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PATTERNS OF GENETIC ARCHITECTURE FOR LIFE-HISTORY TRAITS AND MOLECULAR MARKERS IN A SUBDIVIDED SPECIES

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Abstract.—Understanding the utility and limitations of molecular markers for predicting the evolutionary potential of natural populations is important for both evolutionary and conservation genetics. To address this issue, the distribution of genetic variation for quantitative traits and molecular markers is estimated within and among 14 permanent lake populations of *Daphnia pulicaria* representing two regional groups from Oregon. Estimates of population subdivision for molecular and quantitative traits are concordant, with Q_{ST} generally exceeding G_{ST}. There is no evidence that microsatellites loci are less informative about subdivision for quantitative traits than are allozyme loci. Character-specific comparison of Q_{ST} and G_{ST} support divergent selection pressures among populations for the majority of life-history traits in both coast and mountain regions. The level of within-population variation for molecular markers is uninformative as to the genetic variation maintained for quantitative traits. In *D. pulicaria,* regional differences in the frequency of sex may contribute to variation in the maintenance of expressed within-population quantitative-genetic variation without substantially impacting diversity at the genic level. These data are compared to an identical dataset for 17 populations of the temporary-pond species, *D. pulex.*

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

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The level of genetic variation maintained within natural populations and its distribution among populations for fitness-related characters are of special interest in the contexts of evolutionary (Hartl and Clark 1989; Falconer and Mackay 1996) and conservation genetics (Frankel and Soule 1981; Beardmore 1983; Vida 1994; Lynch 1996). The ability of a population to respond to selective pressures imposed by a changing environment is directly related to an appropriate measure of additive genetic variance. In the absence of complications due to selection on correlated characters, this relationship is defined by the breeder's equation, $R = h^2S$, where R is the evolutionary response to selection, h^2 is heritability, and S is the selection differential (Turelli and Barton 1990; Falconer and Mackay 1996). It has been demonstrated that heritabilities, especially for traits with direct fitness consequences, have practical importance for the adaptability and stability of natural populations over time (e.g., Grant and Grant 1993).

Due to the difficulty in obtaining quantitative-genetic information for the majority of organisms, the genetic variance for quantitative characters is often indirectly inferred from measures of molecular genetic variation (Bonnell and Selander 1974; O'Brien et al. 1985; Avise 1989; Gilbert et al. 1991). The utility of this extrapolation is questionable because empirical evidence demonstrating the nature of the relationship between quantitative and molecular data is limited (e.g., Podolsky and Holtsford 1995; Bonnin et al. 1996; Yang et al. 1996; Lynch et al. 1999; Pfrender and Lynch 2000). In addition, there are theoretical reasons to expect that the correspondence between variation at the molecular and quantitative-trait levels may be weak (Lande and Barrowclough 1987; Lynch 1996).

Variation for polygenic, quantitative characters is introduced by mutation at a higher rate than for single-locus molecular markers (Kimura 1983; Lynch 1988, 1996). Thus, substantial variation for quantitative characters may be present in populations lacking in diversity at the molecular level. Second, the expected level of heterozygosity at neutral molecular markers declines linearly with inbreeding, whereas the pattern for quantitative characters can deviate substantially from this due to nonadditive sources of genetic variance (reviewed in Lynch 1996). Finally, the large sampling error often associated with quantitative and molecular genetic parameter estimates can make any existing relationship difficult to identify.

The genus *Daphnia* offers an ideal model system for the simultaneous study of quantitative and molecular characters for a number of reasons. First, *Daphnia* are easily maintained in the laboratory and have short generation times. Second, most *Daphnia* are cyclical parthenogens and can be maintained clonally for an indefinite period under controlled environmental conditions. Thus, characters of interest can be measured on multiple, genetically identical individuals, allowing for the clean separation of phenotypic variance into its genetic and environmental components. Third, the population structure can be assayed via a number of molecular-marker techniques including allozymes and microsatellites. Finally, the highly fragmented population structure of *Daphnia* species makes it possible to examine molecular and quantitative-genetic characteristics of many replicate populations over a small geographic range.

The primary aim of this study is to characterize and compare quantitative-genetic variation for life-history characters to variation at allozyme and microsatellite loci within and among 14 populations of *Daphnia pulicaria* to make inferences about their evolutionary histories. Much of the existing

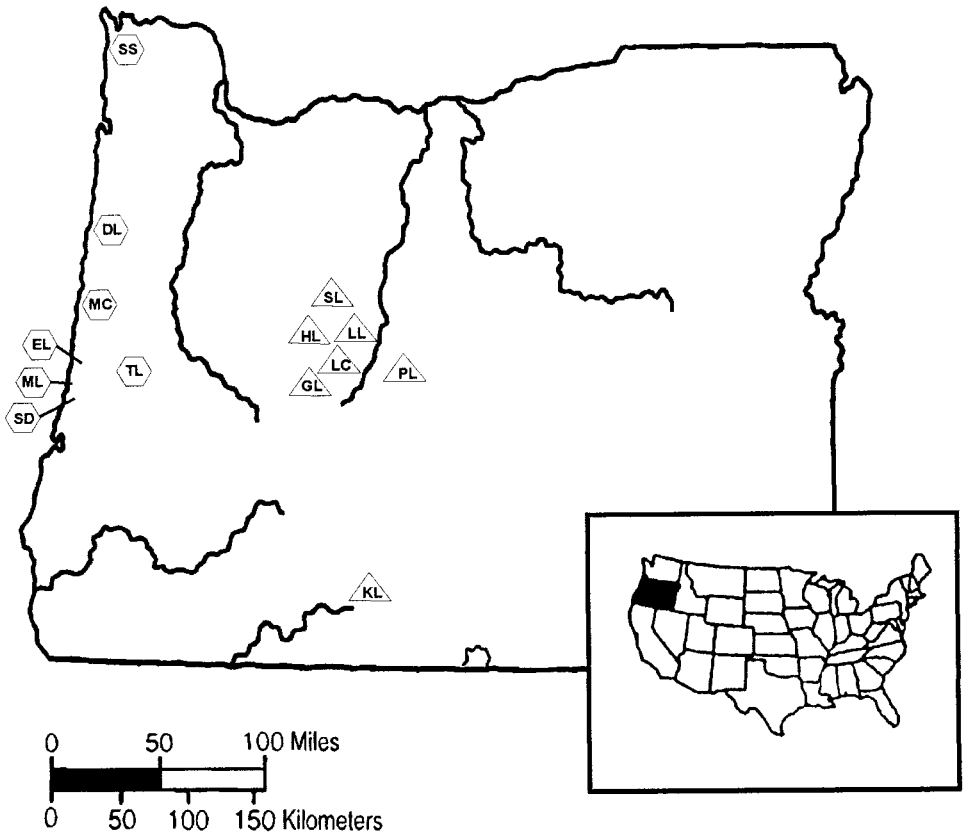

FIG. 1. Distribution of lake populations. Hexagons denote coastal region populations of *Daphnia pulicaria*, and triangles denote mountain populations.

Daphnia literature focuses on species inhabiting temporary-pond environments both in the western and midwestern United States (Lynch 1980, 1984, 1987; Lynch et al. 1989; Crease et al. 1990; Lynch et al. 1999), in which the sexual phase is enforced on an annual basis. This can result in a yearly influx of expressed genetic variation (Deng and Lynch 1996), and such species have been shown to exhibit a wide range of heritabilities for life-history traits (Lynch et al. 1989; Spitze 1993; Lynch et al. 1999). *Daphnia pulicaria*, the focal species of this study, inhabits permanent lakes and therefore experiences a relatively stable environment in which prolonged periods of clonal selection may be frequent. Thus, an additional goal is to explore the generality of the molecular-quantitative genetic relationship by comparing similar species experiencing different environmental pressures. The shift from pond to lake habitats has been posited as an important event in the evolution of morphological characters and ultimately in speciation within the *D. pulex* complex (Colbourne et al. 1997), making the comparison of genetic architectures between closely related pond and lake species of special interest.

METHODS

Study Populations

Individuals were collected from 14 permanent, natural lakes broadly distributed across western Oregon in June and July 1997. Seven of the lakes are located along the coast, and the remaining seven are in the Cascade Range (Fig. 1). Thus, the distribution of populations includes two regions, a coastal and a mountain region.

Life-Table Assay

The life-table assay was conducted in accordance with a standard experimental design (Lynch 1985; Lynch et al. 1989). Sixty individuals (hereafter referred to as clones) were isolated from each of the 14 populations and placed into individual beakers. Immediate isolation of individuals is required to prevent selection in the laboratory from biasing population samples toward one or a few genotypes. All individuals were kept under constant conditions in incubators. Specifically, individuals were kept at 15°C, a 12:12 L:D photoperiod, and in approximately 100 ml of aged, filtered lake water collected from Dexter Reservoir, Lane County, Oregon. Water levels were maintained by periodic addition of double distilled water. *Daphnia* were fed drops of pure culture *Scenedesmus* algae every other day. Because of the ideal conditions maintained in the laboratory, all individuals remained in the clonal phase of reproduction for the duration of the experiment.

Once individuals reproduced clonally, two replicates of each clonal type were extracted from the initial beakers (for a total of 14 populations $\times$ 60 clones $\times$ 2 replicates = 1680 individuals) and placed into separate beakers in a completely randomized design within the controlled environmental

TABLE 1. Mean (SE) phenotypic values, estimates of broad-sense heritability (H^2), and subdivision (Q_{ST}, Q_{SR}) for population assemblages and character types. S_B, size at birth; S_M, size at maturity; S_A, adult size; CS, clutch size; A_M, age at maturity; A_I, average adult instar duration; G_J, juvenile growth rate; G_A, average adult growth rate. Body sizes are in millimeters, ages in days, and growth rates in millimeters per day.

	S_B	S_M	S_A	CS	A_M	A_I	G_J	G_A
				Total				
Mean	0.68 (0.01)	1.76 (0.03)	2.10 (0.04)	8.02 (0.69)	8.68 (0.33)	4.18 (0.04)	0.12 (0.00)	0.02 (0.00)
H^2	0.11 (0.06)	0.26 (0.07)	0.24 (0.09)	0.15 (0.07)	0.07 (0.10)	0.13 (0.10)	0.05 (0.10)	0.06 (0.06)
Q_{ST}	0.21 (0.08)	0.54 (0.11)	0.49 (0.11)	0.62 (0.17)	0.70 (0.13)	0.54 (0.13)	0.69 (0.43)	0.42 (0.11)
				Coast				
Mean	0.67 (0.01)	1.71 (0.05)	2.05 (0.06)	7.94 (1.14)	8.95 (0.70)	4.16 (0.03)	0.12 (0.00)	0.02 (0.00)
H^2	0.03 (0.10)	0.23 (0.07)	0.06 (0.09)	0.01 (0.09)	−0.02 (0.08)	0.02 (0.10)	−0.11 (0.11)	−0.01 (0.10)
Q_{SR}	0.69 (0.26)	0.63 (0.14)	0.67 (0.14)	0.91 (0.42)	0.84 (0.15)	0.98 (0.25)	1.00 (1.0)	0.50 (0.38)
				Mountain				
Mean	0.69 (0.01)	1.80 (0.04)	2.15 (0.05)	8.10 (0.97)	8.47 (0.11)	4.21 (0.05)	0.12 (0.00)	0.02 (0.00)
H^2	0.19 (0.04)	0.29 (0.06)	0.42 (0.05)	0.28 (0.08)	0.15 (0.07)	0.24 (0.07)	0.20 (0.09)	0.12 (0.09)
Q_{SR}	0.15 (0.08)	0.59 (0.14)	0.37 (0.13)	0.46 (0.18)	0.01 (0.05)	0.05 (0.04)	0.70 (0.33)	0.38 (0.07)

chamber. All conditions described above apply here, except that each beaker was examined every other day and all water replaced. This water contained a constant concentration of *Scenedesmus* of about 250,000 cells/ml. The initial clone bank was maintained throughout the experiment so that individuals suffering mortality could be replaced.

Individuals in the life-table assay were taken through two generations of reproduction prior to measurement of life-history characters such that maternal (and grandmaternal) effects contribute to estimates of environmental rather than genetic variance in the final analysis (Lynch 1985). Data were collected for individuals of the third generation from birth to the release of the fourth clutch. Eighteen life-history characters were measured with a Wild (Leica Corp., Wetzlar, Germany) dissecting microscope including body sizes, clutch sizes, ages of reproduction, and growth rates (calculated from changes in body size over days; Table 1). Developmental stages of embryos within each focal individual were noted to refine estimates of the timing of reproductive events (Lynch et al. 1999).

Quantitative Genetic Analysis

Data were analyzed for each population by one-way analysis of variance (ANOVA) in order to partition the total phenotypic variance for each life-history trait into the within- and among-clone components. The within-clone variance provides an estimate of the environmental variance (V_E) and the among-clone variance estimates the genetic variance (V_G). The ratio of genetic variance to total variance is the broad-sense heritability, $H^2 = V_G/(V_G + V_E)$. Another manner of scaling the genetic variance for comparison among characters and populations is the coefficient of genetic variance ($CV_G = V_G^{1/2}/X$, where X is the mean phenotype).

All data were additionally analyzed via nested analysis of variance, both with and without the inclusion of region as a factor, to extract the among-population and among-region genetic variance. Estimates of population subdivision, $Q_{ST} = V_{GB}/(V_{GB} + 2V_{GW})$, where V_{GB} is the proportion of the genetic variance distributed among populations and V_{GW} is the average of V_G given above (Wright 1951), were obtained as described in Spitze (1993) and Lynch and Spitze (1993). Estimates of regional subdivision (Q_{RT}) were also calculated. The level of quantitative genetic subdivision within each region (Q_{SR}) was estimated by conducting independent nested analyses of variance for the two regional population assemblages (coast and mountain). Finally, estimates of subdivision between individual population pairs were obtained for comparison with estimates of molecular subdivision. Standard errors of all estimates were obtained by the delta method (Lynch and Walsh 1998). The significance of Q_{ST}-values was determined by an ANOVA F-test. It should be noted that our populations have experienced an unknown number of clonal generations in the field prior to analysis and therefore do not necessarily represent panmictic populations in Hardy-Weinberg equilibrium. Therefore, our results may have limited applicability to sexually reproducing species.

Allozyme and Microsatellite Methods and Analysis

Allozyme analysis was performed on fresh tissue when available, but in some instances frozen tissue was used. Ten allozyme loci were sampled for variation in all study populations by use of cellulose-acetate electrophoresis (Hebert and Beaton 1989). The relative mobility of allozyme alleles was calibrated against a standard *Daphnia* genotype.

Thirty individuals from each of the 1997 *D. pulicaria* populations were surveyed for microsatellite variation at six microsatellite loci. DNA was extracted from single individuals using the Chelex method (Walsh et al. 1991; Lehman et al. 1995). PCR reactions for the *D. pulicaria* collection were designed for use on a Li-Cor 4200 LongRead IR automated sequencer (Li-Cor Biosciences, Lincoln, NE). The forward microsatellite primer for each of the six loci was redesigned with M13 forward (−29) 19mer sequence at the 5′ end of the primer for use with the IR-labeled M13 forward (−29) genotyping oligonucleotides available from Li-Cor. PCR reactions consisted of a 1× concentration of 10× polymerase chain reaction (PCR) buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.4, 25 mM MgCl, 0.6% w/v BSA), 0.8 mM dNTPs, 0.5 pmol of the forward microsatellite primer, 5.0 pmol reverse microsatellite primer, 0.33 pmol of M13 forward (−29) IRD-labeled oligonucleotide, 0.02 units of *Taq* polymerase, 5 μl pf Chelex DNA template, and water to the final volume of 12.5 μl. PCR was carried out with a 4-min denaturation at 94°C, followed by 15 to 29 cycles (depending on primer

set) for 45 sec at 94°C, 1 min at 53°C, and 1 min at 72°C, finally followed by a 15-min extension step at 72°C. PCR products were stopped with 4 μl formamide loading dye, then denatured at 80°C for 3 min, cooled on ice, and loaded in 7% polyacrylimide gels with a size standard available from Li-Cor.

The pooled molecular dataset was constructed by weighting allozyme and microsatellite data by the inverse of the sampling variance. Estimates of gene diversity (H_E) within each population were obtained by calculating the Hardy-Weinberg expectation for heterozygosity from the allele frequency data. Estimates of the within-population inbreeding coefficient ($G_{IS} = 1 - [H_O/H_E]$, where H_O is the observed heterozygosity) were estimated for each locus.

The degree of population subdivision for allozymes and microsatellites (G_{ST}) was calculated by first estimating the total gene diversity for the group of all 14 populations. Then the average within-population gene diversity ($H_{W/IN}$) was subtracted from the total gene diversity to obtain the among population gene diversity. G_{ST} is then the ratio of the among-population gene diversity to the total gene diversity (H_{AMONG}/H_{TOTAL}). Average G_{ST}-estimates were also calculated independently for the two regional population assemblages and for each paired population comparison.

In addition, a three-way nested analysis of molecular variance (AMOVA) was conducted for allozymes, microsatellites, and the pooled molecular dataset to extract estimates of molecular variation at the within-population, among-population, and among-region levels. Estimates of molecular subdivision among populations within regions and among regions were calculated by this method and tested for significance by *F*-tests.

Trees of relationship for allozyme, microsatellite, and pooled datasets were constructed using the UPGMA method as implemented in MEGA (Kumar et al. 1993). The *D. pulex* dataset from Lynch et al. (1999) was used to root the tree of relationships among *D. pulicaria* populations.

Results

Quantitative Genetic Variation

Phenotypic values were compared by region using a nested ANOVA. None of the characters differ significantly between regions (Table 1). However, individuals from coastal populations generally exhibit reduced fitness-related characters, with smaller body sizes, fewer offspring, and a later age at maturity. The average expressed heritability over all traits and populations is relatively low with a value of 0.13 (SE = 0.03; Table 1). On average, coastal populations exhibit much lower heritabilities (0.03 ± 0.03) than mountain populations (0.24 ± 0.03).

Significant Q_{ST}-values were observed for all characters except juvenile and adult growth rates, with an average value of 0.53 (0.06) among all populations (Table 1). From the nested analysis including region, the average level of subdivision among populations within regions is 0.40 (0.07). There is no significant genetic subdivision among regions for quantitative traits, with average Q_{RT}-estimates of 0.01 (0.01). Coastal populations have a much greater degree of genetic differentiation for quantitative characters than mountain populations, with average Q_{SR}-estimates across characters of 0.78 (0.06) and 0.36 (0.09), respectively (Table 1). This difference is not always due to reduced within-population genetic variance in coastal populations relative to mountain populations.

Table 2. Average gene diversity (H_E), average inbreeding coefficients (G_{IS}), and estimates of molecular subdivision for all populations (G_{ST}) and the two regional population assemblages (G_{SR}) based on 10 allozyme and six microsatellite loci (standard error in parentheses).

	Allozyme	Microsatellite
	Total	
H_E	0.15 (0.07)	0.40 (0.07)
G_{IS}	−0.18 (0.10)	0.01 (0.09)
G_{ST}	0.27 (0.06)	0.39 (0.06)
	Coast	
H_E	0.15 (0.07)	0.40 (0.07)
G_{IS}	−0.38 (0.15)	−0.09 (0.10)
G_{SR}	0.25 (0.06)	0.36 (0.04)
	Mountain	
H_E	0.14 (0.07)	0.39 (0.07)
G_{IS}	−0.11 (0.08)	0.11 (0.07)
G_{SR}	0.13 (0.04)	0.27 (0.05)

Molecular Variation for Allozymes and Microsatellites

Average gene diversity estimates are greater for microsatellite loci than for allozyme loci (Table 2). Pooled gene diversity estimates range from 0.16 to 0.35 among populations, with nearly identical average estimates in coastal and mountain regions for both types of molecular markers (Table 2). Coastal populations exhibit significant heterozygote excesses at allozyme loci with an average G_{IS}-value of −0.38 (0.15). Mountain populations do not differ significantly from Hardy-Weinberg expectations at allozyme loci. The mean microsatellite G_{IS}-values for the coast and mountain populations are −0.09 (0.10) and 0.11 (0.07), respectively, with populations from neither region differing significantly from Hardy-Weinberg expectations.

Measures of population subdivision analogous to those reported above for quantitative traits were estimated for both allozyme and microsatellite frequency data by comparison of gene diversity estimates within and among populations and by nested AMOVA. Estimates of subdivision based on microsatellite loci are typically greater than those based on allozyme loci (Table 2). The estimates of subdivision are greater for coastal relative to mountain populations. The ANOVA estimate of subdivision among populations within regions for the pooled molecular dataset is significant (0.30). This value is quite comparable to estimates reported in Table 2. There is no significant regional subdivision, with only 2% of the molecular variation among regions.

No apparent geographic pattern is observed in the phylogenetic tree of molecular variation at nuclear loci for the Oregon *D. pulicaria* (Fig. 2). Similar results are obtained when the analysis is limited to either microsatellite or allozyme data. The markers do resolve Oregon populations of lake-dwelling *D. pulicaria* and pond-dwelling *D. pulex* as monophyletic clades.

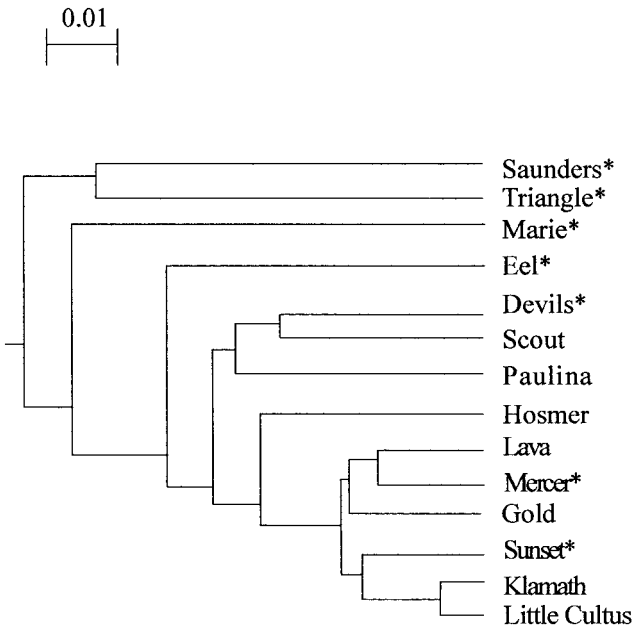

FIG. 2. UPGMA tree of Oregon *Daphnia pulicaria* populations based on pooled data for 10 allozyme and six microsatellite loci. The tree is rooted with Oregon populations of *D. pulex* from a previous study (Lynch et al. 1999). Asterisks indicate coastal populations, unmarked populations indicate mountain populations.

Patterns of Relationship between Genetic Parameter Estimates

Estimates of subdivision for molecular (G_{ST}) and quantitative traits (Q_{ST}) are positively associated such that pairs of populations exhibiting the highest levels of genetic differentiation for life-history traits also exhibit more extreme subdivision for molecular markers (Fig. 3; $r = 0.38$, $P = 0.0002$, not accounting for nonindependence of paired population comparisons). In contrast, there is no correlation between within-population levels of genetic variation for molecular (H_E) and quantitative traits (H^2: $r = -0.07$, ns; CV_G: $r = 0.18$, ns).

Inbreeding coefficients based on microsatellites and allozymes (G_{IS}) are positively correlated (Fig. 4; $r = 0.80$, $P = 0.001$), although allozyme loci have a tendency to deviate from Hardy-Weinberg expectations in the direction of heterozygote excess. There is a significant positive relationship between population inbreeding coefficient (G_{IS} averaged across all allozyme and microsatellite loci) and average H^2 for life-history traits (Fig. 5; $r = 0.62$, $P = 0.02$) such that populations displaying heterozygote excess maintain lower average heritability for fitness traits. A similar pattern is observed between H^2 and G_{IS} for coastal and mountain populations (coast: $r = 0.61$, ns; mountain: $r = 0.43$, ns), although it is not significant for either region alone. All patterns of relationship between quantitative and molecular markers employing the coefficient of genetic variation are consistent with results reported for heritability. There is no correlation between average heterozygosity and mean population phenotype for any life-history character (S_B: $r = 0.13$, ns; S_M: $r = -0.17$, ns; S_A: $r = -0.23$, ns; C_S: $r = -0.17$, ns; A_M: $r = -0.10$, ns; A_I: $r = 0.16$, ns; G_J: $r = -0.23$, ns; G_A: $r = -0.25$, ns).

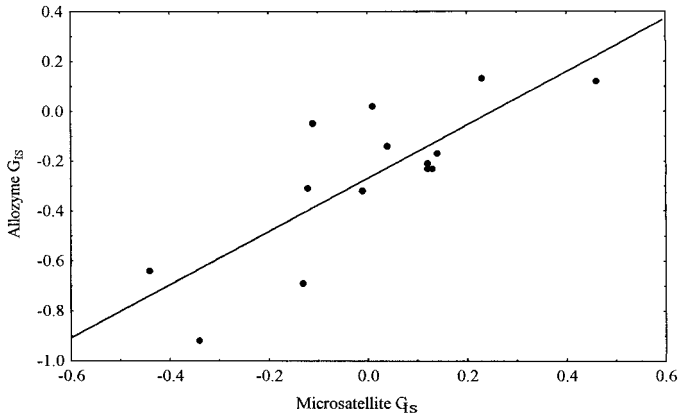

FIG. 4. Correlation between average microsatellite and allozyme inbreeding coefficients (G_{IS}) for each of the 14 populations ($r = 0.80$, $P = 0.006$).

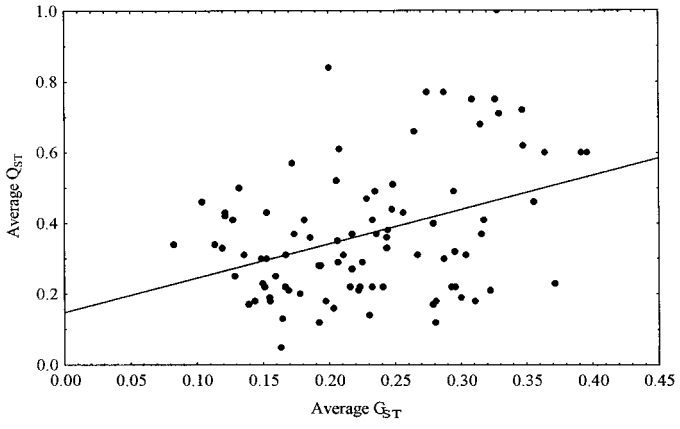

FIG. 3. Correlation between average estimates of subdivision at the quantitative trait (Q_{ST}) and molecular levels (G_{ST}) for all possible population pairs ($r = 0.38$, $P = 0.0002$).

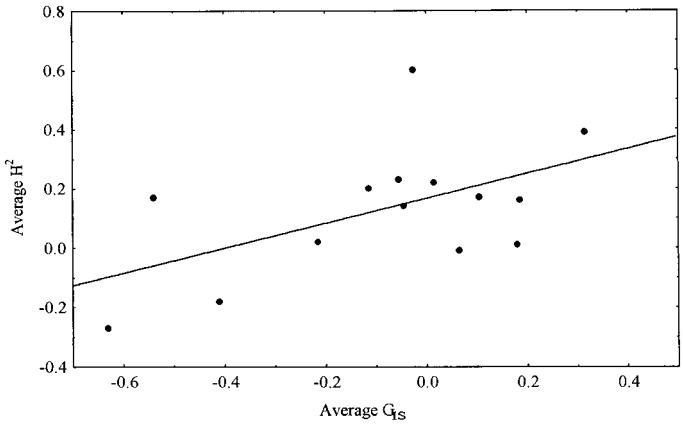

FIG. 5. Relationship between average inbreeding coefficient (G_{IS}) for all loci and average heritability (H^2) for life-history traits ($r = 0.53$, $P = 0.05$). Negative G_{IS}-values indicate heterozygote excess, and positive values indicate homozygote excess.

Discussion

The level of subdivision between populations can be of practical importance for accurately identifying distinct population units (Avise 1989). In the current study, there is a significant correlation between the level of divergence between pairs of populations at the molecular (G_{ST}) and quantitative trait levels (Q_{ST}), with average Q_{ST} exceeding average G_{ST} in most cases. Therefore, our results support the idea that information about the level of subdivision as indicated by neutral molecular markers can provide a conservative expectation for the relative degree of subdivision for adaptive characters (Lynch et al. 1999).

The analysis of molecular and quantitative subdivision should be taken with some caution due to the nonindependence of paired population estimates. However, it is unclear how to adequately address this issue statistically. The high level of significance for the relationship between Q_{ST} and G_{ST} even with large error variance around individual estimates seems an unlikely chance result. A similar pattern of relationship between estimates of subdivision was previously described among distantly related taxa (Lynch et al 1999). Therefore, it appears that the broad-scale pattern of relationship between average Q_{ST} and G_{ST} estimates among widely divergent species, with Q_{ST} generally exceeding G_{ST}, may apply to the level of subdivision between individual pairs of populations within species.

A relationship between genetic variance and heterozygosity is theoretically expected (in the simplest case, $V_A = H_E\alpha^2$, where α represents the average allelic effect; Falconer and Mackay 1996). However, within populations of *D. pulicaria,* no relationship is observed between pooled allozyme and microsatellite gene diversity and heritability or the coefficient of genetic variation for life-history traits. Earlier studies of *D. pulex* and *D. obtusa* populations from Oregon also failed to find a relationship between within-population molecular variation and variation at the quantitative trait level (Spitze 1993; Lynch et al. 1999; Pfrender and Lynch 2000).

Additional evidence is available from studies on cotton-top tamarin populations (Cheverud et al. 1994) and plant populations of the genus *Scabiosa* (Waldmann and Andersson 1998); again, a lack of relationship between genetic variation for neutral, molecular, and adaptive quantitative characters is demonstrated. Thus, it appears that there is generally no correspondence between the level of fitness-related quantitative genetic variation and molecular variation maintained within populations. However, genetic variation for a quasi-neutral quantitative trait, *Drosophila* bristle number, was found to correlate with genetic variation at the molecular level (Briscoe et al. 1992).

The cyclical parthenogenetic life history of our study species makes it possible for populations in stable environments to experience prolonged periods of clonal selection. Such a lack of sexual reproduction can lead to populations that deviate from Hardy-Weinberg expectations and may limit the extrapolation of our results to sexually reproducing species. However, our results, with molecular genetic parameter estimates providing information about the level of genetic subdivision but not about the level of genetic variation within populations for fitness-related traits, appear to be generally consistent with existing evidence from sexual species.

Another important issue relates to the utility of allozymes versus microsatellites in revealing the population structure for characters with direct fitness consequences. Some concern has been raised over the use of microsatellites because of their high levels of polymorphism relative to allozyme loci (Hedrick 1999; Balloux et al. 2000). The current study indicates that microsatellite loci are just as informative as allozymes about the expected level of population subdivision for quantitative characters. The strongest pattern is found between estimates of subdivision based on the pooled molecular markers and quantitative traits, perhaps reflecting the importance of including many loci or that a combination of marker types is preferable. Neither type of molecular marker is sufficient to predict the level of variation maintained at the quantitative trait level within populations.

The fact that molecular genetic variation is uninformative as to the expected level of genetic variation for quantitative characters suggests that the two types of measures can be complementary, relating to different aspects of population histories. Levels of genetic variation for quantitative traits and molecular markers are theoretically expected to differ because polygenic, quantitative characters generally have higher mutation rates and greater exposure to selective pressures (Lynch 1996). Thus, quantitative variation can be informative as to conditions influencing the evolution of populations in the more recent past, whereas neutral molecular markers can be reflective of more long-term evolutionary events. In this case, we have an unusual ability to evaluate both kinds of data in multiple populations occupying two distinct regions as well as in a closely related species, *D. pulex,* for which an identical dataset is available for 17 populations from two regions (also clades; Lynch et al. 1999).

By combining the two types of genetic information along with knowledge about the natural history of the organisms, one can formulate hypotheses about the evolutionary forces that are at work and how those may differ among groups of interest. Between regional population groups of *D. pulicaria,* a distinction is apparent in terms of the amount of genetic variation maintained within populations for fitness-related characters, with coastal populations maintaining almost no variation at this level and mountain populations maintaining an average heritability of 0.24 (Table 3). This difference between regions is not apparent at the molecular level, as indicated by nearly identical gene diversity estimates for both allozyme and microsatellite loci.

The difference in habitat between coastal and mountain populations seems an obvious factor contributing to the difference in the level of maintained genetic variation for quantitative characters. Coastal populations inhabit a milder climate than mountain populations where lakes often freeze over for as much as half the year. Thus, mountain populations may be forced to complete the sexual phase on an annual basis, much like temporary-pond populations of *Daphnia.* Annual bouts of sex can lead to the maintenance of higher levels of expressed genetic variation and heterozygosity levels closer to Hardy-Weinberg expectations; both of these characteristics are exhibited by the mountain populations. The fact that the reduced genetic variation in coastal populations is not re-

TABLE 3. Summary table of quantitative and molecular genetic variation within and among populations of *Daphnia pulicaria* and *D. pulex* including two regional groups for each species (standard errors in parentheses). Molecular parameter estimates are based on pooled allozyme and microsatellite data.

	Daphnia pulicaria	
	Coast	Mountain
H^2	0.03 (0.03)	0.24 (0.03)
H_E	0.26 (0.07)	0.24 (0.07)
Q_{SR}	0.78 (0.06)	0.36 (0.09)
G_{SR}	0.29 (0.04)	0.17 (0.04)
	Daphnia pulex	
	Northern	Southern
H^2	0.24 (0.10)	0.12 (0.11)
H_E	0.45 (0.02)	0.26 (0.02)
Q_{SR}	0.30 (0.09)	0.15 (0.09)
G_{SR}	0.15 (0.02)	0.34 (0.06)

flected at the molecular level suggests that there may be hidden variation for quantitative characters in coastal populations that could be released by genetic slippage following a bout of sexual reproduction (depending on the direction of linkage disequilibrium; Lynch and Deng 1994). The population phylogeny indicates no geographic pattern, which further supports the idea that the difference in genetic architectures between coastal and mountain regions reflects differences in habitat related to the frequency of sexual reproduction rather than more long-term patterns of evolutionary relationship.

In the temporary pond species, *D. pulex,* we see a different pattern of relationship between regional population groups, with the southern populations exhibiting reduced levels of molecular variation relative to northern populations, with no clear distinction between groups in the level of genetic variation maintained at the quantitative trait level (Lynch et al. 1999; Table 3). The low molecular variation in southern populations suggests that they may have experienced a bottleneck at some time in the past or that migration into the southern coastal region is low. This scenario is further supported by the population phylogeny in which it is shown that the regional groups of populations represent distinct clades (Lynch et al. 1999). Similar levels of quantitative genetic variation between *D. pulex* clades suggests that sufficient time has passed to regenerate variation at this level.

Average levels of subdivision for neutral, single-locus molecular markers provide a neutral expectation for the level of genetic subdivision for quantitative traits in the same populations (Felsenstein 1986; Lande 1992; Spitze 1993). Therefore, comparison of character-specific quantitative genetic estimates of subdivision to molecular genetic subdivision can be informative about selection pressures on particular traits among populations. Levels of quantitative genetic subdivision among coastal populations are high, with an average of 78% of the genetic variance distributed among populations. Estimates of quantitative genetic subdivision exceed estimates of subdivision at neutral loci for all life-history characters except size at birth and growth rates, which is consistent with strong divergent selection for the majority of fitness characters. Thus, given prolonged periods of clonal selection apparent on the coast, populations appear to diverge more rapidly for fitness-related characters than for molecular markers. Among mountain populations, estimates of Q_{SR} are lower, with an average value of 0.36. There appears to be divergent selection among mountain populations for body size, growth rates, and clutch size. However, estimates of subdivision for ages at reproduction fall well below those for molecular markers, suggesting purifying selection for reproductive timing in the mountain region. This pattern for mountain populations of *D. pulicaria* is similar to that observed among populations of Oregon *D. pulex* (Lynch et al. 1999).

Estimates of inbreeding coefficients differ between the two marker types with allozyme loci more frequently exhibiting heterozygote excesses, but the G_{IS}-values for allozyme and microsatellite loci are strongly correlated. Allozyme loci have been shown to be quasi-neutral in *Daphnia,* with fluctuating positive and negative selection (Lynch 1994). Fluctuating directional selection could lead to the observed tendency toward heterozygote excesses at allozyme loci (Falconer and Mackay 1996).

The correlation between H^2 and average G_{IS}-values indicates lower levels of expressed genetic variation for quantitative traits in association with stronger heterozygote excesses. In separate studies, both reduced heritabilities (e.g., Lynch 1984) and increased heterozygote excess (Hebert 1974; Young 1979a,b) have been shown to result from periods of clonal selection. Thus, this association between H^2 and G_{IS} may represent the effects of the duration of clonal selection at the quantitative trait and molecular levels. The direction of this relationship is upheld in both coastal and mountain regions, although it is not significant for either in isolation.

Temporary-pond populations of *D. pulex* from Oregon have been shown to deviate from Hardy-Weinberg expectations in the direction of homozygote excess likely as a result of population subdivision within ponds (a Wahlund effect; Lynch et al. 1999). It was also shown that the degree of local inbreeding (as indicated by average heterozygosity levels) was associated with population mean phenotypes for life-history characters in a direction consistent with observed effects of inbreeding in laboratory experiments. In *D. pulicaria,* populations tend to deviate from Hardy-Weinberg expectations in the direction of heterozygote excess, and heterozygosity is not associated with mean phenotype for any life-history trait. This is exemplified by the lack of a significant difference between coastal and mountain populations in terms of mean phenotype, whereas the coastal populations exhibit heterozygote excess for allozyme loci and the mountain populations do not.

An understanding of the utility and limitations of molecular information for predicting the evolutionary potential of natural populations is clearly of importance in both evolutionary and conservation genetics. Although molecular variation appears to be an inadequate measure of genetic variation within populations for characters with direct relevance to fitness, subdivision among populations for molecular markers may be informative as to the expected subdivision for fitness-related quantitative traits. However, this should be put into practical use with some caution, because biologically relevant levels of subdivision can exist for populations that do not

exhibit significant differentiation at the molecular level (Yang et al. 1996; Kremer et al. 1997; Lynch et al. 1999).

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