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Vassiliki Koufopanou; Graham Bell

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# DEVELOPMENTAL MUTANTS OF *VOLVOX*: DOES MUTATION RECREATE THE PATTERNS OF PHYLOGENETIC DIVERSITY?

### VASSILIKI KOUFOPANOU<sup>1</sup> AND GRAHAM BELL Department of Biology, McGill University, 1205 Avenue Dr. Penfield, Montreal, Quebec, H3A 1B1, CANADA

Abstract. - The nature of the variation which is created by mutation can show how the direction of evolution is constrained by internal biases arising from development and pre-existing design. We have attempted to quantify these biases by measuring eight life history characters in developmental mutants of Volvox carteri. Most of the mutants in our sample were inferior to the wild type, but deviated by less than tenfold from the wild-type mean. Characters differed in mutability. suggesting different levels of canalisation. Most correlations between life history characters among strains were positive, but there was a significant negative correlation between the size and the number of reproductive cells, suggesting an upper limit to the total quantity of germ produced by individuals. The most extreme phenotypes in our sample were very vigorous, showing that not all mutations of large effect are unconditionally deleterious. We investigated the effect of developmental constraints on the course of evolution by comparing the variance and covariance patterns among mutant strains with those among species in the family Volvocaceae. A close correspondence between patterns at these two levels would suggest that pre-existing design has a strong influence on evolution, while little or no correspondence shows the action of selection. The variance generated by mutation was equal to that generated by speciation in the family Volvocaceae, the genus Volvox, or the section Merillosphaera, depending on the character considered. We found that mutation changes the volume of somatic tissue independently of the quantity of germ tissue, so that the interspecific correlation between soma and germ can be attributed to selection. The negative correlation between size and number of germ cells among mutants of V. carteri is also seen among the larger members of the family (Volvox spp.), but not among the smaller members, suggesting a powerful design constraint that may be responsible for the absence of larger forms in the entire group.

Key words. – Developmental constraints, germ, life history, mutants, mutation, phylogenetic diversity, soma, speciation, Volvocaceae, *Volvox*.

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Though the importance of mutation in creating variation is widely recognized, the nature of this variation and the extent to which it determines the patterns of phylogenetic diversity observed in nature remain unknown. Mutational variation is obtained when selection is relaxed, and any directionality that it displays can be interpreted to reflect internal biases in the production of phenotypic change. Such biases are often referred to as developmental constraints (Maynard Smith et al., 1985).

Biases in the mutability of characters will indicate that mutation pressure in nature is not isotropic, meaning that it does not have equal strength in all characters and directions, and this may hinder the response to selection. However, when selection is applied over long periods of time, it may override some of the limitations (Zeng, 1988). We may thus inquire whether biases identified by studying the mutational variation have persisted during evolution and therefore represent basic constraints that characterize the design of the entire clade. Close correspondence between the mutational and interspecific diversity would imply that internal biases have been important, while little or no correspondence would mean that selection is mainly responsible for observed patterns.

The phenotypic effects of mutation in eukaryotes are known mostly from studies of spontaneous and induced mutations in *Dro*sophila (reviewed by Crow and Simmons, 1983), crops (e.g., peanuts, reviewed by Gregory, 1968; peas, reviewed by Gottschalk and Wolff, 1983), and a few other organisms (*Daphnia*, Lynch, 1985; protozoans, reviewed by Bell, 1988). The generalizations that have emerged from such studies are that most mutations are of small effect, are generally inferior to the wild type,

<sup>&</sup>lt;sup>1</sup> Present address: Department of Biology, University of California, Santa Cruz, CA 95064 USA.

and are positively pleiotropic, depressing several characters at the same time. It is also generally assumed that the larger the effect of a mutation the more deleterious it is likely to be (Fisher, 1958; Crow and Simmons, 1983). However, major mutations represent nature's experiments with particular designs, and can reveal the potential and limits of these designs. The study of mutations of large effect provides a direct means of measuring the trade-offs that underlie the evolution of life histories and of defining the "option sets" available to different organisms.

The question of whether biases in the mutational variance identified in a single species correspond to those observed in the phylogenetic diversity of related taxa has been addressed only in a qualitative way. In Drosophila melanogaster, genetic variants which affect the expression of the bithorax complex uncover primitive abdominal patterns (Lewis, 1978), and those which affect the achaete-scute complex mimic chaeta patterns found in the Drosophilidae (Garcia-Bellido, 1983). In Caenorhabditis elegans, there is some similarity between cell-lineage patterns of mutants and those of related species of nematodes (Ambros and Fixsen, 1987). Patterns of limb reduction obtained by experimentally manipulating the development of frogs and salamanders were similar to observed phylogenetic trends (Alberch and Gale, 1985); if there are genetic effects which mimic these manipulations, these results would suggest that patterns of diversity in the digital morphology of amphibians reflect basic properties of their developmental system.

This study examines a number of developmental mutants of *Volvox carteri* (Chlorophyta: Volvocaceae). The range of variation in these mutants spans the entire range found in the Volvocaceae with respect to colony size, number of cells, and the extent of differentiation between somatic and reproductive tissues. To test whether mutation is biased, we have compared the variance and covariance of characters among mutants to those of wild-type strains of the same species. To examine the relationship between mutational and phylogenetic diversity, we have compared the genetic variances and covariances of characters in the mutants with those of species in the Volvocaceae at three taxonomic levels: the entire family, the genus *Volvox*, and the section Merillosphaera of which *V. carteri* is a member.

### MATERIALS AND METHODS

*Life Cycle. – Volvox* is haploid. The asexual life cycle of V. carteri (reviewed by Kirk and Harper, 1986) includes a single reproductive episode, during which reproductive cells are produced and develop synchronously and symmetrically. Briefly, a mature germ cell cleaves 12 to 13 times to produce the full adult complement of cells. During the development of the embryo, germ potential is segregated among cells by a series of asymmetric cell divisions, which ultimately give rise to two types of cells: a few large germ cells and many small somatic cells. The embryo then turns inside out, so that the flagellated somatic cells are on the outside of the spheroid, and grows by enlarging its cells and intercellular spaces. Early in growth, the embryos are released from the parent, which dies a few hours later.

Strains and Culture Conditions. - All strains examined in this study belong to V. carteri forma nagariensis, and were kindly supplied by Dr. D. L. Kirk (Washington University at St. Louis, Missouri). Though this species has been studied extensively there are only two sets of natural isolates of this form, one from Japan (strain HK10, male and female) and one from India (strain Poona, male and female). "Wild type" strains have been obtained from the original two strains at different times, and some were subsequently backcrossed to them. "Mutant" strains were derived from the Japanese female strain through chemical mutagenesis (nitrosoguanidine), UV-irradiation or spontaneously. They do not represent a random sample of all mutational effects, but rather a sample of major effect mutations, identified on the grounds of aberrant numbers and sizes of cells (D. L. Kirk, pers. comm.). To the extent that no effort was made to collect mutants which vary in a particular direction, and that the person isolating the mutant strains was not aware of the hypotheses tested here, the sample of mutants is random with respect to the above characters. However, the likelihood that a variant would be detected, and thus included in our sample, partly depends on its fitness, because although natural selection was relaxed during the isolation of the strains it could not be eliminated entirely. For example, a germless mutant could not have been included in our sample, since such a variant cannot be propagated. Apart from such extreme cases, however, the conditions of culture (synchronous cultures, supplied with adequate light and nutrients at near optimal temperatures), allowed the detection of a wide range of aberrations. Although no formal genetic analysis has been made on this particular set of strains, previous work (Huskey et al., 1979, and D. L. Kirk, pers. comm.) suggests that most of the observed phenotypic effects are due to mutations of single genes or tightly-linked groups of genes.

Stock cultures of all strains were maintained axenically in Standard Volvox Medium (SVM, Kirk and Kirk, 1983) at 20°– 23°C and a 48 hr light–dark cycle (32 hr light under fluorescent lamps giving 130  $\mu$ E/ cm<sup>2</sup>/sec, followed by 16 hr dark). Cultures were reinoculated into new medium every 7 to 10 days.

Phenotypic Characterization of Strains. -We performed two experiments, the first employing 13 strains (5 wild type and 8 mutant) and the second using 22 strains (7 wild type and 15 mutant). In both experiments, 500 ml flasks containing 250 ml SVM were inoculated (4 replicates/strain in the first and 2 in the second trial) and incubated in growth chambers under continuous light, in order to enhance any inherent differences in the strains' capacity for growth, at 130  $\mu$ E/cm<sup>2</sup>/ sec (185  $\mu$ E/cm<sup>2</sup>/sec for experiment 2) and  $22^{\circ} \pm 1^{\circ}$ C. Cultures were aerated with sterile-filtered air in the first trial, to stir the medium and to provide an additional source of carbon, and mildly shaken, with no addition of air, in the second.

To measure population growth, 10 ml aliquots were taken from each culture every 3 days (2 days for the second trial), starting at day 0 and continuing until day 27 (19 for the second trial), by which time most cultures were declining. The chlorophyll was extracted from each sample in ethanol and the optical density (measured as Absorbance) scored at 665 nm on a spectrophotometer. To score morphological traits of the adults, 2 additional 10 ml samples were taken on the 12th and 13th days (9th and 10th for the second trial), while cultures were still growing exponentially, and fixed with gluteraldehyde. Twenty mature individuals per strain (5 individuals  $\times$  4 replicate cultures in the first experiment, and 10 individuals  $\times$  2 replicate cultures in the second) were randomly chosen from the fixed samples and measured using a Zeiss light microscope. Mature individuals were defined as those having at least one cleaving reproductive cell. To standardize measurements, only two drops of sample were used on each microscope slide, together with one drop of a Bio-Gel Sephadex bead suspension containing beads of 80  $\mu$  mean diameter, the maximum diameter of germ cells. For each individual, we measured the following characters:

(1) Total number of germ cells (by direct count).

(2) Volumes (in  $\mu^3$ , estimated from cell diameter measured at 400×) of all germ cells, and the number of times that each had cleaved. Only germ cells that had cleaved six times or fewer were included in the volume estimates, to avoid biases due to the formation of an internal cavity in the developing embryo.

(3) Total number of somatic cells, estimated as follows. The number of somatic cells per unit area of the spheroid surface was estimated from cell density counts on two  $62 \ \mu \times 62 \ \mu$  grids (one sample from the top and one from the bottom focal plane, measured at 400×). The total surface area of each sphere was estimated from the spheroid diameter at 100×. For strains with very irregular patterns of development, somatic cell numbers were counted directly.

(4) Volumes of somatic cells (in  $\mu^3$ , estimated from cell diameter measured at 400×), from two random samples (one at top, one at bottom) of five cells in the first experiment, and from all somatic cells within the two grids in the second.

Statistical Analyses.—All volumes and numbers of cells measured were log-transformed prior to any further analysis and all symbols used in this paper refer to these log-transformed values. They were then used to estimate the following parameters for each individual: (1) mean volume of somatic cells (VS): (2) total number of somatic cells (NS): (3) total volume of somatic tissue (S = VS+ NS; (4) mean volume of cleaving germ cells (VG); (5) total number of germ cells (NG); (6) total volume of germ tissue (G = VG + NG; (7) total protoplast volume [T  $= \log(\exp S + \exp G)$ ]. To allow for changes in the volumes of germ cells caused by differences in their stage of development, germ cell volumes were standardized at the 64cell stage of cleavage as follows. The volume of entire embryos during the first six cleavage divisions was regressed on the number of cells present in the embryo ("slope at cleavage"). Separate regressions were computed for wild type and mutant strains in each trial, and were then used to calculate the volumes of 64-cell embryos. A slope at cleavage was also computed separately for each strain. It was observed earlier (Koufopanou, 1990) that the value of this slope indicates whether embryonic cells grow between successive cleavage divisions, since species with no growth had slopes of less than 0.50 and species with growth had slopes greater than 0.50. Even in the absence of growth, slopes tend to be positive due to the formation of more intercellular spaces with increasing number of embryonic cells.

Finally, the maximum value of log(Absorbance) during the growth of a culture was used to express the carrying capacity of each replicate culture of a strain, K. It was found that this quantity correlated well with the density-independent rate of increase, r. estimated as the slope of the linear regression of log(Absorbance) on time (days) during early population growth (correlation coefficient of regressions of K against r, r = 0.69, N = 13 in the first experiment, and r = 0.92, N = 22 in the second). K was preferred to r as an index of overall vigor of strains, because it is derived directly from observation and therefore does not include error due to parameter estimation.

An extreme group of mutants, the *regenerators*, whose somatic cells all redifferentiate as germ cells late in colony growth, was treated separately from the rest of the mutants. In the *regenerators*, there is an inverse relationship between the mean volume of cleaving cells per individual and the proportion of its cells that are cleaving, because redifferentiated somatic cells tend to cleave later and at smaller sizes than do normal germ cells. To obtain a measure of mean cell volume at cleavage in each of the *regenerator* spheroids, independent of the proportion of its cells that were cleaving, the mean volume of cleaving cells was standardized relative to spheroids in which all the cells were cleaving, by regressing the mean log volume of germ cells per *regenerator* spheroid against the proportion of its cells that were cleaving (standard errors in parentheses):

experiment 1:

$$Y = -0.82(0.18)X + 3.90(0.31),$$
  

$$N = 20, \quad r^2 = 0.53$$

experiment 2:

$$Y = -0.94(0.19)X + 3.08(0.41),$$
  

$$N = 20, \quad r^2 = 0.59$$

The model for the phenotype of the l-th cell measured in the k-th individual from the j-th replicate culture of the i-th strain is:

$$P_{ijkl} = S_{i} + C_{j(i)} + I_{k(ji)} + A_{l(kji)}$$

Strains are regarded as random effects with variance  $\sigma_{s}^{2}$ ; culture, individual and cell are nested effects with variances  $\sigma_{\rm C}^2$ ,  $\sigma_{\rm I}^2$  and  $\sigma_{\rm A}^2$ , respectively (Sokal and Rohlf, 1981). Developmental variance, defined as the variance among cells within individuals, is expressed by  $\sigma^2_A$ . The environmental variance is  $\sigma_{\rm E}^2 = \sigma_{\rm C}^2 + \sigma_{\rm I}^2 + \sigma_{\rm A}^2$ . Since the cultures were clonal, the genetic variance is estimated by the variance among strains,  $\sigma^2_G =$  $\sigma^2_{\rm S}$ . Note that not all of the above components contribute to the total variance of all characters, since different characters may refer to different levels of comparison. There is no independent estimate of measurement error within a trial, and so the measurement error is always included in the lowest component of variance for that character. The repeatability of measurements on the same strain in the two experiments was between 0.60 and 0.96, depending on the character.

Comparisons with the Interspecific Patterns. — The data on species characters were obtained from Koufopanou (1990). They represent 30 species of Volvocaceae, belonging to 8 genera: Astrephomene (2 species), Gonium (5 species), Pandorina (2 species), Eudorina (2 species), Platydorina (1 species) Pleodorina (2 species). Eudorina  $\times$ Pleodorina (1 strain), Volvulina (2 species), Volvox [13 species in 4 sections: Merillosphaera (7 species), Janetosphaera (2 species), Copelandosphaera (1 species), Euvolvox (3 species)]. All species were cultured in a common laboratory environment and all the characters mentioned above, except K. were measured on 10 mature individuals per species. The variances of characters were calculated from species means, and were estimated for three taxonomic levels, the family Volvocaceae, the genus Volvox, and the section Merillosphaera. Since no reliable phylogeny exists for the Volvocaceae, hierarchical classifications within the family and within the genus were ignored in the computation of variances at the family and the genus levels, respectively. For consistency of comparison, the genetic variances measured in the mutants were recalculated in the same way, from the strain means obtained in experiment 2 (because this included the greater number of strains), and hence are somewhat higher than the genetic component referred to above.

#### RESULTS

## (A) Is Mutation Isotropic?

Effects on Single Characters. – Most of our discussion concerns the seven morphological attributes of adult spheroids, which we shall refer to as "morphological" characters. Note, however, that these characters also measure the life history. The number of germ cells, for example, is equivalent to fecundity, since all cleaving germ cells will eventually give rise to daughter colonies; total tissue is a measure of body size; and the quantity of somatic tissue may have direct impact on survival in nature, since the flagellated somatic tissue prevents the spheroid from sinking (Koufopanou, 1990). The remaining two characters are the population carrying capacity (K), a "population" character measuring some aspect of the overall fitness of strains in laboratory culture, and the slope at cleavage, a "developmental" character measuring the presence or absence of compensatory growth during cleavage. We also considered briefly three other developmental characters, the variance of germ cells within individual spheroids before and during cleavage, and the variance of somatic cells.

Differences among strains, tested against the variance of replicate cultures, are highly significant in all characters measured (Table 1). All morphological characters are analyzed on a relative scale, so that strain means can be pooled (assuming that changes in different characters are uncorrelated with one another), in order to obtain a general estimate of the magnitude of phenotypic change obtained by mutation. The recovery of extreme mutations depends partly on the extent of effort invested in their detection and maintenance. In our sample, only 10% of all mutational effects created deviations of more than tenfold, and only 2% of more than hundredfold from the wild-type mean (Fig. 1). Mutations of very small effects may be lacking because they are difficult to detect visually.

Figure 2 shows the frequency distributions of wild type and mutant strain means for each character. Means represent the average values of individual spheroids for that strain; for K, the values of replicate cultures have been averaged. All traits measured in wild-type strains have approximately Normal distributions (tests for deviations from normality nonsignificant); among mutants, some characters are normally distributed and some are not. Volume of somatic cells, number of germ cells and slope at cleavage are symmetrically distributed around the wild-type mean, but all other distributions of mutant character values are shifted to the left, indicating that, on average, new mutations do not improve traits that are closely related to fitness.

If novel mutations disturb coadapted gene complexes and destabilize phenotypes, then mutants should have larger developmental and environmental variances. Figure 3 compares the developmental, environmental and genetic variance components of mutant and wild-type strains, and supports this expectation: for most characters, variances among mutants exceed the corresponding variances among the wild-type strains by the same factor, but the number of somatic cells and the total quantity of somatic tissue have exceptionally high levels of genetic variance in the mutants. Note the large effect of the *regenerator* strains on the computation of variances.

The variance of cell size at cleavage is less than the variance before cleavage in both wild type and mutant strains, indicating a fairly stable size threshold for the initiation of cleavage (Fig. 3, left). The same is true for the regenerators, but in these strains the variance of cells at cleavage is much greater than that of other mutants, indicating that this mutation has a large effect not only on the allocation to somatic and reproductive tissues, but also on the size threshold for the initiation of cleavage. The large environmental variance of these strains is probably a consequence of their altered mode of development, which results in a greater asynchrony among individuals, rather than of an inherently unstable phenotype.

The Variability of Characters. - The comparisons of genetic variance in Figure 3 suggest that some characters are highly mutable while others are relatively inert. This is further confirmed by an analysis of variance performed on the deviations of strain values from the mean values of characters (Levene's test for heterogeneity of variances, Van Valen, 1978). This test revealed significant differences between wild type and mutant variances, as well as differences among characters (wild type vs. mutants: F= 21.0, P < 0.001; characters: F = 5.3, P< 0.001). Given the differences in the variability of characters, we may further ask if the genetic and environmental components of character variances are correlated. Characters that are little affected by the environment may be less amenable to change by mutation, if their lack of variation reflects basic developmental or mechanical constraints. Alternatively, environmental variance may itself be an evolved character (Bradshaw, 1965). The first hypothesis predicts a positive correlation between the genetic variance generated by mutation and the environmental variance expressed in the wild type. We might also expect a positive overall correlation between genetic and environmental variances. These correlations in our material are very weak, perhaps due to the small sample size (Fig. 4). Note, however, that the number of somatic cells and the total quantity of somatic tissue have both



FIG. 1. Frequency distribution of mutant character value deviations from the wild-type mean, data for all morphological characters combined (experiment 2); points are deviations of mutant strain means from the mean of wild-type strains, N = 99.

the largest environmental and the largest genetic variances.

Genetic Correlations.—The correlation matrices for all pairwise combinations of characters show that most correlations are positive or zero (Table 2); only one significantly negative correlation was obtained in both experiments, between the number and size of reproductive cells. Note that the *regenerator* strains are not included in the analysis of somatic tissue and its components, since all their somatic tissue is converted into germ at maturity. The prevalence of positive correlations indicates extensive positive pleiotropy of gene action.

We chose to analyze in more detail three independent correlations that are of special interest in the evolution of Volvox, those involving the composition and the coevolution of somatic and germ tissues. Figure 5 (top) shows that total somatic tissue varies by more than three orders of magnitude (and is reduced to zero in the mature regenerator), but germ varies over scarcely one order of magnitude. Soma and germ are weakly positively correlated in wild and mutant strains combined (r = 0.54, P < 0.05, N =20), but this correlation breaks down when mutants are analyzed separately (r = 0.36,P > 0.30, N = 13). Mutation thus appears to change total somatic tissue independently of germ tissue.

TABLE 1. Strain means ( $\pm$  one standard deviation) for morphological, population and developmental characters. *F*-ratios for differences among wild type or mutant strains also shown, their significance tested against the variance of replicate cultures (N refers to all characters to the right of that column; N1: no. of spheroids; N2: no. of replicate cultures; N3: no. of cells at cleavage; \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001; 1: slope and standard error of estimate).

	Somatic cells				Germ cells		
Type Strain	NI	Number	Volume	Somatic tissue	Number	Volume	
Experiment 1							
Wild type							
EVE	20	$2.82 \pm 0.45$	$2.65 \pm 0.16$	$5.48 \pm 0.42$	$0.82\pm0.29$	$5.10\pm0.13$	
JZ	20	$2.80 \pm 0.26$	$2.63 \pm 0.12$	$5.44\pm0.33$	$0.75\pm0.32$	$5.03\pm0.16$	
PF1	20	$2.87\pm0.31$	$2.61\pm0.15$	$5.49\pm0.37$	$0.96 \pm 0.11$	$5.19\pm0.12$	
Poona	20	$3.11 \pm 0.23$	$2.65 \pm 0.14$	$5.75 \pm 0.24$	$1.02 \pm 0.14$	$5.25 \pm 0.16$	
sugZ	14	$3.01 \pm 0.21$	$2.52 \pm 0.15$	$5.54 \pm 0.23$	$0.85 \pm 0.21$	$5.04 \pm 0.14$	
Mutant							
LagK2	17	$2.86\pm0.27$	$2.64 \pm 0.24$	$5.54\pm0.39$	$0.85 \pm 0.21$	$4.90 \pm 0.29$	
157	20	$2.70 \pm 0.35$	$2.51 \pm 0.14$	$5.21 \pm 0.36$	$0.78 \pm 0.11$	$5.07 \pm 0.22$	
7537	20	$1.93 \pm 0.25$	$2.53 \pm 0.19$	$4.45 \pm 0.37$	$1.00 \pm 0.35$	$4.69 \pm 0.19$	
mulH1	20	$1.14 \pm 0.49$	$2.96 \pm 0.24$	$4.26 \pm 0.46$	$0.55 \pm 0.31$	$4.76 \pm 0.31$	
S16	20	$2.64 \pm 0.24$	$2.75 \pm 0.15$	$5.39 \pm 0.34$	$0.95 \pm 0.21$	$5.08 \pm 0.13$	
S7	20	$2.83 \pm 0.49$	$2.66 \pm 0.16$	$5.50 \pm 0.46$	$0.88 \pm 0.10$ 0.63 ± 0.36	$4.97 \pm 0.13$	
8/4	17	$2.10 \pm 0.25$	$2.64 \pm 0.15$	$4.75 \pm 0.26$	$0.03 \pm 0.30$	$4.97 \pm 0.10$	
Regenerator							
regZ	20				$1.99 \pm 0.28$	$3.91 \pm 0.30$	
Tests for differe	nces ar	nong strains:					
Wild type	F	2.5	1.04	1.6	3.9*	3.4*	
Mutant	F	53.4***	7.3***	25.5***	5.8***	4./**	
Experiment 2							
Wild type							
ADM	10	$2.44 \pm 0.46$	$2.56 \pm 0.09$	$4.99 \pm 0.47$	$0.68 \pm 0.29$	$4.78 \pm 0.20$	
EVE	20	$2.87 \pm 0.28$	$2.44 \pm 0.13$	$5.31 \pm 0.31$	$0.81 \pm 0.15$	$4.97 \pm 0.14$	
JZ	20	$2.76 \pm 0.30$	$2.46 \pm 0.12$	$5.22 \pm 0.33$	$0.87 \pm 0.16$	$5.02 \pm 0.12$	
PF1	14	$2.40\pm0.33$	$2.35 \pm 0.17$	$4.77 \pm 0.36$	$0.84 \pm 0.16$	$4.92\pm0.14$	
PF7	12	$3.21 \pm 0.22$	$2.46 \pm 0.12$	$5.67 \pm 0.16$	$0.74 \pm 0.18$	$5.03\pm0.18$	
Poona	12	$3.32 \pm 0.06$	$2.45 \pm 0.16$	$5.77 \pm 0.10$	$0.99 \pm 0.07$	$5.32 \pm 0.13$	
sugZ	20	$2.84\pm0.20$	$2.26 \pm 0.11$	$5.10 \pm 0.24$	$0.85 \pm 0.14$	$4.85 \pm 0.10$	
Mutant							
LagK2	10	$2.58 \pm 0.39$	$2.41 \pm 0.21$	$4.99 \pm 0.50$	$0.48 \pm 0.29$	$4.88 \pm 0.24$	
LagK3	20	$2.79 \pm 0.35$	$2.70 \pm 0.09$	$5.48 \pm 0.37$	$0.82 \pm 0.25$	$4.95 \pm 0.15$	
LagK4	16	$2.11 \pm 0.49$	$2.20 \pm 0.35$	$4.31 \pm 0.62$	$1.27 \pm 0.28$	$4.63 \pm 0.19$	
144	20	$0.98 \pm 0.53$	$2.19 \pm 0.22$	$3.28 \pm 0.53$	$0.42 \pm 0.37$	$4.09 \pm 0.22$	
154	20	$0.19 \pm 0.43$ 1.45 ± 0.37	$1.82 \pm 0.07$ 2.13 ± 0.22	$2.88 \pm 0.10$ $3.59 \pm 0.50$	$1.31 \pm 0.22$ 0.61 ± 0.16	$4.19 \pm 0.23$ 4.64 + 0.18	
161	20	$1.43 \pm 0.37$ 2 73 + 0 32	$2.13 \pm 0.22$ 2 41 + 0 20	$5.39 \pm 0.30$ 5.14 + 0.36	$0.01 \pm 0.10$ $0.72 \pm 0.20$	$4.04 \pm 0.10$ $4.87 \pm 0.14$	
7537	20	$2.75 \pm 0.32$ 2.16 ± 0.17	$2.41 \pm 0.20$ 2 42 + 0.09	$457 \pm 0.30$	$1.11 \pm 0.20$	$4.07 \pm 0.14$ $4.47 \pm 0.22$	
mulH1	20	$1.29 \pm 0.41$	$2.95 \pm 0.10$	$4.32 \pm 0.31$	$0.70 \pm 0.29$	$4.51 \pm 0.28$	
S16	14	$2.56 \pm 0.40$	$2.57 \pm 0.15$	$5.13 \pm 0.43$	$0.96 \pm 0.12$	$4.97 \pm 0.21$	
<b>S</b> 5	19	$2.70 \pm 0.41$	$2.47 \pm 0.12$	$5.18 \pm 0.45$	$0.60\pm0.22$	$4.91 \pm 0.24$	
<b>S</b> 7	20	$2.70\pm0.43$	$2.47 \pm 0.14$	$5.17 \pm 0.44$	$0.70\pm0.30$	$4.86 \pm 0.19$	
S74	20	$2.19 \pm 0.14$	$2.41 \pm 0.24$	$4.60 \pm 0.32$	$0.67\pm0.20$	$4.68 \pm 0.26$	
Regenerator							
regZ	20				$2.18 \pm 0.30$	$3.08 \pm 0.40$	
Ser3	10				$2.19 \pm 0.58$	$3.07 \pm 0.48$	
Tests for differe	nces ar	nong strains:					
Wild type	F	6.7*	15.7**	6.3*	6.0*	8.5**	
Mutant	F	34.5***	15.9***	20.7***	17.8***	6.4**	

Table	1.	Extend	led.
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Germ tissue	Total tissue	N2	K	N3	Slope at cleavage <sup>1</sup>
$5.93\pm0.38$	$6.08\pm0.35$	4	$1.33\pm0.27$	64	$0.12\pm0.06$
$5.78 \pm 0.38$	$5.98 \pm 0.30$	4	$1.29 \pm 0.12$	66	$0.29\pm0.05$
$6.15 \pm 0.18$	$6.24\pm0.20$	4	$1.57 \pm 0.13$	83	$0.23\pm0.05$
$6.27 \pm 0.23$	$6.40 \pm 0.20$	4	$1.50 \pm 0.09$	76	$0.26 \pm 0.04$
$5.90 \pm 0.25$	$6.07 \pm 0.20$	4	$1.02 \pm 0.10$	26	$0.19 \pm 0.05$
$5.75 \pm 0.45$	$5.99 \pm 0.39$	4	$1.20 \pm 0.39$	38	$0.23\pm0.08$
$5.86 \pm 0.28$	$5.95\pm0.28$	4	$0.81 \pm 0.17$	76	$0.17\pm0.05$
$5.69\pm0.40$	$5.71 \pm 0.40$	4	$1.20\pm0.08$	94	$0.37\pm0.06$
$5.31 \pm 0.48$	$5.33 \pm 0.49$	4	$1.26 \pm 0.27$	46	$0.23 \pm 0.14$
$6.03 \pm 0.26$	$6.13 \pm 0.25$	4	$1.24 \pm 0.07$	81	$0.21 \pm 0.04$
$5.85 \pm 0.22$	$6.05 \pm 0.26$	4	$1.12 \pm 0.19$	54	$0.24 \pm 0.04$
$5.60 \pm 0.30$	$5.64 \pm 0.29$	4	$1.21 \pm 0.08$	41	$0.11 \pm 0.06$
$5.90\pm0.47$	$5.89 \pm 0.48$	4	$1.48 \pm 0.12$	268	$0.17 \pm 0.05$
5 3**	4 6**		11 9***		
4.4**	6.7***		3.8**		
5 46 1 0 42	$5.61 \pm 0.41$	2	0.29 + 0.05	24	0.24 + 0.08
$5.40 \pm 0.42$ 5.78 ± 0.22	$5.01 \pm 0.41$ 5.80 ± 0.27	2	$0.38 \pm 0.05$ 0.27 ± 0.10	24	$0.34 \pm 0.08$
$5.78 \pm 0.23$ 5.80 ± 0.10	$5.89 \pm 0.27$ 5.00 ± 0.10	2	$0.37 \pm 0.10$ 0.82 ± 0.16	03 72	$0.22 \pm 0.04$ 0.24 ± 0.02
$5.89 \pm 0.19$ 5.76 ± 0.25	$5.99 \pm 0.19$ 5.70 ± 0.27	2	$0.03 \pm 0.10$ 1.12 ± 0.16	13	$0.24 \pm 0.03$ 0.17 ± 0.04
$5.70 \pm 0.25$ 5.77 + 0.29	$6.06 \pm 0.15$	2	$1.12 \pm 0.10$ $1.21 \pm 0.10$	27	$0.17 \pm 0.04$ 0.31 + 0.07
$6.31 \pm 0.15$	$6.25 \pm 0.15$	2	$1.21 \pm 0.10$ $1.18 \pm 0.02$	47	$0.31 \pm 0.07$ $0.18 \pm 0.04$
$5.69 \pm 0.20$	$5.80 \pm 0.18$	2	$0.69 \pm 0.01$	44	$0.14 \pm 0.03$
$5.36 \pm 0.46$	$557 \pm 039$	2	$0.38 \pm 0.18$	16	$0.25 \pm 0.13$
$5.30 \pm 0.40$ 5.77 + 0.35	$5.96 \pm 0.33$	2	$0.38 \pm 0.18$ 0.92 + 0.03	45	$0.25 \pm 0.15$ 0.10 ± 0.08
$5.90 \pm 0.24$	$5.90 \pm 0.33$ 5.82 + 0.29	2	$0.52 \pm 0.05$ 0.59 + 0.25	106	$0.10 \pm 0.00$ $0.23 \pm 0.05$
$5.12 \pm 0.39$	$5.02 \pm 0.29$ $5.12 \pm 0.39$	$\frac{1}{2}$	$0.24 \pm 0.14$	18	$0.23 \pm 0.03$ $0.24 \pm 0.09$
$5.70 \pm 0.23$	$5.70 \pm 0.22$	$\overline{2}$	$0.71 \pm 0.41$	19	$0.30 \pm 0.14$
$5.25\pm0.28$	$5.27 \pm 0.25$	2	$0.63 \pm 0.10$	63	$0.33 \pm 0.13$
$5.59\pm0.31$	$5.73\pm0.31$	2	$0.52 \pm 0.11$	70	$0.16 \pm 0.05$
$5.58\pm0.32$	$5.62\pm0.30$	2	$1.11 \pm 0.04$	61	$0.37\pm0.06$
$5.21\pm0.39$	$5.33\pm0.28$	2	$0.88\pm0.08$	55	$0.19 \pm 0.11$
$5.93 \pm 0.27$	$6.00 \pm 0.28$	2	$0.83\pm0.02$	43	0
$5.50 \pm 0.32$	$5.71 \pm 0.29$	2	$0.73 \pm 0.10$	38	$0.17\pm0.07$
$5.55 \pm 0.33$	$5.73 \pm 0.31$	2	$0.69 \pm 0.04$	49	$0.12 \pm 0.05$
$5.35 \pm 0.28$	$5.43 \pm 0.27$	2	$0.68 \pm 0.20$	34	$0.24 \pm 0.09$
$5.26\pm0.45$	$5.26\pm0.45$	2	$1.39\pm0.05$	306	$0.29\pm0.07$
$5.26 \pm 0.27$	$5.26 \pm 0.27$	2	$1.44 \pm 0.02$	25	$0.25\pm0.07$
8.5**	4.3		23.0***		
4.4**	4.8**		10.5***		

V. KOUFOPANOU AND G. BELL



FIG. 2. Frequency distributions of mutant and wild-type strain values for nine characters. Data from experiment 2 only are shown, but data from both experiments are comparable; strain means, N = 7 for wild type and N = 15 or 13 for mutant strains.

The effect of mutation on the size and number of somatic cells was also asymmetrical, with a thousandfold change in number and only a tenfold change in size (Fig. 5, middle). There is a weak positive correlation between size and number of somatic cells in the mutants (r = 0.53, P < 0.05, N = 13), but none in the wild-type



FIG. 3. Comparison of developmental, environmental and genetic components of variance for characters measured in mutant and wild-type strains (experiment 2). All points lie above the line of equality, indicating that mutants are equally or more variable than wild-type strains. *Regenerator* strains are shown separately (dark symbols, connected with the respective values for other mutants; genetic variance was calculated with, dark symbols, and without, open symbols, the *regenerator* strains); vgcl: volume of cleaving germ cells, vg: volume of somatic cells; other character symbols defined in methods.



FIG. 4. Comparison of genetic and environmental components of variance. Somatic cell number and total somatic tissue have both high genetic and environmental variances. Points are seven morphological characters and the population carrying capacity, *K*. Top figure, experiment 1 (open squares): r = 0.81, df = 6, P < 0.05; experiment 2 (closed squares): r = 0.55, df = 6, P > 0.05. Bottom figure, experiment 2 only; wild type: r = 0.20, df = 6, P > 0.05.

strains (r = 0, N = 7; see also Table 3 for a list of all the regression lines in this figure).

Germ tissue responded symmetrically, both number and size showing up to a hundredfold change (Fig. 5, bottom). Size correlates positively with number in wild-type strains (r = 0.77, P < 0.05, N = 7). Most mutations (10 out of 15 strains) follow this trend, depressing both size and number of germ cells, but the few mutations that increased the number of reproductive cells beyond that of the wild type induced an overall negative correlation (r = -0.91, P <0.001, N = 15), suggesting an upper limit to the total amount of reproductive tissue produced by individuals. The slope of the regression line is approximately -1 [b = -0.98, SE (b) = 0.13 in the second experiment; b = -0.70, SE (b) = 0.17 in the first], showing that some mutations change the allocation per cell by changing the number of cell divisions involved in the production of germ cells, without affecting the total amount of germ tissue produced.

When each experiment is considered separately, morphological traits correlate very weakly, if at all, with K, possibly because of the large environmental variance in K, unless the *regenerator* strains are included (Table 2). When values from both trials are combined, but the *regenerators* excluded, the size but not the number of both somatic

TABLE 2. Pairwise correlations (Pearson r) between characters; strain means, wild type and mutant strains combined. Experiment 1, left of diagonal, N = 13 or 12; experiment 2, right of diagonal, N = 22 or 20. Bottom row gives the correlations with *regenerator* strains removed from analysis. Diagonal gives repeatabilities of estimates for strains measured in both experiments (N = 13). Correlations in italics are part-whole correlations. (1: correlations calculated using pooled data from both experiments; \* P < 0.05, \*\* P < 0.01.)

	NS	VS	S	NG	VG	G	T	K
NS	0.75**	0.46*	0.97**	-0.22	· 0.87**	0.54*	0.69**	0.29
VS	-0.60*	0.95**	0.61**	-0.39	0.39	-0.02	0.14	0.24
S	0.97**	-0.38	0.60*	-0.20	0.84**	0.53*	0.72**	0.35
NG	0.60*	-0.48	0.52	0.93** 0.58*	$-0.85^{**}$ -0.35	-0.02 0.60 <b>**</b>	-0.18 0.44*	0.66** 0.36
VG	0.77**	-0.24	0.80**	-0.75** 0.36	0.96** 0.82**	0.54** 0.54**	0.65** 0.64**	-0.47* 0.23
G	0.84**	-0.44	0.82**	0.37 0.80**	0.33 0.85**	0.73** 0.79**	0.95** 0.94**	0.17 0.52*
Т	0.93**	-0.45	0.92**	0.23 0.76**	0.45 0.84**	0.96** 0.97**	0.72** 0.76**	0.11 0.53*
K	-0.02	0.03	-0.04	0.35 0.14	$\begin{array}{c}-0.11\\0.42\end{array}$	0.35 0.35	0.16 0.19	0.68** 0.63*
<i>K</i> <sup>1</sup>	0.28	0.49**	0.41*	0.46** 0.26	-0.14 0.46**	0.41* 0.57**	0.35* 0.56**	

and germ cells is correlated with K. suggesting that size of cells might be a better indicator of vigor than number. Somatic and germ tissue and total body mass also correlate positively with K. A better understanding of these correlations can be obtained by examining Figure 6 in which K is graphed against the bivariate plots of number and size of germ cells and of somatic and germ tissues. This figure suggests that the most vigorous strains are the ones with the largest germ cells and the highest total mass. The effect of regenerators on the correlations is also apparent in these figures. Despite their extreme morphology, regenerators are as vigorous as the best wild-type strains.

In short, our analysis showed differences in character mutability, with some character changes correlated to others. These results strongly suggest that mutation is not isotropic. Most mutations deviated by less than tenfold; they did not improve character values, but rather reduced them and increased their variance. There was little correlation between the genetic and environmental components of variance. Character values were mostly positively correlated, but there was a negative correlation between the number and size of germ cells. Correlations between morphological characters and overall vigor of strains were in the predicted direction. The most extreme phenotypes, the regenerators, were very vigorous, suggesting a second peak in the adaptive landscape.

## (B) Do Variances and Covariances among Mutants and Species Correspond?

Our analysis of the mutational variation has indicated biases with respect to the magnitude and direction of change. If developmental constraints have played an important role in evolution, we might expect to observe the same biases among species.

First, we have asked whether mutation has created levels of variation comparable to those observed among species. Figure 7 compares the variance of morphological characters measured in the mutants (strain means) to the variance of the same characters measured in species (species means) of the family Volvocaceae, the genus *Volvox* and the section Merillosphaera; it shows that



FIG. 5. Correlations between somatic and germ tissues and between sizes and numbers of their constituent cells. Points are strain means, experiment 2 only; bars represent one standard deviation; top figure includes line of equality for comparison.

the correspondence of the variance generated by mutation and speciation differs among characters (Levene's test for heterogeneity of variance between mutants and species, Van Valen, 1978). For somatic tissue, mutation has created as much variance as exists among species in the entire family Volvocaceae, species within Volvox and within Merillosphaera being less variable than the mutants. The size of somatic cells is equally variable in all groups. For germ tissue, the variance among mutants is more

			df	r
Tissues: soma vs. germ				
Volvox carteri				
All strains	Y =	1.43(0.53)X - 3.28(0.67)	18	0.54*
Wild type only	Y =	0.93(0.47)X - 0.13(0.29)	5	0.66
Mutant only	Y =	1.13(0.89)X - 1.72(0.79)	11	0.36
Volvox sect. Merillosphaera	Y =	2.16(0.52)X - 7.64(0.17)	5	0.88**
Volvox	Y =	1.22(0.24)X - 2.00(0.16)	11	0.84**
Volvocaceae	Y =	1.95(0.21)X - 6.46(0.28)	14	0.93***
Somatic cells: volume vs. number				
Volvox carteri				
All strains	Y =	0.13(0.06)X + 2.09(0.21)	18	0.46*
Wild type only	Y =	0.003(0.12)X + 2.42(0.10)	5	0
Mutant only	Y =	0.18(0.09)X + 2.03(0.24)	11	0.53*
Volvox sect. Merillosphaera	Y =	-0.29(0.56)X + 3.24(0.31)	5	-0.22
Volvox	Y =	-0.56(0.19)X + 3.97(0.24)	11	-0.67*
Volvocaceae	Y =	-0.28(0.07)X + 3.09(0.24)	14	-0.75***
Germ cells: volume vs. number				
Volvox carteri				
All strains	Y =	-1.01(0.14)X + 5.60(0.30)	20	-0.85**
Wild type only	Y =	1.37(0.50)X + 3.85(0.12)	5	0.77*
Mutant only	Y =	-0.98(0.13)X + 5.47(0.27)	13	-0.91***
Volvox sect. Merillosphaera	Y =	-0.90(0.21)X + 5.87(0.15)	5	-0.88**
Volvox	Y =	-1.07(0.20)X + 6.08(0.21)	11	-0.85***
Volvocaceae-Volvox-Astrephomene <sup>1</sup>	Y =	0.84(0.14)X + 2.75(0.22)	13	0.84***

TABLE 3. Comparison of regressions among strains of *V. carteri* and among species of Volvocaceae. Strains or species means; all regressions performed on the log-transformed values, standard errors in parentheses; \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001.

 $^{1}$  The two species of this genus do not follow the same pattern as the rest of Volvocacean species; some authors assign this genus to a separate family.

comparable to that among species of *Volvox*. For those taxa whose variance is equal to that of the mutants the means are also comparable.

Second, we have compared the covariances generated by mutation and speciation (Fig. 8 and Table 3). Here, we have focused our comparisons on taxa whose variance is comparable to that of the mutants. There are significant correlations between soma and germ among species at all three taxonomic levels, but no significant correlation among mutants (r = 0.93, P < 0.001, for spp. within family, r = 0.84, P < 0.01, for spp. within genus, r = 0.88, P < 0.01, for spp. within section, but r = 0.36, P > 0.30, for mutants). Though there is a negative correlation between size and number of somatic cells among species, the same correlation is weakly positive among mutants. For germ cells, the regression of size on number in mutants and in species of the entire genus or those of the section Merillosphaera are very similar (slopes: b = -0.98  $\pm$  0.13, P < 0.001, for mutants; b = -1.07 $\pm$  0.20, P < 0.001, for species of *Volvox*;  $b = -0.90 \pm 0.21$ , P < 0.01, for species of Merillosphaera; *t*-tests for difference of two slopes: t = 0.40, df = 24, P > 0.50, for mutants vs. spp. of *Volvox*; t = 0.36, df = 18, P > 0.50 for mutants vs. spp. of Merillosphaera). Note, however, the strong positive correlation for species other than *Volvox*.

A similar analysis was performed on the slopes at cleavage, an index of the presence or absence of growth during embryonic development. For *V. pocockiae*, all species of *Volvox* in the section Merillosphaera and all Volvocaceae other than *Volvox*, the slopes at cleavage are less than 0.50, indicating absence of growth during cleavage; in other species of *Volvox*, however, this slope is greater than 0.50, indicating that their embryos do grow during cleavage, perhaps compensating for the small initial sizes of their germ cells (Bell, 1985). Figure 9 shows that, despite the large decrease in the size



FIG. 6. Correlations of morphological characters with the population carrying capacity, K, a measure of the overall vigor of strains. Points are strain means; data from both experiments combined.

threshold for initiation of cleavage, mutation did not produce any compensatory growth of developing embryos as a correlated response.

#### DISCUSSION

Mutational variation, such as that described here, is obtained by relaxing selection so that character variation can be expressed, regardless of its adaptive value. Comparing the variation expressed in the unselected mutant strains to that of the selected natural strains allows us to infer the history of selection in nature. Analysis of the mutational variation per se provides information on the variability of traits and the correlation structure of the system in question, and thus enables us to predict the outcome of selection applied in a different direction. This information is critical in understanding and modeling the evolution of life histories.

## Mutation, Selection, and the Trade-Offs Among Life History Traits

The simplest hypothesis we can formulate about mutational variation is that all kinds of changes are possible and occur independently. This predicts that all traits are equally mutable, mutational effects being symmetrically distributed around the wildtype values, and that all changes are uncorrelated with one another. Our survey of mutational effects showed that characters differ in mutability and that changes in different traits are often correlated, thus rejecting this hypothesis.

An alternative to this null hypothesis is that the distributions of character values generated by mutation are affected by the selective histories of the populations or the characters considered. For example, individuals in a population may be well adapted to their environments, so that random changes in their genotypes will tend to decrease rather than increase their perfor-



FIG. 7. Comparison of variance generated by mutation and speciation (variance of strain or species means). Character symbols defined in methods. F, G, S: variance of species within the family Volvocaceae, the genus *Volvox*, and the section Merillosphaera, respectively. Levene's test for heterogeneity of variance due to mode of derivation \* P < 0.05 or P < 0.01.



FIG. 8. Comparison of correlations generated by mutation and speciation. Regression lines are shown, dashed lines for species, solid lines for mutants (see Table 3 for regression line equations). Size of squares indicates three taxonomic levels: large, for spp. of Merillosphaera; medium, for other spp. of *Volvox*; small, for other Volvocaceae. When somatic tissue is plotted, species lacking such tissue are not shown.

mances; this will lead to positive correlations among character values, since the adverse effects of mutation will be manifest in a number of traits. Also, based on population genetic theory, we can distinguish between two kinds of traits: those that are under persistent directional selection, such as traits highly correlated with fitness, and those that are not; at equilibrium, traits under directional selection will approach their maximum attainable values, so that mutations which tend to increase one or more components of such traits will also tend to



FIG. 9. Comparison of modes of embryonic development (slopes at cleavage) generated by mutation and speciation. Size of squares indicates three taxonomic levels as in Figure 8. All mutants have slopes <0.50, indicating absence of growth during cleavage (broken line: regression line for non-Merillosphaera *Volvox*: b = -0.71, a = 3.50, r = 0.85, df = 4, P < 0.05; no significant correlation among species of Merillosphaera or other Volvocaceae; apparent relationship in mutants is due to mutants having larger intercellular spaces between cleavage products).

decrease others, thus inducing negative correlations among these components.

As it has been found previously in *Drosophila* and crops (Crow and Simmons, 1983; Gregory, 1968; Gottschalk and Wolff, 1983), we also found that most new mutations did not improve the performance of traits, supporting the adaptationist view. A consequence of this is the prevalence of positive correlations among these traits, indicating positive pleiotropy of gene effects. However, a significant negative correlation was obtained between two variables, the size and number of germ cells.

A distinction can be made between the somatic and germ tissues, which allows us to postulate a difference in their selective histories: germ tissue represents the input to the next generation, and is thus presumed to be under directional selection, while somatic tissue is sterile; since its function is to enhance the performance of germ, its quantity in nature may represent an optimal value for a given volume of germ. Thus, we might expect mutational variation in germ tissue to be biased towards values lower than the wild type, while those of somatic tissue to be symmetrically distributed around the wild-type mean. Our data confirm the expectation for germ tissue, particularly since the regenerator mutations, which increased the number of germ cells by as much as 20 times, also decreased their size by an equivalent amount so that total germ was not increased. Our data, however, do not support the expectation for somatic tissue, since the distribution of mutants with respect to this variable was also skewed to the left of the wild-type values and not symmetrical as expected by the hypothesis. This could be attributed to a rarity of mutations that would increase somatic tissue independently of germ, thus resulting in an overall increase in size, or of those that increase soma at the expense of germ. Note, however, that the latter kind of mutations is more difficult to recover due to their very low fitness.

A second alternative hypothesis suggests that biases in the mutational variation will arise directly from the underlying organismal architecture, regardless of selective history. First, the variability of traits may be limited by the present design. We may note, for example, that in our sample of mutants neither the size nor the number of somatic cells was increased to any considerable extent. It is possible that the differentiation of somatic tissue is related to small size (see review by Kirk and Harper, 1986), so that cells larger than some threshold value will not differentiate as somatic, and that the production of many more somatic cells implies a very small size which would be very difficult to cleave effectively under the present design.

Second, the correlation structure of life history may also be determined by organismal architecture. This can be studied by categorizing changes in character values according to whether they represent changes in the total resource available for a function, for example, resource acquisition or overall vigor or fitness, or changes in the allocation of resource among components contributing to that function, for example, alternative reproductive strategies. The likelihood of obtaining positive or negative correlations will depend on whether or not these changes tend to increase the variance in total relative to the variance in allocation (Bell and Koufopanou, 1986; van Noordwijk and de Jong. 1986; Houle, 1991); these are in turn determined by the number of characters in each category, the number of loci affecting each character, and the average magnitude of effect at each locus. Resource acquisition, or fitness, may depend on many physiological, ecological and behavioral attributes and therefore be affected by many loci of small effect, while allocation, or availability of alternative reproductive strategies, may only be induced by a limited number of developmental switches and thus be influenced by fewer loci of larger effect (see also Houle, 1991). Such developmental switches have been identified in animals (for example, a single-gene mutation in Caenorhabditis elegans increases lifespan by 40% and decreases fertility by 75%; Friedman and Johnson, 1987) and plants (reviewed by Hilu, 1983). This analysis thus implies that mutation will supply mostly variation in total rather than allocation and that organismal architecture is dominated by positive correlations, so that trade-offs will be recovered more rarely.

The developmental hypothesis can be distinguished from the selective hypothesis in a case, such as the relationship between the size and number of germ cells, where the two hypotheses make opposite predictions: though a negative correlation was observed in our data, as expected based on the selectionist argument, it was only induced by a few mutations of large effect, while the majority of strains in the sample (10 out of 15 strains) showed positive pleiotropy as predicted by the developmental hypothesis.

The nature of character limitation and the pleiotropy of effects could become more apparent if we considered the likelihood and the average effect of mutations at different stages of the life cycle of V. carteri: mutations that affect the timing of initiation of cleavage will determine the initial investment in the germ cell, and will therefore change all characters in the same direction, creating positive pleiotropy. Those mutations, however, affecting the initial allocation to somatic and germ tissues and the number of cell divisions involved in the formation of their constituent cells will reveal negative pleiotropy. Mutations may also change the absolute or the relative growth rates of somatic and germ cells after development, resulting in positive or negative pleiotropy, respectively. While our paper has

dealt primarily with the manifestations of these effects on the mature adult phenotypes, a set of more detailed measurements of early development would help to identify the origins of this variation.

## Are Mutations of Large Effect Unconditionally Deleterious?

Major gene effects offer an appealing mechanism for rapid speciation, but they are thought to have large and unconditionally deleterious effects which make them unlikely candidates for an important role in evolution (Lande, 1983). Some experimental evidence, however, has suggested the contrary (Templeton and Johnston, 1982). In plants, major mutations affecting traits important in the evolution of major taxonomic groups are known (Hilu, 1983), though fewer such mutations have been found in animals (Raff et al., 1987). Our most extreme strains, the *regenerators*, were very vigorous and seemed to break the correlations observed among all other strains. Their high carrying capacity may partly be explained by an economy in locomotion: once mature, the *regenerators* sink to the bottom, since only somatic cells bear flagella in Volvox. The regenerator mutation is known to occur spontaneously (Huskey et al., 1979; D. L. Kirk, pers. comm.), and our results suggest that it should be successful in environments where sinking is not particularly disadvantageous. Conversely, its rarity in nature demonstrates the selective advantage of bearing somatic tissue. This observation implies a Genotype  $\times$  Environment interaction with respect to the fitness of large-effect mutations.

## Does Mutation Recreate Phylogenetic Diversity?

We addressed this question by testing the null hypothesis that species closely related to *V. carteri* are indistinguishable from the mutants. Failure to reject this hypothesis will mean that developmental constraints, revealed as mutational biases, represent the most economical explanation for observed phylogenetic diversity. Alternatively, selection may be invoked to explain observed patterns. We found that, depending on the character considered, mutation recreated the variance observed in the family, genus or section. Unfortunately, the absence of a detailed phylogeny of the Volvocales prevents us from correlating the mutational inertia with rates of character evolution.

Among species, somatic tissue is highly correlated with germ tissue; this could be due to a lack of potential variation in the soma/germ ratio caused by some developmental constraint, or it could be due to selection continually adjusting the quantity of soma with respect to the quantity of germ. Our analysis of mutants suggested that mutations can at least decrease soma independently from germ, thus providing no evidence of a strict developmental constraint. Furthermore, the interspecific comparisons revealed a negative correlation between the size and number of somatic cells, but the mutants gave a positive correlation. We can therefore attribute the interspecific correlations to the action of selection.

The interspecific relationship between the number and size of germ cells is a complex one: in the smaller members of the Volvocaceae, which include species other than *Volvox*, the correlation is positive, while in the larger members, which only include species of Volvox, it is negative. The non-Volvox species may have been derived by heterochronic changes in the timing of initiation of cell division, which would change the initial size of germ cell, and hence the final size and number of cells of mature individuals, in the same direction (Koufopanou, 1990). Species of Volvox, however, may have resulted from changes in the allocation of germ tissue per individual reproductive cell without much further change in the total germ tissue. The reason for the observed ceiling in total germ among species of Vol*vox* may be that further increases in germ tissue are selectively disadvantageous under the present design, or that there is a lack of potential variation for increased total germ. The finding of a similar negative correlation between the number and size of germ cells among the unselected mutants of V. carteri supports the second alternative. The genus Volvox includes the largest members of the flagellated green algae (Volvocales), and the constraint hypothesis may provide an explanation for the absence of larger forms in this clade (see also Koufopanou, 1990). Note that the mutational relationship does not bend like the interspecific correlation (see Fig. 8, bottom), and thus does not follow

the pattern of the smaller, non-Volvox species. It would be interesting to test whether developmental mutants of these smaller species will behave similarly to the mutants of V. carteri and the species of Volvox, conservative of total germ and showing a tradeoff between cell size and number, or to the non-Volvox species, conservative of the sizenumber ratio, and with most of the variance along the total germ axis.

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Corresponding Editor: R. Lenski