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Quantifying the Decanalizing Effects of Spontaneous Mutations in Rhabditid Nematodes

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Abstract

The evolution of canalization, the robustness of the phenotype to environmental or genetic perturbation, has attracted considerable recent interest. A key step toward understanding the evolution of any phenotype is characterizing the rate at which mutation introduces genetic variation for the trait (the mutational variance, V_M) and the average directional effects of mutations on the trait mean (ΔM). In this study, the mutational parameters for canalization of productivity and body volume are quantified in two sets of mutation accumulation lines of nematodes in the genus *Caenorhabditis* and are compared with the mutational parameters for the traits themselves. Four results emerge: (1) spontaneous mutations consistently decanalize the phenotype; (2) the mutational parameters for decanalization, V_M (quantified as mutational heritability) and ΔM , are of the same order of magnitude as the same parameters for the traits themselves; (3) the mutational parameters for canalization are roughly correlated with the parameters for the traits themselves across taxa; and (4) there is no evidence that residual segregating overdominant loci contribute to the decay of canalization. These results suggest that canalization is readily evolvable and that any evolutionary factor that causes mutations to accumulate will, on average, decanalize the phenotype.

Keywords

canalization; environmental variance; mutation accumulation; mutational variance; robustness

Canalization renders a trait insensitive to perturbations of the environment and/or the genetic background. The importance of canalization has been recognized for a long time (Waddington 1942; Schmalhausen 1949; Lerner 1954) and has been the subject of renewed interest in recent years (for recent reviews, see de Visser et al. 2003; Dworkin 2005a; Hansen 2006). From an evolutionary perspective, canalization is important for two seemingly contradictory reasons. First, canalization will evolve via natural selection whenever the same phenotype is favored in different environments or genetic backgrounds. In contrast, when different phenotypes are favored in different environments or genetic backgrounds, phenotypic plasticity or epistasis is the adaptive outcome. Canalization and plasticity are thus extreme manifestations of the same underlying trait, which is often called variability, that is, the ability to vary. Evolution requires variation, so the less a phenotypic trait is able to vary, depending on environmental or genetic circumstances, the less able it will be to evolve when circumstances change. It is easy to imagine that adaptive canalization within one set of environmental or genetic parameters can lead to extreme loss of fitness (potentially leading to extinction) when circumstances take the environment or genetic

background out of the range to which the phenotype is adapted. Under this scenario, canalization retards the potential for evolution (Ancel and Fontana 2000; de Visser et al. 2003).

Second, and alternatively, canalization can promote variability under certain circumstances (Rendel 1967). This view stems from the observation that traits that are normally highly canalized exhibit increased variability under environmental stress (Waddington 1953) or in the presence of mutations of large effect (Dun and Fraser 1958). Consistent with this view is recent evidence that certain genetic systems may act as capacitors by masking the deleterious effects of mutations and allowing hidden genetic variation to accumulate (Rutherford and Lindquist 1998; Masel and Bergman 2003). Under this scenario, a genetic system (e.g., a molecular chaperone) buffers the effects of deleterious mutations at other loci, allowing alleles that would be deleterious in the absence of the capacitor to accumulate in the population. If the environment changes such that some of the previously deleterious variation is now beneficial, a change in the regulation of the capacitor can release the now-beneficial genetic variation, allowing adaptive evolution to proceed without having to wait for a new beneficial mutation. Whether the mechanisms that canalize the phenotype against environmental perturbations are distinct from those that confer robustness to mutation is of particular interest because theory predicts that environmental canalization will respond to selection much more readily than will genetic canalization (Wagner et al. 1997). If mechanisms that confer environmental robustness also confer robustness to mutation, genetic canalization can readily evolve as a correlated response to direct selection for environmental robustness. It has been argued on logical grounds that environmental and genetic canalization should, in general, employ the same underlying mechanisms (Meiklejohn and Hartl 2002; Proulx and Phillips 2005), and studies of the sensitivity of RNA secondary structure show that environmental and mutational perturbation have similar effects (Ancel and Fontana 2000). Conversely, the mechanisms that control interindividual environmental variance often differ from those that control within-individual environmental variance (developmental stability; Stearns et al. 1995; Milton et al. 2003; Pelabon et al. 2004; Dworkin 2005c; Santos et al. 2005), which argues against the existence of a single global mechanism that canalizes the phenotype against all types of perturbation.

Many attempts to draw a connection between environmental and genetic canalization have focused on the relationship between the mutational variance, V_M , and the environmental variance, V_E (Stearns et al. 1995; Wagner et al. 1997; de Visser et al. 2003; Zhang and Hill 2008). It has been argued that traits with low V_M must be more canalized than traits with large V_M , and an observed correlation between V_M and V_E among traits can be taken as evidence for a common mechanism of canalization (Stearns et al. 1995). That argument confounds canalization with mutational target size, however; all else equal, traits that are influenced by a large number of loci will have a larger V_M than will traits influenced by few loci (Houle 1998). Nevertheless, a fundamental relationship between V_M and V_E has interesting implications. If canalization is adaptive, deleterious mutations should, collectively, decanalize the phenotype against both genetic and environmental perturbations, although individual mutations need not have decanalizing effects on both types of perturbation. A decanalizing effect against genetic perturbation will appear as synergistic epistasis of deleterious mutations (Carter et al. 2005; Azevedo et al. 2006). A decanalizing effect against environmental perturbation will appear as a positive relationship between mutation load and environmental variance.

The relationship between mutation load and V_E has a long history. Lerner (1954) introduced the concept of genetic homeostasis whereby heterozygotes should exhibit greater developmental stability (i.e., be more canalized) than homozygotes. It has long been known that inbred genotypes often have larger V_E than do their outbred progenitors (Lerner 1954;

Mitton and Grant 1984; Whitlock and Fowler 1999), as do individuals carrying mutations of large visible effect (Dun and Fraser 1958; Dworkin 2005*b*). Both situations are compatible with heterozygotes being more highly canalized than homozygotes (i.e., over-dominance for canalization), but they are also compatible with deleterious mutations having simple recessive effects on canalization.

Theoretical analyses of two kinds of biological networks, metabolic (Kacser and Burns 1981; Keightley 1996; but see Bagheri-Chaichian et al. 2003) and transcriptional (Siegal and Bergman 2002; Bergman and Siegal 2003; Azevedo et al. 2006; Kimbrell and Holt 2007), lead to the prediction that robustness to perturbation should emerge as an inherent property of the system even in the absence of direct selection for canalization (i.e., optimizing selection for a particular phenotype). It is therefore tempting to draw the conclusion that decanalization of the phenotype by deleterious mutations would constitute evidence that canalization of the phenotype is itself an adaptation. However, the aforementioned models assume either explicitly or implicitly that the underlying system (the metabolic or transcriptional network) is underoptimizing selection, so it is not clear whether decanalization of the phenotype results from the direct effect of relaxed optimizing selection for a canalized phenotype or from the indirect effects of relaxed optimizing selection for some property of the underlying network.

Here I incorporate results from several mutation accumulation (MA) studies in rhabditid nematodes in an initial attempt to quantify the relationship between spontaneous mutation and environmental canalization. The environmental coefficient of variation (CV_E) is employed as a measure of canalization such that when the same trait is compared among genotypes, the lower the environmental variation, the greater the degree of canalization. The overriding interest is essentially comparative natural history: what is the relationship between mutation load and V_E across taxa? If V_E does not differ between MA lines and their ancestral progenitors (control lines), that would imply that the mechanisms of environmental and genetic canalization are distinct and, further, that the mechanism underlying environmental canalization either is itself highly canalized against mutation or provides a very small mutational target. Moreover, the taxa involved in these experiments accumulate mutation load at characteristically different rates. If the relationship between mutation load and V_E differs among taxa, there are two potential explanations. First, it may be that the size of the mutational target for environmental canalization differs among taxa. Second, and perhaps more interestingly, it allows the possibility that the mechanism of environmental canalization differs among taxa. Finally, are the results consistent with a relationship between canalization and heterozygosity per se, or are the results better explained by additive effects or simple dominance?

Material and Methods

The two sets of MA lines of rhabditid nematodes included in this study are those of Vassilieva and Lynch (VEL; Vassilieva and Lynch 1999; Vassilieva et al. 2000) and those of Baer et al. (CFB; Baer et al. 2005, 2006; Ostrow et al. 2007). Details of the MA protocols and phenotypic trait assays are given in the original articles. The VEL lines were originated from a single highly inbred individual of the N2 strain of *Caenorhabditis elegans*. The CFB lines were originated from a single highly inbred individual from each of two isohermaphrodite strains of three species of self-compatible rhabditid nematodes (*C. elegans*, N2 and PB306 strains; *Caenorhabditis briggsae*, HK104 and PB800; *Oscheius myriophila*, DF5020 and EM435). The VEL and CFB experiments each began with 100 replicate MA lines per strain. The criteria for the choice of CFB strains are given by Baer et al. (2005). Phenotypic data included in this study are productivity (VEL and CFB) and body volume (CFB). Productivity is total reproduction over an individual's lifetime and is highly

correlated with fitness, as estimated by the method of Charlesworth (1994, pp. 116–126; $r \approx 0.9$; C. F. Baer, unpublished result).

Each of these studies included the ancestral progenitor (control) as a reference to which MA lines could be directly compared. The progenitor of each set of lines was cryo-preserved at the time at which MA lines were initiated and was thawed for use in subsequent comparisons. On thawing, multiple control lines were initiated and replicated after one generation, at which time both control and MA lines were propagated for three generations of single-worm descent to remove parental and grandparental effects. Control lines are not expected to have diverged genetically, although residual segregating variation could, in principle, lead to variation among control lines. The difference between the means of MA lines and the means of the ancestral control lines is the cumulative effect of new mutations on the mean phenotype. The difference between the among-line component of variance of MA lines and that of control lines is the genetic variance contributed by new mutations.

The CFB lines were assayed for productivity at 20°C at 100 and 200 generations of MA (all strains) and at 25°C at 220 generations of MA (only *C. elegans* and *C. briggsae*). Generations were assayed at different times, and each assay/generation was divided into two blocks. The design included 34 MA lines and 20 control lines per block and five replicates per line, although the actual number of lines and replicates varied among strains and generations (for details, see Baer et al. 2005, 2006). The CFB lines were assayed for body volume at 20°C at 200 generations of MA, divided into two assay blocks. The design included 40 MA lines and 20 control lines per block, with three replicates per line and 10 individual worms per replicate (Ostrow et al. 2007).

The VEL lines were assayed for productivity at generations 7, 20, 30, 50, 90, 120, 163, and 214. At the outset of the experiment, 100 MA lines were included; by generation 214, there were 73 lines remaining. In each assay, 20 control lines were included; each line was replicated five times, following the same protocol described for the CFB study.

Data Analysis

The within-line component of variance represents V_E in an MA experiment (Lynch and Walsh 1998, pp. 331–333). To quantify V_E independent of the trait mean, we calculated the within-line coefficient of variation ($CV_E = 100 \times \{[\text{Var}(\bar{z})]^{1/2}/\bar{z}\}$) for each line in each assay block, employing Haldane's (1955) correction for sample size $CV^* = CV \times [1 + (1/4n)]$, where n is the within-group sample size and $\bar{z}$ is the trait mean. The change in the mean and the change in the variance of V_E are both of interest (see "Discussion"). The change in the mean CV_E represents the change in the mean phenotype due to MA (ΔM in the MA parlance), where the phenotype is environmental canalization. The cumulative effects of MA on environmental canalization were determined by the regression of CV_E on generations of MA (the regression slope is designated R_m); division of the regression slope by the generation 0 mean (which we designate M_0) provides the percent change per generation (i.e., ΔM). Half the per-generation change in the among-line variance in the CV_E (i.e., the within-line variance) with MA represents the V_M for canalization and was determined by the regression of the among-line component of variance in CV_E against generations of MA; division of V_M by the generation 0 mean (which we designate $V_{E,0}$) provides the mutational heritability h_M^2 (Lynch and Walsh 1998, pp. 328–330). Regression slopes and variance components were calculated using SAS PROC MIXED, version 9.1.3. Details vary for each data set and are described below.

A major goal of this study is to compare the mutational properties for canalization of a trait (i.e., the CV_E) with the mutational properties of the trait itself. For example, the change in

the mean CV_E for productivity is compared with the change in mean productivity. For the sake of notational clarity, statistics relating to the CV_E are left unsubscripted (ΔM , V_M , h_M^2), whereas the same statistic for the trait itself is subscripted with a z , to represent the phenotype (i.e., ΔM_z , $V_{M,z}$, $h_{M,z}^2$).

CFB: Productivity—We first tested each strain individually for a possible effect of assay temperature on the relationship between CV_E and generation by fitting the general linear model CV_E (productivity) = generation + temperature + temp $\times$ gen + block + error. In this case, generation was modeled as a categorical variable with two classes, control and MA. We ignore the 10% difference in generations of MA between the 20° and 25°C treatments. Block was modeled as a random effect; other effects are fixed. The relevant effect is the temp $\times$ gen interaction. In only one case (PB306) was the interaction term marginally significant ($P < .04$; data not shown). At 20°C, PB306 displayed the typical difference in CV_E between control and MA worms, but the difference was much smaller at 25°C. We included data from both temperatures in the full analysis, which is therefore conservative with respect to the ability to detect a main effect of generation. We next fit the full model CV_E (productivity) = generation + strain + generation $\times$ strain + block + residual. Generation was modeled as a continuous variable, where gen = 0 for control lines and 100, 200, or 220 for MA lines, respectively. All class variables are modeled as random effects. Among-line (residual) variance was allowed to vary between strains (SAS PROC MIXED option GROUP = <strain>). The CV_E data are not normally distributed, so hypothesis tests using REML or F -tests are suspect. Instead, we used a bootstrap approach to assign approximate standard errors and/or confidence limits to the regression parameters as follows. We first resampled the data with replacement at the level of line, maintaining the assay structure and the number of MA and control lines per assay. We then fit the linear model as above. The procedure was repeated 1,000 times, and it determined empirical 95% confidence limits for ΔM . Statistics related to the among-line components of variance (V_M , $V_{E,0}$, h_M^2) and their associated standard errors were determined by averaging over the six strains.

We next repeated the analysis for each strain individually. The slope of the regression of CV_E on generation was determined for each strain using the model CV_E (productivity) = generation + block + residual, with the residual variance allowed to vary among generations. Approximate empirical standard errors and/or confidence limits for regression parameters (means and variances) were determined by the same bootstrap protocol as for the full data set. Each analysis was performed twice, once for the full data set and once with low-fitness (productivity < 7) replicates (not lines) excluded; we refer to the latter as quasi-normal data. Justification for the separate analyses of the full data set and the quasi-normal data is given below.

VEL: Productivity—Analysis was analogous to that for the CFB data for individual strains, with one difference. Because of the larger number of fitness assays (eight vs. three), we calculated separate regressions of CV_E against generation MA for control and MA lines; for the control lines, MA generation is a proxy for chronological time. The regression slope of mean CV_E and the among-line variance in CV_E were negative ($P < .05$) for the control lines, so we report the regression parameters for MA lines; the results are thus conservative because the time trend in the controls is in a direction opposite that of the MA lines.

CFB: Body Volume—The analysis was the same as for productivity, except that only one MA generation (200) was included. Up to 10 worms from each of three replicates were measured; the CV_E reported is the sum of the within- and among-replicate components of

variance. We used the uncorrected coefficient of variation (CV) because the relevant sample size correction is a function of the variance components and cannot be determined a priori.

On the CV as a Measure of Variation

In a comparative context, by definition, the variance—the expected squared deviation of an individual from the population mean—is meaningful only with respect to the mean. By way of analogy, many authors have used variations of “the mouse and the elephant” parable (e.g., Sokal and Rohlf 1981, p. 59; Houle 1992); that is, if the variance of a trait (e.g., size) is the same in mice and in elephants, the trait must be much more innately variable in mice than in elephants, given the orders of magnitude of difference in the means. The CV is the most straightforward way of standardizing variances to be independent of the mean, thereby allowing meaningful comparisons between groups with different means. Moreover, the opportunity for selection (I), the variance divided by the mean squared (essentially the CV), has a straightforward interpretation in the context of evolution: the larger I is for a trait, the faster it will respond to a given strength of selection, and I for fitness provides the upper bound on the rate of response to selection (Crow 1958; Houle 1992; Wade 2006).

Nevertheless, the CV has been criticized as a measure of variability in comparative studies (Lande 1977; Schultz 1985; Dworkin 2005a) on two grounds. The first class of objections concerns the reliability of the usual statistical tests for differences between groups in the CV, given particular distributions of the underlying data (Schultz 1985; Dworkin 2005a). In this study, I employ resampling statistics that do not depend on underlying assumptions about the distribution of the data, so the first class of objection is not relevant. The second class of objection concerns the relationship of the CV with the mean. Specifically, it is often observed empirically that the CV is negatively correlated with the mean. That criticism is relevant to this study because in (almost) all cases, the trait mean declines with generations of MA, leading to a negative correlation between the CV and the trait mean. Lande (1977) outlined several situations in which purely mathematical considerations led to an a priori prediction of a negative correlation of the CV with the mean. The only one that applies to this study is the case in which there is an appreciable probability that the trait value is 0. To accommodate the possibility that the observed increase in CV in productivity with generation MA is a statistical artifact owing to the greater frequency of replicates that failed to reproduce in the MA lines relative to the control lines, we analyzed productivity data in which replicates with zero productivity were included and again when replicates with very low productivity (fewer than seven offspring) were excluded. The cutoff value of seven offspring was chosen because in most cases (all but the HK104 strain of the CFB data set), the remaining data were very close to normally distributed (quasi-normal), as determined by an eyeball fit to the normal line of a quantile-quantile plot.

A different possible reason for a negative correlation between the CV and the mean is measurement error, such that small individuals are inherently harder to measure precisely than are large ones. A subset of the replicates in the CFB productivity data set was counted twice (by the same counter). There was no correlation (Pearson's $r = 0.0028$, $n = 525$) between mean productivity and repeatability of the measurement (defined as the absolute value of the difference of the two measurements divided by the mean). We do not have repeatability data for body volume, but if measurement error explained the increase in CV over time, then the smallest species (*C. briggsae*) should have the largest control CV, and the largest species (*O. myriophila*) should have the smallest CV, and that is not the case (table 3).

Some authors (Schultz 1985; Dworkin 2005a) have advocated using Levene's statistic, $L = |\ln(x_i) - \text{Md}[\ln(x)]|$, rather than the CV as a measure of variability. The primary justification for using Levene's statistic rather than the CV is that the traditional hypothesis tests of

variation in L are more robust to deviations of the distribution of the data from the underlying assumptions (e.g., normality), a consideration that is not relevant to this study. Regardless, using L rather than the CV as the measure of variability yields results that are very similar to those using the CV, and the overall conclusions are the same.

Results

Results for productivity including all data (i.e., zeros included) are presented in table 1, and those for quasi-normal data are presented in table 2. Results for body volume of the CFB lines are presented in table 3. Mutational parameters for the trait of interest (ΔM_z , $h^2_{M,z}$, and $CV_{M,z}$) are presented in the last three columns in each table (bold) to facilitate comparison between the mutational parameters for the environmental variance of a trait and the mutational parameters for the trait itself.

Change in the Mean (ΔM)

For the full CFB data set (zeros included), CV_E increased at about 0.16% per generation ($\Delta M = 0.00162$; table 1), an absolute rate of change very comparable to the average rate of change of mean productivity itself ($\Delta M_z = -0.00144$; table 1). For the quasi-normal data, the rate of change in CV_E was about half that of the full data set ($\Delta M = 0.00074$; table 2), but it was still significantly greater than 0 and was again close to the average rate of change of quasi-normal productivity ($\Delta M_z = -0.00106$; table 2).

When considered on a strain-by-strain basis, there is a rough correlation between the rate of change of CV_E and the rate of change of the trait itself. For the full CFB data set, the rate of change for the two *Caenorhabditis briggsae* strains is about twice that of the two *Caenorhabditis elegans* strains for both CV_E (ΔM) and the trait itself (ΔM_z), and neither differs significantly from 0 for the EM435 strain of *Oscheius myriophila* (table 1). However, the rank order of the two strains of *C. briggsae* differs between ΔM and ΔM_z ; ΔM_z is greater in HK104 than in PB800, but the reverse is true for ΔM . A similar pattern holds for the quasi-normal data, except that the discrepancy between ΔM and ΔM_z for the HK104 strain is substantial. The cause of that discrepancy may be because average fecundity in that strain was much lower than in the other strains, so that random environmental perturbations that would have simply reduced fecundity in the other strains resulted in sterility in HK104.

Results for the VEL data (N2 strain of *C. elegans*) are quite consistent with those of N2 from the CFB data set, especially for the quasi-normal data (tables 1, 2). The discrepancy between the full data set and the quasi-normal data is greater for the CFB data and may be a result of the VEL data including many more early generations of MA in which the failure of an individual to reproduce (because of either sterility or death before reproduction) was uncommon.

On average, CV_E for body volume increased significantly with generations of MA (table 3), although the results were more variable than those for productivity. The average increase ($\Delta M \approx 0.06\%$ /generation) was somewhat less than that for the full productivity data set, although it was very comparable to that of the quasi-normal data. Of the individual strains, only PB800 differed significantly from 0. Possible biological reasons why the trend in body volume is weaker than that of productivity are considered further in "Discussion." Possible experimental factors include smaller sample sizes within assays and fewer assays.

Decanalization in an MA experiment can, in principle, result either from the accumulation of new mutations or from the loss of overdominant alleles segregating in the progenitor stock. If decanalization results from the loss of overdominant alleles, the prediction is that the rate of increase of environmental variance should slow over time. To test that hypothesis, we

calculated change per generation (the regression slope, R_m) in the full CFB data set separately at 100 and 200 generations of MA, as above. Over the first 100 generations, R_m was 0.058 ± 0.026 ; over the first 200 generations, R_m was 0.087 ± 0.027 . We performed a similar analysis using the full VEL data set. The data were divided into the first four fitness assays (generations 7, 20, 30, and 50) and the second four assays (generations 90, 120, 163, and 214), and R_m was determined for each subset of the data. Over the first 50 generations, R_m was not significantly different from 0 (-0.035 ± 0.062); over the subsequent 165 generations, R_m was significantly positive (0.067 ± 0.019). The increase in the rate of canalization over time strongly supports the idea that deleterious mutations, not loss of overdominant alleles, act to decanalize the phenotype.

Change in the Among-Line Variance (V_M and h_M^2)

The phrase “mutational variance” has both a general meaning—the input of new genetic variation by mutation—and a specific mathematical meaning; that is, V_M . In what follows, I will use the words “mutational variance” in the former loose sense and the abbreviation V_M in its specific mathematical sense. Because the trait in question is the CV, the unstandardized variance itself (i.e., V_M) provides a meaningful statistic for comparison between the groups included in this study, although it is not useful for broader comparisons with other studies. A more useful statistic for broader comparisons is the mutational heritability. Most important, in every case except one (body volume in the DF5020 strain of *O. myriophila*), the point estimate of the among-line variance in CV_E increased with increasing generations of MA, although in some cases, the rate of increase was not significantly different from 0 (tables 1–3). Several interesting patterns emerge from the results. First, both V_M and h_M^2 are considerably smaller for the quasi-normal data than for the full data set, although V_M is somewhat more sensitive to the inclusion of zeros. Second, the mutational heritabilities for canalization of the trait (i.e., h_M^2) are always of the same magnitude as the mutational heritability of the trait itself ($h_{M,z}^2$) — on the order of 10^{-3} /generation — and the point estimates of h_M^2 often exceed those of $h_{M,z}^2$. Third, V_M for CV_E for body volume is ~20-fold less than V_M for CV_E for productivity for the full data set (0.22 vs. 4.13), but the point estimates of h_M^2 differ less than twofold (0.0025 vs. 0.0043), roughly consistent with the difference between the two traits in both ΔM for CV_E and $h_{M,z}^2$. The relationship between the mutational variance for CV_E and the mutational variance for the trait itself (i.e., between V_M and $V_{M,z}$) is quite variable, but it is noteworthy that the PB800 strain of *C. briggsae* is consistently the most variable by any measure.

Discussion

This study had its origins as a review of the literature, in an attempt to quantify the relationship between mutation load and canalization (i.e., V_E), motivated by a suggestion of Wagner et al. (1997) that the increase in the genetic variance in V_E in an MA experiment would allow the (de)canalizing effects of mutations to be quantified. However, it quickly became apparent that there is very little appropriate published data—hence the resort to our own data, which had previously been observed to fit the predicted relationship at least qualitatively. Most authors do not report environmental variances of control lines, presumably because V_E of the control is rarely of direct interest in an MA experiment. For a data set to be appropriate for the purposes of this study, it must include V_E for both MA lines and control lines. Simply tracking the change in V_E in MA lines over time is not sufficient because environmental variance can change (increase or decrease) over time for reasons other than mutation load.

Previous studies have established that both productivity and body volume decrease about twice as fast in the two strains of *Caenorhabditis briggsae* as in the two strains of *Caenorhabditis elegans* and the DF5020 strain of *Oscheius myriophila* (Baer et al. 2005, 2006; Ostrow et al. 2007; tables 1–3). Similarly, the mutational coefficient of variation (CV_M) for productivity is about twice as great in *C. briggsae* as in *C. elegans* (tables 1, 2). That result is not true for body size, although the trend is in the same direction (table 3). The per-generation change in mean phenotype due to MA is $\Delta M = U$, where U is the genomic mutation rate for the trait in question and $\frac{1}{2}$ is half the average homozygous effect of a new mutation on the trait (Lynch and Walsh 1998). Similarly, the mutational variance is $V_M = UE(2)$. Thus, differences among groups in ΔM and/or V_M could be due to differences in U , $\frac{1}{2}$, or both.

Overall, the results of this study provide strong evidence that, on average, spontaneous (presumably deleterious) mutations decanalize the phenotype against random environmental perturbations. In retrospect, this result is exactly what would have been predicted, given the body of work on the relationship between canalization and inbreeding. What is perhaps surprising is the magnitude of the effect. The per-generation change in the CV_E (ΔM) for productivity is very close to that of productivity itself (ΔM_Z), on the order of 10^{-3} /generation (tables 1, 2). The pattern is very similar for body volume (table 3). Thus, the prediction follows: if selection against deleterious mutations is relaxed, the phenotype, in general, will become decanalized against the effects of random environmental perturbation. In particular, any factor that reduces effective population size to the extent that deleterious mutations begin to accumulate will also tend to increase phenotypic sensitivity to environmental variation.

A separate issue concerns the accumulation of genetic variance for canalization. The circumstances under which canalization (environmental and/or genetic) can evolve have been the subject of considerable discussion (Wagner et al. 1997; Meiklejohn and Hartl 2002; Siegal and Bergman 2002; de Visser et al. 2003; Dworkin 2005a; Proulx and Phillips 2005; Zhang and Hill 2005). The second major result of this study is that genetic variance for canalization accrues at a substantial rate. The mutational heritability of canalization (h_M^2) for both productivity and body volume is on the order of 10^{-3} /generation (tables 1–3), similar to (or greater than) the mutational heritabilities of the traits themselves ($h_{M,z}^2$). Thus, there is abundant genetic variation for environmental canalization on which natural selection can act should it so choose.

Because the CV is dimensionless, direct comparison of V_M for canalization between traits is meaningful. The V_M for canalization increases from body volume (average $V_M = 0.22$; table 3) to quasi-normal productivity (average $V_M = 1.01$; table 2) to productivity in the full data set (average $V_M = 4.13$; table 1). The smaller V_M for body volume is most straightforwardly interpreted as a result of fewer loci affecting canalization of body size than affect canalization of productivity. The fourfold difference in V_M between quasi-normal productivity and productivity in the full data set is almost certainly due to the fact that productivity is a composite of two traits, fecundity and survivorship (Lande 1977). Because the quasi-normal data do not include replicates that failed to reproduce, canalization for survival before first reproduction does not factor into V_M for the quasi-normal data. The discrepancy in V_M between the two traits suggests that more loci contribute to canalization for survivorship than for fecundity, perhaps up to four times as many if the effects are equal.

An alternative explanation for the differences in V_M between traits is that the susceptibility to decanalizing mutations is the outcome of adaptive evolution. Wagner et al. (1997) demonstrated that traits under strong selection are more difficult to canalize genetically than

are traits under weaker selection because natural selection will efficiently weed out deleterious alleles affecting traits under strong selection. It is reasonable to assume that productivity is under stronger selection than body volume, and the observed results are in the predicted direction. However, the mutational target size argument seems more parsimonious.

A third prominent result is the observation that the mutational properties (change in mean and variance) of canalization are at least roughly correlated with those for the trait itself. That result is consistent with genetic and environmental canalization having similar underlying mechanisms. Unfortunately, the existence of such a correlation does not provide a strong test of the hypothesis of similar mechanism. Mutational input for a trait and for canalization of the trait may covary for two reasons. First, it may be that any allele that affects environmental canalization pleiotropically affects genetic canalization (i.e., robustness to mutation) in a similar way. By way of illustration, suppose that high alleles have strong canalizing effects and that low alleles have weak canalizing effects. In such a case, if lines are initially fixed for different alleles, there will be a positive correlation between the rate of accumulation of mutational variance for a trait and the rate of accumulation of mutational variance for canalization of the trait such that lines that are fixed for high alleles (strong canalization) will accumulate mutational and environmental variance more rapidly than lines fixed for low alleles. Perhaps more parsimonious, however, is the possibility that the mechanisms underlying environmental and mutational canalization are independent of each other. If the mutation rate differs between taxa, then, all else equal, taxa with high mutation rates will accumulate mutations that affect a trait and mutations that affect environmental canalization of the trait more rapidly than taxa with low mutation rates. Implicit in this argument is the idea that the mutational target sizes (i.e., number of loci) of both the trait and environmental canalization for the trait are relatively consistent across taxa. The evidence from this study suggests that such is likely to be the case, but it need not necessarily be that way, particularly for taxa that have been separated by tens or even hundreds of millions of years.

Ideally, one would like to know the within-taxon genetic correlations between the trait and canalization for the trait. A positive mutational correlation between the trait mean and CV_E would provide concrete evidence that alleles that directly affect the trait also indirectly affect canalization of the trait. However, one cannot calculate a legitimate genetic correlation between the change in the mean and the change in the within-line variance from our data because the CV is not independent of the mean (the same holds true for other measures of variation, such as Levene's statistic). In principle, the way to determine the genetic correlation is via cross-validation of a partitioned sample, but the within-line sample sizes in these data sets are too small to permit meaningful cross-validation. Resolution of the question awaits a much larger study.

Finally, as noted in the introduction to this article, it has been suggested that heterozygosity per se may play an important role in phenotypic canalization (Lerner 1954; Mitton and Grant 1984). Whether overdominance *sensu strictu* is common in natural populations is debatable, but even a few overdominant loci with typical effect sizes can provide a substantial fraction of the standing genetic variance (Charlesworth and Hughes 2000). If our control lines had even a few residual segregating overdominant loci, we would expect to see a decrease in CV_E over time, as the residual segregating loci fixed via drift. Although these experiments were not designed to explicitly measure heterozygous effects, the consistent increase of CV_E with generations of MA is not consistent with residual segregating overdominant loci, nor is the strong and consistent positive relationship of CV_E with increasing mutation load consistent with overdominance being necessary for environmental

canalization, although we cannot rule out that overdominant loci play some role in canalization.

Conclusions

Three primary results emerge from this study. First, and most important, spontaneous mutations have a consistent decanalizing effect on two biologically important phenotypic traits, productivity and body size, such that the phenotype becomes progressively less robust to random environmental variation as mutations accumulate. That result extends and generalizes what has been learned about canalization from studies comparing inbred and outbred genotypes. Second, the magnitude of the decanalizing effects is perhaps surprisingly large— ΔM and h_M^2 are of the same order as for the traits themselves. Thus, there is abundant raw material for the evolution of canalization should natural selection favor a change in the extent of canalization. The magnitude of variation available for the evolution of canalization has not previously been quantified. Third, comparison across taxa reveals that the mutational properties (ΔM and V_M) for canalization are at least roughly correlated with the mutational properties for the traits themselves (ΔM_z and $V_{M,z}$). That result is consistent with mutational and environmental canalization operating via similar underlying mechanisms, although the simpler explanation of similar mutational targets accumulating mutations at characteristically different rates cannot be rejected.

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Table 1

Productivity (all data, zeros included)

Species and strain	R_m (SE)	M_0 (SE)	$\Delta M \times 10^3$ (CL)	V_M (SE)	$V_{E,0}$ (SE)	$h^2_M \times 10^3$ (CL)	$\Delta M_z \times 10^3$	$h^2_{M,z} \times 10^3$	$CV_{M,z}$
<i>Caenorhabditis briggsae</i> :									
HK104	.164 (.026)	77.37 (3.14)	2.13 (1.38, 2.96)	2.35 (.86)	1,679.7 (233.7)	1.48 (.34, 3.13)	-2.81	1.07	2.62
PB800	.154 (.018)	37.98 (1.72)	4.08 (3.03, 5.26)	3.68 (.61)	459.4 (112.7)	8.78 (4.06, 17.97)	-2.51	2.12	2.65
<i>Caenorhabditis elegans</i> :									
N2 (CFB)	.072 (.015)	41.11 (2.13)	1.78 (.95, 2.68)	1.82 (.43)	351.5 (70.05)	5.59 (2.37, 11.66)	-1.09	1.00	1.47
N2 (VEL)	.092 (.015)	33.69 (1.17)	2.75 (1.76, 3.77)	1.72 (.41)	794.5 (137.5)	2.27 (.93, 3.87)	-1.45	1.50	1.50
PB306	.054 (.019)	52.06 (2.79)	1.06 (.34, 1.90)	1.69 (.70)	786.8 (198.4)	2.46 (.34, 6.32)	-1.32	1.97	1.32
<i>Oscheius myriophila</i> :									
DF5020	.093 (.028)	31.07 (2.37)	3.04 (1.08, 5.28)	2.50 (1.03)	433.4 (156.9)	7.15 (1.24, 23.89)	-1.20	2.46	1.71
EM435	-.052 (.048)	77.15 (5.91)	-.65 (-1.68, .72)	.36 (1.49)	1,868.8 (329.9)	.31 (-.98, 2.68)	.08	...	...
All strains	.086 (.010)	53.24 (1.17)	1.62 (1.19, 2.05)	2.07 (.45)	929.9 (274.9)	4.30 (1.38)	-1.48	1.72	1.95

Note: R_m = slope of the regression of the within-line coefficient of variation (CV) against generations of mutation accumulation; M_0 = mean within-line CV of generation 0 (control) lines; ΔM = per-generation change in the within-line CV scaled to the generation 0 mean; V_M = half the per-generation increase in the among-line variance of the within-line CV; $V_{E,0}$ = the among-line variance in the within-line CV at generation 0 (control); h^2_M = the mutational heritability of the within-line CV; ΔM_z = per-generation change in mean productivity; $h^2_{M,z}$ = mutational heritability of productivity; $CV_{M,z}$ = mutational coefficient of variation for productivity. CFB = lines from Baer et al. (2005, 2006; Ostrow et al. 2007); VEL = lines from Vassilieva and Lynch (1999; Vassilieva et al. 2000). Values in the last three columns (bold) have been previously published (Baer et al. 2005, 2006) and are included for purposes of comparison.

Table 2

Quasi-normal productivity

Species and strain	R_m (SE)	M_0 (SE)	$\Delta M \times 10^3$ (CL)	V_M (SE)	$V_{E,0}$ (SE)	$h_M^2 \times 10^3$ (CL)	$\Delta M \times 10^3$	$h_{M,z}^2 \times 10^3$	$CV_{M,z}$
<i>Caenorhabditis briggsae</i> :									
HK104	.021 (.023)	58.02 (2.70)	.37 (-.40, 1.20)	.59 (.56)	899.8 (144.7)	.75 (-.40, 2.42)	-2.21	1.07	1.69
PB800	.069 (.012)	34.27 (1.33)	2.01 (1.28, 2.83)	1.06 (.28)	336.0 (74.3)	3.45 (1.21, 7.48)	-2.05	2.12	1.89
<i>Caenorhabditis elegans</i> :									
N2 (CFB)	.029 (.011)	35.84 (1.46)	.82 (.16, 1.55)	.71 (.29)	292.9 (45.9)	2.61 (.56, 5.79)	-1.07	1.00	.94
N2 (VEL)	.027 (.012)	29.82 (.95)	.93 (.18, 1.84)	.76 (.24)	267.5 (36.2)	2.96 (.91, 5.71)	-.77	.14	.32
PB306	.033 (.013)	38.36 (1.72)	.88 (.17, 1.63)	.49 (.29)	456.2 (69.3)	1.17 (-.13, 3.10)	-1.00	1.97	1.35
<i>Oscheius myriophila</i> :									
DF5020	.036 (.015)	27.2 (1.44)	1.34 (.26, 2.47)	.58 (.33)	227.3 (48.9)	2.95 (.031, 8.64)	-.95	3.12	1.68
EM435	-.043 (.028)	51.05 (4.11)	-.81 (-1.74, .26)	-.40 (.70)	872.8 (193.9)	-.32 (-1.50, 1.75)	-.35	...	...
All CFB Strains	.030 (.008)	40.73 (.96)	.74 (.33, 1.16)	.51 (.20)	514.2 (121.6)	1.77 (.60)	-1.27	1.86	1.51

Note: See note for table 1 for definitions of variables and abbreviations. Values in the last three columns (bold) have been previously published (Baer et al. 2005, 2006) and are included for purposes of comparison.

Table 3

Body volume

Species and strain	R_m (SE)	M_0 (SE)	$\Delta M \times 10^3$ (CL)	V_M (SE)	$V_{E,0}$ (SE)	$h_M^2 \times 10^3$ (CL)	$\Delta M \times 10^3$	$h_{M,z}^2 \times 10^3$	CV _{M, z}
<i>Caenorhabditis briggsae</i> :									
HK104	.0061 (.023)	27.11 (3.04)	.28 (-1.23, 2.21)	.44 (.66)	280.4 (151.4)	2.97 (-1.61, 15.98)	-1.71	2.05	1.52
PB800	.044 (.013)	18.56 (1.69)	2.44 (1.30, 3.77)	.33 (.21)	107.0 (50.5)	5.35 (-.47, 25.45)	-1.41	2.55	1.57
<i>Caenorhabditis elegans</i> :									
N2 (CFB)	.013 (.012)	23.58 (2.03)	.60 (-.42, 1.77)	.20 (.12)	94.5 (30.0)	2.60 (-.29, 7.54)	-.75	3.46	1.16
PB306	-.0022 (.014)	20.11 (2.08)	-.059 (-1.23, 1.43)	.18 (.22)	139.7 (39.1)	1.66 (-1.33, 6.71)	-.71	9.59	1.42
<i>Oscheius myriophila</i> :									
DF5020	.014 (.022)	31.40 (2.94)	.50 (-.79, 2.06)	-.011 (.18)	221.7 (49.6)	.086 (-1.28, 2.45)	-.89	2.91	1.86
EM435	.0079 (.018)	35.78 (2.23)	.24 (-.72, 1.23)	.18 (.22)	146.5 (60.0)	2.21 (-1.02, 10.36)	-1.36	...	...
All strains	.015 (.006)	25.82 (.91)	.59 (.09, 1.10)	.22 (.06)	165.0 (29.4)	2.48 (.71)	-1.14	4.11	1.51

Note: See note for table 1 for definitions of variables and abbreviations. Values in the last three columns (bold) have been previously published (Ostrow et al. 2007) and are included for purposes of comparison.