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Lack of concordance between genetic diversity estimates at the molecular and quantitative-trait levels

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Abstract

In many applications of population genetics, particularly in the field of conservation biology, estimates of molecular diversity are used as surrogate indicators of less easily acquired measures of genetic variation for quantitative traits. The general validity of this approach to inferring levels of quantitative genetic variation within populations is called into question by the demonstration that estimates of molecular and quantitative-genetic variation are essentially uncorrelated in natural populations of *Daphnia*, one of the few organisms for which multiple estimates of both quantities are available. On the other hand, molecular measures of population subdivision seem to give conservatively low estimates of the degree of genetic subdivision at the level of quantitative traits. This suggests that although molecular markers provide little information on the level of genetic variation for quantitative traits within populations, they may be valid indicators of population subdivision for such characters.

Introduction

A fundamental mission of programs in endangered species management is the maintenance of genetic variation for characters that are likely to be the targets of current or future selective pressures. In a changing environment, populations that are depauperate with respect to genetic variation for adaptive characters have a heightened vulnerability to extinction (Lynch and Lande 1993; but see Lande and Shannon 1996). While adaptations may be due to mutations of large effect, the divergence of quantitative traits associated with differences between populations and the adaptive response to selective pressures within populations often has a polygenetic basis (Wright 1968; Lande 1981; Coyne 1985). Since the ability of a population to respond to novel selective pressures is proportional to the additive genetic variation underlying the traits that are the target of selection (Falconer and Mackay 1996), maintenance of additive genetic variation is a worthy objective of any conservation program. Unfortunately, direct quantification of the level of genetic variation for traits underlying life histories, morphology, physiology, and behavior is technically difficult. Statistical considerations indicate that such characters are often a function of dozens to hundreds of loci (Wright 1968; Lande 1981), but in only a few cases is the identity of even a single quantitative-trait locus known in a natural population (Long et al. 1998; Lyman and Mackay 1998). To complicate matters further, the phenotypic expression of most polygenic characters has a large environmental component (Wright 1968; Falconer and Mackay 1996). Methods based on the resemblance between relatives can be used to partition the phenotypic variance of a trait into its genetic and environmental components, and these have long been used with success in studies of domesticated species (Wright 1968; Falconer and Mackay 1996). However, almost all empirical studies in conservation genetics

rely on surrogate molecular markers, whose expression is unmodified by the environment, as general indicators of genetic variation (Bonnell and Selander 1974; O'Brien et al. 1985; Avise 1989; Gilbert et al. 1991; Wayne et al. 1991; Ciofi and Bruford 1999).

Molecular genetic techniques have taken a prominent role in the analysis of endangered or threatened species. In particular, there are three areas where information on the patterns of molecular genetic variation has a demonstrated utility. The application of these techniques has played an invaluable role in the identification of ESUs (Waples 1991) and MUs (Moritz 1994). They provide quantitative measures of admixture and hybridization (Allendorf and Waples 1995; Wayne 1995), and in the context of captive breeding programs, molecular genetic techniques form the basis of pedigree analysis used to minimize inbreeding between founders (Haig et al. 1994, 1995). However, the degree to which measures of molecular genetic diversity map to measures of variation for quantitative traits has seldom been evaluated empirically and theoretical considerations suggest that the concordance may be weak (Lande and Barrowclough 1987; Lynch 1996). Recent studies on a captive Cotton-top tamarin population (Cheverud et al. 1994) and an endangered plant (Waldmann and Andersson 1998) are consistent with this expectation.

Although molecular variants are sometimes subject to weak selective forces (Gillespie 1991), they are often effectively neutral (Kimura 1983), in which case standing levels of molecular heterozygosity and population subdivision primarily reflect the outcome of the joint operation of random genetic drift, migration, and mutation. Letting N be the effective population size, the expected heterozygosity of a neutral locus is reduced by a factor of 1/2N each generation by random genetic drift. It is replenished at the rate of 2μ, where μ is the genic mutation rate per generation, such that the average heterozygosity at equilibrium is approximately $4N\mu$ (Kimura 1983). For a character with an additive basis, the genetic variance is reduced by drift at exactly the same rate as heterozygosity. However, the expected heritability (fraction of the phenotypic variation that is attributable to genetic causes) of such a quantitative character is replenished at the rate of approximately V_m / V_e per generation, where V_m is the rate at which mutation introduces new quantitative variation (Hill 1982; Lynch and Hill 1986; Burger et al. 1989; Houle 1989) and V_e is the environmental component of the phenotypic variance of the trait. Since μ is often on the order of 10^{-6} or smaller, while V_m/V_e is on the order of 10^{-4} to 10^{-2} (Lande 1975; Lynch 1988; Keightley and Hill 1992; Lynch and Walsh 1998), when the effective population size is small (as it is in populations of endangered species), the heterozygosity of most loci may be essentially undetectable (less than 0.001), when the heritability for quantitative traits is above the detectable level (greater than about 0.10). For quantitative traits with a non-additive genetic basis, such as many fitness-related traits (Falconer and Mackay 1996; Lynch and Walsh 1998), the relationship between heterozygosity and heritability becomes even less predictable, since in this case components of genetic variance can often increase with random drift (Robertson 1952; Goodnight 1988).

A good correspondence between allozyme heterozygosity and heritability has been reported for laboratory populations of *Drosophila* (Briscoe et al. 1992), but the character investigated (bristle number) is known to have an additive genetic basis and to behave in an essentially neutral fashion. Numerous studies have considered the relationship between genetic variation at the molecular level and phenotypic variation within natural populations, but such studies have little bearing on the issues that concern us here. Phenotypic variation is often largely a function of environmental and developmental noise, and substantial levels of such nonheritable variation can exist in the complete absence of genetic variation for the trait. Moreover, arguments have been made that the environmental component of variance often increases with decreasing genetic variation, due to the reduction of developmental homeostasis with increasing homozygosity (Lerner 1954; Soule 1981). These and other factors virtually insure that measures of phenotypic variation will bear little resemblance to levels of genetic variation underlying a quantitative trait (Willis et al. 1991).

This paper takes an empirical look at this issue, drawing on parallel studies of levels of genetic variation for enzymes, microsatellites, body-size, and life-history characters in natural populations of the aquatic microcrustacean *Daphnia*. We have reported previously on allozyme variation and heritabilities of body-size and life-history traits for three populations of midwestern *D. pulex* (Lynch 1984; Lynch et al. 1989) and eight populations of *D. obtusa* (Spitze 1993). In this report, we examine data obtained more recently from seventeen populations of western *D. pulex* (Lynch et al. 1999), and fourteen of western *D. pulicaria*.

Methods

Quantitative genetic analysis

The methods employed in these studies are essentially the same as those described in detail elsewhere (Lynch 1984; Lynch et al. 1989, 1999; Spitze 1993). Briefly, multiple clones were collected from natural pond-dwelling populations of *D. pulex* (Lynch et al. 1999) and lake-dwelling populations of *D. pulicaria* (Morgan KK in preparation) in western and central Oregon, USA. 75–100 individuals from each population were isolated and propagated in the laboratory by clonal reproduction. From each population 50–60 clones were randomly selected for a standard life-table experiment. Two replicate individuals from each clone were grown under controlled temperature and light conditions on a fixed food concentration, and monitored daily for growth and reproductive characters including age at first reproduction, instar-specific adult body size, and instar-specific clutch size. Each replicate line was started with a single immature female. All replicates were maintained under the assay conditions for two generations prior to measurement of life history and morphological characters. This procedure insures that any maternal (and grandmaternal) effects contribute to the within- rather than the among-clone component of variance (Lynch 1985). A one-way analysis of variance was used to partition the phenotypic variance for individual traits into within- and among-clone components, the latter being equivalent to the total genetic variance (σ_g^2) and the former to the environmental variance (σ_e^2) of the trait. The broadsense heritability of a trait is defined to be $H^2 = \sigma_g^2 / \sigma_t^2$, where $\sigma_t^2 = \sigma_g^2 + \sigma_e^2$ is the total phenotypic variance. The degree of population subdivision for each trait, Q_{st}, was estimated following Lynch et al. (1999) after extracting estimates of the among-population genetic variance by nested analysis of variance. Standard errors of the genetic parameter estimates were obtained by use of expressions derived by the delta method (Lynch and Walsh 1998).

Molecular genetic analysis

From each population information was gathered on two nuclear sets of markers. 24–30 individuals from each population were characterized with six polymorphic microsatellite and thirteen allozyme loci following the methods described in Lynch et al. (1999). For the microsatellite analysis, DNA was extracted from single *Daphnia* by the Chelex method (Lehman et al. 1995) using either fresh or ethanol preserved individuals. PCR reactions were carried out in 12.5 μl volumes using primers designed for midwestern *D. pulex* (Lynch et al. 1999). To visualize the microsatellite alleles a single primer from each pair was either endlabled with ^{32}P or modified to include a M13 sequence. Endlabled PCR products were run on 6% polyacrylamide gels and visualized by autoradiography. PCR products including the M13 sequence were IR labeled and visualized on a Li-Cor 4200 LongRead IR automated sequencer. Representative genotypes were visualized on both systems to standardize scoring.

Allozyme loci were scored for all populations by cellulose-acetate electrophoresis (Hebert and Beaton 1989). Alleles were characterized by relative mobility, calibrated against a standard *Daphnia* genotype.

Gene diversity estimates at the molecular level were obtained for each nuclear gene locus as the within-population heterozygosity (H_w) expected under random mating and averaged over all observed loci (Nei 1987). 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. Final estimates of G_{st} (and their standard errors) were obtained by averaging over each of the locus specific estimates.

Results

We observed no significant relationship between heritability and gene diversity estimates from seventeen populations of western *D. pulex* and fourteen populations of western *D. pulicaria* (Figure 1). The least square regressions of population mean heritability and gene diversity (based on combined allozyme and microsatellite loci) for body size, clutch size, and age at reproduction were all non-significant and accounted for little of the variation in either *D. pulex* (p = 0.73, 0.46, and 0.22; r^2 = 0.008, 0.038, and 0.097) or *D. pulicaria* (p = 0.69, 0.75, and 0.77; r^2 = 0.013, 0.008, 0.008). Separate analyses of microsatellite and allozyme loci yielded qualitatively similar results. We observed no significant linear relationships between population mean heritability and gene diversity.

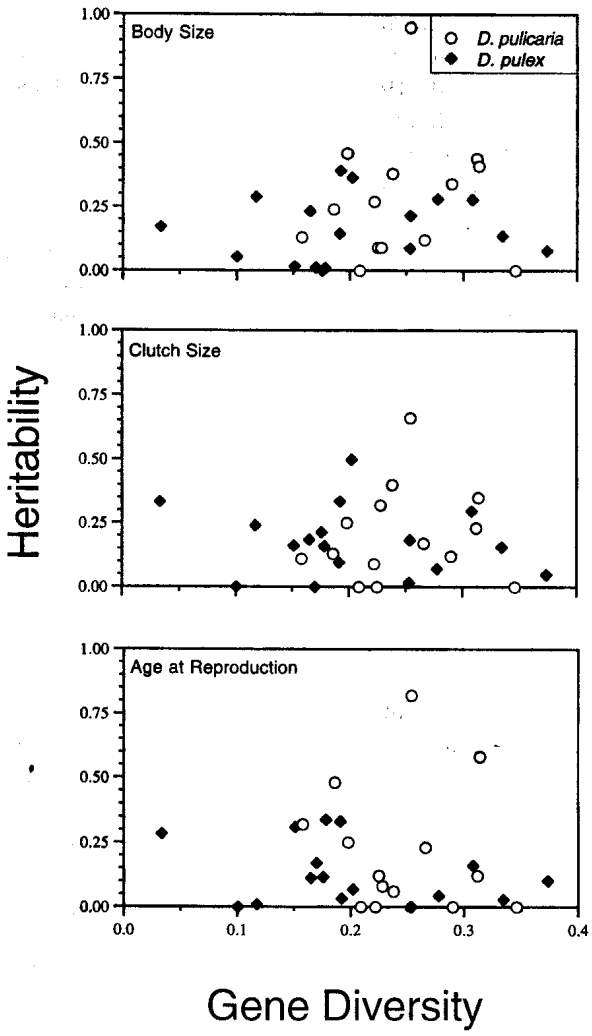

Figure 1. Relationship between heritabilities for quantitative traits and within- population gene diversities (heterozygosity) for molecular markers. Each point represents a population. The estimates of heritability for body size are the averages of the heritabilities estimated for 5 instar-specific lengths, while those for clutch size and age at reproduction are averages of instar-specific estimates for the first four adult instars.

Since our heritability estimates are each based on 50–60 clones they are only estimates of the population parameters, so the true relationship between H^2 and H_w is likely to be better than what we have estimated. However, after removing the average sampling variance of the heritability estimates from the residual variance about the regressions, the r^2 values for the regression of body size, clutch size and age at reproduction against gene diversity are still only 0.009, 0.041, and 0.103 respectively, for *D. pulex* and 0.014, 0.009, 0.008 respectively, for *D. pulicaria*. An additional source of bias in the regression is sampling variance due to sampling of loci. Estimates of H_w based on other sets of loci would almost certainly be different. Accounting for this source of uncertainty, in addition to that due to the heritability estimates, still only raises the r^2 values to 0.010, 0.043, and 0.109 for pond- and 0.016, 0.010, and 0.009 for lake-dwelling species. These values are well below any reasonable level of significance for 16 and 13 degrees of freedom.

Table 1. Estimates of population subdivision at the levels of nuclear molecular markers (G_{st}) and quantitative-genetic (Q_{st}) variation. G_{st} and Q_{st} are the fractions of the total genetic diversities that are attributable to the among-population component of variation. Standard errors are given in parentheses

	D. pulex	*D. pulicaria*
Q_{st}	0.350 (0.070)	0.449 (0.062)
G_{st}	0.295 (0.035)	0.390 0(.060)

Estimates of population subdivision based on nuclear molecular markers (G_{st}) and quantitative genetic variation (Q_{st}) are shown in Table 1. We observed no significant differences between these measures of subdivision in either *D. pulex* or *D. pulicaria*.

Discussion

The most striking feature of these studies is the lack of correlation observed between genetic diversity indices. These results are consistent with those obtained previously for *D. obtusa* (Spitze 1993) in which the regressions for body size, clutch size, and age at reproduction explained 0.9, 2.6, and 3.6% of the variation respectively. Because our heritability measures are based on 50–60 clones, and our gene diversity measures are based on a small subset of the genome, they are only estimates of the population parameters. Thus, the true relationship between H^2 and H_w is likely to be different than what we have estimated. However, after accounting for possible downward bias of the regressions due to sampling of individuals and loci, we maintain our conclusion that measure of within-population genetic diversity for quantitative characters are essentially unrelated to those at the molecular level. The concordance of these separate studies indicates that for *Daphnia* populations this result may be a general one. Since *Daphnia* populations are typically large and are likely at or near

equilibrium the question is, what can be said about taxa that are threatened or endangered and have small effective populations sizes?

In small or recently bottlenecked populations, as is the case for many threatened and endangered taxa, genetic variation for both molecular markers and quantitative traits may be influenced more by the effects of drift than selection. The influence of drift on quantitative traits will act to reduce the correlation between estimates of molecular and quantitative-genetic variation in a number of ways. First, for the case of a quantitative trait with a purely additive genetic basis, the components of quantitative-genetic variance can drift substantially above or below their expected values (López-Fanjul et al. 1989). Second, when there is substantial nonadditive genetic variation underlying the expression of quantitative traits, the frequencies of alleles in interacting genes may be altered leading to an increase in expected additive genetic variance. This effect has been observed in bottlenecked populations of house flies (Bryant et al. 1986; Bryant and Meffert 1993) and fruit flies (López-Fanjul and Villaverde 1989). Taken together these factors suggest that unless molecular marker loci are tightly associated with quantitative trait loci (QTLs) they will provide little information regarding genetic variation underlying quantitative traits. There is a recent emphasis on applications of a QTL approach in conservation settings (Marmiroli et al. 1999), and further development of this approach will certainly aid our ability to correlate relatively easily measured molecular variation with genetic variation for quantitative trait expression in small populations.

Measures of molecular diversity among populations may be more informative. In three separate experiments involving 8 populations of *D. obtusa* from the midwest US (Spitze 1993), 14 western *D. pulicaria* and 17 populations of *D. pulex* from eastern Oregon (Lynch et al. 1999) we performed simultaneous life-table analyses of multiple clones, and then partitioned the total phenotypic variance into components due to populations, clones within populations, and individuals within clones. For the same sets of populations, we estimated the degree of population subdivision at the molecular level by use of the index G_{st}, which measures the fraction of the total gene diversity in a sample of populations that is attributable to the among-population component (Nei 1987). Q_{st}, the analogous measure for quantitative characters, is the fraction of the total genetic variation (that among populations plus that among clones within populations) that is attributable to the among- population component (Spitze 1993; Lynch et al. 1999). Note that this estimate of subdivision for a quantitative character is not a function of micro- environmental sources of variation, which contribute only to the within-clone component of variance. In principle, however, Q_{st} can be biased either upwardly or downwardly by genotype x environment interaction at the level of population mean phenotypes.

The estimates of Q_{st} are essentially equal to G_{st} (Table 1). Thus, for *Daphnia*, estimates of population subdivision based on molecular markers tend to be equivalent to estimates of population divergence at the quantitative-trait level. Since the divergence of allozyme frequencies in *Daphnia* is actually greater than the neutral expectation, due to the cumulative influence of fluctuating selection (Lynch 1987), these results suggest that selection contributes to divergence of mean phenotypes in the study populations.

Our quantitative-genetic analyses have been designed to eliminate confounding factors by raising all individuals in a common garden environment and isolating genetic from environmental sources of variation by analysis of variance. Although this study is based on a relatively small number of experiments within a single group of organisms, we can only conclude that measures of molecular heterozygosity provide little information about genetic variation for quantitative traits within natural populations. This does not rule out the need for molecular markers in conservation-genetic analysis, but rather points to an alternative application. For numerous endangered species, particularly in captive breeding programs, molecular markers are now used routinely in pedigree analysis. Once acquired, information on relatedness, combined with phenotypic measures can be used to estimate levels of genetic variance for quantitative traits in precisely the same way the heritability estimates are routinely obtained in domesticated species (Henderson 1984; Ritland 1990; Hedrick 1996; Lynch 1996; Storfer 1996). Such analyses are essential to evaluate whether species, such as the cheetah and the elephant seal, that are obviously depauperate in molecular variation are also devoid of genetic variation for adaptive characters. These considerations suggest that for small populations the relative amounts of molecular genetic variation may not be good criteria for management considerations when the objective is future adaptability. Since rate of loss of genetic variation in a small population is an inverse function of the effective population

size, larger populations have larger evolutionary potential. Additionally, as population size increases, the efficiency of selection increases and the role of drift as an evolutionary force is diminished. As a result, larger populations have an increased potential to adapt to environmental challenges.

Significant among-population divergence at the molecular level indicates that an opportunity exists for population subdivision at the level of quantitative characters. Other joint analyses of population divergence at the molecular and quantitative-genetic level reveal a strong correlation between Q_{st} and G_{st} ($r = 0.88$, $df = 8$, $P < 0.01$) and in every case the level of subdivision for quantitative traits is equal to or exceeds that for molecular markers (Lynch et al. 1999). These results suggest that the levels of population subdivision for molecular markers may generally underestimate the levels of population subdivision for quantitative traits, and indicate that measures of divergence based on neutral (or quasi-neutral) molecular markers may provide a conservative indication of the levels of genetic divergence for quantitative traits. That is, significant subdivision at the relatively neutral molecular level may provide a strong indication that genetic subdivision of an equal or greater magnitude exists at the level of quantitative traits upon which selection is likely to be acting. This provides some support for the use of molecular divergence as a guide in listing specific populations under the US Endangered Species Act (Waples 1991; Hedrick 1996).

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