Evolutionary Genetics of *Daphnia*

*(with Ken Spitze)*

**Introduction**

As a science, ecological genetics has developed in a rather fragmented fashion. Numerous studies on a diversity of species have evaluated the levels of molecular variation existing within and among populations as well as among species (for recent reviews, see Kimura 1983; Nei 1987; Selander et al. 1991). Most of these studies have assumed, either implicitly or explicitly, that the molecular markers under consideration (usually allozymes or restriction fragment length polymorphisms) are neutral enough to allow their use as tools for estimating aspects of population structure, divergence times, etc. (Slatkin, chaps. 1, 2, this volume).

Recently, a great deal of attention has also been focused on the estimation of levels of variation and covariation for polygenic characters in natural populations, using quantitative-genetic techniques that have been established firmly in the realm of plant and animal breeding programs (Travis, chaps. 9, 10; Via, chaps. 3, 4, this volume). Usually, these types of studies have been performed with populations for which molecular data are not available, so our understanding of the relationships between patterns of evolution at the two levels is essentially undeveloped.

Over the past decade or so, our laboratory has been examining genetic variation at the molecular and quantitative-trait levels at both the intraspecific (within and among populations) and interspecific levels. This work utilizes planktonic microcrustaceans in the genus *Daphnia* as a model system. We are still a long way from a full understanding of the evolutionary genetic properties of the genus, but enough progress has been made in some areas that it now seems worthwhile to attempt a synthesis of the results. This chapter summarizes some of the more interesting patterns that have begun to emerge.

Although some *Daphnia* have dropped the sexual phase of the life cycle (Hebert 1981; Hebert et al. 1988), most reproduce by cyclical parthenogenesis, and our attention will be focused entirely on populations with that type of life cycle. Cyclically parthenogenetic *Daphnia* normally reproduce by asexual means so long as the environment remains favor-
able. When conditions deteriorate, males are produced, as are haploid eggs, which when fertilized give rise to diapausing embryos (ephippia). For ponds that dry completely, annual recruitment is entirely from these sexually reproduced eggs. However, populations inhabiting permanent lakes have the potential to reproduce indefinitely by parthenogenesis.

Evolution at the Molecular Level

Since the pioneering studies of Hebert (1974a,b), dozens of isozyme surveys have been performed on *Daphnia* populations. Most of these studies have involved ten or fewer loci, and attention has often been restricted to those loci that exhibit the greatest degree of polymorphism. Thus it is still somewhat difficult to make precise statements as to the average levels of biochemical diversity within and among populations. However, some generalizations are now possible.

Our surveys of three species of cyclically parthenogenetic *Daphnia* are based on nine to twelve loci selected only for the ease of resolution of their banding patterns on gels. The observed allele frequencies have been used to estimate $\nu_w$, the average gene diversity within populations (Nei 1987). This statistic is equivalent to the average (over all loci) heterozygosity that would be observed in the progeny if the sampled population were to undergo random mating. Being based on Hardy-Weinberg expectations, $\nu_w$ provides a useful measure of isozyme variation that is unbiased by aspects of population structure, local inbreeding, clonal selection, and so forth. For seven central Illinois *D. pulex* populations, we estimate the average $\nu_w$ to be 0.11 (0.05). (Throughout, numbers in parentheses are standard errors.) An identical estimate has been obtained for nine *D. obtusa* populations in the same general region, whereas the average $\nu_w$ for eight western Oregon populations of *D. pulicaria* is 0.15 (0.07).

For Czechoslovakian populations of *D. magna*, *D. longispina*, and *D. galeata*, using the six-locus survey of Hebert et al. (1989b), we estimate $\nu_w$ to be 0.12 (0.06), 0.13 (0.07), and 0.17 (0.10), respectively. Benzie's (1986a) extensive survey of Australian *D. cephalata* and *D. carinata* populations yields rather higher $\nu_w$ estimates—0.16 (0.06) and 0.21 (0.07)—whereas Korpelainen (1986a) obtains estimates ranging from 0.04 to 0.09 for four European species of *Daphnia*. Taken as a group, these data suggest that *Daphnia* harbor substantial levels of variation at the level of nuclear genes—an average individual appears to be heterozygous at 5% to 20% of its protein-coding loci. These could very well be underestimates since it is well known that protein electrophoresis is unable to discriminate a large fraction of actual variants. To resolve this issue, studies need to be performed at the DNA level.
Hebert (1974a,b) pointed out an interesting feature of the temporal dynamics of genotype frequencies in British populations of *D. magna*. Populations inhabiting intermittent ponds exhibited fairly stable genotype frequencies within and between years. The genotype frequencies were generally in good agreement with Hardy-Weinberg expectations, and gametic-phase disequilibria between loci were not observed. In contrast, genotype frequencies in permanent populations exhibited dramatic temporal instabilities, often deviating far from Hardy-Weinberg expectations and showing marked gametic-phase disequilibria. The observed changes did not repeat themselves annually; rather, the dominant multilocus genotypes varied from year to year. These observations were corroborated by Young (1979a,b), and similar results have been noted in permanent-pond populations of *D. pulex* (Lynch 1983; Weider 1985) and permanent-lake populations of *D. pulicaria* (Lynch et al., in prep.).

Variation in the reproductive mode provides a potential explanation for the dramatic differences in population structure in the two types of environments (Hebert 1974a,b). In intermittent environments, populations are transient, and sexual reproduction is enforced on an annual basis. Recombination every few generations produces an enormous number of new clones each year, and a short growing season does not provide sufficient time for selection to advance appreciably a small number of multilocus genotypes. On the other hand, in permanent environments, periods of purely clonal reproduction can extend for a dozen or more generations. If uninterrupted by significant recruitment of new clones from resting eggs, this can provide ample time for a small fraction of the initial pool of clones to come to dominance (Spitze 1991). Once the total number of clones has been reduced to a small number, there will be a high probability of the chance development of gametic-phase disequilibria, and the multilocus genotypes that are advanced will be simply those that are associated fortuitously with the most favorable clones at the time. A repeatable seasonal cycle of multilocus genotypes is not to be expected if a population is experiencing a low level of recruitment from sexually produced eggs, since that would occasionally produce new associations between the electrophoretic markers and the genes underlying the selected characters.

Unfortunately, the general patterns outlined above have not held up to close scrutiny in other taxa. For example, some large-lake populations of *D. cucullata* and *D. galeata* exhibit a high degree of temporal stability in genotype frequencies with few significant deviations from Hardy-Weinberg expectations (Mort and Wolf 1985, 1986). On the other hand, Finnish rock-pool populations of *D. magna* and *D. pulex* are characterized by large temporal fluctuations of genotype frequencies (Korpelainen 1986b,c). Taken at face value, these discrepancies seem to be inconsistent
with Hebert's hypothesis, but they do not necessarily rule it out. Although the rarity of males and ephippial (sexual) females in large-lake populations has led to the general feeling that sexual reproduction is uncommon, it is possible that on a lakewide basis there is enough sexual recruitment to keep the populations in a state similar to that of Hebert's intermittent *D. magna* populations. It is also conceivable that the annual recruitment of sexually produced propagules is quite low in the small rock pools studied by Korpelainen. If that were the case, pronounced gametic-phase disequilibria might arise even in a relatively short growing season.

When isozyme frequencies are available for multiple populations of the same species, it is possible to partition the total gene diversity into within- and between-population components, \( v_w \) and \( v_b \) (Nei 1987). \( v_b \) is equivalent to the expected gene diversity (heterozygosity assuming random mating) between populations that is in excess of that within populations. The index

\[
G_n = \frac{v_b}{v_w + v_b},
\]

which scales from 0 to 1, provides a useful index of population subdivision.

For our surveys of *D. pulex*, *D. pulicaria*, and *D. obtusa*, \( G_n = 0.18 \) (0.07), 0.31 (0.08), and 0.29 (0.03), respectively. These results do not necessarily imply that *D. pulex* has an unusually low degree of population subdivision. The ponds surveyed for this species are no more than 80 km apart, whereas the maximum distances between the *D. pulicaria* and *D. obtusa* populations are 240 and 560 km, respectively. So, in principle, the differences in \( G_n \) are compatible with an isolation-by-distance hypothesis. Indeed, for *D. pulex* we have shown that \( G_n \) increases from approximately 0.05 for populations 1 km apart to 0.3 for populations separated by 1000 km (Crease et al. 1990). Korpelainen (1984) has also observed an isolation-by-distance relationship in *D. magna*. Figure 6.1 summarizes data from surveys of a number of *Daphnia* species. Although there is considerable noise in the relationship, it can be seen that in general there is a slow increase in \( G_n \) with distance.

Provided the allelic variants are effectively neutral, data on spatial and temporal variation of isozyme frequencies can be used to estimate aspects of population structure such as effective population size, migration rates, and so on (Nei 1987; Weir 1990). Although such analyses are now quite common in the literature, they generally have been carried out in the absence of any direct evidence that the influence of selection on the isozyme variants is in fact negligible. Since a large number of *Daphnia* surveys involve long (up to eight-year) temporal sequences of data, it has been possible to employ statistical procedures to evaluate this assumption.
Fig. 6.1. The relationship between the degree of population subdivision and maximum distance among sites involved in a survey for a variety of Daphnia species. In addition to our own results, data are given from Korpelainen (1984, 1986a), Benzie (1986a), Mort and Wolf (1986), and Hebert et al. (1989c).

(Lynch 1987). Analyses from several populations lead to the conclusion that the isozyme variants are quasineutral. In no case does the long-term average selection coefficient on an isozyme variant deviate significantly from zero, but on specific dates the observed changes in allele frequencies often exceed the expectations resulting from sampling error alone. Thus the data suggest that periods of positive selection on the isozyme variants are balanced roughly with periods of negative selection. The variants are neutral with respect to each other in the long term but not in the short term.

It has long been known that fluctuating selection of this sort leads to a driftlike phenomenon (Wright 1948; Kimura 1954), causing gene frequencies to diverge among isolated populations more rapidly than expected under neutrality and also influencing the level of heterozygosity that can be maintained within populations. Therefore, the use of isozyme variants to infer properties of population structure in Daphnia needs to be tempered with a great deal of caution.

The selection analyses in Lynch (1987) provide insight into some previously puzzling observations. For example, the permanent D. magna populations of Hebert (1974a) often exhibited dramatically different gene frequencies even when only meters apart, whereas the more geographi-
cally distant, large-lake populations studied by Mort and Wolf (1985, 1986) were quite homogeneous with respect to gene frequencies. Why do the permanent D. magna populations exhibit so much more differentiation despite being subject to greater gene flow? A possible answer lies in the pattern of selection. The variance in the selection coefficients for the allelic variants in the D. magna populations is relatively high, whereas that for the large-lake populations of Mort and Wolf seems to be essentially zero (Lynch 1987). The observed differences in the geographic structure of gene frequencies in these two groups of populations are qualitatively consistent with the expectations based on differences in fluctuating selection intensity. In no case do we have evidence that selection is operating on the allelic markers themselves. More likely, as noted above, certain aspects of population structure, such as the incidence of sex, influence the associations between the markers and other loci upon which selection is acting. Presumably, the inevitable development of gametic-phase disequilibrium in response to clonal selection causes the variance in selection intensity on isozyme variants to be unusually high in Daphnia relative to sexual species.

Through the use of restriction-site mapping, we have extended our work on population-genetic structure in D. pulex to the mitochondrial DNA (Crease et al. 1990). Within populations, the average nucleotide diversity is approximately 0.002. In other words, random pairs of mitochondria sampled from an individual population differ at about 0.2% of their nucleotide sites. Estimates of this statistic for a diversity of other species range from 0.03% to 0.5% (Lynch and Crease 1990), so D. pulex is not particularly noteworthy in this regard. On the other hand, extension of the nucleotide diversity analysis to the between-population level has revealed this species to be among the most highly subdivided taxa ever observed at the level of mitochondrial DNA.

We measure population subdivision at the level of nucleotides by use of the index \( N_m \), which has a zero-to-one scale as in the case of \( G_a \) (Lynch and Crease 1990). \( N_m \) is on the order of 0.2 for populations less than 1 km apart, and approaches an asymptote of approximately 0.7 for populations 100–1000 km apart. The latter value exceeds the differentiation for the entire New World population of Drosophila melanogaster (Lynch and Crease 1990).

The degree of geographic differentiation at the level of mitochondrial genes in D. pulex is approximately three times that of nuclear genes. Such a pattern is in rough accord with theoretical expectations. Since the effective population size for the mitochondrial genes is only one-quarter of that for genes in the nucleus, they are approximately four times as vulnerable to random genetic drift. Recall, however, that the between-population divergence of isozyme frequencies occurs more rapidly than expected.
on the basis of random genetic drift alone. This will almost certainly be true for the mitochondrial variants as well, since they will be subject to the same random processes that lead to disequilibrium with selected quantitative trait loci.

Recently, we extended this type of analysis to a multigene family—the 18s, 28s ribosomal DNA (Crease and Lynch 1991). Among only ninety clonal isolates of D. pulex, thirty-seven distinct repeat types were identified, and depending on the population, individuals carried a minimum of two to four repeat types. The average nucleotide diversity for random repeats within populations was approximately 0.003, slightly greater than that observed for the mitochondrial genome. The degree of population subdivision was slightly less than the level observed for single-copy nuclear genes (isozymes). These results were somewhat surprising. It has been suggested that homogenizing forces such as gene conversion, unequal crossing-over, and replication slippage will tend to eliminate within-population diversity in multigene families while driving isolated populations to fixation for alternative copy types (Dover 1982; Ohta and Dover 1984). If that were true, then \( N_{eq} \) for multigene families should be high compared to that for single-copy loci.

To evaluate the phylogenetic relationships between our three study species, we have computed Nei’s (1972) generic distances (\( D \)) for all combinations of populations. This statistic estimates the average number of allelic substitutions separating random pairs of genes sampled from two populations or species. For neutral markers, \( D \) is expected to increase linearly with time, provided the mechanisms underlying the molecular evolution remain constant throughout the phylogeny and the taxa under consideration remain isolated reproductively. When populations are connected via migration, \( D \) is expected to approach an equilibrium value defined by the balance between drift, mutation, and migration (Slatkin, chap. 1, this volume).

The fitted tree in figure 6.2 indicates that the distance of D. obtusa to D. pulex–D. pulicaria is approximately five times that between the latter two species. For the most part, the distance among populations of the same species is relatively small compared to that between taxa. However, there are some striking exceptions, leading to the impression that there is nearly a continuous distribution of genetic distances ranging from the within-species to the between-species level. For example, an Indiana population of D. pulex (PA in the figure) is quite distinct from five Illinois populations despite the fact that they are quite close geographically. An Oregon population (AMZ in the figure) that appears to be quite similar to D. pulex morphologically lies well outside of the D. pulex-pulicaria group.

Despite the fact that D. pulex and D. obtusa are separated by approxi-
Fig. 6.2. Phylogenetic relationships between various populations of D. obtusa, D. pulex, and D. pulicaria. The total genetic distance between any two populations is equal to twice the distance to the common ancestor linking them. The phylogenetic tree was estimated by UPGMA. Each branch tip represents a population with the U.S. state abbreviation appearing after the comma (ON denotes Ontario, Canada).

mately 1.0 substitutions per isozyme locus, they can still be induced to hybridize in the laboratory (Agar 1920; Pojman, pers. obs.). However, the offspring of such matings do not appear to be capable of reproduction. On the other hand, D. pulex and D. pulicaria do appear to be cross-fertile. Extensive isozyme and mitochondrial DNA evidence indicates that such hybridizations have given rise to large numbers of obligately parthe-

Other Daphnia species are known to hybridize in nature—D. hyalina, D. galeata, and D. cucullata in Europe (Wolf 1987), D. carinata and D. cephalata in Australia (Benzie 1986b; Hebert 1985), and D. galeata and D. rosea in North America (Taylor and Hebert 1992). In each of these cases, the genetic distance between parental species is on the order of 0.3 to 0.4. Thus it appears that a genetic distance (based on isozymes) of 0.4 or so must be exceeded for reproductive isolation to be firmly established between Daphnia species. Such divergence may require a very long time. Based on empirical data in Nei (1987), time (in years) \( \approx 5 \times 10^6 \times D \), suggesting that reproductive isolation in Daphnia species requires at least a few million years of isolation.

Life-History Evolution

Genetic diversity can be characterized in several ways at the level of quantitative traits. Within populations, the fraction of the total phenotypic variance attributable to genetic differences among clones is known as the broad-sense heritability,

\[
H^2 = \frac{\sigma^2_{\bar{x}}}{\sigma^2_{\bar{x}} + \sigma^2_x},
\]

where \( \sigma^2_{\bar{x}} \) is the total expressed genetic variance (the variance between clones) and \( \sigma^2_x \) is the environmental component of variance for the trait (the variance within clones).

Our general approach to estimating \( H^2 \) in Daphnia has been to isolate random clones from a population and subsequently raise them in the laboratory under defined conditions. Prior to analysis, each clone is maintained as two or more sublines for two to three generations. This treatment insures that, in the final analysis, maternal effects and/or container effects do not contribute to the between-clone component of variance, as this would lead to overestimates of the genetic variation (Lynch and Ennis 1983; Lynch 1985). The experimental assay is performed on one or more replicates from each subline, with the entire collection of individuals being maintained in a randomized design in an environmental chamber. In the studies reported here, individuals were maintained at 20°C on a 12:12 light:dark cycle and fed a saturating level of a green alga. The genetic and environmental components of variance were obtained by equating the mean squares of an analysis of variance to their expectations. Further details of the laboratory and analytical methods can be found in Lynch (1985) and Lynch et al. (1989).
TABLE 6.1  
Genetic diversity statistics for life-history characters

<table>
<thead>
<tr>
<th>Character*a,b</th>
<th>(L_{o2} )</th>
<th>(G_j )</th>
<th>(G_a )</th>
<th>(C_{1.2} )</th>
<th>(C_{3.4} )</th>
<th>(k_1 )</th>
<th>(L_{k-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daphnia pulex:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(H^2 ) PA</td>
<td>0.44**</td>
<td>0.32**</td>
<td>0.36**</td>
<td>0.32**</td>
<td>0.42**</td>
<td>0.04</td>
<td>0.33**</td>
</tr>
<tr>
<td>KA</td>
<td>0.50**</td>
<td>0.52**</td>
<td>0.59**</td>
<td>0.41**</td>
<td>0.54**</td>
<td>0.43**</td>
<td>0.40**</td>
</tr>
<tr>
<td><strong>Daphnia pulicaria:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(H^2 ) HO</td>
<td>0.25</td>
<td>0.01</td>
<td>-0.10</td>
<td>0.08</td>
<td>0.14</td>
<td>0.53**</td>
<td>-0.16</td>
</tr>
<tr>
<td>OD</td>
<td>0.31</td>
<td>0.09</td>
<td>0.55**</td>
<td>0.00</td>
<td>-0.01</td>
<td>0.03</td>
<td>0.18</td>
</tr>
<tr>
<td>KL</td>
<td>0.29</td>
<td>0.44*</td>
<td>0.13</td>
<td>0.69**</td>
<td>0.71**</td>
<td>-0.12</td>
<td>0.30**</td>
</tr>
<tr>
<td><strong>Daphnia &quot;amazon&quot;a,d</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(H^2 ) AMZ</td>
<td>0.32**</td>
<td>0.38**</td>
<td>0.79**</td>
<td>0.22</td>
<td>0.34**</td>
<td>0.38**</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Daphnia obtusa:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(H^2 ) QH</td>
<td>0.48**</td>
<td>0.22*</td>
<td>0.05</td>
<td>0.31**</td>
<td>0.37**</td>
<td>0.30</td>
<td>0.17*</td>
</tr>
<tr>
<td></td>
<td>0.31**</td>
<td>0.17**</td>
<td>0.41**</td>
<td>0.19**</td>
<td>0.18**</td>
<td>0.20**</td>
<td>0.58*</td>
</tr>
</tbody>
</table>

*a Key to characters: \(L_{o2} \) is the mean length (mm) of offspring produced in the second clutch; \(G_j = \ln(L_{o2}/L_{o1})/t \) is the juvenile growth rate, with \(L_{o2} \) and \(L_{o1} \) being the lengths at birth and at the time of carrying the first clutch, and \(t \) the time between these points; \(G_a = \ln(L_{a+1}/L_{a})/t \) is the adult growth rate, with \(L_{a+1} \) being the length in the fourth adult instar; \(C_{1.2} \) refers to the number of offspring released in the first and second clutches (statistics were computed separately for each trait and then pooled); \(C_{3.4} \) is defined similarly for the third and fourth clutches; \(k_1 \) is the age at first release of live progeny; and \(L_{k-1} \) is the length in the instar prior to the first appearance of a clutch.

b Key to significance levels: ** denotes \(P \leq 0.01 \), * denotes \(0.01 < P \leq 0.05 \).

c The different study populations are PA (Portland Arch, Indiana), KA (Kickapond, Illinois), HO (Horsmer Lake, Oregon), OD (Ode Lake, Oregon), KL (Klamath Lake, Oregon), and AMZ (Amazon Pack, Oregon).

d The Amazon population is electrophoretically distinct enough from the other species in this survey that it probably is a unique, but undescribed, species. We therefore refer to it as \(D. "amazon" \) throughout.

The heritability estimates given in table 6.1 for \(D. pulex \), \(D. "amazon" \), and \(D. obtusa \) were obtained from collections of clones taken from temporary ponds early in the spring, shortly after the hatching of resting eggs. (The heritability estimates for \(D. obtusa \) are pooled results from a simultaneous analysis of eight populations.) The heritabilities of most size, growth, and reproductive traits in these populations are highly significant, ranging from 0.2 to 0.8. Qualitatively similar results were obtained earlier, with slightly different methods, for another temporary pond population of \(D. pulex \) (Lynch 1984b).

We have performed another assay of this sort with animals from a \(D. pulex \) population known to be reproducing by obligate parthenogenesis. Although the experiment was comparable in power to those described
above, none of the twenty life-history traits examined exhibited significant heritability (Lynch et al. 1989). Similarly, when assays were performed on collections of clones taken from a cyclically parthenogenetic population toward the end of the growing season, the heritabilities were found consistently to be much lower than those found several weeks earlier in the same pond (Lynch 1984b). Both observations are in agreement with the idea that prolonged clonal selection is very effective at eliminating most of the genetic variation for life-history traits from a population. This hypothesis is supported further by results from laboratory experiments involving electrophoretically marked populations of clones (Spitze 1991).

The D. pulicaria populations that we have examined occupy large permanent lakes in the Oregon Cascades, and it is likely that individuals overwinter in the water column. The analyses were again comparable in size and design to those noted above, yet for two of the populations surveyed (Hosmer and Odell), only a single character exhibits significant heritability (table 6.1). It seems likely that prolonged periods of clonal selection in the absence of significant recruitment from resting eggs may serve to keep quantitative-genetic variation low in these populations, similar to the situation in populations reproducing by obligate parthenogenesis. Both populations exhibited significant deviations from Hardy-Weinberg and gametic-phase equilibria for electrophoretic markers (Lynch et al., in prep.), consistent with the expectation for populations that have not been homogenized recently by sexual reproduction. Interestingly, a third D. pulicaria population, from Klamath Lake, harbored substantial genetic variation for life-history traits (table 6.1). Unlike the Hosmer and Odell populations, this population was in Hardy-Weinberg equilibrium, suggesting that it may have recently experienced a phase of sexual reproduction.

At the species level, the total genetic variation can be partitioned into within- and between-population components. The fraction of the total genetic variation attributable to differences among populations, which we denote as $Q_{tr}$, provides a useful phenotypic analog of $G_{tr}$ and $N_{tr}$. $Q_{tr}$ takes a slightly different form than our measures of gene and nucleotide diversity. For quantitative characters with an additive genetic basis, Wright (1951) showed that the mean additive genetic variance within populations is $\sigma_{tr}^2 = (1 - Q_{tr})\sigma_{a}^2$, where $\sigma_{a}^2$ is the additive genetic variance that would exist if all populations were joined by random mating into one panmictic unit. The expected between-population variance is $\sigma_{tr}^2 = 2Q_{tr}\sigma_{a}^2$. It follows that

$$Q_{tr} = \frac{\sigma_{tr}^2}{2\sigma_{tr}^2 + \sigma_{tr}^2}.$$
For a character with an additive-genetic basis in a diploid species, $Q_{st}$ has exactly the same expected value that $G_{st}$ would have if the latter were computed on the basis of the allele frequencies for the quantitative-trait loci. The two in front of $\sigma^2_{st}$ is due to the fact that $Q_{st}$ is based on a comparison of genotypes, whereas $G_{st}$ and $N_{st}$ are based on comparisons of genes; the between-population variance for a quantitative trait is magnified due to the statistical association of identical genes within individuals in subdivided populations.

Since the *D. obtusa* study was based on eight populations, it was possible to estimate $Q_{st}$ for life-history traits. Population differentiation was significant for all of the characters, even for the ones with negligible variation within populations (table 6.1). Remarkably, the average value of $Q_{st}$, 0.29(0.06), is the same as the value of $G_{st}$ obtained for this species with isozymes. Since, as noted above, the population divergence of isozyme frequencies in *Daphnia* almost certainly is inflated above the neutral expectation by fluctuating selection, these results suggest that similar phenomena may be playing a role in diversifying the mean phenotypes of different populations. This is supported by recent results of a clonal analysis of populations of *D. pulicaria* and *D. galeata* in several Michigan lakes. Using the data of Leibold and Tessier (1991), the estimates of $Q_{st}$ are 0.46(0.05) and 0.61(0.24), respectively. Again, compared to the estimates of $G_{st}$ reported above for broader geographic regions (fig. 6.1), this amount of population subdivision is quite high. Leibold and Tessier (1991) provide compelling evidence that the divergence is due to the local adaptation of populations to selection by vertebrate predators.

We have attempted to put the observed divergence among species in a population-genetic context by computing for each trait the divergence statistic of Lynch (1990),

$$\Delta = \frac{\sigma^2_{st}}{2\sigma^2_{m}}$$

where $T$ is the total number of generations of divergence between a pair of species. $\Delta$ is a useful measure because of its relationship to the neutral expectation. In the absence of natural selection, the variance among the mean phenotypes of isolated taxa is expected to increase at the rate $2\sigma^2_{m}$ per generation, where $\sigma^2_{m}$ is the rate at which new variation enters a population per generation via mutation (Lynch, chap. 5, this volume). Estimates of $\sigma^2_{m}/\sigma^2_{s}$, where as above $\sigma^2_{s}$ is the environmental variance for the trait, range from $10^{-4}$ to $10^{-2}$ for a wide range of characters and species, including *Daphnia* (Lynch 1985, 1988). Thus, observed values of $\Delta$ below $2 \times 10^{-4}$ are consistent with the hypothesis that natural selection
TABLE 6.2
Mean phenotypes for the species, and the divergence statistic for
D. obtusa–D. pulex/pulicaria

<table>
<thead>
<tr>
<th>Charactera</th>
<th>L02</th>
<th>G1</th>
<th>G2</th>
<th>C1</th>
<th>C3</th>
<th>k1</th>
<th>L4–1</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. pulex</td>
<td>0.628</td>
<td>0.242</td>
<td>0.038</td>
<td>14.5</td>
<td>35.6</td>
<td>6.78</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>(0.025)</td>
<td>(0.006)</td>
<td>(0.002)</td>
<td>(2.5)</td>
<td>(1.2)</td>
<td>(0.08)</td>
<td>(0.01)</td>
</tr>
<tr>
<td>D. pulicaria</td>
<td>0.643</td>
<td>0.188</td>
<td>0.039</td>
<td>8.9</td>
<td>19.0</td>
<td>7.39</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>(0.020)</td>
<td>(0.026)</td>
<td>(0.003)</td>
<td>(1.8)</td>
<td>(4.1)</td>
<td>(0.39)</td>
<td>(0.14)</td>
</tr>
<tr>
<td>D. “amazon”</td>
<td>0.549</td>
<td>0.135</td>
<td>0.030</td>
<td>7.7</td>
<td>18.3</td>
<td>10.04</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>(0.007)</td>
<td>(0.004)</td>
<td>(0.002)</td>
<td>(0.5)</td>
<td>(1.3)</td>
<td>(0.26)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>D. obtusa</td>
<td>0.599</td>
<td>0.228</td>
<td>0.047</td>
<td>13.2</td>
<td>31.7</td>
<td>6.64</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>(0.008)</td>
<td>(0.003)</td>
<td>(0.001)</td>
<td>(0.4)</td>
<td>(1.7)</td>
<td>(0.08)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Δ (×10^-8)</td>
<td>3.2</td>
<td>0.00</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

a See table 6.1 for character descriptions.
b All Δ values reported as 0.0 were actually less than zero; the variance between observed means was less than the sampling variance of the mean.

The ability of natural selection to maintain species differentiation has prevented species’ mean phenotypes from diverging as fast as would be likely if random drift and mutation were the sole evolutionary forces.

Since D. pulex and D. pulicaria are not completely reproductively isolated, and the taxonomic status of Daphnia “amazon” is undetermined, we will simply consider the split between D. obtusa and D. pulex/pulicaria (averaging the mean phenotypes of the latter two species). Assuming that the populations average about four generations per year, then the genetic distance estimates given in figure 6.2 imply that the D. obtusa–D. pulex/pulicaria split occurred roughly $T = 2 \times 10^7$ generations in the past. The estimates of Δ given in table 6.2 were obtained by using half the squared difference between means, minus the sampling variance of the means, as an estimate of $\sigma^2_\Delta$. For each character, the environmental variance was taken to be the average value of the within-clone variances taken over populations and species.

Averaging over all seven characters, the mean value of Δ is $5.5(4.4) \times 10^{-9}$, which is roughly five orders of magnitude below the minimum neutral expectation. Such an extremely low rate of evolution strongly suggests that stabilizing selection plays a major role in preventing the diversification of size, growth, and reproductive characters among Daphnia species. Thus, while the high levels of genetic variation observed within and between populations of the same species indicate that these characters can diverge rapidly in response to local selection pressures, the
TABLE 6.3
Genetic correlations with adult size ($L_{t-1}$)

<table>
<thead>
<tr>
<th>Character</th>
<th>$L_{01}$</th>
<th>$G_i$</th>
<th>$G_s$</th>
<th>$C_{1,2}$</th>
<th>$C_{3,4}$</th>
<th>$k_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Daphnia pulicaria</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>0.60*</td>
<td>0.62*</td>
<td>-0.65*</td>
<td>0.33</td>
<td>0.18</td>
<td>-0.90</td>
</tr>
<tr>
<td>KA</td>
<td>0.57*</td>
<td>0.58**</td>
<td>-0.04</td>
<td>0.37*</td>
<td>0.71**</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Daphnia Obtusa</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KL</td>
<td>0.29</td>
<td>0.44*</td>
<td>0.13</td>
<td>0.69**</td>
<td>0.71**</td>
<td>-0.12</td>
</tr>
<tr>
<td>AMZ</td>
<td>0.14</td>
<td>0.26</td>
<td>0.03</td>
<td>0.48**</td>
<td>0.64**</td>
<td>-0.56</td>
</tr>
</tbody>
</table>

*Key to significance levels: ** denotes $P \leq 0.01$, * denotes $0.01 < P \leq 0.05$.  
See table 6.1 for a description of the characters.

amount of additional divergence that occurs after speciation is relatively small.

Body size has long been regarded as a fundamental determinant of the ecological and evolutionary properties of planktonic cladocerans (Brooks and Dodson 1965; Lynch 1980). To evaluate the degree to which natural selection on body size is expected to influence the evolution of other life-history characters, we have computed the genetic correlations between length at maturity ($L_{t-1}$) and the other characters in table 6.1. The genetic covariances between characters are estimated in a manner analogous to the univariate procedure for variance component estimation—by equating the mean cross-products of a multivariate analysis of variance to their expectations. Significant correlations at the genetic level imply that the traits are not free to evolve independently, either because of pleiotropic effects of segregating alleles or because of gametic-phase disequilibrium between variants at different loci.

For most of the populations we have observed, size at maturity is positively genetically correlated with size at birth, juvenile growth rate, and clutch sizes, and negatively genetically correlated with the age at first reproduction (table 6.3). On the other hand, the genetic correlation between adult size and adult growth rate appears to be evolutionarily labile; it is significantly positive in *D. Obtusa* and significantly negative in *D. pulicaria* from Portland Arch. For *D. Obtusa*, it was also possible to compute genetic correlations at the between-population level. These were
all qualitatively consistent with the within-population estimates (table 6.3).

These results bear out the conclusion of a more extensive study of the
genetic covariances of several life-history traits in _D. pulex_ (Spitze et al.
1991)—contrary to the usual expectation (Travis, chap. 9, this volume),
genetic trade-offs between life-history characters appear to be rare in
_Daphnia_.

Discussion

A pattern that emerges from our studies of quantitative variation in
_Daphnia_ is that, for populations that go through an annual bout of sexual
reproduction, pronounced levels of genetic variance for life-history
characters early in the growing season are followed by nearly undetect-
able levels only a few weeks later. Such cycles of genetic variation can be
repeated on an annual basis (Lynch 1984b). These observations are
concordant with the predictions of a quantitative-genetic model for phenot-
typic evolution in cyclical parthenogens under stabilizing selection
(Lynch and Gabriel 1983). Clonal selection effectively eliminates deviant
phenotypes, thereby rapidly reducing the level of expressed genetic var-
ance. But because individual genotypes are discriminated solely on the
basis of their phenotypic properties, irrespective of the underlying geno-
type, hidden genetic variance builds up throughout the period of clonal
selection. For polygenic characters, the same phenotype can be obtained
with many different genetic constitutions. Thus, in principle, it is possible
for a population to exhibit no variation at the phenotypic level despite the
existence of substantial variation at the molecular level. Our empirical
observation of relatively stable genotype frequencies at the isoyme level
(close to Hardy-Weinberg expectations) during a period of rapid loss of
quantitative genetic variation confirms the idea that populations can con-
sist of large numbers of ecologically equivalent clones at least for moder-
ate periods of time (Lynch 1984a,b).

So long as a population is reproducing parthenogenetically, the pool of
hidden genetic variance is evolutionarily inert, despite the fact that it con-
tinues to grow. However, a single bout of sex is sufficient to convert a
large fraction of the hidden genetic variance into the expressed form
(Lynch and Gabriel 1983). Thus, populations that abstain from sex for
very long periods of time are expected to achieve much higher levels of
expressed genetic variance, and hence higher short-term evolutionary poten-
tial, following the event than can ever be obtained in a purely sexual
population.
If the prevailing form of selection is either diversifying or directional with a sufficiently concave fitness function, the results outlined above can be altered. Under these conditions, clonal selection will actually favor the coupling of genes of like effects. When segregation and recombination remove such positive associations, the variance among progeny clones can then be less than that among the parents. Such changes have been documented recently in the D. "amazon" population (Lynch and Deng, in prep.).

Under certain conditions, recruitment of sexually produced progeny can induce a change in the mean of a quantitative trait as well as in the variance, leading to genetic slippage from the end of one year to the beginning of the next. This can happen if a portion of the flush of genetic variance at the outset of each growing season is a consequence of the hatching of resting eggs produced over a period of several years. There is good evidence that the sediments in permanent lakes do contain a cladoceran "seed bank" (Herzig 1983; Carvalho and Wolf 1989). Thus, if ecological conditions are such that different mean phenotypes are favored in different years, while enhancing the amount of variation upon which selection can act, recruitment from multiple cohorts of resting eggs could significantly erode the selective progress made in the preceding year.

A second issue that remains to be resolved at both the empirical and theoretical levels is the extent to which nonadditive gene action contributes to the evolutionary dynamics of phenotypic means and variances. As noted above, clonal selection operates on the total genotypic properties of individuals, whereas selection in a sexual population advances alleles primarily on the basis of their additive effects. If significant nonadditive gene combinations are favored by selection, recombinational breakdown will occur upon sexual reproduction. Thus, depending upon the mode of gene action, long-term clonal selection can facilitate the evolution of coadapted gene complexes, only to be followed by a sort of outbreeding depression upon sexual reproduction. The fact that Daphnia exhibit inbreeding depression (Banta 1939; Innes 1989), which is not possible with purely additive gene action, implies that these issues warrant further exploration. Recently in the D. "amazon" population, we observed genetic slippage in the means for life-history traits averaging about 10% of the phenotypic standard deviations (Lynch and Deng, in prep.). Future work is needed to evaluate the extent to which the magnitude of genetic slippage builds up with the length of the asexual phase and to determine the degree to which genetic slippage erodes the response to directional selection during the clonal phase.

So far, our results indicate that the genetic architectures (heritabilities and genetic correlations) of different populations of the same species are
qualitatively similar (Spitze et al. 1991). This conclusion is upheld for the most part when the genetic correlations of different species are compared, suggesting that the microevolutionary responses of different species to the same selection pressures will be qualitatively similar as well. With the possible exception of adult growth rate, the entire constellation of characters that we have examined appears to evolve in a highly coordinated fashion. It remains to be seen whether these parallel patterns are a consequence of similar pleiotropic constraints throughout the entire assemblage or of the overriding influence of selection favoring specific allometric relationships.

A common observation is that whereas microevolutionary change among different populations of the same species can proceed quite rapidly, there is a progressive slowdown in the rate of phenotypic divergence as an established phylogenetic group ages (Lynch 1990). Our results are certainly consistent with this pattern. Individual populations harbor substantial genetic variation for quantitative traits, and this permits a rather high degree of divergence among isolated populations. However, the evolutionary changes in life-history features among species appear to be within the evolutionary potential of individual species. Reproductive isolation has done essentially nothing to facilitate the divergence of life-history characters among the species we have studied.

Acknowledgments

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References

Benzie, J.A.H. 1986a. The ecological genetics of freshwater zooplankton in Aus-


