Kibota and Lynch 1996).

We have previously obtained estimates of  $U_{min}$  for clutch size in *Daphnia* by use of the method of Deng and Lynch (1996), which infers the properties of deleterious mutations from observations on the mean and variance of fitness of parental clones (from a natural population) and their selfed derivatives under the assumption that the distribution of fitness is a consequence of selection-mutation balance. This procedure yielded similar results for both the *D. pulex* population that is the subject of this study and for a lake-dwelling population of *Daphnia pulicaria* (Deng and Lynch 1997). Averaging over the results for both species,  $U_{min}$  for clutch size is inferred to be 0.87 (0.14), which is significantly higher than the average value that we obtained by direct observation in the current study, 0.20 (0.10). However, not too much should be made of this difference, because a number of assumptions underlie the field study of Deng and Lynch (1997), including the assumption that individual fitness scales linearly with clutch size. When one further considers that the laboratory estimate may be biased downwardly somewhat by the selective extinction of low-fitness clones, while the field technique actually accounts for selection, then the two estimates are reasonably compatible. If the difference is, in fact, due to selective line extinction, then this result suggests that our mutation-accumulation estimates of  $U_{min}$  for other characters may be downwardly biased as well. Thus, the total weight of evidence suggests that the genomic mutation rate for fitness characters in *Daphnia* is in excess of 0.1 per generation and perhaps as high as 1.0.

The primary exception to the observation that the genomic deleterious mutation rate for fitness in multicellular organisms is on the order of one per individual per generation is the recent report that genomic mutation rates for total productivity and longevity in the nematode *Caenorhabditis ele-*

TABLE 3. Estimates of the standing levels of total genetic variance in the natural population ( $V_G$ ) and the average persistence time ( $\bar{t}_p$ ). Standard errors are given in parentheses.

Character	$V_G$	$\bar{t}_p$
Size at maturity (mm)	0.00843 (0.00268)	46.6 (21.0)
Age at maturity (days)	2.81694 (2.54885)	57.1 (49.8)
Adult instar duration (days)	0.10026 (0.04780)	35.6 (17.6)
Size at birth (mm)	0.00051 (0.00012)	24.1 (8.1)
Clutch size:		
1st	3.09267 (1.38332)	21.9 (6.9)
2nd	10.34448 (4.62696)	23.8 (9.1)
3rd	12.64713 (5.65690)	59.1 (48.1)
4th	17.96256 (8.03443)	33.8 (10.9)
5th	23.18091 (10.36854)	71.6 (43.9)

*gans* are in the range of 0.001–0.01 per character per generation (Keightley and Caballero 1997). To explain this discrepancy, Keightley and Caballero (1997) favor the idea that the previous estimates of  $U$  in *Drosophila melanogaster* are inflated by an unusually high activity of transposable elements in this species and also potentially flawed by the occurrence of adaptive evolution in the control lines. Such arguments are made more plausible by reanalyses of the published *Drosophila* data by Keightley (1996) and Garcia-Dorado (1997), but one could argue that assumptions made in the latter studies (including assumptions involving the form of the distribution of mutational effects) may have introduced biases that do not arise with the Bateman-Mukai technique. The controversy has by no means been laid to rest, as a recent empirical study with *D. melanogaster*, quite different in design from previous studies, has yielded results that are consistent with the early empirical estimates (Shabalina et al. 1997). Finally, it is possible that selection against new mutations was unusually strong in the experiment of Keightley and Caballero (1997), where about one in fourteen individuals typically did not even reproduce. A recently completed mutation-accumulation experiment with *C. elegans* in which such reproductive problems did not arise (Vassieleva and Lynch, unpubl.) has yielded estimates of  $U_{min}$  that are higher than those reported by Keightley and Caballero (1997).

The average effects of mutations that we detected are quite small. Averaging over all characters, the Bateman-Mukai technique yielded an upper-bound estimate of the heterozygous effect (as a proportion of the mean phenotype) of only 0.024. These results are also qualitatively consistent with previous observations on other organisms. For example, the average heterozygous effect of a mutation influencing egg-to-adult viability in *Drosophila* is less than 0.02 (Crow and Simmons 1983), whereas that influencing total fitness in *Arabidopsis* is less than 0.10 (Schultz et al., unpubl.) An upwardly biased estimate of the average selective effect of deleterious mutations influencing cell division rate in *E. coli* is 0.01 (Kibota and Lynch 1996).

In the absence of information on the functional relationship between the characters that we measured and total fitness, a direct translation cannot be made from our estimates of average mutational effects on phenotypes and their selective consequences in nature. However, the magnitudes of the persistence times that we have estimated provide indirect support for the idea that most mutations in natural populations of *Daphnia* reduce fitness by only a few percent. That the reciprocal of persistence time is equivalent to the average selection coefficient against a mutant allele in the heterozygous state can be seen by the following simple selection-mutation model,

$$V_G(t + 1) = (1 - s)V_G(t) + V_m, \quad (1)$$

where  $s$  is the fraction of genetic variance removed as a consequence of selection against rare alleles each generation (equivalent to the selection coefficient). Noting that the equilibrium solution to this equation is  $\hat{V}_G = V_m/s$ , an estimator for  $s$  is  $V_m/\hat{V}_G - 1/t_p$ . Taking the reciprocals of the persistence times in Table 3 yields  $s$  in the narrow range of 0.014–0.056, with an average value of 0.030 (0.005).

The solution of equation (1) can also yield insight into the

potency of polygenic mutation as a mechanism for generating the standing pool of genetic variation for life-history characters in natural populations. Starting with a population completely lacking in genetic variation,  $V_G(0) = 0$  the expected level of genetic variance after  $t$  generations of mutation and selection is

$$V_G(t) = \frac{V_m}{s}(1 - e^{-st}). \quad (2)$$

Recalling the definition of persistence time as  $\bar{t}_p = \hat{V}_G/V_m$ , the time for a population initially lacking in genetic variance to accumulate new genetic variance equivalent to 50% and 90% of the equilibrium expectation is approximately  $0.7\bar{t}_p$  and  $2.3\bar{t}_p$  generations, respectively. Thus, returning to Table 3, it can be seen that the values of  $\bar{t}_p$  for the study population imply that no more than a few tens to 100 generations are required for a population to achieve the equilibrium level of genetic variance expected under selection-mutation balance. Such conditions are likely to have been met for our field population, which inhabits a natural woodland pond.

Expressions for the dynamics of genetic variance in the form of equations (1) and (2) can be somewhat misleading, as a broader interpretation simply takes  $s$  to be a loss coefficient. For example, for a finite sexual population with effective size  $N_e$  and in the absence of any selection, the dynamics of the expected genetic variance are described by equation (1) with the drift coefficient  $1/(2N_e)$  substituted for  $s$ . The equilibrium level of genetic variance is then  $2N_e V_m$  (Clayton and Robertson 1955; Lande 1976; Lynch and Hill 1986), and the persistence time is  $2N_e$  generations. For an asexual population,  $\bar{t}_p = N_e$  generations under this model. Given the small persistence times that we have observed, this neutral model is clearly incompatible with the observed data, because the effective size of our field population is at least thousands, if not millions, of individuals.

Thus, our favored interpretation of the data is that standing levels of genetic variance for life-history traits are largely a reflection of the recurrent introduction of mildly deleterious alleles by mutation. This position is supported by the fact that estimates of the degree of inbreeding depression for fitness in *Drosophila* are in very good agreement with expectations based on the estimates of mutation rates, selection coefficients, and coefficients of dominance obtained from mutation-accumulation experiments (Simmons and Crow 1977; D. Charlesworth and Charlesworth 1987; Lynch et al. 1995; Lynch and Walsh 1998). A recent analysis by Charlesworth and Hughes (in press) also suggests that a large fraction of the standing genetic variance for fitness in *Drosophila* is accounted for by mutation-selection balance. If this view is generally true, then differences in levels of genetic variance for fitness-related characters may largely reflect differences in mutational properties and past population history rather than yield insight into differences in future evolutionary potential. An empirical way to resolve this issue would be to subject natural populations with different standing levels of genetic variance for life-history characters (and ideally the same mean phenotypes) to similar selection pressures and to monitor the response. If the majority of genetic variation is simply a consequence of the segregation of unconditionally

deleterious alleles, then there should be little correspondence between the level of variation and the response to selection.

An unresolved question that is fundamental to our understanding of the role of mutation in both evolution and extinction is the extent to which mutations for quantitative characters have fitness effects that are contextually dependent as opposed to being unconditionally deleterious or beneficial. One extreme view is that mutations with minor to mild effects on the phenotype have effects on fitness that depend on both the genetic background within which they arise and on the form of the fitness function. Many of the quantitative-genetic models that treat characters as being under stabilizing selection make this assumption explicitly (see reviews in Turelli 1984; Lande 1988). Under these models, a mutation is beneficial if it moves the phenotype toward the optimum and is deleterious otherwise. Alternatively, a large fraction of mutations may be unconditionally deleterious, such as those that alter physiological performance in a way that is detrimental regardless of the environmental setting. Some models for mutationally driven extinction focus entirely on such mutations (Lynch and Gabriel 1990; Lande 1994; Lynch et al. 1995). Recent studies with *Drosophila* have shown that the fitness effects of spontaneous mutations are strongly environment dependent and exhibit strong genotype  $\times$  environment interaction (Kondrashov and Houle 1994; Fry et al. 1996; Fernandez and Lopez-Fanjul 1997; Wayne and Mackay 1998), although none of these studies has revealed a class of mutations that are clearly deleterious in one environment and beneficial in another.

A reasonable interpretation of our data is that at least some of the mutations that arose have the potential to be advantageous in specific ecological settings, although most mutations might be disadvantageous within Amazon Pond if the resident population is highly adapted to that environment. For our study population, mutation had average directional effects, increasing clutch sizes, but decreasing body sizes, development time, and viability. Body size and clutch size are examples of characters whose fitness effects are likely to be quite contextually dependent in *Daphnia*, because size-selective predation is a very significant source of mortality for members of this genus (Lynch 1980). Changes in body size are beneficial or detrimental depending on the dominant form of predation (vertebrates selectively consume large individuals, while invertebrate predators are confined to small prey) and on whether the current genotypic value is above or below the target prey size. In a predator-free world, large clutches may almost always be advantageous, but in an environment dominated by visually feeding predators, a large egg mass may be nearly lethal because of the increased visibility that it imposes on the mother. Our study population, which is exposed to intense predation from salamander larvae, is confronted with this situation. However, it is also exposed to intense predation from invertebrate (*Chaoborus*) larvae, so it is possible that large body sizes and small clutch sizes are jointly selected for.

All species have definable ranges of phenotypic variation, beyond which no individual is found in any population. Such boundaries may evolve passively as a consequence of ecological circumstances, with the selective pressures within the center of the range being reinforcing and those at the pe-

riphery (where only transient migrants occur and contribute little to the next generation) being of little relevance (Kawecki et al. 1997; Kirkpatrick and Barton 1997). In that case, mutations that are advantageous in the center of the range but deleterious in the periphery will accumulate, imposing a genetic barrier to range expansion. Alternatively, the internal genetic architecture of species may impose limits to the degree to which mutation can expand the range of phenotypic variation. Further work will be required to establish whether the directional effects of mutations that we observed for the study population are general, or whether clones from other populations (with different initial mean phenotypes) will respond in different ways to mutation accumulation.

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