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## SPONTANEOUS MUTATIONS FOR LIFE-HISTORY CHARACTERS IN AN OBLIGATE PARTHENOGEN

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*Abstract.*—By allowing mutations to accumulate spontaneously in different lines derived from a single female of an obligately parthenogenetic *Daphnia*, it has become possible to estimate the rate at which new genetic variance for life-history characters arises as well as to identify the average pleiotropic effects of mutant polygenes. The estimated polygenic mutation rates are quite compatible with those available for sexual organisms. The results are therefore in conflict with the hypothesis that parthenogens compensate for the loss of recombination by elevating the mutation rate. Based on these results, it is argued that the rate of phenotypic evolution may be enhanced as much as five-fold by sexuality. However, if dominance or epistatic gene interactions are of major importance, or if the sensitivity to environmental effects is reduced or the rate of polygenic mutation enhanced under asexuality, the full advantage of sex will not be attained and may even be reversed. Regardless of these conditions, it is clear that the mutational rate of production of polygenic variation is sufficient to allow significant rates of phenotypic evolution in purely asexual organisms.

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For the past several years, a considerable controversy has been brewing over the selective pressures that facilitated the evolution of sexuality and that are responsible for its current maintenance as a dominant reproductive mode (Ghiselin, 1974; Williams, 1975; Maynard Smith, 1978; Bell, 1982; Shields, 1982). Most of the debate has focused on the temporal and spatial scale of environmental variation. Most, but not all (Thompson, 1976), agree that sex will enhance the rate of phenotypic evolution by facilitating the creation of novel genotypes through recombination and segregation. Such evolutionary change is deemed essential for the maintenance of a viable population in the face of potential competitors, predators, and/or pathogens that are themselves capable of adaptive evolution (Levin, 1975; Selander and Hudson, 1976; Glesener and Tilman, 1978; Jaenike, 1978; Hamilton et al., 1981; Rice, 1983). Of course, unless inbreeding is intense (Shields, 1982), obligatory sex has the added disadvantage of breaking up the very same co-adaptive complexes of genes that it creates (Thompson, 1976).

A critical component of the controversy that has been largely ignored is the creative role of recurrent polygenic mutation. Most of the theory explicitly assumes that mutations are predominantly or entirely disadvantageous and that their accumulation in obligately asexual lineages ultimately results in extinction (Muller, 1964). The validity of this notion, for example, is absolutely critical to Shield's (1982) hypothesis that inbreeding sex, rather than asexuality, has evolved as a mechanism to maintain a relatively stable adaptive genotype while minimizing the accumulation of deleterious mutations.

Whereas it is true that most major mutations have either direct or correlated effects that are disadvantageous and often unconditionally so (Gustafsson, 1947; Stevenson and Kerr, 1967; Wright, 1968, 1977), mutations with relatively minor effects are often distributed evenly in positive and negative directions (Oka et al., 1958; Gregory, 1965). Because most of the continuously distributed traits upon which natural selection operates are based on multiple loci with small individual

effects (Bulmer, 1980; Falconer, 1981; Lande, 1981), and because the mutation rate for such genes is much greater than that for macromutations (Gregory, 1965; Mukai, 1979), polygenic mutation cannot be ignored as a major factor in adaptive evolution (Lande, 1976, 1983).

One possible explanation for the success of many obligately asexual organisms is compensation for the absence of recombination by an elevated rate of polygenic mutation. In the case of characters with a purely additive genetic basis, the expected rate of phenotypic evolution under sexuality can be matched by an otherwise similar asexual population whose mutational rate of input of genetic variance ( $V_M$ ) is elevated by approximately  $2n$ , i.e., twice the number of effective loci for the character in the sexual population (Lynch and Gabriel, 1983). This critical factor may be substantially lower for characters whose expression is dependent upon complex dominance or epistatic interactions, since clonal propagation provides a mechanism for immediately fixing gene complexes that would otherwise be left reassorting in a sexual population.

In order to determine whether an obligately parthenogenetic clone of *Daphnia* has an abnormally high rate of polygenic mutation or high mutational effects, I have allowed spontaneous mutations for life-history polygenes to accumulate in a lineage derived from a single mother. A multivariate analysis of divergent lines provides estimates of  $V_M$  for single traits as well as information on the average pleiotropic effects of mutant polygenes. To my knowledge, no other such data are available for an organism that has been unaltered by intentional inbreeding, artificial selection, or other modifications of the genetic background. The *Daphnia* data, when appropriately scaled, appear to be completely congruent with estimates of  $V_M$  derived indirectly for other sexual organisms (Lynch, unpubl.) and, hence, fail to reveal any evidence of enhanced mutability under parthenogenesis.

#### MATERIALS AND METHODS

The stem mother for this experiment was taken from a laboratory culture of an obligately parthenogenetic race of *Daphnia pulex* (Group A) found in Busey Pond, Urbana, Illinois (Lynch, 1983). In March 1981, 50 lines were initiated over a period of three days. All lines were derived from progeny of a single well-fed mother, with a single offspring being used to initiate each line. These lines were continuously maintained in isolation from each other using procedures designed to minimize selection within and between lines. Each line was maintained in several 50 ml beakers (single animals/beaker) in an environmental chamber, the animals being transferred by pipette every other day to a clean beaker containing a fresh food supply (below). At this same time the beakers were examined for progeny. When offspring appeared in a line, 2–4 progeny of a single mother of that line were removed by pipette to begin the next generation.

Despite efforts to maintain the individual lines under as uniform conditions as possible and to minimize the occurrence of selective deaths, the operation of some selection cannot be ruled out. After two years of clonal divergence, only eight of the original 50 lines remained. Many of the losses were obviously non-selective deaths, being due to accidental spills or deaths caused by careless manipulation of animals. This was especially true early in the experiment, when less care was taken in maintaining the lines in replicate. However, a large number ( $\sim 30$ ) of the lines were lost during a 3–4 week period of greatly elevated mortality for which I have no explanation. In the event that the agent causing this intense clonal mortality acted selectively upon measured traits, the true mutational rates of input of genetic variance ( $V_M$ ) will be underestimated in the following analyses. Regardless of the number of surviving lines at the time of analysis, it would not have been physically possible to assay more than 8–10 of them with the following experimental design.

The experiment reported here was designed to partition the phenotypic variance ( $V_T$ ) of the experimental lineage into its genetic ( $V_G$ ) and environmental ( $V_E$ ) components and to further dissect  $V_E$  into two subcomponents—the within-clutch variance ( $V_c$ ) and the residual environmental variance ( $V_r$ ).  $V_c$ , the phenotypic variance between progeny taken from the same clutch of the same mother, is the baseline environmental variance.  $V_r$  is the additional environmental variance in a population that results from differential transmission of environmental effects between generations (maternal, grand-maternal, great-grand-maternal effects). While  $V_r$  potentially includes some variance between clutches within mothers, that possibility was eliminated in this experiment.

A general problem with unequivocally quantifying the genetic variance resulting from a mutation accumulation experiment is the inevitable inclusion of some environmental effects in the between-lines component of variance. This can result in a severe overestimate of the mutational rate of input of genetic variance, especially when  $V_G$  is small. The problem can be avoided by subdividing the main lines into equal numbers of sublines prior to an assay and performing a nested analysis of variance. As shown in the Appendix, the line, subline, and error mean square terms of such an analysis can be decomposed into the variance components  $V_c$ ,  $V_r$ , and  $V_G$  (Eq. A3). By factoring out the between-sublines component of variance from the between-lines mean square, all sources of environmental variance can be removed from the latter term. An estimate of  $V_M$  is obtained by dividing  $V_G$  by the number of generations of line divergence (A2). Similarly, the components of covariance can be dissected from the mean cross-product terms of a nested multivariate analysis of variance and normalized to provide estimates of the subcomponent correlations between traits (A4). Approximate expressions for the sampling variances of all the statistics dealt with in this paper are derived in the Appendix.

The analysis of lines was performed in March 1983, two years after their initial isolation. Eight sublines of each of the eight remaining lines were maintained on a controlled food supply for three generations prior to further subdividing them into five replicates (Fig. 1). Passages between generations only involved progeny from their mother's first clutch in order to eliminate between-clutch maternal effects. Individuals were grown singly in 50 ml beakers in the same environmental chamber set at 20°C, 12:12 photoperiod. The food supply consisted of a standard suspension of *Scenedesmus* and *Chlamydomonas* (ratio 80:16  $\times 10^3$  cells·ml<sup>-1</sup>, equivalent to 1.54  $\mu\text{g C}\cdot\text{ml}^{-1}$ ) in a chemically defined zooplankton medium (see Lynch et al., 1985 for details). Each animal was transferred with a wide-bore pipette to a clean beaker containing 40 ml of fresh food every other day. When they reached maturity, the 320 animals for this experiment were monitored daily for growth (nearest 0.01 mm), survival, and reproduction (clutch size carried and/or number and size of progeny released) through their first three adult instars under a Wild M-8 microscope equipped with an ocular micrometer. This procedure generated estimates of several life-history traits that are analyzed in detail below:  $B_{o1}$ ,  $B_{o2}$ , and  $B_{o3}$ —mean length of newborns from the first, second, and third clutches;  $B_{k1}$ ,  $B_{k2}$ , and  $B_{k3}$ —body length of the mother in the first, second, and third adult instars;  $k$ —age at first reproduction (first release);  $C_1$ ,  $C_2$ , and  $C_3$ —size of the first, second, and third clutches; and  $w$ , a composite measure of relative fitness estimated from

$$w_i = \sum_{x=1}^y e^{-rx} l_{xi} m_{xi}$$

(Charlesworth, 1980) where  $r$  is the instantaneous rate of increase determined for the pooled population of 320 clones using the stable age equation (in this case  $r = 0.358 \text{ day}^{-1}$ ),  $y$  is the age at release of the third clutch, and  $l_{xi}$  ( $=1$  or  $0$ ) and  $m_{xi}$  are the survivorship and reproductive rates of individual  $i$  at age  $x$ .

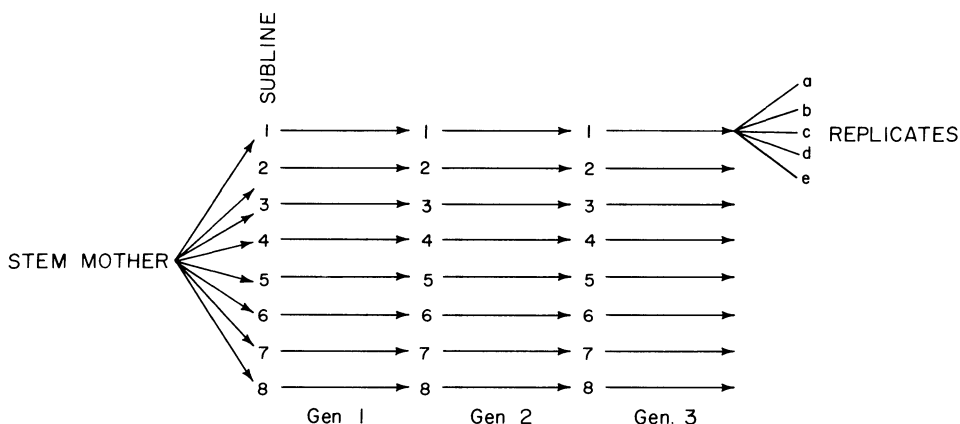


FIG. 1. The experimental design. For clarity only one of the eight stem lines and replicates for one of its eight sublimes are denoted. Arrows denote the release of an animal's first clutch. Thus, the replicate animals that were measured in the life history analysis are the great-great-granddaughters of the stem mother.

An inevitable complication in the analysis of any mutation accumulation experiment is the near impossibility of synchronizing the starting date of all experimental animals because of genetic and environmental variance for age at first reproduction. Any uncontrolled variation between days in the treatment of animals will inflate the total phenotypic variance, and if the lines are differentially assorted between dates, much of this additional variance may appear in the between-lines component of variance and result in an inflated estimate of  $V_M$ . The starting dates of the animals assayed in this experiment spanned a period of six days with >95% of them initiating in the first four days. In order to eliminate this potential source of environmental heterogeneity, the date effect was factored out by treating it as a main effect in the final analysis of data.

It is unlikely that microenvironmental variation within the environmental chamber contributed to the phenotypic variance observed in this experiment. The environmental chamber was equipped with two powerful fans to minimize temperature variation, and lighting was maintained at a uniformly low level by placing a large piece of filter paper between the animals and light. As a further

buffer to microenvironmental variation, all of the beakers were housed in covered polycarbonate boxes. Although individual beakers tended to be aggregated in boxes according to starting date and subline, the boxes were haphazardly rearranged each day. Any positional effects that might have existed despite these precautions would be factored out of the final analysis in the date term or would appear in  $V_e$ .

There is no evidence that the genetic variance accumulated during this experiment has resulted from anything other than mutation. Production of males has never been detected in Group A *Daphnia pulex* in the field or in the laboratory. Although many resting eggs have been produced in our laboratory stock cultures, we have not detected any segregation at heterozygous enzyme loci (*Lap* and *Got*) over a period of five years. Such results argue against the existence of any meiotic activity or mitotic recombination in this obligately parthenogenetic lineage. Long-term field studies on Group A *Daphnia* provide further support for this position (Lynch, 1983).

After examining the effects of a variety of transformations on the univariate distributions, I decided that untransformed data best approximated normality for all

TABLE 1. Mean phenotypic values ( $\bar{z}$ ), genetic, within-clutch, and residual environmental components of variance ( $V_G$ ,  $V_c$  and  $V_r$ ), and broad sense heritabilities ( $H^2$ ) for a lineage of *Daphnia pulex* following two years (~75 generations) of clonal divergence. Standard error estimates are given in parentheses. Trait symbols are defined in the text.

Trait	$\bar{z}$	$V_c$	$V_r$	$V_G$	$H^2$	$V_r/V_c$	$V_r/V_G$	$V_M/V_E$
$B_{01}$	0.65	0.000452 (0.000047)	0.000079 (0.000130)	0.000039 (0.000038)	0.068 (0.069)	0.175	2.026	0.00098
$B_{02}$	0.68	0.000594 (0.000100)	0.000208 (0.000121)	0.000037 (0.000091)	0.045 (0.109)	0.350	5.622	0.00062
$B_{03}$	0.69	0.000478 (0.000090)	0.000468 (0.000184)	0.000096 (0.000157)	0.092 (0.152)	0.978	4.875	0.00135
$B_{k1}$	1.85	0.004677 (0.000427)	0.001378 (0.000505)	0.001260 (0.000747)	0.172 (0.105)	0.295	1.094	0.00277
$B_{k2}$	2.03	0.006679 (0.000611)	0.001325 (0.000596)	0.002170 (0.001134)	0.213 (0.115)	0.198	0.611	0.00361
$B_{k3}$	2.16	0.007354 (0.000672)	0.001178 (0.000599)	0.002165 (0.001132)	0.202 (0.109)	0.160	0.544	0.00338
$k$	7.4	0.521348 (0.047690)	0.089704 (0.042873)	0.061176 (0.046259)	0.091 (0.070)	0.172	1.466	0.00133
$\sqrt{C_1}$	3.0	0.096690 (0.008882)	0.017089 (0.007954)	0.000920 (0.003867)	0.008 (0.034)	0.177	18.575	0.00011
$\sqrt{C_2}$	1.6	1.920692 (0.177190)	0.542863 (0.201711)	0.378367 (0.252119)	0.133 (0.091)	0.283	1.435	0.00205
$\sqrt{C_3}$	1.7	2.263196 (0.207904)	0.447439 (0.198967)	0.414066 (0.262906)	0.133 (0.086)	0.198	1.081	0.00204
$w$	1.00	0.111439 (0.008262)	0.031361 (0.007624)	0.008917 (0.007911)	0.059 (0.052)	0.281	3.517	0.00083

of the characters except clutch sizes, which were square root transformed. The mean squares and cross products for the complete data set were simultaneously generated with the MANOVA subroutine of the SAS general linear model (Freund and Littell, 1981) by sequentially adding the factors date, lines within dates, and sublimes within lines to the model (Table A1).

## RESULTS

*Univariate Statistics.*—Univariate analyses of variance revealed highly significant ( $P < 0.005$ ) date, line, and subline effects for all characters except for the line effect on  $\sqrt{C_1}$  ( $P = 0.14$ ). The components of variance extracted from the ANOVA tables are presented in Table 1. In all cases, the majority of the phenotypic variance is accounted for by the within-clutch component ( $V_c$ ). However, the residual component of environmental variance ( $V_r$ ) was also substantial for all characters, falling in the range of 15 to 35% of  $V_c$  (except in the case of  $B_{03}$ ). Without the incorporation of sublimes into the experimental design, it would not have been possible to factor  $V_r$  out of the between-lines component of variance, and the estimates of  $V_G$  and  $H^2$  (broad-sense heritability =  $V_G/V_T$ ) would have been inflated by a factor of  $(1 + V_r/V_G)$ . The significance of such a potential oversight is outlined in Table 1.  $V_r/V_G$  ranged from 0.5 to 18.6.

The nested experimental design reveals that two years of clonal divergence was sufficient to generate genetic variance for life-history traits on the order of 1 to 21% of the total phenotypic variance. The mean heritability for all of the characters (0.11) has a fairly narrow standard error of 0.02. With the exception of  $\sqrt{C_1}$ , the range of the ratio  $V_M/V_E$  is also fairly narrow (0.0008–0.0036). Thus, mutation generates new genetic variation each generation equivalent to ~0.2% of  $V_E$ . There does appear, however, to be some variation in  $V_M/V_E$  between characters. For example,  $V_M/V_E$  is consistent among the

TABLE 2. Correlation coefficients for bivariate relationships. Above the diagonal, the upper and lower entries are  $r_c$  and  $r_r$ . Below the diagonal, the upper and lower entries are  $r_T$  and  $r_G$ . \* and \*\* denote significance at the 0.05 and 0.01 levels.

	$B_{o1}$	$B_{o2}$	$B_{o3}$	$B_{k1}$	$B_{k2}$	$B_{k3}$	$k$	$\sqrt{C_1}$	$\sqrt{C_2}$	$\sqrt{C_3}$	$w$
$B_{o1}$	—	0.023	-0.053	0.575**	0.515**	0.371**	0.326**	0.127	0.207**	0.073	-0.085
	—	1.156	0.937	0.746**	0.532*	0.426	0.591*	-0.116	-0.201	-0.111	-0.425
$B_{o2}$	0.283**	—	0.080	0.106	0.069	0.124	0.165	-0.145	-0.310**	0.041	-0.244*
	1.113	—	0.407	1.055**	1.072**	0.999**	0.080	0.664	1.323	0.323	0.112
$B_{o3}$	0.198	0.348**	—	0.153	0.168	0.421**	-0.079	0.210	0.001	-0.404**	-0.113
	0.500	1.215*	—	0.232	0.515*	0.600*	0.020	-0.462	0.172	0.079	0.118
$B_{k1}$	0.621**	0.459**	0.319**	—	0.826**	0.701**	0.291**	0.423**	0.267**	0.232**	0.124
	0.970**	0.595	1.537	—	0.720**	0.524**	0.454	0.498*	-0.295	-0.310	-0.376
$B_{k2}$	0.525**	0.411**	0.475**	0.827**	—	0.897**	0.295**	0.289**	0.622**	0.503**	0.361**
	0.736*	0.420	1.587	0.967**	—	0.966**	0.582*	0.295	0.372	0.396	0.193
$B_{k3}$	0.388**	0.388**	0.621**	0.694**	0.917**	—	0.226**	0.238**	0.652**	0.583**	0.448**
	0.545	0.034	1.563	0.863**	0.981**	—	0.463	0.097	0.519	0.496	0.338
$k$	0.395**	0.226*	0.123	0.391**	0.409**	0.339**	—	0.147*	0.453**	0.376**	-0.221**
	0.909*	2.842	1.504	1.103**	1.049**	0.989**	—	0.173	0.268	0.113	-0.368
$\sqrt{C_1}$	0.090	0.088	-0.026	0.313**	0.204**	0.122*	0.072	—	0.126	0.065	0.244**
	0.463	0.876	3.290	-0.654	-0.345	-0.138	0.649	—	-0.296*	-0.316*	-0.255
$\sqrt{C_2}$	0.140*	-0.041	0.172	0.209**	0.598**	0.649**	0.422**	0.057	—	0.820**	0.526**
	0.215	-0.182	0.305	0.756*	0.803**	0.905**	0.577	-0.055	—	1.019**	0.695**
$\sqrt{C_3}$	0.052	0.060	-0.024	0.186**	0.519**	0.602**	0.345**	0.011	0.870**	—	0.472**
	0.179	-0.838	0.423	0.666	0.792**	0.914**	0.545	-0.343	1.008**	—	0.872**
$w$	-0.189**	-0.258**	-0.105	-0.053	0.228**	0.329**	-0.289**	0.191**	0.521**	0.512**	—
	-0.797	-1.533	-1.734	-0.834*	-0.749	-0.489	-0.852*	-0.292	-0.018	0.112	—

offspring size measures as well as among the adult size measures, but the mean ratio in the latter group is  $\sim 3$  times higher than in the former, perhaps because size late in life is a product of direct and indirect effects of many more genes than size at birth.

*Bivariate Statistics.*—The majority of characters measured in this study were significantly correlated at the phenotypic, genetic, and/or environmental levels (Table 2). Several of the genetic and residual environmental correlation coefficients had very high absolute values but are not recorded as being significant because of their large standard errors.

Sizes of all three adult instars were highly positively correlated at all levels, whereas the three offspring size measures tended to be positively associated at the genetic and residual environmental levels but uncorrelated at the within-clutch level. There were also positive relations between offspring and adult sizes. In fact, at the phenotypic level all such relationships were highly significant. All of the component correlations ( $r_G$ ,  $r_c$ , and  $r_r$ ) between  $B_0$  and  $B_k$  were positive, but not all were significant.

Age at first reproduction ( $k$ ) is positively related to both progeny and adult sizes, largely because of strong genetic correlations. Correlations between  $k$  and body size measures also tend to be positive at the environmental levels but much less than at the genetic level. All of the clutch size measures are positively associated with  $k$  at all levels, although only the phenotypic and within-clutch correlation coefficients are statistically significant.

There were no significant correlations between the sizes of the first and second or first and third clutches at the within-clutch, genetic, or phenotypic levels, but both relationships were significantly negative at the residual environmental level. On the other hand,  $\sqrt{C_2}$  and  $\sqrt{C_3}$  behave as though they are measures of the same character with nearly perfect correlation at all levels.

There is no evidence for a genetic trade-

off between offspring size and offspring number in this population, seven of nine such correlations being positive, and all being insignificant. At the within-clutch level, significantly negative correlations did appear for ( $B_{02}$ ,  $\sqrt{C_2}$ ) and ( $B_{03}$ ,  $\sqrt{C_3}$ ), indicating that environmental effects may contribute to such a trade-off. However, the within-clutch effect on ( $B_{02}$ ,  $\sqrt{C_2}$ ) is strongly counteracted by a positive residual environmental effect. The net result of the various effects on offspring size–offspring number relationships is a negligible amount of correlation at the phenotypic level.

On the other hand, the phenotypic correlations between all adult size and clutch size measures were positive and highly significant. In general, these same relations are found at the genetic and environmental levels. However, the genetic correlations for adult size measures and  $\sqrt{C_1}$  were uniquely negative, although each of them was insignificant.

Under the assumption that there are no correlations between genetic and environmental effects across characters,

$$r_{T,xy} = H_x H_y r_{G,xy} + C_x C_y r_{c,xy} + R_x R_y r_{r,xy} \quad (1)$$

where  $C_x = (V_{c,x}/V_{T,x})^{1/2}$  and  $R_x = (V_{r,x}/V_{T,x})^{1/2}$ . (See Falconer [1981] for a derivation of the two component case.) Some indication that this assumption (i.e.,  $Cov_{G_x,c_y}$ ,  $Cov_{G_x,r_y}$ ,  $Cov_{c_x,r_y} \approx 0$ ) is approximately true for this population is provided in the lower half of Figure 2. Taken over all characters, the correlations between  $r_G$ ,  $r_c$ , and  $r_r$  are significant, but the coefficients of determination are very low.

The approximate linearity and homoscedasticity of the relations of  $r_G$ ,  $r_c$ , and  $r_r$  to  $r_T$  (Fig. 2) support the use of Equation (1) to derive estimates of  $H_x H_y$ ,  $C_x C_y$ , and  $R_x R_y$ , averaged over all characters. The fitted equation,

$$r_T = 0.098r_G + 0.628r_c + 0.228r_r + 0.027,$$

is highly significant ( $r^2 = 0.95$ ,  $P < 0.00001$ ) with an intercept close to the expected value of 0 and a sum of coef-

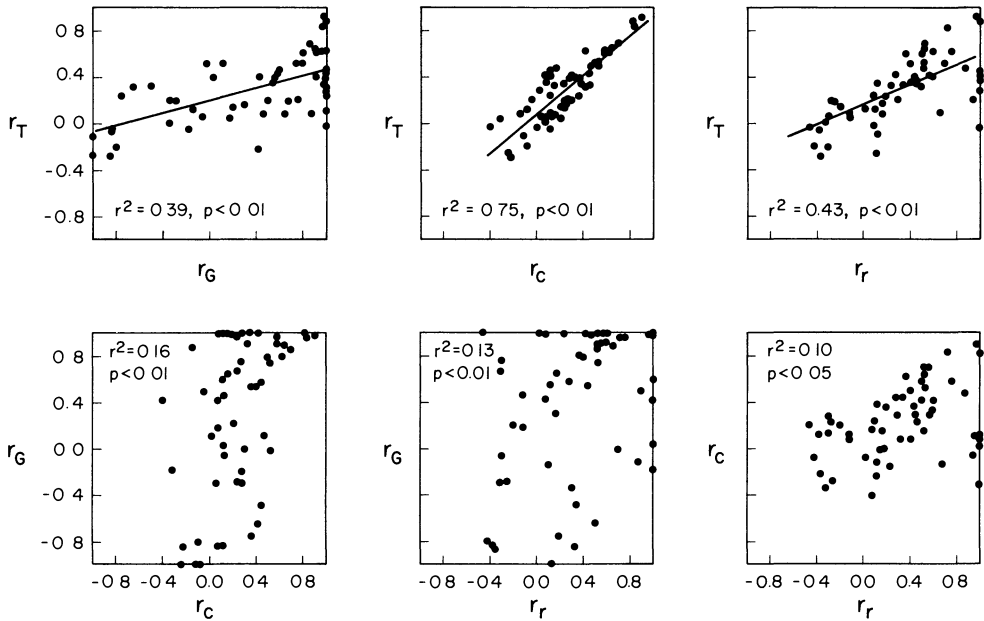


FIG. 2. Least-squares relations between phenotypic, genetic, within-clutch, and residual environmental correlations ( $r_T$ ,  $r_G$ ,  $r_c$ , and  $r_r$ , respectively), taken from Table 2.

ficients (0.954) close to the expected value of 1. The coefficient, 0.098, based on bivariate relations provides an estimate of  $\bar{H}^2$ , which is very close to the mean (0.11) determined previously with univariate analyses.

*Expected Response to r Selection.*—Most of the life-history traits measured in this study also exhibit significant correlations with the composite fitness measure,  $w$  (Table 2). At all covariance levels, the offspring sizes are either negatively or insignificantly correlated with  $w$ . The relations of adult size and  $w$  are more variable, but in general they are strongly negative at the genetic level and positive at the environmental and phenotypic levels. Age at first reproduction is negatively associated with  $w$  at all levels. Finally, whereas the clutch sizes tend to be only weakly correlated with  $w$  at the genetic level, they have a strong positive association at the environmental and phenotypic levels.

The covariances of characters with  $w$  and with each other ultimately determine

the dynamics of multivariate evolution that would result if the clones were released from the structured experimental setting and allowed to differentially expand. Under the assumption of multivariate normality the vector of responses to selection by the various characters is

$$\Delta \bar{z} = \mathbf{G} \cdot \mathbf{P}^{-1} \cdot \mathbf{S}$$

where  $\mathbf{G}$  is the genetic covariance matrix,  $\mathbf{P}^{-1}$  is the inverse of the phenotypic covariance matrix, and  $\mathbf{S}$  is the selection differential (Lande, 1982).  $\mathbf{S}$  is the vector of changes in means of the characters after selection but before reproduction and is equivalent to the vector of phenotypic covariances of the characters with  $w$ ,  $\text{Cov}_T[\mathbf{w}\mathbf{x}]$ .

The selection differential is defined by the effects of selection acting directly upon the characters, as well as by indirect effects of selection upon phenotypically correlated traits:

$$\mathbf{S} = \mathbf{P} \cdot \nabla \ln \bar{W} \quad (2)$$

where  $\nabla \ln \bar{W}$ , the selection gradient, is

the vector of partial differentials  $\partial \ln \bar{W} / \partial \bar{z}_x$  (Lande, 1979). Rearranging (2) and substituting from above, we find the selection gradient,

$$\nabla \ln \bar{W} = \mathbf{P}^{-1} \cdot \text{Cov}_T[\mathbf{w}\mathbf{x}] \quad (3)$$

and the response to selection,

$$\Delta \bar{z} = \mathbf{G} \cdot \nabla \ln \bar{W}. \quad (4)$$

Through the use of (4), the response to selection can be partitioned into two components that resolve the direct and indirect effects of selection on character  $x$

$$\Delta \bar{z}_x = \left\{ V_{G,x} \cdot \frac{\partial \ln \bar{W}}{\partial \bar{z}_x} \right\} + \left\{ \sum_{y \neq x} \text{Cov}_{G,xy} \cdot \frac{\partial \ln \bar{W}}{\partial \bar{z}_y} \right\}.$$

Thus, the variance components in Table 1 and the covariances underlying the correlation coefficients in Table 2 can be used to examine the potential evolutionary consequences of pleiotropic effects for mutationally derived life-history polygenes in this population. In the following, I assume that the selection differential estimated by  $\text{Cov}_T[\mathbf{w}\mathbf{x}]$  is representative of that which would be experienced if the clones were suddenly allowed to differentially expand in an otherwise similar laboratory setting. Based on the similarity of their covariance relationships, I also assume  $B_{o1}$ ,  $B_{o2}$ , and  $B_{o3}$  to be measures of the same trait; the same assumption is made for  $B_{k1}$ ,  $B_{k2}$ , and  $B_{k3}$  and for  $\sqrt{C_2}$  and  $\sqrt{C_3}$ . This reduces  $\mathbf{P}$  and  $\mathbf{G}$  to  $5 \times 5$  matrices. The new variance and covariances for  $B_o$ ,  $B_k$ , and  $\sqrt{C_{2,3}}$  were taken to be the averages of the individual traits.

The solution of equations (3) and (4) (Table 3) indicates that, under the expected selection regime (S), size at birth would decline as a consequence of direct selection but even more so because of selection on correlated characters; this is largely due to the positive genetic co-

variance of  $k$  and  $B_o$  and the negative selection gradient on  $k$ . There are also indirect selection effects on  $B_k$ , but they tend to balance each other so that the response to selection for this character is almost entirely a product of direct selection on it. On the other hand, while direct selection on age at first reproduction is expected to reduce it, selection on correlated characters (especially  $\sqrt{C_{2,3}}$ ) has a strong positive effect and will substantially diminish the net selection response. There are also conflicting direct and indirect selection pressures on both clutch size measures. As expected, both have direct positive effects on fitness. However,  $\sqrt{C_1}$  is negatively influenced by selection on all other characters (especially  $k$ ) and has a negative net selection response. All of the selection responses listed in Table 3 can be modified by changing the selection gradient.

#### DISCUSSION

While the loss of many lines prior to the completion of this two-year experiment is a potential source of error in the estimation of  $V_M$ , the estimates obtained for this obligately parthenogenetic lineage of *Daphnia* appear to be quite compatible with those available for other organisms. The mean value of  $V_M/V_E$  ( $\bar{x} \pm 2 \text{ SE} = 0.0017 \pm 0.0006$ ) obtained in this study is similar to the mean for bristle numbers in *Drosophila melanogaster* ( $0.0026 \pm 0.0014$ ) resulting from the pooling of 26 independent estimates based on a variety of strains, bristle patches, and experimental techniques (Lynch, unpubl.). A slightly elevated value is expected for *Drosophila* since its generation time is somewhat longer than that of *Daphnia* under laboratory conditions. When estimates of  $V_M/V_E$  for *Daphnia* and six other organisms are related to generation time, the mean for *Daphnia* falls almost perfectly on the fitted regression (Lynch, unpubl.).

Although a comparable experiment with a cyclically parthenogenetic *Daphnia* has not yet been done, these results lead to the tentative conclusion that the

TABLE 3. Estimated selection differential (S), selection gradient ( $\nabla \ln \bar{W}$ ), and response to selection/generation ( $\Delta \bar{z}$ ) for five composite life-history traits for the laboratory population based on the covariance relationships after two years of clonal divergence. The response to selection is subdivided into components resulting from direct selection and from selection on correlated characters.

Trait	S	$\nabla \ln \bar{W}$	$\Delta \bar{z}$		
			Direct	Indirect	Net
$B_{o1}, B_{o2}, B_{o3}$	-0.0020	-1.8616	-0.0001	-0.0003	-0.0004
$B_{k1}, B_{k2}, B_{k3}$	0.0062	0.3743	0.0007	0.0000	0.0007
$k$	-0.0923	-0.2653	-0.0162	0.0145	-0.0017
$\sqrt{C_1}$	0.0252	0.2267	0.0002	-0.0023	-0.0021
$\sqrt{C_2}, \sqrt{C_3}$	0.3378	0.1521	0.0603	-0.0171	0.0432

mutational rate of input of genetic variance for this obligate parthenogen is no greater than that to be expected for an otherwise similar sexual species. Parthenogenesis appears to be ameiotic in all *Daphnia*. There has been one report of a sort of meiosis (endomeiosis) during the maturation of parthenogenetic eggs in *Daphnia pulex* (Bacci et al., 1961), but it is inconsistent with the results of other, much more thorough cytological studies (Mortimer, 1936; Ojima, 1958; Zaffagnini and Sabelli, 1972), and repeated attempts to detect recombination at heterozygous enzyme loci during parthenogenesis in cladocerans have failed to identify any meiotic activity (Hebert and Ward, 1972; Manning et al., 1978; Lynch, 1983).

The possibility that some parthenogenetic organisms do have elevated rates of mutation cannot be ruled out, since many (all known parthenogenetic vertebrates) are known to have hybrid origins (Lynch, 1984a). Interracial hybridization often results in a release of mutator activity in *Drosophila* through a syndrome known as hybrid dysgenesis (Woodruff and Thompson, 1980). As this phenomenon appears to extend to other organisms (cf. Shaw et al., 1983) and to mutations of small effects (Mackay, 1984), it is feasible that the evolution of parthenogenesis may be accompanied by a rise in  $V_M$  at least early in its transitional stages.

The results of this experiment support the contention of Lynch and Gabriel

(1983) that  $V_M$  is sufficiently large to allow the short-term accumulation and maintenance of enough polygenic variation in purely asexual lineages for a significant degree of phenotypic evolution. If, however,  $V_M$  is approximately independent of sex, the rate of phenotypic evolution for an asexual population is likely to be lower than that for an otherwise comparable sexual population. For large populations and mutations of small effects (Turelli, 1984), the rate of evolution (per generation) for a character under weak Gaussian selection and unconstrained by pleiotropic effects is approximately

$$\Delta \bar{z} = \frac{V_G(\theta - \bar{z})}{V_G + V_E + V_w}$$

where  $V_G$  is the total genetic variance under asexuality and the additive genetic variance under sexuality,  $V_w$  is a measure of the width of the fitness function (decreasing as the intensity of selection increases), and  $(\theta - \bar{z})$  is the deviation of the mean from the optimal phenotype (Lande, 1976). Assuming equal  $V_E$ ,  $V_w$  and  $(\theta - \bar{z})$ , the relative rate of phenotypic evolution for sexual and asexual populations is then

$$\Delta \bar{z}_s / \Delta \bar{z}_a = \frac{V_{G,s}}{V_{G,a}} \cdot \frac{V_{G,a} + V_E + V_w}{V_{G,s} + V_E + V_w}$$

For large populations that have been established for more than a few hundred generations, the expected levels of genetic variance under these circumstances are

$$\hat{V}_{G,a} = \frac{V_M + \sqrt{V_M[V_M + 4(V_E + V_w)]}}{2}$$

and

$$\hat{V}_{G,s} = \frac{2nV_M + \sqrt{2nV_M[2nV_M + 4(V_E + V_w)]}}{2}$$

where  $n$  is the effective number of loci segregating for the character in the sexual population (Lande, 1976; Lynch and Gabriel, 1983). These are the equilibrium levels of genetic variance that are expected to result from selection-mutation balance. Under these conditions, the upper limit to  $\Delta\bar{z}_s/\Delta\bar{z}_a$ ,  $\sqrt{2n}$ , is approached when  $2nV_M \ll (V_E + V_w)$ .

Two sets of circumstances indicate that the condition,  $2nV_M \ll (V_E + V_w)$ , may often be approached. First,  $V_M/V_E$  is almost always substantially less than 0.1 for short-lived organisms (generation times <1 year) and, even for long-lived organisms, is unlikely to ever exceed 1 (Lande, 1976; Hill, 1982; Lynch, unpubl.). Second, except for cases of extremely stringent selection that are likely to result in extinction,  $V_w$  will often be of the same order of magnitude as  $V_E$  or greater (Lynch and Gabriel, 1983; Turelli, 1984); for the one case in which estimates of  $V_w$  are available for *Daphnia* life-history characters in a natural population (Lynch, 1984b),  $V_w/V_E$  ranges from 4.0 to 12.2. It appears, therefore, that the relative effect of recombination on the rate of evolution will often be highly dependent on the number of effective loci contributing to the character. Since minimum estimates of  $n$  are typically on the order of 5–10 or greater (Lande, 1981; Turelli, 1984), an elevation of the rate of evolution under sexuality by a factor of 3–5 is not incon-

ceivable. It must be emphasized, however, that the full advantage of sexuality will not be reached, and may even be reversed, if dominance or epistasis plays a major role in character expression, if  $V_{M,a} > V_{M,s}$ , and/or if  $V_{E,a} < V_{E,s}$  (Lynch and Gabriel, 1983).

Another factor that may impede the response of a character to selection in both asexual and sexual populations is the existence of negative genetic correlation with other characters that are similarly related to fitness. Negative genetic correlations between fitness characters are commonly observed in both natural and domesticated populations (Falconer, 1981; Lande, 1982; Istock, 1983), but it is rarely possible to discriminate pleiotropy from linkage effects. In the long run, genetic correlations based on linkage can be altered by recombination, but pleiotropy is more persistent and can permanently restrict the rate of evolution.

By allowing mutations to randomly accumulate on an initially uniform genetic background, this study has permitted the identification of the average pleiotropic effects of mutant polygenes influencing the life history characters of *Daphnia*. Nearly all of the characters examined were interrelated through pleiotropy in ways that could either facilitate or detract from their response to selection. Thus, the evolution of daphnid life histories is a highly integrated process, and the response of any single character to selection will often be greatly modified by the action of selection on correlated characters.

Finally, the residual environmental effects revealed by this study deserve some consideration. Even after being grown in a common laboratory environment for three generations, significant differences that cannot be attributed to mutation appeared between sublines within lines. On average, these residual environmental effects accounted for 19% of the phenotypic variance and 23% of the phenotypic correlation between characters. They are likely to be equally or more important under natural conditions where parental age effects may exist and environmental

heterogeneity may be greater. Although the number of generations through which residual environmental effects persist cannot be determined from this study, previous investigations (Agar, 1913; Lynch and Ennis, 1983) have demonstrated the transmittance of major maternal effects over single generations, and some suggestive evidence indicates that reduced effects may extend to two generations but not more (Agar, 1913). Unless they are positively correlated with genetic effects, residual environmental effects will reduce the efficiency of the selection process by depressing the correlation between genotype and phenotype.

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#### APPENDIX

The following derivations for a nested, multivariate analysis of variance provide estimators for the components of variance and covariance and their standard errors based on the mean squares and cross-products. The analysis is applied specifically to a mutation accumulation experiment utilizing an asexual organism in which  $a$  lines have diverged from a common ancestor for  $\Delta T$  generations, each line having been subdivided into  $b$  sublines for the last  $t$  generations of the experiment. During the analysis each subline is replicated by raising  $n$  progeny from the same clutch of a single mother. The goal is to partition the phenotypic variances and covariances into their genetic and environmental components.

The linear model used for the analysis is

$$X_{ijk} = \mu + L_i + S_{ij} + \epsilon_{ijk}; \\ i = 1 \dots a, j = 1 \dots b, k = 1 \dots n$$

where  $X_{ijk}$  is the phenotypic value of the  $k$ th replicate of the  $j$ th subline of the  $i$ th line and  $\mu$  is the grand mean.  $L$ ,  $S$ , and  $\epsilon$  refer to line, subline, and error effects, assumed to be mutually independent. Under these conditions, the expected mean squares and cross-products are linear functions of the between-replicates ( $V_c$ ), between-sublines ( $V_s$ ), and between-lines ( $V_l$ ) components of variance (Table A1).

The error mean square ( $M_e$ ) provides an estimate of the variance between replicates, which in this case is equivalent to the within-clutch component of environmental variance. The mean square term for sublines within lines ( $M_s$ ) contains, in addition to  $V_c$ , the between-sublines component of variance which consists of variance due to residual environmental effects ( $V_e$ ) and genetic variance resulting from mutations over the period of subline isolation:

$$V_s = V_e + tV_M \quad (A1)$$

where  $V_M$  is the mutational rate of input of genetic variance/generation. For this experimental design,  $V_e$  contains all sources of environmental variance not included in  $V_c$ . Finally, the line mean square ( $M_l$ ) contains a third source of variance, the between-lines variance, which is a function of the ge-

TABLE A1. Analysis of variance for a nested experimental design (Snedecor and Cochran, 1967). The date effect (see main text) does not enter into the final analysis and is excluded from the table. For a bivariate analysis of variance, substitute mean cross product for mean square and *Cov* for *V*. Terms are defined in the appendix.

Source of variation	<i>df.</i>	Mean square	Expected mean square
Lines within dates	$a - 1$	$M_l$	$V_c + nV_s + bnV_l$
Sublines within lines	$a(b - 1)$	$M_s$	$V_c + nV_s$
Error	$ab(n - 1)$	$M_e$	$V_c$

netic variance ( $V_G$ ) that has accumulated among the lines since their separation,

$$V_l = V_G = \Delta T \cdot V_M \tag{A2}$$

Substituting (A1) and (A2) into the expressions for the expected mean squares in Table A1, we obtain expressions for the components of phenotypic variance for character  $x$ :

$$\begin{aligned} V_{c,x} &= M_{e,x} \\ V_{G,x} &= (M_{l,x} - M_{s,x})/bn, \\ V_{r,x} &= [(M_{s,x} - M_{e,x})/n] - tV_{M,x} \end{aligned} \tag{A3}$$

Heritability in the broad sense is defined by

$$H_x^2 = V_{G,x}/V_{T,x}$$

where

$$V_{T,x} = V_{G,x} + V_{c,x} + V_{r,x}$$

By use of the delta technique (Taylor expansion), approximate expressions for the variance of these parameter estimates are found to be

$$\begin{aligned} \text{Var}(V_{c,x}) &\approx \text{Var}(M_{e,x}), \\ \text{Var}(V_{G,x}) &\approx (bn)^{-2} \cdot [\text{Var}(M_{l,x}) + \text{Var}(M_{s,x})], \\ \text{Var}(V_{r,x}) &\approx n^{-2} \cdot [\text{Var}(M_{s,x}) + \text{Var}(M_{e,x}) \\ &\quad + [(t/\Delta T)^2 \cdot \text{Var}(V_{G,x})], \\ \text{Var}(V_{T,x}) &\approx \text{Var}(V_{G,x}) + \text{Var}(V_{r,x}) + \text{Var}(V_{c,x}), \\ \text{Var}(H_x^2) &\approx \frac{1}{V_{T,x}^2} [\text{Var}(V_{G,x}) + H_x^2 \text{Var}(V_{T,x})]. \end{aligned}$$

Under the assumption of normality, the mean squares are independent and have variances that are estimated by  $2(M_{i,x})^2/(df. + 2)$  where  $i = e, s,$  or  $l$  (Bulmer, 1980).

In a parallel fashion, the covariance between characters due to within-clutch environmental effects, residual environmental effects, and genetic effects can be dissected from the mean cross products of a multivariate analysis of variance. Correlations between characters at these three levels are then given by

$$r_{c,xy} = \frac{V_{c,xy}}{(V_{c,x} \cdot V_{c,y})^{1/2}} = \frac{M_{e,xy}}{(M_{e,x} \cdot M_{e,y})^{1/2}} \tag{A4}$$

and

$$\begin{aligned} r_{r,xy} &= \frac{V_{r,xy}}{(V_{r,x} \cdot V_{r,y})^{1/2}} \\ &\approx \frac{M_{s,xy} - M_{e,xy}}{[(M_{s,x} - M_{e,x})(M_{s,y} - M_{e,y})]^{1/2}}, \end{aligned}$$

when  $t$  is small, and by

$$\begin{aligned} r_{G,xy} &= \frac{V_{G,xy}}{(V_{G,x} \cdot V_{G,y})^{1/2}} \\ &= \frac{M_{l,xy} - M_{s,xy}}{[(M_{l,x} - M_{s,x})(M_{l,y} - M_{s,y})]^{1/2}} \end{aligned}$$

where the  $M_{i,xy}$  terms represent the mean cross products for characters  $x$  and  $y$ . The combined effects of genetic and environmental covariance between characters can be assessed by direct regression of individual phenotypic values without respect to lineage,

$$r_{T,xy} = \frac{V_{T,xy}}{(V_{T,x} \cdot V_{T,y})^{1/2}}.$$

Under the assumption of bivariate normality, the hypotheses that  $E(r_{T,xy}) = 0$  and  $E(r_{E,xy}) = 0$  can be evaluated with standard significance tables based on the known distribution of  $r$  when  $E(r) = 0$ . However, due to the indirect nature of their computation, the sampling distributions of  $r_{r,xy}$  and  $r_{G,xy}$  are unconstrained by the theoretical limits of  $(-1, +1)$ , and it is more difficult to attach a specific level of confidence to them. However, the sampling variances of  $r_{r,xy}$  and  $r_{G,xy}$  can be estimated as follows.

By the delta technique, the variance of a correlation coefficient (dropping the subscript  $i$  for the time being) is found to be

$$\begin{aligned} \text{Var}(r_{xy}) &\approx r_{xy}^2 \{ [\text{Var}(V_{xy})/V_{xy}^2] + [\text{Var}(V_x)/4V_x^2] \\ &\quad + [\text{Var}(V_y)/4V_y^2] \\ &\quad - [\text{Cov}(V_x, V_{xy})/(V_x V_{xy})] \end{aligned}$$

$$- [\text{Cov}(V_y, V_{xy})/(V_y V_{xy})] \\ + [\text{Cov}(V_x, V_y)/(2V_x V_y)]$$

(Kendall and Stuart, 1976). The terms  $\text{Var}(V_{xy})$ ,  $\text{Var}(V_x)$ , and  $\text{Var}(V_y)$  have been defined above. The terms  $\text{Cov}(V_x, V_{xy})$ ,  $\text{Cov}(V_y, V_{xy})$ , and  $\text{Cov}(V_x, V_y)$  can also be expressed in terms of observed mean squares and mean cross products. By the delta technique, it can be shown that

$$\text{Cov}(V_{G,j}, V_{G,k}) \approx (bn)^{-2} \cdot [\text{Cov}(M_{i,j}, M_{i,k}) \\ + \text{Cov}(M_{e,j}, M_{e,k})]$$

and that for small  $t$

$$\text{Cov}(V_{r,j}, V_{r,k}) \approx n^{-2} \cdot [\text{Cov}(M_{s,j}, M_{s,k}) \\ + \text{Cov}(M_{e,j}, M_{e,k})]$$

where  $j, k = x, y$ , or  $xy$ . Further noting from Bulmer (1980) that

$$\text{Var}(M_{i,xy}) = (M_{i,xy}^2 + M_{i,x}M_{i,y})/(d.f. + 2), \\ \text{Cov}(M_{i,x}, M_{i,y}) = 2M_{i,xy}^2/(d.f. + 2), \\ \text{Cov}(M_{i,x}, M_{i,xy}) = 2M_{i,x}M_{i,xy}/(d.f. + 2), \\ \text{Cov}(M_{i,y}, M_{i,xy}) = 2M_{i,y}M_{i,xy}/(d.f. + 2),$$

the variance estimator for  $r_G$  and  $r_r$  is found by back substitution to be

$$\text{Var}(r_{xy}) \approx r_{xy}^2 \left\{ D_{xy}^{-2} \left[ \frac{A_{xy}^2 + A_x A_y}{\alpha} + \frac{B_{xy}^2 + B_x B_y}{\beta} \right] \right. \\ + (1/2)D_x^{-2} \left[ \frac{A_x^2}{\alpha} + \frac{B_x^2}{\beta} \right] \\ + (1/2)D_y^{-2} \left[ \frac{A_y^2}{\alpha} + \frac{B_y^2}{\beta} \right] \\ - 2(D_x D_{xy})^{-1} \left[ \frac{A_x A_{xy}}{\alpha} + \frac{B_x B_{xy}}{\beta} \right] \\ - 2(D_y D_{xy})^{-1} \left[ \frac{A_y A_{xy}}{\alpha} + \frac{B_y B_{xy}}{\beta} \right] \\ \left. + (D_x D_y)^{-1} \left[ \frac{A_{xy}^2}{\alpha} + \frac{B_{xy}^2}{\beta} \right] \right\}.$$

In the estimation of the genetic correlation coefficient,  $\alpha, \beta, A_x, B_x$ , and  $D_x$  are respectively equal to  $(a + 1)$ ,  $[a(b - 1) + 2]$ ,  $M_{i,x}, M_{e,x}$ , and  $(M_{i,x} - M_{e,x})$ . For  $r_r$  they are respectively  $[a(b - 1) + 2]$ ,  $[ab(n - 1) + 2]$ ,  $M_{s,x}, M_{e,x}$  and  $(M_{s,x} - M_{e,x})$ . The square root of this expression provides an estimate of the standard error for  $r_G$  and  $r_r$ . In the text, I treat  $r_G$  and  $r_r$  as being significant at the 0.05 level when their absolute values exceed 1.96 standard errors and as being significant at the 0.01 level when their absolute values exceed 2.58 standard errors. These should be considered to be approximations in the absence of information on the actual sampling distribution of  $r_G$  and  $r_r$ .