Population Structure in *Daphnia obtusa*: Quantitative Genetic and Allozymic Variation

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ABSTRACT

Quantitative genetic analyses for body size and for life history characters within and among populations of *Daphnia obtusa* reveal substantial genetic variance at both hierarchical levels for all traits measured. Simultaneous allozymic analysis on the same population samples indicate a moderate degree of differentiation: $G_{ST} = 0.28$. No associations between electrophoretic genotype and phenotypic characters were found, providing support for the null hypothesis that the allozymic variants are effectively neutral. Therefore, G_{ST} can be used as the null hypothesis that neutral phenotypic evolution within populations led to the observed differentiation for the quantitative traits, which I call Q_{ST}. The results of this study provide evidence that natural selection has promoted diversification for body size among populations, and has impeded diversification for relative fitness. Analyses of population differentiation for clutch size, age at reproduction, and growth rate indicate that neutral phenotypic evolution cannot be excluded as the cause.

A logical consequence of FISHER's fundamental theorem of natural selection is that populations should exhibit low levels of genetic variance for traits that are closely related to relative fitness, such as key life history traits (FISHER 1958; FALCONER 1981). This conclusion applies to populations that experience prolonged selection in a relatively stable environment. However, several processes can act to seriously impede this loss of genetic variation, including mutation (LANDE 1976a; TURELLI 1984), heterozygote superiority (FALCONER 1981), frequency-dependent selection (BULMER 1980), temporally variable selection (EWING 1979), antagonistic pleiotropy (WILLIAMS 1957; ROSE 1982, 1983, 1985), and gene flow (FELSENSTEIN 1976; LANDE 1992).

Recent reviews of the literature on the quantity of genetic variance for various types of characters have revealed that life-history traits are often significantly heritable, although not to the degree exhibited by physiological, behavioral, and particularly by morphological traits (MOUSSEAU and ROFF 1987; ROFF and MOUSSEAU 1987; ROFF 1992). One interpretation of this observation is that populations may seldom be subjected to constant selection for long enough periods to effectively eliminate genetic variation for traits closely related to fitness, and indeed for relative fitness itself. If substantial genetic variance for quantitative traits exists, then detectable evolutionary change within such populations is possible. Such change can occur due to genetic drift in small populations, resulting in neutral phenotypic evolution, or due to natural selection in populations of any size.

Since the advent of protein electrophoresis, the results of a large number of studies have been published that detail the comparative divergence of phenotypic and allozymic characteristics. The subjects of such studies have encompassed a variety of plants (*e.g.*, RITLAND and JAIN 1984), arthropods (*e.g.*, ALLEGRUCCI, CESARONI and SBORDONI 1987), fish (*e.g.*, TURNER 1974), birds (*e.g.*, KARL, ZINK and JEHL 1987), and mammals (*e.g.*, SCHNELL, BEST and KENNEDY 1978). Most of these have reported significant differences between analyses of phenotypic and electrophoretic data, and have suggested that natural selection is responsible for the different pattern of diversification of phenotypic characters. LEWONTIN (1984) argued that it should be easier to detect divergence for quantitative traits (*e.g.*, morphological, life history) than for single loci (*i.e.*, allozymes). However, ROGERS (1986) and FELSENSTEIN (1986) both pointed out errors in LEWONTIN's conclusions and indicated that WRIGHT's (1951) model of neutral evolution of quantitative characters might be used to examine whether interpopulational divergence of polygenic traits and allozymes were caused by similar evolutionary processes.

Most of these previous empirical studies have compared the concordance of phenotypic and allozymic diversification by examining the correlation between pairwise among-population estimates of phenotypic divergence (or some multivariate analog, such as Mahalanobis distance) and genetic distance [*e.g.*, NEI's (1972)]. However, essentially no study has compared the among-population variation for quantitative traits with the expectation of neutral phenotypic evolution. Such expectations rely on estimates of values such as

the rate of input of variation via spontaneous mutation, time since divergence, and population size (CHAKRABORTY and NEI 1982; ROGERS and HARPENDING 1983; LYNCH and HILL 1986; LANDE 1992). Although there are estimates for the former (LYNCH 1988b), it is generally difficult to estimate reliably the latter quantities in the absence of detailed historical information. However, if information on population differentiation exists for genes that are effectively neutral, then the relative degree of among-population variation for quantitative traits can be examined against an appropriate neutral expectation (FELSENSTEIN 1986; LANDE 1992). It is essential to compare the degree of population differentiation to the expectation of neutral phenotypic evolution when evaluating the role of natural selection as the cause of the observed differentiation.

Previous studies of cyclically parthenogenetic populations of *Daphnia pulex* inhabiting temporary ponds indicate that populations harbor substantial amounts of genetic variation for life history traits, due to their initiation each year by the hatch of resting eggs, that are produced during the previous year(s) by sexual reproduction (LYNCH 1983, 1984a,b; LYNCH, SPITZE and CREASE 1989; CREASE, LYNCH and SPITZE 1990). After initiation, the populations go through an extended period of clonal reproduction. During this period of clonal selection, early season broad-sense heritabilities for quantitative traits (life-history characters) of 0.3–0.5 decrease to nearly undetectable levels (LYNCH 1984b; LYNCH, SPITZE and CREASE 1989; SPITZE 1991), while allele frequencies for enzymes assayed by protein electrophoresis remain stable (LYNCH 1983, 1987; CREASE, LYNCH and SPITZE 1990). At seasons' end, the populations engage in bouts of sexual reproduction before the ponds become desiccated or frozen. Due to recombination and the release of hidden genetic variance, the populations exhibit high heritability at the beginning of the following year (LYNCH 1984b; LYNCH and GABRIEL 1984; LYNCH, SPITZE and CREASE 1989).

Although the frequency of recruitment of new genetic variants via sexual reproduction is not well understood in permanent populations of Daphnia, quantitatively similar patterns of genetic variation have been observed. Several lake populations of *Daphnia pulicaria* and *Daphnia galeata mendotae* exhibited average heritabilities of 0.31–0.60 for body size at birth and at maturity [values extracted from ANOVA tables in LEIBOLD and TESSIER (1991) and in TESSIER, YOUNG and LEIBOLD (1992)]. Clutch size was also significantly heritable, but at a reduced level ($H^2 = 0.18$). In one of the populations of *D. galeata mendotae*, these high initial values for heritability were reduced to undetectable levels over a period of 2 months.

Whether in temporary ponds or permanent lakes, the phase of clonal reproduction can result in a significant evolutionary change in quantitative traits due to selective pressures, such as predation (SPITZE 1991; TESSIER, YOUNG and LEIBOLD 1992). These rates of evolutionary change and loss of genetic variance greatly exceed that explainable by genetic drift (LYNCH 1984b; SPITZE 1991). These observations imply that natural selection can be a potent force in shaping evolutionary change in quantitative characters within years. An unanswered question is the degree to which natural selection is responsible for divergence among populations for such traits. The goals of this project are to answer the following questions: What is the degree of genetic variability within and among populations of *D. obtusa* for allozymes and for quantitative traits? Are allozymic variants effectively neutral? Is the degree of within-population genetic variance greater for morphological characters than for important fitness components? Is there evidence that natural selection has affected the degree of among-population variability for quantitative traits?

MATERIALS AND METHODS

Study species, populations: *D. obtusa* is a species of cladocera that is found primarily in temporary ponds. It is widely dispersed in the United States and is known from Ontario (SCHWARTZ, INNES and HEBERT 1985; INNES, SCHWARTZ and HEBERT 1986; INNES 1989; HEBERT, SCHWARTZ and WEIDER 1989). Unlike its congener *D. pulex*, in which some clones are known to reproduce by obligate parthenogenesis, all previous studies have indicated that *D. obtusa* reproduces entirely by cyclical parthenogenesis (LYNCH 1983, 1984a; INNES, SCHWARTZ and HEBERT 1986, HEBERT, SCHWARTZ and WEIDER 1989).

This study concerns eight populations of *D. obtusa* from midwestern United States and southern Canada. All have been the subject of previous study utilizing protein electrophoresis (LYNCH 1983, 1984a; LYNCH and SPITZE 1993). Some of the populations have been the subject of preliminary mitochondrial DNA restriction site analysis (T. J. CREASE, personal communication). All available information indicates that the study populations belong to a single species. The populations, with 2–3 letter abbreviations, are: Coal Tipple Pond (CT, located near Portsmouth, Ohio), Nobody's Home Pond and Nothing Pond (NH and NP, located in Morton, Illinois), Ojibway Pond (OJ, located near Windsor, Ontario), Buffalo Wallow Pond (BUF, located in Urbana, Illinois), Hey-A-Pond (HAP, located near St. Joseph, Illinois), May-Bab Pond (MAY, located in Urbana, Illinois), and Trelease Center Pond (TCP, located in the University of Illinois Trelease Woods natural area, northeast of Urbana, Illinois). Clonal isolates were obtained from each population, representing early-season samples shortly after recruitment from resting eggs (LYNCH 1984b; LYNCH, SPITZE and CREASE 1989; SPITZE, BURNSON and LYNCH 1991). Each isolate was maintained in the laboratory under well fed conditions to eliminate the possibility of sexual reproduction.

Electrophoretic methods: Each clonal isolate (60–125 per population) was assayed by protein electrophoresis for seven

structural enzymes: Pgm (phosphoglucomutase; EC 2.7.5.1), Pgi (phosphoglucose isomerase; EC 5.3.1.9), Got (glutamate oxaloacetate transaminase; EC 2.6.1.1), Pep (peptidase; EC 3.4.13), Fh (fumarate hydratase; EC 4.2.1.2), Mpi (mannosephosphate isomerase; EC 5.3.1.8) and Ldh (lactate dehydrogenase; EC 1.1.1.27). Horizontal starch gel electrophoresis was used for Pgm, Pgi, Got and Pep (LYNCH 1983), while cellulose acetate electrophoresis was used for Fh, Mpi and Ldh (HEBERT and PAYNE 1985).

Life table methods: Life table methods used in this study have been detailed extensively elsewhere (LYNCH, SPITZE and CREASE 1989; SPITZE, BURNSON and LYNCH 1991). Briefly, two replicates were established from each clonal isolate, and were acclimated to experimental conditions for one generation, to eliminate environmental maternal effects as a contributor to between-clone variance (LYNCH and ENNIS 1983; LYNCH 1985). Each population was represented by 20–28 clones, haphazardly chosen from among the initial isolates. All animals were maintained in 200 ml of medium in a controlled temperature and light cabinet at 20° with a 12L/12D light cycle. The medium consisted of aged tap water to which 300,000 cells of Scenedesmus were added per ml. After the initial generation of acclimation, a single, randomly selected individual served as the experimental individual for each replicate. Fresh medium was provided daily to each individual as life-history characters were assessed. Life-history characters included body length (mm, excluding tailspine), clutch size carried, and number of young released. The experiment was concluded when every surviving individual released its sixth clutch of offspring.

From the raw data collected from each experimental individual, values for the following characters were determined for analysis: L_i (mean body length in instar i), G_i (growth increment following instar i, equivalent to $L_{i+1} - L_i$), C_i (clutch size carried in clutch i), K_i (age at release of ith clutch), and w (relative fitness). Although individuals were examined only once per day, the age at release of a clutch could be estimated with greater precision by observing the egg development stage of the succeeding clutch (LYNCH, SPITZE and CREASE 1989). Relative fitness was estimated as $w = \sum l_x m_x \exp(-rx)$ where l_x and m_x represent age-specific survival and reproduction of the individual, and r is the intrinsic rate of population growth obtained from the entire data set (CHARLESWORTH 1980).

Analytical methods: Genotype frequencies within each population were first compared to Hardy-Weinberg (HW) expectations. When no systematic departure was observed, estimates of allozymic variability are reported as the HW expectations. Standard errors are reported under the assumption that each locus represents an independent assessment of allozymic variation. Among-population diversity for allozymes is represented as G_{ST}, following well defined procedures (CROW 1986).

Components of variance attributable to genetic and environmental sources were obtained by equating observed mean squares from one-way ANOVA with their expectations (LYNCH 1984b; LYNCH, SPITZE and CREASE 1989). Variance due to measurement error was subtracted using techniques outlined previously (LYNCH 1988a; LYNCH, SPITZE and CREASE 1989). Reported values for the heritability of each character from each population are the means of 1000 bootstrap iterations involving resampling across clones, a procedure that reduces the bias inherent in obtaining estimates from the ratio of variance components. Hypotheses concerning the magnitude of heritabilities were evaluated using the distribution of the bootstrap iterations (SPITZE, BURNSON and LYNCH 1991). The component of variance for quantitative traits representing among-population (σ^2_{GB}) and average within-population (σ^2_{GW}) were obtained as means of 1000 bootstrap iterations, each involving resampling across clones within populations for the entire data set, using nested ANOVA. The degree of population differentiation is represented as the proportion of variance that is among-populations, Q_{ST}, which is analogous to G_{ST}, but has a slightly different form. For characters with a purely additive basis that are evolving neutrally, WRIGHT (1951) showed that $\sigma^2_{GW} = (1 - Q_{ST})\sigma^2_T$, where σ^2_T is the total genetic variance that would be exhibited were all individuals part of a panmictic population. He further showed that $\sigma^2_{GB} = 2Q_{ST}\sigma^2_T$. It follows that $Q_{ST} = \sigma^2_{GB}/(\sigma^2_{GB} + 2\sigma^2_{GW})$ (LANDE 1992). Although WRIGHT's (1951) formulation described completely isolated populations, the same result can be shown from LANDE's (1992) formulae for an island model with migration, providing that the number of populations is large, and the number of migrants per generation is low (less than one per generation). The neutral expectation for Q_{ST} is the value of G_{ST} for neutral single-locus genes in the same populations (FELSENSTEIN 1986; LANDE 1992). Hypotheses concerning Q_{ST} were evaluated using the distribution obtained from the 1000 bootstrap iterations mentioned above.

Data from each population were examined to determine whether any difference among clones was attributable to their electrophoretic genotype. Discovery of such a relationship would constitute evidence that the electrophoretically detectable alleles were non-neutral, either through direct action or linkage. Failure to find such a relationship would provide support for the null hypothesis that the alternative alleles are effectively neutral. To examine this, nested ANOVA on 10 key life-history traits was performed for each assayed locus, using the model: population, genotype within population, clone within genotype. SAS Proc GLM and type III SS (SAS Institute, Inc.) were used throughout, because of unbalanced design (unequal number of clones per genotype, some missing data).

Finally, the degree of within-population genetic variation for allozymes was compared to that for quantitative characters. A positive association is the expectation if similar evolutionary forces are acting on both types of characters, whereas no such association is expected if the evolutionary dynamics of the two types of characters are governed by different forces.

RESULTS

Genetic variation within and among populations, allozymes: Six of the seven loci assayed electrophoretically were polymorphic in at least one population; only Ldh was fixed for the same allele in all populations. Of 56 population loci assayed (8 populations × 7 loci), 22 were polymorphic. When the genotype frequencies for these 22 polymorphisms were compared to Hardy-Weinberg expectations, only two revealed nominally significant deviations: Mpi in population CT, and Pgi in population OJ. Since slightly over one nominally significant departure was expected due to chance, the data set was adequately described by Hardy-Weinberg expectations. The expected heterozygosity for each population locus is presented in Table 1. The average heterozygosity across the study populations was 0.12.

The proportion of genetic diversity that is among

TABLE 1

Expected heterozygosity for each locus in each population, based on observed allelic proportions

Population	Pgm	Pgi	Got	Pep	Fh	Mpi	Ldh	Mean (SE)
CT	0.098	0.000	0.000	0.000	0.000	0.476	0.000	0.082 (0.067)
NH	0.000	0.336	0.000	0.000	0.000	0.498	0.000	0.119 (0.079)
NP	0.000	0.422	0.000	0.000	0.000	0.622	0.000	0.149 (0.099)
OJ	0.454	0.500	0.046	0.117	0.000	0.157	0.000	0.182 (0.079)
BUF	0.000	0.000	0.000	0.000	0.217	0.306	0.000	0.075 (0.049)
HAP	0.096	0.020	0.123	0.000	0.078	0.585	0.000	0.129 (0.079)
MAY	0.000	0.000	0.472	0.000	0.000	0.576	0.000	0.150 (0.097)
TRE	0.000	0.076	0.091	0.000	0.000	0.401	0.000	0.081 (0.055)
Mean	0.081	0.169	0.092	0.015	0.037	0.453	0.000	0.121 (0.059)
G_{st}	0.399	0.590	0.181	0.320	0.112	0.133		0.276 (0.106)

TABLE 2

Broad-sense heritabilities and the degree of population divergence for quantitative characters, averaged across character types, for each population

Population	Body size	Growth	Clutch size	Reprod. age	Relative fitness
CT	0.51*	0.32†	0.28†	0.31†	0.05
NH	0.30*	0.15	0.21	−0.04	0.52****
NP	−0.14	0.23†	−0.21	0.03	0.06
OJ	0.46*	0.44***	0.42*	0.28	0.47****
BUF	0.45*	0.22	0.13	−0.02	0.08
HAP	0.56**	0.50****	0.37**	0.83****	0.67****
MAY	0.29*	0.29*	0.55****	0.72*	0.66****
TRE	0.02	0.33*	0.32†	0.39†	0.40†
Total	0.42****	0.35****	0.35****	0.47****	0.42****
Q_{ST}	0.40****,[a]	0.29*	0.21*	0.18*	0.08*,[b]

Note: † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; **** $P < 0.001$.

[a] Significantly greater than 0.28, $P = 0.03$.

[b] Significantly less than 0.28, $P = 0.002$.

populations for each polymorphic locus, represented by G_{ST}, is summarized at the bottom of Table 1. Locus-specific estimates ranged between 0.11 and 0.59. The average across loci is 0.28.

Genetic variation within and among populations, quantitative characters: Estimates of genetic variance for quantitative characters are presented as the average value for 1000 bootstrap iterations, each estimating the broad-sense heritability (H^2) for each character in each population. These population-specific values are presented as the mean across sets of like characters in Table 2. That is, the average H^2 for body size is the average across 11 instar-specific estimates (L_i) within each population. Similar means are presented across instar-specific growth increments (G_i), clutch sizes (C_i), clutch-specific estimates of age at reproduction (K_i). A single composite value of relative fitness (w) is also presented. The final line in Table 2 represents the mean across characters in each group for 1000 bootstrap estimates when the entire data set was combined in a nested ANOVA and a single within-population variance component was extracted. For this assessment, resampling was performed across clones within populations.

The broad-sense heritability for each type of character was significantly greater than zero in about half of the population-specific estimates. Body size was significantly heritable in six of the eight populations, with values ranging from −0.14 to 0.56. The average within-population estimate is 0.42, and was highly significant ($P > 0.001$). Four of the population-specific estimates were significant for instar-specific growth increment. The estimates ranged from 0.15 to 0.50, with an average of 0.35 ($P < 0.001$). Clutch sizes and ages at reproduction were also significantly heritable in nearly half of the populations, with average heritabilities of 0.35 and 0.47, respectively (both $P < 0.001$). Relative fitness was significantly heritable in four populations, with estimates ranging from 0.05 to 0.67, and an average of 0.42 ($P < 0.001$).

The greatest Q_{ST} is found for body size (0.40, $P < 0.001$), and for growth increments (0.29, $P < 0.05$). Clutch size (0.21, $P < 0.05$) and ages at reproduction (0.18, $P < 0.05$), also exhibited significant among-population variance, although at a reduced level. Relative fitness displayed the least population differentiation (0.08), although the value was significantly greater than zero ($P < 0.05$).

Heritability of morphological *vs.* life history traits: If body size and growth increments are categorized as morphological traits, and clutch size, age at reproduction, and relative fitness as life-history traits, there was no significant difference in average heritability of character types within the study populations. Morphological traits had values of 0.42 and 0.35, while life history traits exhibit heritabilities of 0.35, 0.47 and 0.42.

Selective value of allozymes: Ten key life history characters were analyzed by nested ANOVA to determine whether single-locus genotype was related to the quantitative traits, either by direct action or linkage. The characters were: body size at birth (L_1), body size

at maturity (L_{mat}), the first four clutch sizes (C_i, $i = 1$–4), age at first reproduction (K_1), combined growth increments during immature instars (sum of G_i, $i = 1$–4), combined growth increments during mature instars (sum of G_i, $i = 5$–8), and relative fitness. Although perhaps only clutch size, age at reproduction, and relative fitness may be considered "true" fitness components, body size and growth increment characters were also examined to determine whether any phenotypic effect of the allozyme variants could be found. Of 36 tests for "true" life history characters, two were nominally significant at $P < 0.05$. For the total of 60 tests for all characters, only three were nominally significant, exactly the number expected by chance. No nominal significance level was less than 0.02, indicating that adjustment for multiple comparisons would render all tests nonsignificant. These results offer no reason to reject the null hypothesis that allozymic variants are effectively neutral.

Neutral expectations for among-population variance: If allozymes can be considered effectively neutral, the estimate of proportion of diversity among populations for the electrophoretic data (0.28) can be used as the null hypothesis for testing among-population variance for quantitative traits. The values for proportion of variance for quantitative traits that is among-population (Q_{ST}) were reevaluated against this value by determining the proportion of the bootstrap estimates that exceeded this value, in one direction or the other. If Q_{ST} is significantly greater than 0.28 for a type of character, this indicates that diversifying selection is responsible. If the value is indistinguishable from the observed G_{ST}, then the hypothesis that among-population variance is due to neutral phenotypic evolution cannot be rejected. If the value of Q_{ST} is significantly less than 0.28, then selection must be constraining among-population divergence.

For average body size (mean Q_{ST} across 11 instar-specific measures), the Q_{ST} value of 0.40 (Table 2) is significantly greater than 0.28 ($P = 0.03$). Q_{ST} for growth increments (0.29) is indistinguishable from 0.28 ($P = 0.53$). Values of Q_{ST} for clutch sizes (0.21, $P = 0.83$), and for ages at reproduction (0.18, $P = 0.82$) are also indistinguishable from 0.28. However, the estimate of Q_{ST} for relative fitness (0.08) is significantly less than 0.28 ($P = 0.002$). That is, 99.8% of the bootstrap iterations resulted in an estimate of Q_{ST} that was less than 0.28.

Allozymic *vs.* quantitative genetic variation: Linear regression of average heritability for each character (Table 2) on population heterozygosity (Table 1) across the study populations revealed no significant association. All regression coefficients were negative, although none approached statistical significance. The greatest variance explained by a regression was 29%; the average variance explained was 15%.

DISCUSSION

Although it is relatively easy to demonstrate that interpopulation and interspecific variation for quantitative traits is non-zero [reviewed in ENDLER (1986)], this is not the appropriate null hypothesis for detecting the action of natural selection in leading to population differentiation (LANDE 1976b, 1977; LYNCH and HILL 1986). Indeed it has proven difficult to demonstrate that variation among species exceeds the minimum rate of neutral phenotypic evolution (LYNCH 1990). When assessment of interpopulation divergence for quantitative traits is accompanied by similar assessments for effectively neutral genes, an appropriate null hypothesis for the action of selection as the cause can be constructed by comparing G_{ST} for effectively neutral genes with Q_{ST} for the quantitative traits (WRIGHT 1951; FELSENSTEIN 1986; LANDE 1992). The advantage of this approach for testing the null hypothesis of genetic drift is that estimates of divergence time, population size, and heritability are unnecessary. Such estimates are necessary for many other tests for neutral evolution [*e.g.*, LANDE (1977) and LYNCH (1990)], but are typically quite hard to obtain. In this study, the null hypothesis of neutral phenotypic evolution as a sufficient explanation of interpopulational divergence could be rejected for body size and for relative fitness (biotic potential). Since Q_{ST} for body size significantly exceeded G_{ST} for effectively neutral allozymes, the action of diversifying selection can be invoked as the cause of interpopulational divergence. On the other hand, variation in relative fitness among populations was significantly less than the neutral expectation, suggesting that selection has impeded diversification. Interpopulational divergence for clutch size, age at reproduction, and growth rate were indistinguishable from the neutral expectation, although all were significantly less than zero.

Similar patterns of Q_{ST} for body size have been observed for permanent lake populations [values extracted from ANOVA tables in LEIBOLD and TESSIER (1991) and in TESSIER, YOUNG and LEIBOLD (1992)]. Values of 0.32–0.46 were observed for body size at birth, and 0.41–0.86 for size at maturity. Although no allozyme data are available for these populations, values of G_{ST} for populations of Daphnia occupying similar geographical ranges rarely exceed 0.35 (LYNCH and SPITZE 1993).

What can account for this pattern? It has been well established that survival in Daphnia is profoundly affected by predation. Indeed predation has been implicated as responsible for more than 90% of all mortality experienced by a population of *Daphnia rosea* (DODSON 1972). Most predation is of a size-selective nature, but different types of predators prey selectively on different ranges of prey size. Vertebrate

predators, such as fish and salamander larvae, selectively prey on large bodied individuals, whereas many invertebrate predators, such as insect larvae, levy greatest mortality on the smallest individuals (DODSON 1970; LYNCH 1979; SPITZE 1985). Since the intensity of these different types of predation can vary spatially and temporally among populations, this source of mortality can act as a potent source of diversifying selection among Daphnia populations. Significant evolutionary change in body size in as few as a half-dozen generations has been experimentally demonstrated in response to predation by an invertebrate (SPITZE 1991), and predation has been implicated in such changes in a natural population (TESSIER, YOUNG and LEIBOLD 1992).

Selection on relative fitness itself, however, is always directional towards increase (LANDE 1979, 1982a). Such cosmopolitan patterns of selection should seriously impede diversification [but see COHAN (1984) and LYNCH (1986)]. The result of this study conforms to this expectation. However, while consistent directional selection should impede the accumulation of interpopulational differences in relative fitness, key components of fitness may evolve more freely. Although clutch sizes and the ages at reproduction may be under rather consistent directional selection, the intensity of such selection will be less than on fitness, to an extent governed by the genetic correlation of each component of fitness with relative fitness itself (LANDE 1982a,b; LANDE and ARNOLD 1983). The results of this study indicate that clutch size and age at reproduction exhibited greater interpopulational differences than did relative fitness. However, the degree of among-population diversification for these traits is consistent with the expectation of neutral phenotypic evolution. Therefore, no selection need be invoked to explain the divergence.

These results provide further evidence for substantial genetic variance for quantitative traits in populations of Daphnia, consistent with earlier work on *D. pulex* (LYNCH 1984b; LYNCH, SPITZE and CREASE 1989), and permanent lake Daphnia populations (LEIBOLD and TESSIER 1991; TESSIER, YOUNG and LEIBOLD 1992). The average heritabilities for body size and growth obtained here (0.42 and 0.35) are quite consistent with those for temporary pond populations of *D. pulex* for body size [0.37 in the Busey Pond population; LYNCH (1984b)], and growth [0.41 and 0.34, for populations PA and KA, respectively; LYNCH, SPITZE and CREASE (1989)]. They are also consistent with values obtained for body size in lake populations (0.31–0.60) [values extracted from ANOVA tables in LEIBOLD and TESSIER (1991) and TESSIER, YOUNG and LEIBOLD (1992)]. Even for important fitness components such as clutch size and age at reproduction, more than half of the assayed populations in this study exhibited significant heritabilities. The average heritabilities for these traits was 0.35 and 0.47, respectively, which are quite similar to previously published values of 0.39 (LYNCH 1984b) and 0.32–0.45 (LYNCH, SPITZE and CREASE 1989) for clutch size, and values of 0.48 (LYNCH 1984b) and 0.14–0.28 (LYNCH, SPITZE and CREASE 1989) for age at first reproduction. In this study, even the measure of relative fitness (biotic potential) also displayed substantial genetic variance, with an average heritability of 0.42.

These estimates for average heritability of life history traits are higher than are often found. Previously published summaries of heritabilities for life history, physiological, behavioral, and morphological traits indicate that life history traits are the least heritable (average $h^2 = 0.26$), whereas morphological traits on average exhibit the highest heritability (average $h^2 = 0.46$) (MOUSSEAU and ROFF 1987). In this study, the average heritability of all types of traits exceeded 0.35 (Table 2). Morphological traits (body size, growth rate) were no more heritable than life history traits (clutch size, age at reproduction, biotic potential). It is important to note, however, that the heritability estimated in this study is the broad-sense heritability (appropriate for clonal reproduction). If substantial non-additive gene action is contributing to these values, then the narrow-sense heritabilities will be brought more in line with previously published values.

As with most studies that have reported on the selective value of allozymes, the results reported here show no association between any phenotypic trait and any assayed locus. In fact, very few studies have found such relationships [see KOEHN, ZERA and HALL (1983)]. Previous studies have failed to detect any consistent relationship between electrophoretic profile and phenotype in Daphnia (LYNCH 1984a; LYNCH, SPITZE and CREASE 1989). However, there is some evidence that allozymic variants are not always effectively neutral. Analysis of long term studies have suggested that the temporal pattern of allozyme frequencies may be influenced by fluctuating selection (LYNCH 1987). Chance association between marker loci (allozymes) and loci with significant effects on phenotypic characters can lead to periods when particular allozymes are not effectively neutral, although long term average selection coefficients are essentially zero. In large populations, where drift plays essentially no role in allele frequency dynamics, fluctuating selection leads to a driftlike phenomenon, resulting in allele frequency changes that mimic the effects of genetic drift. Effectively, the long term consequences of drift and of fluctuating selection are similar. However, the degree of population differentiation will be greater when drift and fluctuating selection both contribute, than when drift acts alone. If fluctuating

selection is important in *D. obtusa*, then G_{ST} cannot be used as an unbiased null hypothesis for evaluation of Q_{ST}. It does, however, provide a conservative benchmark for the action of diversifying selection, because G_{ST} will be slightly greater under the action of fluctuating selection, than for a purely neutral situation. Therefore, the conclusion of this study that interpopulational divergence for body size has been enhanced by diversifying selection is strengthened. However, the conclusion that selection is constraining diversification of biotic potential is weakened, to an extent determined by the relative contribution of fluctuating selection to the observed value of G_{ST}.

One of the central questions facing evolutionary biologists is the relative degree to which random processes and natural selection lead to evolutionary change. Since allozyme data is relatively inexpensive and easy to obtain, it will be useful if such assays are incorporated into studies that assess natural selection as the agent of interpopulational divergence. It is interesting to note that after submission of this paper, the results of a similarly designed study on population differentiation in Drosophila was published (PROUT and BARKER 1993). Their conclusion was identical to that reported here: population differentiation for body size was significantly greater than the expectation of neutral phenotypic evolution (as derived from allozymic F_{ST}), implicating the action of some type of diversifying selection. Rejection of the appropriate null hypothesis of genetic drift will strengthen future conclusions on the importance of natural selection in promoting evolutionary change within and among populations.

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