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QUANTITATIVE GENETIC VARIATION IN *DAPHNIA*: TEMPORAL CHANGES IN GENETIC ARCHITECTURE

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Abstract.—Nonadditive genetic variation and genetic disequilibrium are two important factors that influence the evolutionary trajectory of natural populations. We assayed quantitative genetic variation in a temporary-pond-dwelling population of *Daphnia pulex* over a full season to examine the role of nonadditive genetic variation and genetic disequilibrium in determining the short-term evolutionary trajectory of a cyclic parthenogen. Quantitative traits were influenced by three factors: (1) clonal selection significantly changed the population mean phenotype during the course of the growing season; (2) sexual reproduction and recombination led to significant changes in life-history trait means and the levels of expressed genetic variation, implying the presence of substantial nonadditive genetic variation and genetic disequilibrium; and (3) Egg-bank effects were found to be an important component of the realized year-to-year change. Additionally, we examined the impact of genetic disequilibria induced by clonal selection on the genetic (co)variance structure with a common principal components model. Clonal selection caused significant changes in the (co)variance structure that were eliminated by a single bout of random mating, suggesting that a build-up of disequilibria was the primary source of changes in the (co)variance structure. The results of this study highlight the complexity of natural selection operating on populations that undergo alternating phases of sexual and asexual reproduction.

Key words.—*Daphnia*, genetic slippage, life-history evolution, linkage disequilibrium, parthenogenetic, quantitative traits.

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The evolutionary dynamics of quantitative characters in populations that reproduce both sexually and asexually may differ substantially from those in a purely sexual population. Evolutionary theory predicts that the response to natural selection in sexual populations is determined largely by the additive effects of segregating loci affecting the expression of quantitative traits (Fisher 1930; Falconer and Mackay 1996). In contrast, for asexual populations the genotype of an individual is essentially a single linkage group and selection operates on the joint additive and nonadditive effects underlying the genetic architecture of quantitative traits.

Directional and/or stabilizing selection during prolonged periods of asexual reproduction can build up substantial genetic disequilibria for polygenic traits. The levels of genetic disequilibria, either gametic-phase or Hardy-Weinberg, affect the genetic architecture of natural populations in two ways. First, during periods of asexual reproduction, directional and/or stabilizing selection can erode the expressed genetic variation for quantitative traits. However, when genetic disequilibria exist, a substantial portion of the total genetic variation may be ''hidden'' by the disequilibria. Depending on the prevailing phase of disequilibria, whether in coupling or repulsive phase, the expressed genetic variation can be either greater or less than the genetic variation that would be expressed in the absence of disequilibria. A single bout of random mating is sufficient to eliminate Hardy-Weinberg disequilibrium and reduces the levels of gametic-phase disequilibrium. For a polygenic trait with a purely additive basis this reduction in disequilibrium converts 50–75% of the ''hidden'' genetic variation to expressed genetic variation (Lynch and Gabriel 1983). In populations that undergo periodic bouts of sexual reproduction, as in cyclical parthenogens like most *Daphnia* species, this can result in cycles of expressed genetic variation with the amplitude of the cycle decreasing with increasing frequency of sex (Lynch and Gabriel 1983).

Second, when there are nonadditive genetic effects in disequilibrium, the genotypic mean of a quantitative character can change upon sexual reproduction and recombination (Lynch and Deng 1994; Deng and Lynch 1996; Via and Shaw 1996). This phenomenon has been referred to as ''genetic slippage'' because the break-up of favorable gene combinations promoted by prior selection results in a change of the genotypic mean following recombination in the direction contrary to previous selection. A detailed theory has been developed to describe the effects of disequilibria on traits with a purely additive genetic basis (Lynch and Gabriel 1983), nonadditive genetic basis (Lynch and Deng 1994), and for the multivariate case where genetic correlations exist among traits (Deng and Lynch 1996).

Prior investigations of cyclically parthenogenetic populations of *Daphnia* have demonstrated a pattern consistent with the expectation that genetic variation is eroded by clonal selection during periods of asexual reproduction and restored to relatively high levels following sexual reproduction and recombination. Lynch (1984a) monitored quantitative genetic variation over a two-year period in a population of *D. pulex* and found high levels of expressed genetic variation for size and fitness-related characters early in the season, with a rapid erosion of the levels of variation after a few generations of asexual reproduction. High levels of genetic variation were reestablished when hatchlings, which were the result of sexual reproduction in the prior year, emerged at the beginning of the next year. Similar results have been reported by Tessier et al. (1992) for a permanent-lake population of *D. galeata mendotae*. In that study, expressed genetic variation for life-history traits was completely eliminated during a two-month period of asexual reproduction. An increase in expressed ge-

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netic variation following sexual reproduction has also been observed in a laboratory study of permanent-lake-dwelling *D. pulicaria* (Deng and Lynch 1996). In contrast, Lynch and Deng (1994) observed a reduction in the level of expressed genetic variation following sexual reproduction in a population of *D. pulex*. Interestingly, the results of these prior studies are in accord with the theoretical prediction that recombination can lead to either an increase (lake-dwelling *D. pulicaria*) or a decrease (pond-dwelling *D. pulex*) of expressed genetic variation depending on the prevailing phase of disequilibria. These results suggest that the selective regime in different populations may promote alternate phases of gametic phase disequilibrium. After sexual reproduction, the mean genotypic value for the suite of life-history traits assayed in these studies changed an average of approximately one-tenth and three-tenths of a phenotypic standard deviation in *D. pulex* and *D. pulicaria*, respectively. These observations suggest that selection prior to the sexual phase led to the build-up of gametic-phase and/or Hardy-Weinberg disequilibria between genes with nonadditive effects.

Three factors are important in determining the short-term evolutionary trajectory of quantitative characters in cyclically parthenogenetic populations. First, the patterns of nonadditive genetic variation have a large potential influence on the response to selection during periods of asexual reproduction. Second, the pattern of genetic slippage will influence the realized response to selection by altering the population mean phenotype and level of expressed genetic variation. Third, because *Daphnia* populations are established from a bank of diapausing eggs that are the result of sexual reproduction in prior years, egg-bank effects are likely to contribute to changes in quantitative characters from season to season if hatchlings arise from diapausing eggs deposited in multiple years.

Although it is possible to infer the direction of prior selection by an examination of genetic slippage, no direct comparison of observed and inferred selection was made in earlier studies. This study utilizes the unique properties of cyclically parthenogenetic *Daphnia* to examine the relative contribution of selection, genetic slippage, and egg-bank effects on the short-term evolutionary trajectory of a single natural population. Because *Daphnia* are capable of reproducing either sexually or asexually, unique genotypes can be maintained indefinitely in the laboratory by clonal propagation. Unlike the situation for most sexual species, this property allows for direct comparison of individuals from different populations or different sampling dates in a common environment without the confounding elements of varying laboratory conditions and maternal effects. Unbiased estimates of genetic parameters can be obtained by partitioning phenotypic variation into within- and among-clone components. Because genotypes can be replicated, it is possible through a simple analysis of variance (ANOVA) to generate an unbiased estimate of the environmental variation from the within-clone component of variation. The broad-sense heritability (H^2) is then the ratio of the among-clone variance to the total within- and among-clone variance. Narrow-sense heritability (h^2), the proportion of total variance due to additive genetic effects, can be estimated as twice the covariance between parental and sexually produced offspring mean values scaled relative to the phenotypic variance in the parental generation (Lynch and Deng 1994).

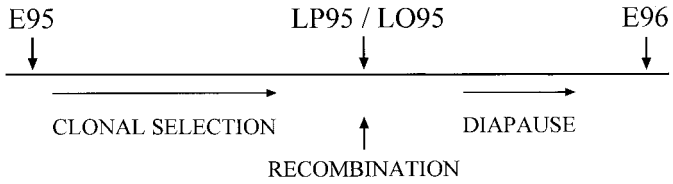

FIG. 1. Sampling scheme used in this study. *Daphnia* were collected at three intervals covering a full year period. The first early season collection (E95) was made at the initial appearance of *Daphnia* in the pond in year 1. After a prolonged period of clonal reproduction, a second collection was made as the population entered a phase of sexual reproduction. This collection (LP95) comprised female *Daphnia* carrying fertilized eggs produced by sexual reproduction and recombination. Their offspring were reared to form a late season offspring collection (LO95). Finally, after summer diapause, at the initial appearance of *Daphnia* the following year a second early season (E96) collection was made.

In this study we assessed the response to selection during the asexual phase by comparing the genotypic population means for morphological and life-history characters at the beginning of the season with those observed at the end of the growing season when the population entered a sexual phase. Similarly, we examined the impact of genetic slippage following recombination by comparing parents with their sexually produced offspring. Finally, we examined the contribution of egg-bank effects by comparing the sexually produced offspring cohort to the early season hatchlings of the following year.

METHODS

All of the *D. pulex* clones in this study were obtained from an ephemeral pond located in the Willamette Valley in western Oregon. This population, which inhabits a small woodland pond located in Amazon Park in Eugene, Oregon, has been the subject of intensive study (Lynch and Deng 1994; Lehman et al. 1995; Deng 1996; Lynch et al. 1998, 1999). Temporary ponds in western Oregon typically fill with water at the onset of the first winter rains in late November to early December. *Daphnia pulex* in these temporary ponds hatch from an egg bank of diapausing eggs soon after the ponds fill to establish the populations. Amazon Pond, which dries up each year sometime between early May and early June, has a maximum surface area of approximately 160 m^2 and reaches a maximum depth of 1 m. Important predators present are invertebrate dipteran larvae (*Chaoborus*) and vertebrate salamander larvae (*Ambystoma*).

To examine the temporal patterns of quantitative genetic variation, *Daphnia* were collected on three occasions covering a full year (Fig. 1). The first collection was made at the initial appearance of *Daphnia* in the pond in late November 1994, referred to as the early 1995 (E95) collection. Early collection was made to maximize the levels of quantitative genetic variation present because early clones were the recent product of ephippial hatching from the sediments and therefore minimally exposed to selection (Lynch 1984a). A total of 160 mature females were isolated into single 250-ml beakers and maintained in the laboratory by clonal reproduction.

At the end of the growing season a second collection was made when the pond entered a sexual phase. This collection comprised 500 mature females carrying outcrossed sexually produced diapausing eggs. The females were isolated into individual beakers and the sexual eggs collected when shed. These diapausing eggs were hatched by exposing them to repeated cycles of light and dark following the procedure outlined by Pfrender and Deng (1998). Parental females and their hatchlings were maintained by clonal reproduction yielding 100 parent-offspring pairs. These two groups were referred to as the late parental 1995 (LP95) and late offspring 1995 (LO95) collections. At the first appearance of *Daphnia* the following year, a final collection of 100 individuals was made, referred to as the early 1996 (E96) collection. All laboratory clone stocks were maintained in an environmental chamber at 15°C with a photoperiod of 12:12 L:D prior to the experimental assay. Clones were housed in 250-ml beakers containing 200 ml of aged filtered pond water supplemented with the green alga *Scenedesmus* as a food resource.

Quantitative Genetic Analysis

Life-history trait data were obtained by a standard life table experimental design (Lynch 1985; Lynch et al. 1989). The assay contained 100 individuals randomly selected from the clonal stock for each of the four groups (E95, LP95, LO95, and E96). In all, 400 clonal lines were assayed with each genotype represented by two replicate lines. Each line was established with a single immature female taken from its clonal culture. Experimental lines were maintained by asexual reproduction under the assay conditions for two generations prior to measurement. This protocol insures that any maternal (and grandmaternal) effects contribute to the within- rather than the between-clone component of variance (Lynch 1985). In the third generation, individuals were examined daily under a Wild (Heerbrugg, Switzerland) dissecting microscope to obtain estimates of the life-history traits: size at birth, instar-specific body sizes, instar-specific ages, and clutch sizes. Size at birth and instar-specific body size were measured as the length from the top of the head to the base of the tail spine. The number of offspring released at each adult instar was recorded.

To enhance the precision of estimates of age at first reproduction, time of birth, and duration of adult instars, the developmental stage of immature eggs in the brood chamber was recorded. *Daphnia* exhibit characteristic developmental stages in their asexually produced eggs, which can be easily distinguished by morphological criteria. A frequency distribution of egg stages was generated over the entire experiment to estimate the relative duration of each developmental stage. These stages can be used to obtain more precise estimates of age parameters by subtracting the expected time to reach the embryonic stage in a developing clutch from the time at which a released clutch was first observed (Lynch et al. 1989). This indirect approach is possible because female *Daphnia* extrude a new clutch into the brood chamber almost immediately upon the release of the previous clutch. We also used these modified age estimates to calculate growth rate parameters, computed as $[\ln(B_{i+1}) - \ln(B_i)]/t$, where B_i is the size and t is the duration of the ith instar (Lynch et al. 1989). All individuals were followed from birth to the release of the fourth clutch.

During the experimental assays, each individual was maintained in 100 ml of aged, filtered water taken from the source pond, supplemented with a pure laboratory culture of the green alga *Scenedesmus* to a density of approximately 3×10^5 cells/ml. The food/water mixture was replaced every other day. The experiment was conducted in Percevil (Boone, Iowa) incubators maintained at 12°C with a 10:14 L:D photoperiod.

Mortality during the experimental assay led to some missing data, so a one-way ANOVA for unbalanced data was used to partition the phenotypic variance into within- and among-clone components of variance (Searle et al. 1992). Because replicates within clonal lines are genetically identical, the variance within a clone is a direct measure of environmental sources of variation and the variance among clones is a measure of expressed genetic variation. Prior to computing variance components from the observed mean squares, measurement error variance was eliminated from the within-clone component of variance as described by Lynch et al. (1989). Broad-sense heritability (H^2) was estimated as the proportion of total variation due to among-clone variation. Narrow-sense heritability (h^2) was estimated as twice the slope of the regression between parents and offspring (Falconer and Mackay 1996). In all cases, the standard errors of genetic parameter estimates were obtained by use of expressions derived by the delta method (Lynch and Walsh 1998).

Genetic disequilibrium can have an impact on the form of the genetic variance-covariance matrix (**G**). Differences between matrices can be assessed in a number of ways, and Flury (1987) has pointed out that matrix association can be significant in a hierarchical series of relationships. The most inclusive level is matrix equality. Less inclusive is matrix proportionality, where matrix elements differ by some scalar. Matrices may also share a common principle component structure. Matrix similarity can be assessed for each of these properties beginning with the least inclusive level in the hierarchy and proceeding to the most inclusive—matrix equality. This approach has been used to compare phenotypic variance-covariance matrices (Steppan 1997) and genetic variance-covariance matrices (Phillips and Arnold 1999).

Comparisons of **G**-matrix structure between collection groups were made using the program CPCRAND (Phillips 1994). CPCRAND conducts multiple iterations of matrix comparison by first randomizing clones with respect to group (in this case collection date or parent-offspring) and then comparing the two ''new'' matrices. The observed comparison statistic of the actual matrices is compared to the distribution obtained from the randomized populations to estimate the probability of obtaining a statistic that large simply by chance. Matrix comparisons between the E95 and LP95, LP95 and LO95, and E95 and LO95 collections were each made with 1000 iterations used to generate the comparison statistic distribution.

RESULTS

Life-history trait means for the four experimental groups are given in the Appendix. A subset of seven traits showed

TABLE 1. Broad- and narrow-sense heritability estimates. Broad-sense heritability (H^2) estimates are the ratio of the between-clone variance to the total variance. Narrow-sense heritability (h^2) is estimated as twice the covariance between late season parents (LP95) and offspring (LO95) divided by the mean phenotypic variance. Early season collections are designated by E95 and E96.

Trait	h^2	H^2_{E95}	H^2_{LP95}	H^2_{LO95}	H^2_{E96}
Instar-specific size	0.31	0.56**	0.41**	0.57**	0.62**
Size at birth	0.25	0.18	0.32**	0.37**	0.26
Size at maturity	0.44	0.41**	0.28*	0.54**	0.43**
Juvenile growth rate	0.65*	0.58**	0.71**	0.09	0.34
Adult growth rate	0.23	0.20	0.39*	0.29	0.36
Age at first reproduction	0.42	0.61**	0.62**	0.38**	0.06
Clutch size	0.02	0.45**	0.34*	0.32	0.31

* $P < 0.05$, ** $P < 0.01$.

significant expressed genetic variation (Table 1). These characters including, instar-specific size, size at birth, size at maturity (defined as size during the instar when eggs are first deposited into the brood pouch), juvenile and adult growth rate, age at first reproduction, and clutch size were the focus of further analysis.

Broad-sense heritability (H^2) estimates indicated significant expressed genetic variation for five of the seven life-history traits examined in the E95 sample. All seven life-history characters in the LP95 generation and four of seven in LO95 generation showed significant levels of expressed genetic variation. The E96 collection showed significant expressed genetic variation for only two traits, instar-specific size and size at maturity. In contrast, only one of the narrow-sense heritability (h^2) estimates was significant (throughout, values are given followed by their standard errors in parentheses). Averaging over all traits in the late season parental generation, the mean broad-sense heritability was 0.44 (0.06). The estimated average narrow-sense heritability (h^2_{PO}) of 0.33 (0.07) was approximately 75% as large. Because the difference ($H^2_P - h^2_{PO}$) is all due to nonadditive genetic variance, this result suggests that approximately 11% of the phenotypic variance and 25% of the genetic variance in this population was due to nonadditive genetic effects.

The mean phenotypes for three of the seven traits showed a significant increase between the beginning of the growing season and the sexual phase at the end of the growing season (Table 2). In units of phenotypic standard deviations, the average change due to clonal selection was 0.20 (0.07). The general trend was for individuals to become larger, have larger clutches, and reproduce later. Following sexual reproduction, four of the seven traits decreased in mean phenotype, significantly so for age at first reproduction. The average absolute change of the mean phenotype following sexual reproduction was 0.10 (0.03) phenotypic standard deviations. The general trend was for trait means to change in the opposite direction from that observed over the asexual phase. The total realized absolute change in average mean phenotype across a full year (i.e., from the early season collection in 1995 to the early season collection in 1996) was 0.27 (0.05) phenotypic standard deviations. Trait means that changed significantly across years were in all cases the same as those that changed significantly during the asexual phase. Comparison of the LO95 and E96 collections revealed a significant decrease in the mean adult growth rate and an average absolute change of 0.17 (0.03) phenotypic standard deviations. This amount represents the residual change not accounted for by change due to clonal selection or genetic slippage following sexual reproduction.

Sexual reproduction did not lead to an increase in expressed genetic variation. In five of the traits there was a decrease in expressed genetic variation (scaled relative to the phenotypic variance), significantly so for juvenile growth rate and age at first reproduction (Table 3). The average broad-sense heritability across all seven traits in the offspring generation, 0.37 (0.06), was 17% less than that in the parental generation, 0.44 (0.06).

TABLE 2. Change in life-history trait means scaled relative to the phenotypic standard deviation. LP95 − E95 is the change during the clonal phase of population growth. LO95 − LO95 is the change following sexual reproduction (genetic slippage). E96 − LO95 is the residual change attributable to egg-bank effects and E96 − E95 is the realized change in the mean value from the first to the second year.

Trait	LP95 − E95	LO95 − LP95	E96 − LO95	E96 − E95
Instar-specific size	0.54*	0.04	−0.13	0.43*
Size at birth	0.06	−0.01	0.06	0.11
Size at maturity	0.36*	−0.05	0.09	0.41*
Juvenile growth rate	0.13	−0.22	−0.13	−0.24
Adult growth rate	0.02	0.19	−0.34*	−0.12
Age at first reproduction	0.09	−0.14*	0.27	0.22
Clutch size	0.19*	0.06	0.17	0.39*

* $P < 0.05$.

TABLE 3. Change in expressed total genetic variance following sexual reproduction measured in units of the mean phenotypic variance in the parent and offspring generations. The change in genetic variance (ΔV_g) is the genetic variance in the offspring generation minus the genetic variance in the parent generation. Data for *Daphnia pulex* are from this study and Lynch and Deng (1994). Data for *D. pulicaria* are from Deng and Lynch (1996).

	Pond		Lake
Trait	*D. pulex* 1996 ΔV_g	*D. pulex* 1994 ΔV_g	*D. pulicaria* ΔV_g
Instar-specific body size	0.07	−0.19**	0.26*
Size at birth	−0.06	−0.21*	0.46*
Size at maturity	0.04	0.13**	0.31*
Juvenile growth rate	−0.75*	−0.23**	0.13
Adult growth rate	−0.22	−0.40	0.00
Age at first reproduction	−0.37*	−0.45**	0.06
Clutch size	−0.13	−0.11	0.02

* $P < 0.05$, ** $P < 0.01$.

TABLE 4. Matrix comparisons using the Flury hierarchy. E95 refers to the early season 1995 collection; LP95 and LO95 refer to the late season parent and offspring generations, respectively. Significance tests are conducted to test for equality and proportionality of matrix structure as well as testing for common principal component (CPC) and partial common principal component (CPC_i) structure. Values are the *P*-value for the test of difference at the level of comparison.

Comparison	Equality	Proportionality	CPC	CPC(3)	CPC(2)	CPC(1)
E95 vs. LP95	0.016	0.011	0.430	0.440	0.230	0.440
LP95 vs. LO95	0.033	0.003	0.029	0.026	0.011	0.320
EP95 vs. LO95	0.260	0.230	0.130	0.100	0.000	0.720

The results of log-likelihood-ratio tests for differences between **G**-matrices are given in Table 4. Comparison of the **G**-matrix before and after clonal selection, that is, E95 versus LP95, revealed a significant difference at the levels of matrix equality and proportionality. The null hypothesis of no significant difference could not be rejected for either common principle component structure or any of the partial common principal components. Recombination led to a significant difference between **G** measured in the LP95 and LO95 collections. Finally, no significant difference was detected between the structure of **G** measured at the beginning of the year (E95) and that measured after sexual reproduction and recombination (LO95).

DISCUSSION

Genetic Variation and Disequilibrium

In the present study we investigated the temporal dynamics of quantitative genetic variation for life-history traits in a natural population of *D. pulex.* The study population showed high levels of expressed broad-sense heritability for life-history characters. The average ranged from 0.43 (0.06) in the early season 1995 group to 0.34 (0.06) in the early season 1996 group. These estimates were within the range of values typically observed for temporary-pond- and lake-dwelling *Daphnia.* For temporary-pond populations of *D. pulex* and *D. obtusa*, the average estimates for the same suite of traits were 0.40 (0.04) and 0.27 (0.05), respectively (Lynch et al. 1989; Spitze 1993). In a previous examination of the Amazon population (the focal population in this study), the average ranged from 0.43 (0.04) to 0.51 (0.06) in the parent and offspring generations, respectively (Lynch and Deng 1994).

Substantial levels of nonadditive genetic variation were revealed by differences between estimates of broad- and narrow-sense heritabilities and significant genetic slippage of life-history trait means following sexual reproduction. Comparison of the LP95 and LO95 groups showed a significant difference between broad-sense heritability in the parental generation and narrow-sense heritability estimated from the covariance between parent and offspring phenotypes. This difference suggests that approximately 11% of the phenotypic variance and 25% of the expressed genetic variance is due to nonadditive genetic variation. Additional support for the presence of nonadditive genetic effects is provided by the observation that there was a general decrease in the genotypic means following sexual reproduction. This result is in accord with that of prior studies of genetic slippage in *Daphnia* (Lynch and Deng 1994; Deng and Lynch 1996) and aphids (Via and Shaw 1996).

The theoretical results of Lynch and Deng (1994) predict that the changes in genotypic mean values will almost always be in the opposite direction to that promoted by selection during the clonal phase. Interestingly, in our study, the observed pattern of slippage following sexual reproduction was sometimes contrary to the prediction based on the observed response to selection. The mean value of all seven traits increased over the clonal phase of population growth, leading to the prediction that genetic slippage would result in a decrease in the phenotypic means for these characters. In contrast, we observed an increase in the mean phenotype following recombination for four of the seven traits shown in Table 2. This observation was consistent with selective pressure acting to decrease, not increase, the mean values of these traits. Thus, the observed pattern of genetic slippage seems in conflict with the theoretical predictions. However, as pointed out by Lynch and Deng (1994), genetic slippage of the mean reflects the long-term accumulation of genetic disequilibria, not simply the events of the previous generation. Fluctuating selection from year to year may lead to prevailing disequilibria that are not reflective of the selective forces observed in any one year. In natural populations, variation in the predation regime from year to year may cause fluctuating selection favoring larger body size and early maturation when invertebrate predators dominate (Dodson 1974; Kerfoot 1977) and the opposite when vertebrate predators are the prevailing selective agent (Werner and Hall 1975; Zaret and Kerfoot 1975). The results of our study suggest that the observed direction of slippage alone is not sufficient to infer the direction of selection immediately prior to sexual reproduction.

Selection during the clonal phase can lead to the build-up of gametic-phase and Hardy-Weinberg disequilibrium, and in this study disequilibria were revealed by a change in expressed genetic variation following sexual reproduction and recombination. Contrary to the typical expectation that sexual reproduction leads to an increase in expressed genetic variation, there was a general decrease of expressed genetic variance, significantly so for size at birth, juvenile growth rate and age, at first reproduction. Thus, the prevailing genetic disequilibria in this population prior to recombination were in coupling phase. This result is consistent with the results from prior examination of the Amazon population (Lynch and Deng 1994) and opposite to that observed for a lake-dwelling population of *D. pulicaria* (Deng and Lynch 1996) and other temporary-pond-dwelling populations of *D. pulex* (Lynch 1984b). Taken together, these results imply that there may be a fundamental difference in the way selection acts to establish genetic disequilibria in different populations and highlights the complexity of natural selection among different populations (Deng and Lynch 1996).

There is evidence that within a single season this temporary-pond population of *Daphnia* is under directional selection. The significant change in mean values for life-history traits over the clonal phase is consistent with directional selection. Additionally, there is a positive relationship between broad-sense heritability and the change in the mean trait val-

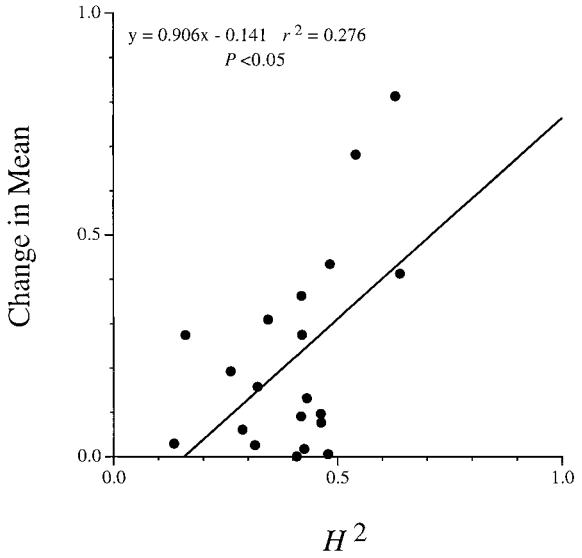

FIG. 2. Relationship between the change in life-history trait means during the asexual phase and broad-sense heritability. Life-history trait means are absolute values scaled relative to the average phenotypic standard deviation for each trait. Broad-sense heritabilities are the average of early season and late season heritabilities.

ues observed across the period of clonal reproduction (Fig. 2). This relationship was indicative of directional selection operating on life-history traits in this population because, all other things being equal, the magnitude of the response to directional selection increases with increasing heritability (Falconer and Mackay 1996). The positive relationship between the response to selection and the level of expressed genetic variation suggests that although a large portion of the standing genetic variation may be due to the segregation of slightly deleterious mutations (Houle et al. 1996; Lynch et al. 1998), at least some of the standing genetic variation is adaptive.

Covariance and Correlation

The signs of phenotypic and genetic correlations between traits were generally in agreement with one another. For example, the correlations between size at maturity and four other life-history traits (Table 5) were of the same sign when measured on a phenotypic or genetic scale. There was a positive correlation between size at maturity and both clutch size and juvenile growth rate. Negative phenotypic and genetic correlations were observed between size at maturity, age at maturity, and adult growth rate. In other words, at both the phenotypic and genetic level some clones in this population attain larger body size at an earlier age and have larger clutch sizes. Evaluating the relationships of these correlations to fitness is difficult because in natural populations of *Daphnia* the selective forces operating on life-history traits are dependent on conditions like the predation regime, which may vary widely. These observations indicate that there are no trade-offs between life-history characters during the juvenile period. However, the negative genetic correlation between size at maturity and adult growth rate suggests that there may be a trade-off between early and late growth.

The stability of the genetic variance-covariance matrix (**G**) has been the subject of a growing body of work. Because a combination of the multivariate effects of selection and the polygenic input of variation by mutation determine the form of **G,** it is reasonable to ask whether **G** is altered by prolonged clonal selection. A comparison of matrices using a common principal component approach revealed that the form of **G** is indeed altered during clonal selection. As shown in Table 4, the null hypotheses of equality and proportionality are rejected for the comparison of G between the E95 and LP95 groups. One interesting question is whether this difference reflects a permanent genetic change or whether, in a fashion similar to the univariate case, these differences are the result of a build-up of genetic disequilibria. Clonal selection can induce changes in expressed genetic covariances in the same way that genetic variances are influenced (Deng and Lynch 1996). These data suggest that the differences in covariance structure observed after clonal selection are the result of a build-up genetic disequilibria because there are no differences between the E95 **G** and **G** measured in the LO95 generation after a bout of sex.

The results of this study demonstrate the complexity of the evolutionary dynamics of temporary-pond-dwelling populations of cyclically parthenogenetic populations of *Daphnia.* The evolutionary trajectory in these populations is determined by the interplay between the response to clonal selection, genetic slippage, and egg-bank effects. The significant and typically high broad-sense heritabilities indicate that during periods of clonal selection, both additive and nonadditive genetic variation contribute to the response to selection. Yearly bouts of sexual reproduction can act to either enhance or retard the advance in mean phenotype gained during the clonal phase and can also alter the levels of expressed genetic variation. Perhaps even more important is the alteration of the genetic (co)variance structure during the

TABLE 5. Phenotypic, genetic, and environmental correlations between life-history traits. E95, early season 1995 collection; LP95, late season parent 1995.

	Phenotypic		Genetic		Environmental	
	E95	LP95	E95	LP95	E95	LP95
Size at maturity versus:						
Clutch size	0.35***	0.25	0.11	−2.51	0.49***	0.71*
Age at maturity	−0.11	−0.31*	−0.03	−0.84*	0.06	0.03
Juvenile growth rate	0.26*	0.40***	0.51*	0.99**	0.07	0.11
Adult growth rate	−0.14	−0.58*	−0.77	−1.11	0.34	−0.52

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

clonal phase. Genetic correlations may have a profound impact on the evolutionary trajectory of a population and even transient changes in the (co)variance structure may impact this trajectory.

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Appendix

Estimates of phenotypic means for early season 1995 (E95), late season parent (LP95) and offspring (LO95), and early season 1996 (E96) collections. The units for body size are millimeters, for ages at reproduction are days, and for growth rates are days^{-1}.

	E95		LP95		LO95		E96	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body sizes:								
Birth	0.624	0.003	0.627	0.003	0.626	0.030	0.628	0.004
Maturity	1.775	0.017	1.827	0.015	1.821	0.016	1.834	0.021
First adult instar	2.024	0.021	2.093	0.019	2.088	0.020	2.051	0.026
Second adult instar	2.183	0.026	2.258	0.024	2.284	0.025	2.238	0.036
Third adult instar	2.309	0.032	2.431	0.023	2.451	0.028	2.413	0.039
Fourth adult instar	2.397	0.038	2.558	0.031	2.561	0.029	2.551	0.043
Clutch sizes:								
First	5.027	0.557	5.833	0.451	5.953	0.503	6.375	0.477
Second	10.565	0.813	12.415	0.747	11.627	0.704	12.913	1.204
Third	14.018	1.257	14.286	1.017	15.333	1.012	16.513	1.381
Fourth	15.878	1.655	18.576	1.537	18.909	1.378	20.806	1.833
Ages at reproduction:								
Maturity	16.228	0.499	16.152	0.513	16.032	0.470	17.265	0.454
Release of first clutch	23.080	0.678	23.512	0.588	22.902	0.532	24.001	0.520
Release of second clutch	30.609	0.829	30.640	0.693	29.982	0.551	31.286	0.665
Release of third clutch	38.458	0.844	37.963	0.764	38.031	0.597	39.143	0.691
Release of fourth clutch	46.096	0.752	46.428	0.728	45.102	0.901	46.402	0.692
Growth rates:								
Juvenile	0.068	0.002	0.071	0.002	0.066	0.002	0.064	0.002
First adult instar	0.022	0.002	0.022	0.002	0.021	0.001	0.018	0.001
Second adult instar	0.013	0.001	0.013	0.001	0.015	0.001	0.012	0.001
Third adult instar	0.009	0.001	0.011	0.001	0.010	0.001	0.010	0.001
Fourth adult instar	0.010	0.001	0.011	0.000	0.011	0.001	0.011	0.001