Evolution, 53(1), 1999, pp. 100–110

THE QUANTITATIVE AND MOLECULAR GENETIC ARCHITECTURE OF A SUBDIVIDED SPECIES

MICHAEL LYNCH,[1,2] MICHAEL PFRENDER,[1] KEN SPITZE,[3] NILES LEHMAN,[4] JUSTIN HICKS,[1] DEBORAH ALLEN,[1] LEIGH LATTA,[1] MARCOS OTTENE,[3] FARRIS BOGUE,[1] AND JOHN COLBOURNE[5]

[1]*Department of Biology, University of Oregon, Eugene, Oregon 97403*

[2]*E-mail: mlynch@oregon.uoregon.edu*

[3]*Department of Biology, University of Miami, Coral Gables, Florida 33124*

[4]*Department of Biological Sciences, University at Albany, State University of New York, Albany, New York 12222*

[5]*Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada*

Abstract.—In an effort to elucidate the evolutionary mechanisms that determine the genetic architecture of a species, we have analyzed 17 populations of the microcrustacean *Daphnia pulex* for levels of genetic variation at the level of life-history characters and molecular markers in the nuclear and mitochondrial genomes. This species is highly subdivided, with approximately 30% of the variation for nuclear molecular markers and 50% of the variation for mitochondrial markers being distributed among populations. The average level of genetic subdivision for quantitative traits is essentially the same as that for nuclear markers, which superficially suggests that the life-history characters are diverging at the neutral rate. However, the existence of a strong correlation between the levels of population subdivision and broadsense heritabilities of individual traits argues against this interpretation, suggesting instead that the among-population divergence of some quantitative traits (most notably body size) is being driven by local adaptation to different environments. The fact that the mean phenotypes of the individual populations are also strongly correlated with local levels of homozygosity indicates that variation in local inbreeding plays a role in population differentiation. Rather than being a passive consequence of local founder effects, levels of homozygosity may be selected for directly for their effects on the phenotype (adaptive inbreeding depression). There is no relationship between the levels of variation within populations for molecular markers and quantitative characters, and this is explained by the fact that the average standing genetic variation for life-history characters in this species is equivalent to only 33 generations of variation generated by mutation.

Key words.—*Daphnia pulex*, genetic architecture, life-history evolution, microsatellite loci, mitochondrial DNA, quantitative genetics.

Received March 3, 1998. Accepted October 19, 1998.

A comprehensive understanding of phenotypic evolution at the species level requires information on the evolutionary forces operating on populations and on the relative consequences of such forces for phenotypic divergence within and among populations. Numerous studies have surveyed the distribution of molecular genetic variation within and among natural populations of plants and animals (reviewed in Avise 1994), but while a few exceptions exist (e.g., Hollocher et al. 1992; Lai et al. 1994), such assays generally yield very little *direct* insight into the process of phenotypic evolution. Molecular markers do, however, provide *indirect* insight into the evolutionary process. Markers such as allozymes, microsatellites, and RFLPs generally have weak enough associations with the phenotype that, as a first approximation, their evolutionary dynamics are often essentially immune to natural selection on short time scales. This effective neutrality is an asset in that the theory of neutral genes can be used to estimate various aspects of gene flow, population subdivision, and relationships between individuals. The latter information can then provide an empirical basis for constructing null hypotheses to test for the significance of natural selection as a determinant of phenotypic evolution.

Quantitative genetics provides a theoretical framework for quantifying the degree to which phenotypic variation within and among populations is a consequence of genetic versus environmental causes. Substantial work has also been done in this area (reviewed in Roff 1997; Lynch and Walsh 1998). However, sample size requirements for the procurement of accurate estimates of quantitative-genetic variation are much more demanding than for molecular variation. This is because most phenotypic traits are products of genes, environment, and developmental noise, whereas most molecular marker profiles are insensitive to variation in the environment. As a consequence, only a few attempts have been made to comprehensively investigate patterns of molecular and quantitative-genetic variation at both the within- and among-population levels (Prout and Barker 1993; Spitze 1993; Long and Singh 1995; Podolsky and Holtsford 1995; Bonnin et al. 1996; Yang et al. 1996; Kremer et al. 1997; Kuittinen et al. 1997). Thus, we still have only a crude understanding of the forces that determine the range of phenotypic variation at the level of species.

The purpose of this study is to investigate the genetic architecture of a highly subdivided species, the cladoceran *Daphnia pulex*, which is a common member of the zooplankton community of temporary ponds throughout the Holarctic. The results are largely of a descriptive nature, and are meant to provide a basis for future hypothesis tests concerned with the genetic mechanisms that influence evolutionary processes at the species level. Our primary goal here is to demonstrate how a combination of molecular and quantitative-genetic analyses can yield insights into evolutionary processes that neither of these approaches can achieve by themselves.

We have focused on *D. pulex* as a model system for several reasons. First, because individuals can be maintained indefinitely in a clonal state, it is possible to cleanly separate the phenotypic variance into genetic and environmental components, a partitioning that can only be approximated in sex-

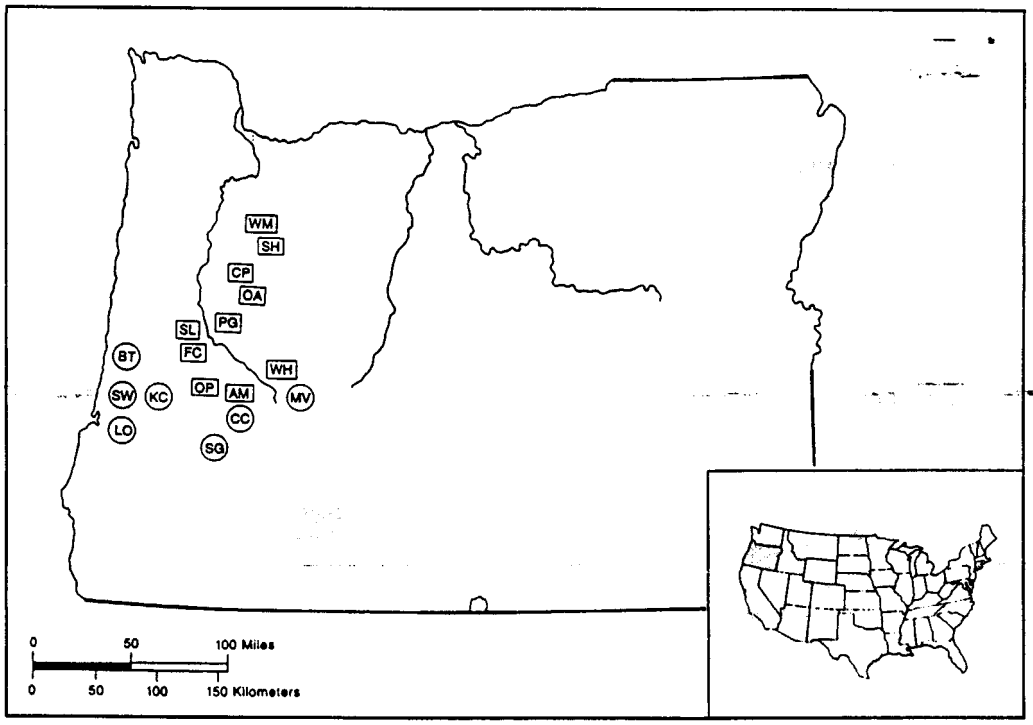

FIG. 1. Map of the sampling sites. The circled sampling sites denote populations contained within the southern clade referred to in the text, while the rectangles denote the northern clade populations.

ually reproducing species. Second, individuals are easily raised in the laboratory, so that representatives of multiple populations can be evaluated in a common environment. Third, generation times are relatively short—on the order of one to two weeks at moderate temperatures. Fourth, an array of molecular-marker techniques are available for assays of population structure. Finally, while the focus of the current study is on western U.S. populations (relatively close to the Pacific coast), a substantial amount of work has been done previously with midwestern (more interior) populations (Lynch 1983, 1984, 1987; Lynch et al. 1989; Crease et al. 1990), so this project may also provide some insight into the genetic properties of peripheral versus central populations.

METHODS

Study Populations

This study involves 17 populations of *D. pulex* derived from temporary ponds in western Oregon (Fig. 1). Samples were obtained from all of the populations in January and February 1996, and 75 clones from each pond were maintained in the laboratory for the next six months as stock for the various analyses reported in this paper. Early sampling was done intentionally to insure that the collected clones were recently hatched from resting eggs, and therefore minimally exposed to selection.

The study populations should comprise a reasonably random sample from the species range in western Oregon. They were obtained over a series of 12 full days of exploration of potential sampling sites in the Willamette Valley, ranging from its origin south of Eugene to north of Salem, in the western foothills of the Cascades, in the coast range, and along the Pacific Coast. No populations were found in the Cascades, and only one (KC, Fig. 1) was located in the coast range. Three populations were found along the coast, and the remaining ones are confined to the Willamette Valley.

To the best of our knowledge, all of the study populations consist entirely or predominantly of cyclical parthenogens, that is, of populations that reproduce by ameiotic parthenogenesis during the growing season and produce resting eggs sexually before the ponds dry in the spring/early summer. This conclusion is based on molecular analyses that indicate segregation of marker alleles and genotype frequencies close to their Hardy-Weinberg expectations (below).

Three populations which subsequently turned out not to be cyclically parthenogenetic *D. pulex* were inadvertently included in our study. One of these appeared to consist largely of two obligately parthenogenetic lineages of *D. pulex*, whereas two others were determined by molecular analyses to be the close relative *D. pulicaria*. We do not report the results on these three populations here, although the molecular data on *D. pulicaria* were used to root the trees of relationship that we report below.

Quantitative-Genetic Analysis

Life-history trait data were obtained with a standard experimental design (Lynch 1985; Lynch et al. 1989). The assays were carried out in two overlapping blocks, with each block containing approximately 25 random clones from each of the populations, and all clones represented by two replicates. In total, approximately 2000 individuals were assayed.

Each line was started with a single immature female taken from its clonal culture, and all replicates were subsequently maintained as single asexually produced progeny under the assay conditions for two generations prior to the actual assay generation. This protocol insures that any maternal (and grandmaternal) effects contribute to the within- rather than the among-clone component of variance (Lynch 1985). In the third generation, individuals were examined daily under a Wild dissecting microscope to obtain estimates of the life-history traits: size at birth, instar-specific body sizes, instar-specific ages, and clutch sizes. All individuals were followed from birth to the release of the fourth clutch.

During the assays, each individual was maintained in 100 ml of aged, filtered water taken from the Amazon Pond (AM) locality, supplemented with a pure laboratory culture of the green alga *Scenedesmus* to maintain a density of approximately 150,000 cells/ml. New water was supplied every other day. The experiment was conducted in a large environmental chamber maintained at 15°C with a 12L:12D photoperiod. All clonal lines were randomized with respect to placement in the environmental chamber and rotated daily to reduce any effects of microenvironmental variation within the chamber.

The developmental stages of asexually produced eggs in *Daphnia* can be discriminated by morphological criteria, providing a basis for attaining relatively precise estimates of times of birth and ages at reproduction. We used a frequency distribution of the observed developmental stages over the entire experiment to estimate the relative duration of each stage. Because mothers extrude a new clutch of eggs into their brood chambers almost immediately upon release of a previous clutch, these stages can be employed to greatly refine the estimates of adult instar durations. We also used these modified age estimates to calculate growth rate parameters, computed as the rate of change in length from the base of the tail spine to the top of the head (natural log-transformed) (Lynch et al. 1989).

In the final analysis of data, one-way analysis of variance was used to partition the phenotypic variance within each population into within- and among-clone components of variance, respectively measures of the genetic and environmental sources of variation. The degree of genetic population subdivision for each trait, Q_{ST}, was estimated as described in Spitze (1993) and Lynch and Spitze (1994) after extracting estimates of the among-population genetic variance by nested analysis of variance. In all cases, standard errors of the genetic parameter estimates were obtained by use of expressions derived by the delta method (Lynch and Walsh 1998).

Molecular-Marker Analyses

Information on three types of molecular markers was obtained from all populations: allozymes, microsatellite loci, and mitochondrial RFLP haplotypes. Allozyme analysis was generally performed on fresh tissue, but frozen individuals were used occasionally. Thirteen allozyme loci were scored for all populations by use of cellulose-acetate electrophoresis (Hebert and Beaton 1989). Alleles were characterized by relative mobility, calibrated against a standard *Daphnia* genotype.

For purposes of microsatellite and mitochondrial analyses, DNA was extracted from single *Daphnia* by the Chelex method (Walsh et al. 1991; Lehman et al. 1995), often using animals that had been preserved in 100% ethanol prior to analysis. For the microsatellite analysis, we relied on six dinucleotide- and trinucleotide-repeat loci isolated and characterized from midwestern *D. pulex* (Colbourne, unpubl. ms.) Using primers designed from the flanking regions of these loci, PCR reactions were carried out in 12.5 μl volumes using 0.035 units of Taq polymerase, 1.3 μl reaction buffer (10 mM Tris-HCl (8.3 pH), 1.5 mM $MgCl_2$, 50 mM KCl), 0.8 μl of 8 mM dNTPs, 0.64 μl of 10 μM forward and reverse primers, 5 μl of DNA extract and water. A fraction of the forward primer in each reaction (0.04 μM of the final volume) was radio-labeled at the 5′-end with γ–^{32}P ATP using T4 polynucleotide kinase. PCR was carried out with a 4 min denaturation at 95°C, followed by 20 cycles for 1 min at 94°C, 1 min at 53°C, and 1 min at 72° C, finally followed by a 30-min extension step at 72°C. Labeled PCR products were denatured at 95°C for 5 minutes, cooled on ice, and then run through 6% polyacrylamide gels with a known sequence as a size standard. The microsatellite alleles were then visualized by autoradiography.

Gene diversity estimates were obtained for each nuclear gene locus within each population as the expected fraction of heterozygotes under random mating. Pairwise estimates of between-population gene diversity were then computed by first estimating the raw gene diversity between populations as the expected fraction of allele pairs (one gene from each population) that are heterozygous, and then subtracting the average within-population gene diversity for the two populations. This yields an estimate of between-population gene diversity that is in excess of that expected on the basis of within-population variation. For some purposes in this paper, we have pooled the average estimates of gene diversity for both the allozyme and microsatellite analyses by weighting each by the inverse of its sampling variance.

To estimate the degree of population subdivision for nuclear gene markers (G_{ST}) we estimated the total gene diversity for each locus from the average population-specific allele frequency estimates. The among-population gene diversity was then obtained by subtracting the average diversity within populations. The final estimates of G_{ST} (and their standard errors) were obtained by averaging over each of the locus-specific estimates. In a similar manner, we obtained estimates of the average degree of inbreeding within local populations (G_{IS} by computing for each locus the fraction by which the observed homozygosity exceeds the Hardy-Weinberg expectation.

RFLP analysis was performed on the control region of the mitochondrial genome. After amplifying the entire region by use of primers described previously by Lehman et al. (1995), the PCR products were digested with a panel of seven restriction endonucleases (*Bam* HI, *Dra* I, *Hha* I, *Mse*I, *Pal*I, *Rsa* I, *Sau* 96I), and the resulting fragments were separated on 2% or 3% agarose gels and visualized by ethidium bromide staining and uv transillumination. Eight to 15 individuals were examined per population.

For each of the unique haplotypes revealed by RFLP analysis, a complete nucleotide sequence was obtained by direct sequencing of the PCR product from at least one individual.

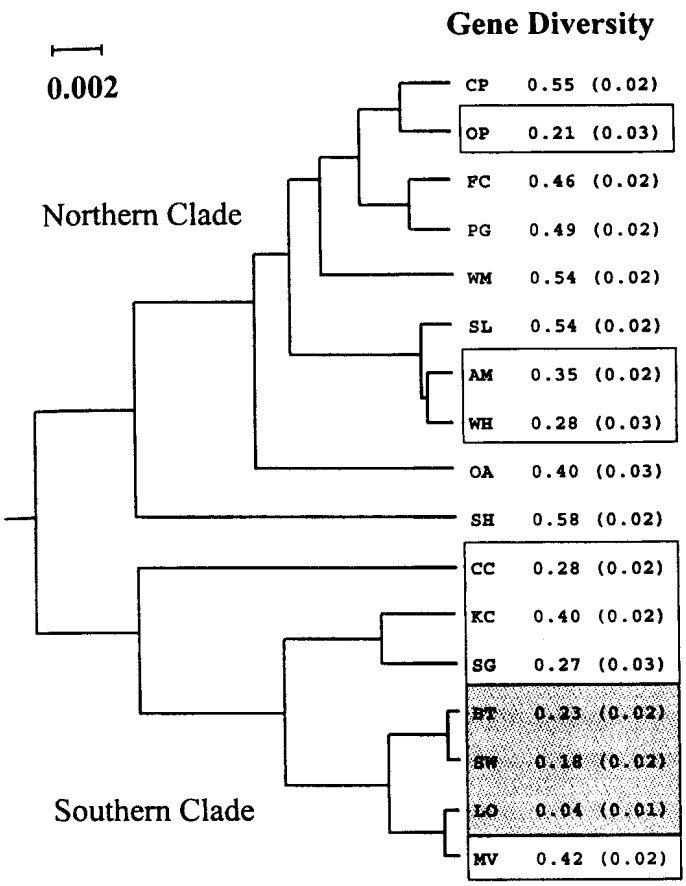

FIG. 2. UPGMA tree for the 17 populations of Oregon *Daphnia pulex*, based on pooled estimates of the among-population nuclear gene diversities obtained from the surveys of allozyme and microsatellite loci. Populations contained within boxes are from the southern valley and the single coastal range population (KC), whereas the shaded boxes refer to coastal populations. Estimates of the within-population gene diversities (and their standard errors) are given on the right. The tree was rooted by including two populations of the closely related species *D. pulicaria* in the analysis (data not shown).

The sequences enabled the construction of restriction maps with high certainty, and the maps were subsequently used to produce a presence-absence matrix of restriction sites for individuals genotyped by the RFLP technique. Entry of this matrix and the haplotype incidences for each population into the program HAPLO2 of Lynch and Crease (1990) yielded estimates of the average number of nucleotide substitutions per site between pairs of individuals within and among populations and of the fraction of total nucleotide diversity that is due to population differentiation (N_{ST}).

Trees of relationship were constructed by applying the UPGMA method, as implemented by MEGA (Kumar et al. 1993) to the between-population gene diversity estimates for both the mitochondrial data and the pooled nuclear-gene data. To evaluate the degree of confidence that can be attached to the branching patterns of such trees, the standard errors of the branch lengths were obtained by use of the least-squares technique of Rheztsky and Nei (1992).

TABLE 1. Estimates of the index of homozygosity in excess of Hardy-Weinberg expectations (with standard errors in parentheses).

Population	G_{is}
Northern Clade	
AM	0.084 (0.034)
CP	0.143 (0.017)
FC	0.280 (0.042)
OA	0.311 (0.051)
OP	0.124 (0.037)
PG	0.074 (0.027)
SH	0.079 (0.031)
SL	0.291 (0.028)
WH	0.014 (0.024)
WM	0.121 (0.029)
Southern Clade	
BT	0.005 (0.034)
CC	0.044 (0.033)
KC	0.085 (0.060)
LO	−0.016 (0.029)
MV	0.217 (0.060)
SG	0.054 (0.033)
SW	0.233 (0.129)

RESULTS

Nuclear Genome Diversity.—There is an approximately ten-fold range in levels of pooled within-population gene diversity among the 17 populations, with a low of 0.04 (0.01) for coastal population LO to a high of 0.58 (0.02) for valley population SH (Fig. 2). Because the standard errors of these estimates are all on the order of 0.01 to 0.03, these differences are highly significant. The degree of population subdivision is essentially the same for both types of nuclear markers: G_{ST} is equal to 0.307 (0.022) for allozyme loci and to 0.293 (0.016) for microsatellite loci.

Considerable geographic patterning exists for the distribution of molecular variation in the nuclear genome, both within and among populations. The phylogenetic tree based on the pooled between-population gene diversity estimates subdivides the collection of populations into two clades—one containing all of the coastal populations (LO, SW, and BT), the coastal range population (KC) and three of the most southern valley populations (CC, SG, and MV), and the other consisting entirely of valley populations (Fig. 2). Hereafter, we will refer to these two groups of populations as the southern and northern clades, respectively. The patterns outlined in Figure 2 appear to be quite robust. All of the internal branch length estimates for this tree exceed two standard errors, except for the very short branch below the AM–WH grouping. Moreover, essentially the same tree is obtained with analyses restricted to allozymes or to microsatellite loci. Most notable is the difference in average within-population gene diversity between the two major clades, 0.26 (0.05) in the southern clade and 0.44 (0.04) in the northern clade. The three coastal populations have the lowest diversity, averaging only 0.15 (0.06).

Analyses with the microsatellite loci revealed a general excess of homozygotes within individual populations (Table 1). For 16 of the 17 populations, G_{IS} is positive, the overall average being 0.126 (0.025). Here too, there is a substantial difference between the two major clades, the average value

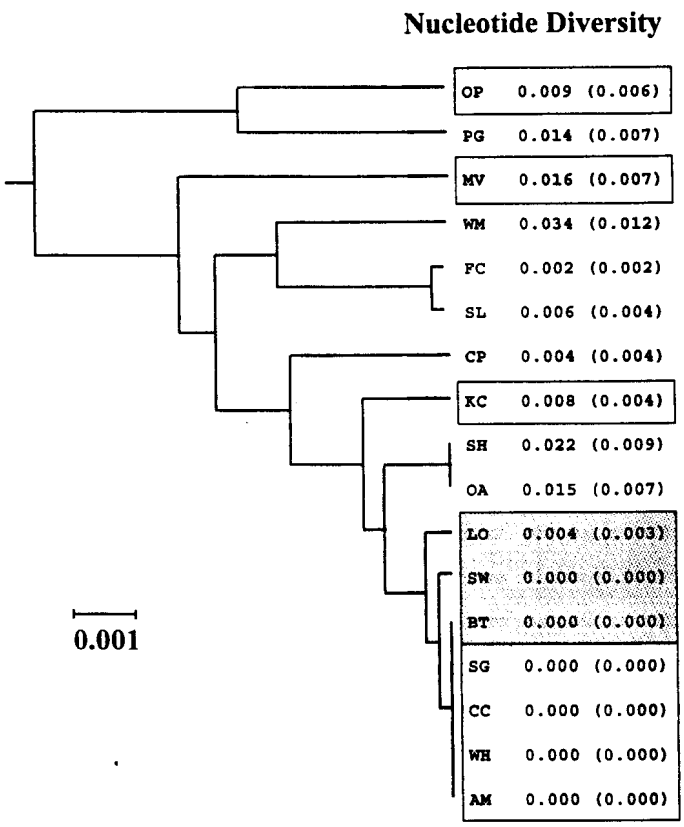

FIG. 3. UPGMA tree for the 17 populations of Oregon *Daphnia pulex*, based on the among-population estimates of nucleotide diversity obtained from the survey of the mitochondrial genome. Details are as in Figure 2.

of G_{IS} being 0.089 (0.037) for the southern clade populations and 0.152 (0.033) for the northern clade populations.

Mitochondrial Variation.—Not as much confidence can be attached to the details of the phylogeny based on mitochondrial DNA analyses (Fig. 3), as most of the pairwise estimates of between-population nucleotide diversity had values that were less than two standard errors. Nevertheless, there are some striking resemblances with the results obtained with nuclear genes. All of the coastal populations and four of the southern valley populations are contained in a single clade, the depth of which is extremely shallow (0.08% nucleotide divergence) as a consequence of being nearly fixed for a single mitochondrial haplotype. The remaining populations are much more diverse, with the maximum nucleotide diversity among pairs of populations being approximately 1.5%, and the average within-population nucleotide diversity being 1.3%. The fraction of nucleotide diversity that is distributed among populations is N_{ST} = 0.516 (0.135).

Quantitative-Genetic Variation.—The average broadsense heritabilities for life-history traits in west coast *D. pulex* are relatively low, all but one of them being less than 0.30 (Table 2). A substantial fraction of the genetic variation for these traits is distributed among populations, with 12 of the 18 traits having estimates of Q_{ST} in excess of 0.30 and an average value of 0.35 (0.07) over all traits (Table 2). There is a fairly strong positive correlation between Q_{ST} and heritabilities (H^2) for the various traits (Fig. 4).

Molecular Variation and Phenotypic Variation.—There is essentially no relationship between molecular measures of within-population variation for nuclear genes and the standing levels of variation for quantitative characters. When population estimates of heritability for the various life-history characters were regressed on the measures of within-popu-

TABLE 2. Estimates of phenotypic means, genetic standard deviations (square roots of the total genetic variance), broad-sense heritabilities, and quantitative-genetic subdivision for life-history traits. The units for body sizes are mm, for ages at reproduction are days, and for exponential growth rates are days^{-1}. Standard errors are given in parentheses.

	Mean	Genetic SD	Heritability	Q_{st}
Body sizes:				
Birth	0.623 (0.004)	0.0114 (0.0029)	0.099 (0.054)	0.671 (0.179)
First adult instar	1.794 (0.016)	0.0591 (0.0076)	0.167 (0.040)	0.539 (0.094)
Second adult instar	2.046 (0.020)	0.0466 (0.0148)	0.108 (0.045)	0.685 (0.117)
Third adult instar	2.232 (0.024)	0.0876 (0.0133)	0.202 (0.047)	0.599 (0.089)
Fourth adult instar	2.388 (0.027)	0.1090 (0.0115)	0.272 (0.052)	0.568 (0.087)
Clutch sizes:				
First	8.105 (0.215)	1.2610 (0.2096)	0.120 (0.044)	0.313 (0.125)
Second	16.400 (0.363)	3.2943 (0.3878)	0.209 (0.045)	0.170 (0.073)
Third	20.776 (0.713)	2.7981 (1.3254)	0.107 (0.053)	0.571 (0.477)
Fourth	22.591 (0.973)	6.2900 (0.9481)	0.188 (0.059)	0.372 (0.125)
Ages at reproduction:				
Maturity	8.684 (0.095)	2.2240 (1.0801)	0.381 (0.031)	0.542 (0.884)
Release of first clutch	12.976 (0.093)	0.4667 (0.3415)	0.003 (0.046)	0.095 (0.348)
Release of second clutch	17.346 (0.122)	0.6162 (0.2206)	−0.105 (0.053)	−0.082 (0.093)
Release of third clutch	22.001 (0.146)	0.5894 (0.4168)	−0.065 (0.058)	0.034 (0.138)
Growth rates:				
Juvenile	0.128 (0.002)	0.0064 (0.0058)	0.052 (0.055)	0.235 (0.135)
First adult instar	0.033 (0.001)	0.0047 (0.0038)	0.058 (0.047)	0.322 (0.211)
Second adult instar	0.021 (0.001)	0.0010 (0.0090)	−0.113 (0.057)	−0.293 (0.274)
Third adult instar	0.014 (0.001)	0.0036 (0.0014)	0.075 (0.062)	0.411 (0.292)
Fourth adult instar	0.016 (0.001)	0.0023 (0.0007)	0.112 (0.059)	0.598 (0.284)

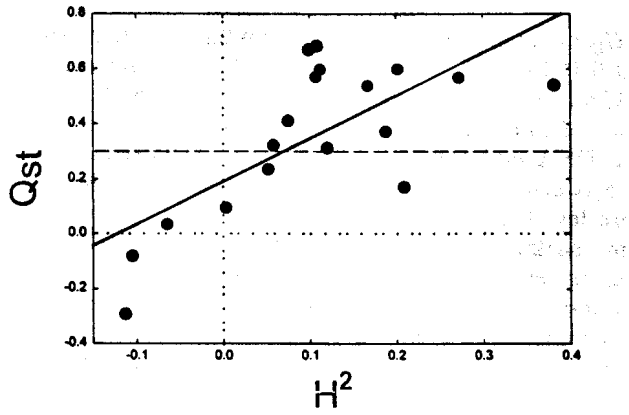

FIG. 4. Relationship between the degree of quantitative-genetic subdivision among populations (Q_{ST}) and the average heritability within populations (H^2) for the 18 characters listed in Table 1. The regression is highly significant ($r = 0.709$, df = 16, $P < 0.01$). The dashed horizontal line denotes the level of subdivision for nuclear molecular markers.

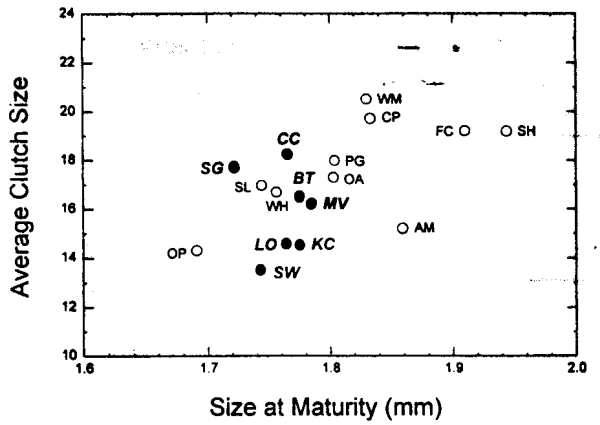

FIG. 5. Mean values for clutch size (averaged over the first four clutches) and size at maturity for the 17 populations. The southern clade populations are denoted with the large filled in circles and bold italics; the northern populations with open circles and nonitalicized labels.

lation gene diversity given in Figure 2, all of the regressions were nonsignificant. Indeed, the average slope of these regressions is −0.03 (0.28), and the average fraction of the variation explained by them is only 0.03. The three coastal populations, which as noted above have exceptionally low levels of gene diversity, have an average heritability over all traits that is essentially identical to the grand mean for all populations (0.11).

In contrast to the situation for levels of quantitative-genetic variation, there is an association between average within-population heterozygosity and population mean phenotypes (Table 3). On average, individuals from populations with high levels of heterozygosity at the molecular level grow faster, have larger sizes at birth and maturity, have larger clutch sizes, and attain maturity at an earlier age. As an example of this gradient in mean phenotype and heterozygosity, the bivariate relationship between average clutch size and size at maturity is shown in Figure 5. Notice that the southern clade populations, which on average have low levels of heterozygosity, tend to be below the grand mean in both characters. The population with the smallest size at maturity and smallest clutch sizes (OP) is a member of the northern clade, but it has the lowest level of heterozygosity of any population in that clade (Fig. 2).

All of the changes in the mean phenotype with increasing homozygosity are in the same direction as those observed when populations of *Daphnia* are directly inbred by self-fertilization (Lynch and Deng 1994; Deng and Lynch 1997), suggesting the possibility that the differences in mean phenotypes among populations may represent a response to local inbreeding depression. To evaluate this possibility further, we have computed from the regressions the average changes in mean phenotypes that are expected when a population is reduced from the average level of nuclear gene heterozygosity (0.40) to complete homozygosity. In Table 3, these empirical observations are compared with the expected re-

TABLE 3. Regressions of population mean phenotypes for life-history traits on average heterozygosity; estimates of the average response to complete inbreeding for two populations (as described in the text); and the range of population mean phenotypes. The various characters are denoted as: B_0, size at birth; B_{mat}, size at maturity; G_j, juvenile growth rate; G_a, adult growth rate; C, clutch size; and k, age at first reproduction. The units for body sizes are mm, for ages at reproduction are days, and for growth rates are days^{-1}. Standard errors are given in parentheses. For the population mean phenotypes, the standard errors (not shown) are typically on the order of 1% of the mean.

	B_0	B_{mat}	G_j	G_a	C	k
Regression coefficient:	0.040	0.248	0.022	0.008	8.300	−0.955
	(0.021)	(0.076)	(0.012)	(0.004)	(2.313)	(0.546)
r	0.45	0.64	0.42	0.48	0.68	0.41
Significance	NS	0.010	NS	0.050	0.010	NS
Projected changes under complete inbreeding:						
Empirical response to homozygosity	0.017	0.100	0.009	0.003	3.337	−0.387
	(0.009)	(0.031)	(0.004)	(0.001)	(0.938)	(0.221)
Observed response to inbreeding:	0.035	0.112	0.028	0.003	8.081	−4.138
	(0.023)	(0.046)	(0.009)	(0.002)	(1.664)	(0.517)
Range of population mean phenotypes:						
Minimum	0.600	1.691	0.116	0.015	13.508	7.936
Maximum	0.658	1.991	0.148	0.025	20.506	9.388

sponses to complete inbreeding that are obtained from laboratory experiments on inbreeding depression, that is, to twice the response to self-fertilization observed in the experiments of Lynch and Deng (1994) and Deng and Lynch (1997), because selfing reduces heterozygosity by 50%. One of the two inbreeding depression experiments was performed on *D. pulex* population AM, which has an average heterozygosity (0.39) that is essentially the same as the average observed in the current study (0.40), whereas the other experiment involved the closely related species *D. pulicaria*. All of the estimates obtained from the regressions on heterozygosity are of similar magnitude or less extreme than those obtained from direct observations of the response to inbreeding, and in only two of the cases (clutch size and age at first reproduction) can the differences be considered significant.

Discussion

All three types of molecular markers employed in this study support the idea that *Daphnia pulex* has a highly subdivided population structure within Oregon. Both the allozyme and microsatellite analyses indicate that about 30% of the total diversity for nuclear markers is distributed among populations, whereas the mitochondrial analysis suggests that the total nucleotide diversity is equally distributed within and among populations. Our estimates of the degree of subdivision for nuclear genes are very similar to those obtained previously for populations of *D. pulex* in the midwestern U.S. (0.32; Crease et al. 1990), for populations of *D. pulicaria* in western Oregon (0.31; Lynch and Spitze 1994), and for populations of *D. obtusa* in the midwestern U.S. (0.29; Spitze 1993).

An elevated level of subdivision for the mitochondrial genome has been observed previously for midwestern *D. pulex*, where $N_{ST} = 0.66$ for the mitochondrial genome and $G_{ST} = 0.32$ for allozymes (Crease et al. 1990). This greater magnitude of population differentiation at the level of the mitochondrial genome may be largely a consequence of the approximately 50% reduction in effective population size experienced by organelle genes in *Daphnia*. As in most animals, the mitochondria of *Daphnia* seem to be maternally inherited. Sex determination in *Daphnia* is environmental, such that each clone surviving to the phase of sexual reproduction is potentially represented by males and females. Thus, for every mitochondrial genome in a mating pool, there are an average of two haploid nuclear genomes.

If, as a crude approximation, we assume that *Daphnia* population structure is analogous to the island model (Wright 1951) and that the molecular markers behave in an effectively neutral manner, then the expected level of subdivision for nuclear genes is $G_{ST} = 1/(1 + 4Nm)$, where N is the effective population size, and m is the migration rate. Setting G_{ST} equal to the observed value, 0.3, leads to an estimate of $Nm = 0.58$. For *Daphnia*, the migration rate should be identical for both nuclear and mitochondrial genomes, because the propagule is always a resting egg, which develops into a clone capable of transmitting both types of genes. If we then estimate the level of subdivision that would result from a 50% reduction in N, that is, with $Nm = 0.29$, we obtain $F_{ST} = 0.46$, which is not significantly different from the observed value of subdivision for the mitochondrial genome ($N_{ST} = 0.52$).

Thus, all three types of molecular analyses are consistent in suggesting that the average number of effective migrants among our study populations is on the order of one every two years (a year being the duration of the life cycle for *Daphnia* inhabiting temporary ponds). However, two cautionary notes are in order here. First, the island model approach to estimating Nm assumes a network of populations that are in drift-migration equilibrium. Given the observed geographic structuring of the within-population gene diversity, most notably the reduction in molecular variation in the southern clade, it seems unlikely that this can be strictly true. This may not greatly bias the results obtained from the island model, because the ratio G_{ST} appears to often attain an equilibrium much more rapidly than does either of its components (the absolute within- and among-population gene diversities) (Crow and Aoki 1984; Slatkin 1993). Second, in previous work, we have found that allozymes are quasineutral in *Daphnia*, that is, the alleles are neutral on average, but with significant positive or negative selection from time to time (Lynch 1987). Such selection most likely results from random associations that develop as a consequence of linkage disequilibrium in clonal populations, rather than from direct selection on the markers themselves. In any event, because fluctuating selection causes the among-population dispersion of allele frequencies to exceed the expectation for a neutral marker, its occurrence will tend to lead to downwardly biased estimates of the migration rate. The strong geographic patterning for molecular variation in our study populations argues against the idea that fluctuating selection is a dominant determinant of allele-frequency distributions in the western Oregon populations, as this would be expected to randomize the patterns of variation within and among populations. If the actual migration rates were in excess of one individual per generation, it is unlikely that there would be much geographic patterning to the relationships among populations.

Our conclusion that the rate of gene flow among populations is quite low on average, with particularly low gene transmission between the coast and the Willamette valley, is supported further by the mitochondrial haplotype distribution. Of the 17 haplotypes identified in this study, all but four are confined to single populations. Such restricted mitochondrial haplotype distributions have been reported previously for *D. pulex* in the midwest (Crease et al. 1990). On the other hand, the single mitochondrial haplotype that comprises all of the coast populations (Fig. 3) is also scattered throughout the range of populations studied, including the northern-most populations. This observation suggests that the southern clade has experienced a colonizing bottleneck involving this single haplotype recently enough that little mitochondrial diversity has been generated in situ by mutation. (A rare derivative haplotype, unique to the coast, was detected in population LO). On the other hand, we cannot rule out the possibility that the entire western Oregon metapopulation of *D. pulex* was historically comprised largely of the dominant coastal haplotype, with the northern clade recently experiencing an influx of haplotypes from other areas. If one considers the six allozyme loci that were examined in the

current study and in the earlier study by Crease et al. (1990), the mean gene diversity within midwest populations (0.17 ± 0.04) is intermediate to that in the southern (0.11 ± 0.04) and northern clade (0.22 ± 0.08) populations, so this latter hypothesis is not unreasonable. In either case, the southern clade clearly exhibits the molecular signature of being on the edge of the range of *D. pulex*

Our analyses using microsatellite loci revealed that within-population homozygosity in excess of Hardy-Weinberg expectations is a common feature of the study populations. All but one of the populations have positive values of G_{IS}, and in ten cases, these are significant. Although temporary pond populations of *Daphnia* often have genotypic frequencies at allozyme loci that are in close agreement with Hardy-Weinberg expectations (Hebert 1974; Lynch et al. 1989; Innes 1991), we have previously recorded significant homozygote excesses in a midwestern *D. pulex* population (Lynch 1984). The mechanism responsible for this pattern remains unclear, although there are several possibilities. First, the presence of null alleles can lead to the false impression of excess homozygosity. However, none of our assays with microsatellites failed to yield an amplifiable product, so if null alleles are present at these loci, they must be quite rare and probably do not present a significant source of bias. Second, excess homozygosity could result if some of our clones underwent a self-fertilization event in the lab prior to the molecular analyses. However, because we have almost never been able to hatch *D. pulex* resting eggs without taking them through a cold/dark treatment, this seems very unlikely, and it cannot explain the results of other studies that are based on direct analysis of field collected samples. The most likely explanation for the homozygote excesses is the presence of some type of population subdivision (a Wahlund effect). This could result from the hatching of multiple cohorts of resting eggs within a given year or from assortative mating due to the sexual maturation of different clones at different times during the sexual phase. It is less clear why such effects would be twice as pronounced in the northern than in the southern clade, and why they are apparently absent in the coastal populations.

Averaging over all characters and populations in this study, the observed broadsense heritability for life-history characters, 0.11, is quite low. For temporary pond populations of midwestern *D. pulex* and *D. obtusa*, the average estimates for the same sets of traits are 0.40 (0.04) and 0.27 (0.05), respectively (Lynch et al. 1989; Spitze 1993), whereas the average for permanent lake populations of Oregon *D. pulicaria* is 0.20 (0.06) (Lynch and Spitze 1994). Given the relative levels of molecular variation observed in these different studies, it seems rather unlikely that the low levels of genetic variation in the western Oregon *D. pulex* populations is a consequence of an extraordinary degree of population bottlenecking. Moreover, as noted above, there is essentially no correlation between the levels of molecular heterozygosity and broadsense heritabilities within populations, that is, populations with low levels of molecular diversity, such as those along the coast, do not have unusually low levels of genetic variation for quantitative traits.

This lack of correspondence between measures of genetic diversity at the levels of quantitative traits and molecular markers is expected on theoretical grounds (Lande and Barrowclough 1987; Lynch 1996). Because the rate at which new molecular variants are generated by mutation is on the order of 10^{-6} to 10^{-3} per generation, while the rate of replenishment of heritability by mutation is on the order of 10^{-3} to 10^{-2}, the genetic variation for quantitative traits is expected to recover from a population bottleneck (or a strong purifying-selection event) much more rapidly than molecular heterozygosity. We have recently estimated the rate at which spontaneous mutation generates new variation for several life-history traits in one of our field populations (AM) (Lynch et al. 1998). Comparing these results with the genetic variance for quantitative traits within the populations in this study, the average standing level of genetic variance for size at birth is found to be equivalent to seven generations of mutational variance, whereas that for size at maturity is equivalent to 22, for age at maturity to 90, and for the first four clutch sizes to 7, 18, 38, and 52 generations of mutational production, respectively. Thus, a bottleneck in genetic variance would have to be very recent, extreme, and geographically widespread to account for the low levels of genetic variation for life-history traits in the Oregon *D. pulex* populations.

A remarkable outcome of our quantitative-genetic analysis is the finding that the average level of genetic subdivision for quantitative traits (Q_{ST}) is essentially identical to that for molecular markers for the nuclear genome (G_{ST}). This result may have arisen by chance, but virtually the same conclusion was reached in a study of another temporary pond species, *Daphnia obtusa* (Spitze 1993). Thus, if the allele-frequency distributions for the nuclear gene markers in our study populations have diverged largely as a consequence of random genetic drift, then on this basis, we cannot formally reject the hypothesis that the level of population divergence for quantitative traits is also a simple consequence of drift. It should be noted, however, that failure to reject the neutral hypothesis is not equivalent to accepting an absence of selection, as there are innumerable ways in which selection can lead to any particular value of Q_{ST}. Moreover, if the level of G_{ST} for marker loci is significantly inflated by fluctuating selection in *Daphnia*, then a comparison of Q_{ST} and G_{ST} is not a strict test of neutrality.

Over the past few years, several other studies have reported joint estimates of Q_{ST} for life-history and growth-related traits and G_{ST} for nuclear marker loci, and these reveal a striking pattern (Fig. 6). There is a strong correlation between the two measures ($r = 0.880$, $df = 8$, $P < 0.01$), and in every case the level of subdivision for quantitative characters is greater than or equal to that for molecular markers, with the magnitude of inflation increasing with decreasing level of molecular subdivision. Given the diversity of organisms (annual and perennial plants, and several invertebrates) underlying this relationship, these results suggest the generalization that measures of subdivision at the level of nuclear markers provide conservative estimates of the degree of genetic subdivision for quantitative characters. Such asymmetry is expected if molecular markers tend to be selectively neutral such that their evolutionary dynamics are governed primarily by the forces of gene flow, random genetic drift, and mutation, and if the divergence of quantitative traits is exacerbated

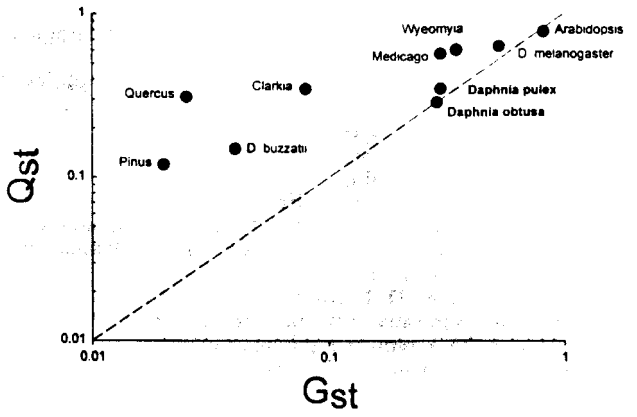

FIG. 6. Estimates of genetic subdivision at the level of quantitative characters (Q_{ST}) versus that at the level of isozymes (G_{ST}). Most of the quantitative traits are size and reproductive characteristics. References: *Arabidopsis thaliana*, Kuittinen et al. (1997); *Daphnia obtusa*, Spitze (1993); *Daphnia pulex*, this study; *Drosophila buzzatii*, Prout and Barker (1993); *Drosophila melanogaster*, Long and Singh (1995); *Clarkia dudleyana* (a flowering annual plant), Podolsky and Holtsford (1995); *Medicago truncatula* (alfalfa), Bonnin et al. (1996); *Pinus contorta* (pine), Yang et al. (1996); *Quercus petraea* (sessile oak), Kremer et al. (1997); *Wyeomyia smithii* (pitcher-plant mosquito), P. Armbruster and J. Hard (pers. comm.). The dashed line denotes the points at which $Q_{ST} = G_{ST}$.

by local adaptation to diverse ecological challenges. Because quantitative traits are subject to the same nonselective forces as molecular markers, then one would expect Q_{ST} to be less than G_{ST} if selection promotes similar phenotypes in all populations, which is contrary to what we observe. Strictly speaking, however, the pattern exhibited in Figure 6 can result in the absence of any selection at all—if there is significant epistatic genetic variance, relative levels of divergence for neutral quantitative traits can exceed that for molecular markers (Lynch 1994).

A closer inspection of the data helps to clarify the nature of the among-population divergence for life-history traits in this study. Although there is a substantial amount of variation among traits for Q_{ST}, much of this variation appears to be associated with variation in broadsense heritability (Fig. 4). This relationship may extend to other species. For the three other studies in which both Q_{ST} and H^2 have been estimated for six or more traits (*Pinus contorta*, pine [Yang et al. 1996]; *Medicago truncatula*, alfalfa [Bonnin et al. 1996]; and *Clarkia dudleyana*, a flowering annual plant [Podolsky and Holtsford 1995]), the regression between these two indices is also positive, although it is only significant in the case of *Pinus*. Under the neutral hypothesis, the average level of heritability of a quantitative trait in a subdivided population is expected to increase with the rate of input of new variation by mutation (Lande 1992; Lynch 1994). However, the magnitude of Q_{ST} for neutral characters is independent of the mutational variance (because both the within- and between-population components of variance are proportional to the mutation rate), and depends only on properties of population structure (effective population size and migration rates) that equally affect all characters. Thus, neutral theory predicts Q_{ST} to be independent of H^2. On the other hand, all other things being equal, the magnitude of response of characters to directional selection increases with increasing heritability (Falconer and Mackay 1996), so the observed scaling of Q_{ST} with H^2 is at least qualitatively consistent with the hypothesis that population differentiation in *D. pulex* is being driven by divergent selection operating in different environments.

In *D. pulex*, the average levels of Q_{ST} and heritability for ages at reproduction are both essentially zero (Table 2). This suggests that reproductive timing is under strong purifying selection for similar phenotypic values in all populations. At the other extreme are instar-specific body sizes, which have nearly uniformly significant heritabilities, averaging to 0.17, and substantial levels of population subdivision, averaging to $Q_{ST} = 0.61$. This level of population divergence is approximately double that observed for nuclear marker genes (0.30), which suggests that body size is under strong diversifying selection among different local environments. In a study with *D. obtusa*, Spitze (1993) also found levels of Q_{ST} for body size to be substantially higher than those for other traits. The maintenance of higher levels of genetic variance within populations for this character, perhaps fostered by temporal changes in optimal body size, presumably promotes the ability of these characters to diverge among populations.

Finally, we note that some of the divergence among mean phenotypes in our study populations appears to be associated with variation in the degree of local inbreeding. For every character examined, the relationship between mean phenotype and population homozygosity is in the same direction as that observed when individual populations are directly inbred by self-fertilization (Table 3). However, the absolute magnitude of the response to population homozygosity is always less than or equal to that observed from inbreeding depression studies. This is not surprising because laboratory experiments that impose inbreeding on a population minimize the effectiveness of selection on the inbred individuals by eliminating the possibility of competition between lines, whereas in nature, one would expect there to be some selection against individuals that exhibit the most negative consequences of inbreeding. Although these results strongly suggest that local inbreeding depression plays a role in determining the mean phenotype of individual populations, homozygosity only explains an average of 27% of the variation among populations for the characters evaluated in this study. Thus, the response to local selective pressures can still move the mean phenotype to a substantial degree from the expectation based on inbreeding alone. In general, the range of variation in population mean phenotypes is on the order of two to five times greater than that which can be accounted for by a simple response to inbreeding (Table 3).

Although it is tempting to conclude that the gradient of mean phenotypes with population homozygosity is simply a passive and maladaptive response to occasional population bottlenecks, it is also worth considering the possibility that the level of homozygosity observed within populations is more intrinsically associated with natural selection. Zooplankton communities are strongly influenced by size-selective predators (Kerfoot 1980; Kerfoot and Sih 1987), and there is no question that variation in predation can cause radical changes in species composition. Predation on temporary pond *Daphnia* by salamander (*Ambystoma*) and dip-

teran (*Chaoborus*) larvae is often extremely intense, with entire *Daphnia* populations being decimated in a matter of a few days when a cohort of predators matures. When predation focuses on large visible individuals, as is the case with vertebrate predators, there is likely to be a strong selective advantage to small individuals carrying relatively small (less visible) clutches. The most rapid way to achieve such a phenotype is through inbreeding (Table 3). Thus, we cannot rule out the possibility that the high levels of homozygosity (and associated small body sizes, slow growth rates, and small clutch sizes) in some populations are consequences of periodic situations in which highly inbred individuals are the only survivors of bouts of vertebrate predation. Similarly, populations with high levels of heterozygosity (and associated large body sizes, high growth rates, and large clutch sizes) may owe such attributes to situations in which invertebrate predators predominate and select strongly against small individuals. For populations experiencing a gradient of intense size-selective predation, there may be situations in which the phenotypic response to inbreeding, normally referred to as inbreeding depression, is actually adaptive.

Acknowledgments

This work was supported by NSF grant DEB-9629775 to ML and NSF grants DEB 92-20634 and 94-24595 to KS. We are very grateful to J. Blanchard, A. Lynch, E. Lynch, E. Martins, T. Siemens, and D. Weiss for assistance with various phases of the project, and to P. Armbruster and J. Hard for providing unpublished data.

Literature Cited

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, New York.

Bonnin, I., J.-M. Prosperi, and I. Olivieri. 1996. Genetic markers and quantitative genetic variation in *Medicago truncatula* (Leguminosa): a comparative analysis of population structure. Genetics 143:1795–1805.

Crease, T., M. Lynch, and K. Spitze. 1990. A hierarchical analysis of population genetic variation in nuclear and mitochondrial genes in *Daphnia*. Mol. Biol. Evol. 7:444–458.

Crow, J. F., and K. Aoki. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. Proc. Natl. Acad. Sci. USA 81:6073–6077.

Deng, H.-W., and M. Lynch. 1997. Inbreeding depression and inferred deleterious-mutation parameters in *Daphnia*. Genetics 147:147–155.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman, Harlow, U.K.

Hebert, P. D. N. 1974. Enzyme variability in natural populations of *Daphnia magna*. II. Genotypic frequencies in intermittent populations. Genetics 77:335–344.

Hebert, P. D. N., and M. J. Beaton. 1989. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories, Beaumont, TX.

Hollocher, H., A. R. Templeton, R. DeSalle, and J. S. Johnston. 1992. The molecular through ecological genetics of *abnormal abdomen* in *Drosophila mercatorum*. III. Components of genetic variation in a natural population of *Drosophila mercatorum*. Genetics 130:355–366.

Innes, D. J. 1991. Geographic patterns of genetic differentiation among sexual populations of *Daphnia pulex*. Can. J. Zool. 69: 995–1003.

Kerfoot, W. C., ed. 1980. Ecology and evolution of zooplankton communities. Univ. of New England Press, Hanover, NH.

Kerfoot, W. C., and A. Sih, eds. 1987. Predation: direct and indirect impacts on aquatic communities. Univ. of New England Press, Hanover, NH.

Kremer, A., A. Zanetto, and A. Ducousso. 1997. Multilocus and multitrait measures of differentiation for gene markers and phenotypic traits. Genetics 145:1229–1241.

Kuittinen, H., A. Mattila, and O. Savolainen. 1997. Genetic variation at marker loci and in quantitative traits in natural populations of *Arabidopsis thaliana*. Heredity 79:144–152.

Kumar, S., K. Tamura, and M. Nei. 1993. MEGA: molecular evolutionary genetics analysis. Vers. 1.01. Pennsylvania State University, University Park, PA.

Lai, C., R. F. Lyman, A. D. Long, C. H. Langley, and T. F. C. Mackay. 1994. Naturally occurring variation in bristle number associated with DNA sequence polymorphisms at the *scabrous* locus of *Drosophila melanogaster*. Science 266:1697–1702.

Lande, R. 1992. Neutral theory of quantitative genetic variance in an island model with local extinction and colonization. Evolution 46:381–389.

Lande, R., and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. Pp. 87–124 *in* M. E. Soule, ed. Viable populations for conservation. Cambridge Univ. Press, Cambridge, U.K.

Lehman, N., M. E. Pfrender, P. A. Morin, T. J. Crease, and M. Lynch. 1995. A hierarchical molecular phylogeny of the genus *Daphnia*. Mol. Phylogenet. Evol. 4:395–407.

Long, A. D., and R. S. Singh. 1995. Molecules versus morphology: the detection of selection acting on morphological characters along a cline in *Drosophila melanogaster*. Heredity 74: 569–581.

Lynch, M. 1983. Ecological genetics of *Daphnia pulex*. Evolution 37:358–374.

———. 1984. The genetic structure of a cyclical parthenogen. Evolution 38:186–203.

———. 1985. Spontaneous mutations for life history characters in an obligate parthenogen. Evolution 39:804–818.

———. 1987. The consequences of fluctuating selection for isozyme polymorphisms in *Daphnia*. Genetics 115:657–669.

———. 1994. The neutral theory of phenotypic evolution. Pp. 86–108 *in* L. Real, ed. Ecological genetics. Princeton Univ. Press, Princeton, NJ.

———. 1996. A quantitative-genetic perspective on conservation issues. Pp. 471–501 *in* J. Avise and J. Hamrick, eds. Conservation genetics: case histories from nature. Chapman and Hall, New York.

Lynch, M., and T. Crease. 1990. The analysis of population survey data on DNA sequence variation. Mol. Biol. Evol. 7:377–394.

Lynch, M., and H.-W. Deng. 1994. Genetic slippage in response to sex. Am. Nat. 144:242–261.

Lynch, M., and K. Spitze. 1994. Evolutionary genetics of *Daphnia*. Pp. 109–128 *in* L. Real, ed. Ecological genetics. Princeton Univ. Press, Princeton, NJ.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Lynch, M., K. Spitze, and T. Crease. 1989. The distribution of life-history variation in *Daphnia pulex*. Evolution 43:1724–1736.

Lynch, M., L. Latta, J. Hicks, and M. Giorgianni. 1998. Mutation, selection, and the maintenance of life-history variation in a natural population. Evolution 52: 727–733.

Podolsky, R. H., and T. P. Holtsford. 1995. Population structure of morphological traits in *Clarkia dudleyana*. I. Comparison of F_{ST} between allozymes and morphological traits. Genetics 140: 733–744.

Prout, T., and J. S. F. Barker. 1993. *F*-statistics in *Drosophila buzzatii*: selection, population size and inbreeding. Genetics 134: 369–375.

Roff, D. A. 1997. Evolutionary quantitative genetics. Chapman and Hall, New York.

Rzhetsky, A., and M. Nei. 1992. A simple method for estimating and testing minimum-evolution trees. Mol. Biol. Evol. 9:945–967.

Slatkin, M. 1993. Gene flow and population structure. Pp. 3–18

in L. Real, ed. Ecological genetics. Princeton Univ. Press, Princeton, NJ.

SPITZE, K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. Genetics 135:367–374.

WALSH, P. S., D. A. METZGER, AND R. HIGUCHI. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506–513.

WRIGHT, S. 1951. The genetical structure of populations. Ann. Eugen. 15:323–354.

YANG, R.-C., F. C. YEH, AND A. D. YANCHUK. 1996. A comparison of isozyme and quantitative genetic variation in *Pinus contorta* ssp. *latifolia* by F_{ST}. Genetics 142:1045–1052.

Corresponding Editor: J. Neigel