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MOLECULAR AND QUANTITATIVE TRAIT VARIATION WITHIN AND AMONG POPULATIONS OF THE INTERTIDAL COPEPOD *TIGRIOPUS CALIFORNICUS*

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Abstract.—While molecular and quantitative trait variation may be theoretically correlated, empirical studies using both approaches frequently reveal discordant patterns, and these discrepancies can contribute to our understanding of evolutionary processes. Here, we assessed genetic variation in six populations of the copepod *Tigriopus californicus*. Molecular variation was estimated using five polymorphic microsatellite loci, and quantitative variation was measured using 22-life history and morphometric characters. Within populations, no correlation was found between the levels of molecular variation (heterozygosity) and quantitative variation (heritability). Between populations, quantitative subdivision (Q_{ST}) was correlated with molecular subdivision when measured as F_{ST} but not when measured as R_{ST}. Unlike most taxa studied to date, the overall level of molecular subdivision exceeded the level of quantitative subdivision ($F_{ST} = 0.80$, $R_{ST} = 0.89$, $Q_{ST} = 0.30$). Factors that could contribute to this pattern include stabilizing or fluctuating selection on quantitative traits or accelerated rates of molecular evolution.

Key words.—F_{ST}, genetic architecture, life-history characters, microsatellite loci, morphology, Q_{ST}, R_{ST}.

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The ability of a population to respond to a changing environment depends on additive genetic variation for ecologically relevant quantitative characters. However, data on such characters are rare for most species due to the technical difficulties of partitioning phenotypic variation into its genetic and environmental components. Instead, the vast majority of studies rely on molecular markers as a surrogate for adaptive genetic variation. This has led to considerable recent interest in the extent to which molecular and quantitative genetic variation are correlated (Pfrender et al. 2000; Merilä and Crnokrak 2001; Reed and Frankham 2001; McKay and Latta 2002).

Within populations, heterozygosity is theoretically expected to be linearly related to additive genetic variance (Falconer and McKay 1996). If this relationship is reliable, then molecular heterozygosity could be used as a convenient predictor of a population's ability to respond to new environmental challenges. A variety of complicating factors can erode this relationship, however, including nonadditive gene action, conversion of genetic variance components by population bottlenecks, differential selection, different mutation rates, and environmental effects (Reed and Frankham 2001). Although heterozygosity may correlate with heritability in those few cases that meet all the assumptions (e.g., Brisoe et al. 1992), many empirical studies show little correspondence. In fact, a recent meta-analysis of 71 datasets found no significant correlation between heterozygosity and heritability with a mean regression coefficient of −0.08 (Reed and Frankham 2001).

The extent to which molecular markers reflect quantitative genetic subdivision between populations is also disputable. A literature review by Merilä and Crnokrak (2001) found a strong positive relationship between molecular (F_{ST}) and quantitative (Q_{ST}) subdivision across studies of a variety of species. However, a subsequent review by McKay and Latta (2002) found no significant correlation and detailed a number of theoretical reasons (e.g., selection, mutation, epistasis) why no such relationship should be expected. This prompted a debate over the appropriate choice of studies and methods (Crnokrak and Merilä 2002; Latta and McKay 2002). Relationships between F_{ST} and Q_{ST} might be expected to be weaker in intraspecific studies, where the range of values is typically smaller than interspecific studies. Of the very few intraspecific studies reported to date, positive correlations between molecular and quantitative subdivision have been found in the annual plant *Senecio vulgaris* (Steinger at al. 2002) and in two species of the microcrustacean *Daphnia* (Lynch et al. 1999; Morgan et al. 2001) but not in the common frog *Rana temporaria* (Palo et al. 2003).

Also at issue is the magnitude of F_{ST} versus Q_{ST}. The majority of studies find that Q_{ST} exceeds F_{ST}, suggesting the effects of diversifying selection (Lynch et al. 1999; Merilä and Crnokrak 2001; McKay and Latta 2002; Rogers et al. 2002; Storz 2002; Palo et al. 2003). Some have suggested that inflated quantitative divergence is less likely in recently diverged populations where selection has had little time to act (Whitlock 1999). However, others have noted this inflation is reduced in highly subdivided populations (Lynch et al. 1999), possibly because there is little room for Q_{ST} to exceed F_{ST} as both measures approach unity (Hendry 2002). Clearly, further work on the empirical relationships between molecular and quantitative genetic variation is warranted.

The copepod *Tigriopus californicus* is a particularly interesting candidate for studies of genetic architecture because its populations show extreme molecular subdivision, with mitochondrial sequence divergence in some cases exceeding 20% (Burton and Lee 1994; Burton 1998; Edmands 2001). Although levels of quantitative variation had never been reported, the species short generation time and ease of husbandry makes it amenable to quantitative trait measurement under controlled laboratory conditions. In addition to assessing the relationship between molecular and quantitative trait variation, the present study focuses on comparisons between northern and southern regions. A mitochondrial phy-

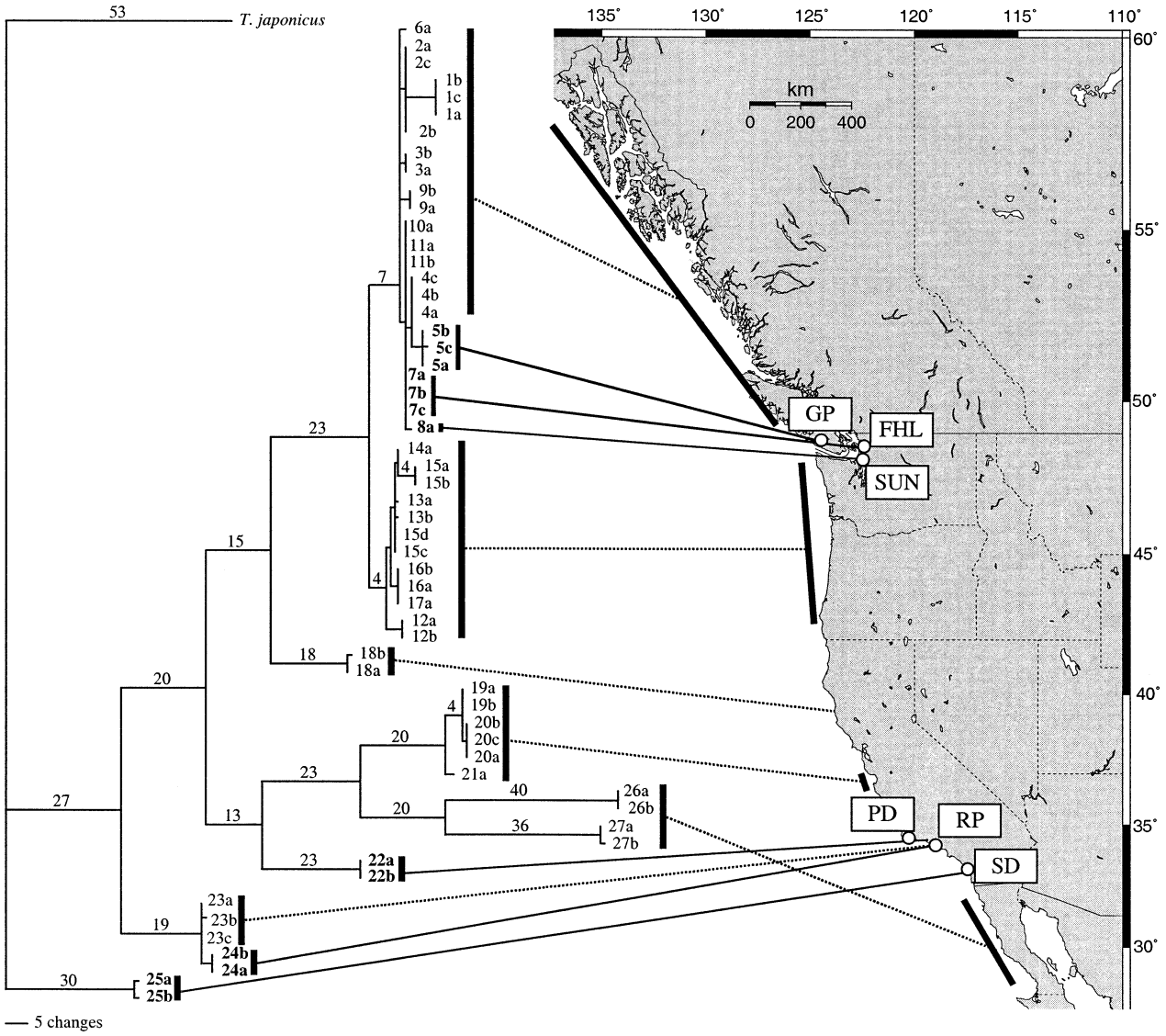

FIG. 1. Phylogeny based on a 395-base segment of cytochrome oxidase I showing reduced interpopulation differentiation at higher latitudes. The tree shown is one of eight equally parsimonious tress of length 440 and consistency index 0.555. Populations are numbered from north to south, and numbers along branches indicate the number of substitutions (values less than four were omitted for visual clarity). Data come from Edmands (2001) except for the SUN population (D. Peterson and S. Edmands, unpubl. data) and the *Tigriopus japonicus* sequence used as an outgroup (Machida et al. 2002). The six populations used in the present study are shown in bold type with solid lines.

logeny for the entire species range shows that populations from Oregon northward are derived and have interpopulation divergences five times lower than those between southern populations (Edmands 2001; Fig. 1). Reduced differentiation at higher latitudes is a pattern common to a variety of taxa and may be driven by recent colonization of high-latitude areas following glaciation (Reid 1990; Hewitt 1996; Armbruster et al. 1998; Avise and Walker 1998). Here, we look at whether northern populations also have reduced subdivision for quantitative characters, and whether either molecular or quantitative variation is reduced within populations. Previous molecular work on *T. californicus* relied primarily on allozymes and mitochondrial DNA sequences (e.g., Burton and Lee 1994), both of which show little variation within populations. In this study we present the first data on microsatellite variation in the species.

MATERIALS AND METHODS

Population Sampling and Culture Maintenance

Copepods were collected from high intertidal pools at three northern locations in British Columbia and Washington and three southern locations in southern California (Fig. 1). The sampling locations were Grappler Point, Vancouver Island, British Columbia (GP, 48°50′N, 125°08′W); Friday Harbor Laboratories, San Juan Island, Washington (FHL, 48°33′N, 123°00′W); Sunset Beach, Anacortes, Washington (SUN, 48°30′N, 122°42′W); Point Dume, Malibu, California (PD,

34°00′N, 118°48′W); Royal Palms, Palos Verdes, California (RP, 33°42′N, 118°19′W); and San Diego, California (SD, 32°45′N, 117°15′W). Samples from pools within the same rock outcrop were combined because previous work shows extensive gene flow within outcrops (Burton and Swisher 1984). Samples were collected in October and November 2001 and were shipped to the laboratory within three days of collection. A subset of the animals was immediately frozen at −80°C for microsatellite analyses and the remaining animals were cultured under common conditions.

Cultures were established by placing 50 gravid females in a 250-ml beaker containing 200 ml filtered (37 μm) seawater supplemented with 0.04 g finely ground *Spirulina* algae flakes. All cultures were kept at 20°C with a 12:12 L:D photoperiod. Every two weeks, half of the water in each beaker was replaced by fresh seawater supplemented with *Spirulina*. All cultures were maintained under these conditions for 7–10 weeks (about two generations) before the crossing experiments began.

Microsatellite Analyses

An enriched DNA library from the Royal Palms population was used for microsatellite isolation and primer development (S. Harrison, D. Peterson, and S. Edmands, unpubl. data). Twelve microsatellite loci were initially screened in all six populations. Five of these loci were found to be polymorphic within at least one population and these five were then surveyed in 50 individuals from each population (these were not the same individuals used for analyses of quantitative traits). The five loci used were di- and tetranucleotide repeats, with total fragment sizes ranging from 100 to 223 bp.

DNA for microsatellite assays was extracted from individual copepods by incubation in 25 μl proteinase-K cell-lysis buffer for 1 h at 65°C followed by 15 min at 99°C (see protocol in Lee and Frost 2002). Polymerase chain reactions (PCRs) were carried out in 10-μl final volumes with 2.5 mM $MgCl_2$. Reactions were composed of a 4-min denaturation at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 55–60°C (depending on the locus), and 30 sec at 72°C, finally followed by a 5-min extension at 72°C.

PCR products (2–3 μl + 0.4 μl loading dye) were run on small (15cm × 17 cm × 0.8 mm), nondenaturing TAE polyacrylamide gels, with the lower portion of the gel supplemented with the polymer Spreadex (Elchrom Scientific, Jamaica Estates, NY). Conditions were optimized for different fragment ranges. Fragments ranging from 100 to 150 bases were run on 7% gels, whereas fragments between 160 and 223 bases were run on 6% gels. Gels were run for 2 h at 250 V and were then stained with ethidium bromide and visualized on a UV light box.

Observed and expected heterozygosities were calculated for each locus in each population and deviations from Hardy-Weinberg equilibrium were tested following the procedure described in Guo and Thompson (1992). Molecular subdivision among populations was calculated in two ways. First, F_{ST} was estimated using both ARLEQUIN (Schneider et al. 2000) and FSTAT version. 2.9.3 (Goudet 1995). Second, a program by Goodman (1997) was used to estimate R_{ST}, an analog of F_{ST} that assumes loci evolve according to the stepwise mutation model. Standard errors for both parameters were calculated by averaging over variance components. Molecular subdivision was assessed among all populations, among populations within regions, between each pairwise combination of populations, and between regions (F_{RT}, Weir and Cockerham 1984).

Quantitative Trait Measurement

A paternal half-sibling design was used to avoid maternal effects. In *T. californicus*, mature males clasp prereproductive females as a form of mate-guarding behavior (Egloff 1967; Burton 1985). Males and virgin females are therefore easily obtained by teasing these pairs apart using a fine probe. One male and two randomly assigned virgin females from the same population were placed in each petri dish containing 15 ml filtered seawater with 3 mg finely ground *Spirulina*. Fifty of these ''families'' were set up for each of the six populations. Each dish was monitored daily. The single male would fertilize both females once they reached maturity. Two to four days later the female would produce her first egg sac. On the day this egg sac was produced, the female was placed in a new petri dish containing fresh seawater with *Spirulina*. On the day the clutch of eggs hatched, the number of live nauplii (larvae) was counted under a dissecting microscope by individually pipetting each nauplius into a new dish. Seven days later, the number of live nauplii and copepodids (metamorphosed juveniles) was counted by pipetting individuals into a new dish. Dishes continued to be monitored until the first female produced an egg sac. These observations resulted in five life-history measurements: hatching time, hatching number, survivorship number, metamorphosis number, and minimum generation time.

On the day the first female in a brood produced an egg sac, the female and a haphazardly chosen male were removed for morphometric measurements. Animals were anesthetized in 14% $MgCl_2$, and egg sacs were separated from females before measurements were done. All measurements were made on a Leica (Buffalo, NY) MZ12 dissecting microscope at a magnification of 32X. Video images were captured and measured using Optimas 5.2 (http: //www.optimas.com/optimas.htm). Absolute size was calibrated using a stage micrometer. The following seven measurements were taken in both males and females: cephalothorax length and width, urosome length and width, telson width, first antennule width, and caudal seta length. In addition, clasper width was measured in males and diameter and area was measured in egg sacs. Landmarks for the morphometric measurements are available from the authors upon request. Because of losses caused by mortality, sample sizes for quantitative traits varied, and the mean number of individuals scored for each trait in each population was 70.9.

Analyses of quantitative trait data were done using Statistica 5.5 (StatSoft, Tulsa, OK). Phenotypic variation among populations was assessed by analysis of variance (ANOVA) with populations nested within regions. To calculate heritabilities, one-way ANOVA was performed for each population. Following Lynch and Walsh (1998), the paternal half-sibling estimator of heritability is $4V_S/V_T$, where V_S is variance among sires and V_T is total variance. Variance com-

TABLE 1. Expected heterozygosity (SE) for each locus in each population.

Population	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	All loci
GP	0.358	0.000	0.519	0.203	0.000	0.216 (0.101)
FHL	0.265	0.000	0.728	0.000	0.000	0.199 (0.142)
SUN	0.000	0.000	0.000	0.000	0.000	0.000 (0.000)
PD	0.000	0.000	0.000	0.641	0.000	0.128 (0.128)
RP	0.480	0.569	0.000	0.449	0.185	0.337 (0.106)
SD	0.134	0.655	0.203	0.000	0.000	0.198 (0.121)
All populations	0.206	0.204	0.242	0.216	0.031	0.180 (0.038)
Northern populations	0.208	0.000	0.416	0.068	0.000	0.138 (0.079)
Southern populations	0.205	0.408	0.068	0.363	0.062	0.221 (0.072)

ponents were obtained by equating observed mean squares (MS) with their expectations such that $V_S = (MS_{SIRE} - MS_{ERROR})$/family size and $V_T = V_S + MS_{ERROR}$. Data were also analyzed by nested ANOVA with individuals nested within families within populations. The degree of genetic population subdivision, Q_{ST}, was calculated as $V_{GB}/(V_{GB} + 2V_{GW})$ following Spitze (1993), where V_{GB} is the genetic variance distributed among populations, and V_{GW} is the genetic variance within populations, which is calculated as four times the mean V_S given above. Subdivision within regions, between regions, and between each pair of populations was estimated by independent nested analyses of variance. Coefficients for expected mean square were adjusted for inequalities caused by mortality.

RESULTS

Molecular Variation

Microsatellite variation within populations (Table 1) was generally low, with mean expected heterozygosities ranging from 0.0 in population SUN to 0.337 ± 0.106 (SE) in population RP. No significant deviations from Hardy-Weinberg expectations were detected. Levels of expected heterozygosity tended to be lower in the north (0.138 ± 0.079) than in the south (0.221 ± 0.072), although the difference was not significant (unpaired, two-tailed *t*-test; $t_{28} = -0.92$, $P = 0.366$). Substantial variation was found among populations (Table 2), with a mean F_{ST} of 0.803 ± 0.044 and a mean R_{ST} of 0.890 ± 0.000. Subdivision among populations within regions was significantly lower (nonoverlap of either mean within two SE of the other mean) in the north for the parameter R_{ST} but not for F_{ST}. Subdivision between regions was 0.350 ± 0.161 for F_{ST} and 0.439 ± 0.009 for R_{ST}.

Quantitative Variation

Phenotypic subdivision was assessed by nested ANOVA (Table 3). Of the five life-history characters, four differed significantly among populations and three differed significantly between regions, with individuals from the northern region generally developing faster (shorter hatching and generation times and higher metamorphosis numbers). Of the 17 morphometric characters, all differed significantly among populations and all but one differed significantly between regions, with northern individuals generally being larger.

Heritability averaged over all traits and populations was 0.28 ± 0.08 (Table 4). Differences between regions and trait types were tested by unpaired two-tailed *t*-tests. Heritabilities in northern populations were significantly lower than those in southern populations ($t_{130} = -2.34$, $P = 0.021$). This difference was due to substantially reduced heritabilities for life-history characters ($t_{28} = -3.19$, $P = 0.004$), but not for morphometric characters ($t_{100} = -0.96$, $P = 0.340$). Across all populations, heritabilities for life-history characters were significantly higher than for morphometric characters ($t_{130} = 2.09$, $P = 0.038$).

The average level of quantitative genetic subdivision was 0.30 ± 0.07 (Table 4). Subdivision among populations within the northern region was equivalent to that among populations in the southern region (paired, two-tailed *t*-test; $t_{21} = 0.103$, $P = 0.919$). Population subdivision for life-history characters (0.11 ± 0.08) tended to be lower than that for morphometric characters (0.35 ± 0.08), although the difference was not significant (unpaired, two-tailed *t*-test; $t_{20} = -1.39$, $P = 0.181$). Similarly, overall subdivision between regions (0.26 ± 0.07) was composed of weaker differentiation for life-history characters (0.07 ± 0.05) than for morphometric characters (0.31 ± 0.09), although the difference was not significant (unpaired, two-tailed *t*-test; $t_{20} = -1.46$, $P = 0.160$).

Relationships between Parameter Estimates

Within populations, expected heterozygosity was not significantly correlated with overall heritability ($r = -0.369$, $P = 0.472$) or with heritability decomposed into life-history characters ($r = 0.224$, $P = 0.670$) or morphometric characters ($r = 0.204$, $P = 0.697$). Between populations (Fig. 2), subdivision for quantitative traits (Q_{ST}) was correlated with subdivision for molecular traits when measured as F_{ST} ($r = 0.691$, $P = 0.004$) but not when measured as R_{ST} ($r = 0.414$,

TABLE 2. Molecular subdivision (SE) among populations (F_{ST}, R_{ST}) and regions (F_{RT}, R_{RT}).

	F_{ST}	R_{ST}	F_{RT}	R_{RT}
All populations	0.803 (0.044)	0.890 (0.000)	0.350 (0.161)	0.439 (0.009)
Northern populations	0.327 (0.154)	0.191 (0.003)		
Southern populations	0.811 (0.065)	0.913 (0.001)		

TABLE 3. Mean phenotypic values (SE) for life-history and morphometric characters in three northern populations and three southern populations. The units for time are days and for size are millimeters. Effects of population and region were tested by nested analysis of variance. F, female; M, male.

	Northern populations			Southern populations			Effect of	
	GP	FHL	SUN	PD	RP	SD	Population	Region
Life-history characters								
Hatching time	3.533 (0.126)	3.897 (0.113)	4.010 (0.079)	3.974 (0.099)	3.753 (0.113)	4.562 (0.090)	***	***
Hatching number	26.756 (1.215)	28.044 (1.258)	22.048 (1.019)	25.228 (1.036)	21.014 (0.831)	32.008 (1.198)	***	ns
Survivorship number	24.689 (1.085)	24.242 (1.409)	19.010 (0.929)	20.956 (1.143)	17.229 (0.951)	23.592 (1.069)	***	ns
Metamorphosis number	12.933 (1.888)	9.394 (1.585)	8.218 (0.920)	7.737 (1.279)	5.357 (0.890)	6.742 (1.078)	ns	**
Generation time	23.591 (0.555)	23.690 (0.319)	24.242 (0.292)	26.721 (0.922)	24.559 (0.530)	26.638 (0.332)	***	***
Morphometric characters								
F cephalothorax length	0.447 (0.007)	0.430 (0.005)	0.452 (0.004)	0.409 (0.005)	0.424 (0.006)	0.436 (0.003)	***	***
M cephalothorax length	0.445 (0.005)	0.438 (0.006)	0.457 (0.004)	0.401 (0.007)	0.416 (0.004)	0.436 (0.003)	***	***
F cephalothorax width	0.374 (0.002)	0.362 (0.003)	0.389 (0.002)	0.336 (0.003)	0.352 (0.002)	0.362 (0.002)	***	***
M cephalothorax width	0.364 (0.002)	0.359 (0.002)	0.381 (0.002)	0.332 (0.003)	0.341 (0.003)	0.354 (0.002)	***	***
F urosome length	0.303 (0.009)	0.287 (0.005)	0.292 (0.004)	0.273 (0.005)	0.287 (0.006)	0.291 (0.003)	***	*
M urosome length	0.306 (0.005)	0.305 (0.006)	0.298 (0.004)	0.269 (0.005)	0.297 (0.006)	0.296 (0.004)	***	***
F urosome width	0.199 (0.002)	0.194 (0.002)	0.207 (0.001)	0.182 (0.002)	0.186 (0.002)	0.188 (0.001)	***	***
M urosome width	0.174 (0.002)	0.168 (0.002)	0.176 (0.001)	0.162 (0.002)	0.163 (0.002)	0.165 (0.001)	***	***
F telson width	0.041 (0.001)	0.039 (0.001)	0.042 (0.000)	0.039 (0.001)	0.040 (0.001)	0.040 (0.000)	***	**
M telson width	0.039 (0.001)	0.040 (0.001)	0.042 (0.000)	0.037 (0.001)	0.039 (0.001)	0.040 (0.000)	***	***
F caudal seta length	0.658 (0.008)	0.650 (0.011)	0.706 (0.007)	0.587 (0.009)	0.594 (0.011)	0.628 (0.008)	***	***
M caudal seta length	0.772 (0.010)	0.762 (0.012)	0.793 (0.006)	0.667 (0.007)	0.698 (0.010)	0.719 (0.008)	***	***
F antennule width	0.045 (0.001)	0.042 (0.001)	0.046 (0.001)	0.041 (0.001)	0.041 (0.001)	0.045 (0.001)	***	ns
M antennule width	0.057 (0.001)	0.054 (0.001)	0.057 (0.001)	0.051 (0.001)	0.053 (0.001)	0.053 (0.001)	***	***
M clasper width	0.072 (0.001)	0.070 (0.001)	0.072 (0.001)	0.063 (0.002)	0.070 (0.001)	0.071 (0.001)	***	***
Egg sac area	0.106 (0.003)	0.105 (0.002)	0.125 (0.002)	0.101 (0.002)	0.110 (0.002)	0.111 (0.001)	***	*
Egg sac diameter	0.440 (0.007)	0.437 (0.006)	0.487 (0.080)	0.451 (0.007)	0.469 (0.006)	0.469 (0.004)	***	*

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, $P > 0.05$.

TABLE 4. Narrow-sense heritabilities (h^2) and quantitative-genetic subdivision among populations (Q_{ST}) and between regions (Q_{RT}). Estimates were averaged over five life-history characters and 17 morphometric characters (SE).

	All characters			Life-history characters			Morphometric characters		
	h^2	Q_{ST}	Q_{RT}	h^2	Q_{ST}	Q_{RT}	h^2	Q_{ST}	Q_{RT}
All populations	0.28 (0.08)	0.30 (0.07)	0.26 (0.07)	0.52 (0.14)	0.11 (0.08)	0.07 (0.05)	0.21 (0.09)	0.35 (0.08)	0.31 (0.09)
Northern populations	0.14 (0.10)	0.13 (0.05)		0.11 (0.15)	0.07 (0.05)		0.15 (0.13)	0.14 (0.05)	
Southern populations	0.42 (0.10)	0.12 (0.05)		0.93 (0.18)	0.12 (0.08)		0.28 (0.09)	0.12 (0.06)	

$P = 0.125$). When Q_{ST} is divided into two components, morphometric traits are significantly correlated with F_{ST} ($r = 0.672$, $P = 0.006$), whereas life-history traits are not ($r = 0.107$, $P = 0.705$). The magnitude of subdivision tends to be higher for molecular characters than for quantitative characters, whether evaluated among all populations ($F_{ST} = 0.80$, $R_{ST} = 0.89$, $Q_{ST} = 0.30$), among populations within the northern region ($F_{ST} = 0.33$, $R_{ST} = 0.19$, $Q_{ST} = 0.13$), among populations within the southern region ($F_{ST} = 0.81$, $R_{ST} = 0.91$, $Q_{ST} = 0.12$), or between regions ($F_{RT} = 0.35$, $R_{RT} = 0.439$, $Q_{RT} = 0.26$). Finally, the 22 quantitative characters show no correlation between Q_{ST} and h^2 ($r = 0.309$, $P = 0.162$).

DISCUSSION

Like previous studies of the species using allozymes and DNA sequences (e.g., Burton et al. 1979; Burton and Lee 1994), our microsatellite data reveal extensive differentiation among populations. However, the average level of population subdivision for microsatellites ($F_{ST} = 0.80$, $R_{ST} = 0.89$) was somewhat lower than that previously found for mitochondrial DNA over a similar geographic range ($F_{ST} = 0.98$; Edmands 2001). This difference may be due both to the higher effective population size for nuclear genes and to the elevated mutation rates at microsatellite loci.

Both morphological and life-history characters differed significantly between regions, with northern copepods generally being larger and developing faster. A positive correlation between latitude and body size (i.e., Bergmann's rule) has long been noted in mammals, where it is generally attributed to regulating heat retention. In ectotherms, gradients in both body size and development rate have been attributed to factors such as temperature or the availability of food or oxygen (Arnett and Gotelli 1999; Spicer and Gaston 1999; Ashton 2002). Further experiments are needed to determine what factors triggered regional differences in our study. Temperature is a particularly likely candidate for the cause of differences in development rate. Because development is closely tied to temperature, countergradient selection might cause copepods from colder, northern climates to develop more rapidly than southern copepods when raised under common-garden conditions.

An earlier phylogenetic study of the species using mitochondrial DNA showed reduced differentiation among northern populations relative to southern populations (Edmands 2001). This pattern might be caused by postglacial expansions and contractions during the Pleistocene ice ages some 20,000 years ago (e.g., Hewitt 1996; Avise and Walker 1998). In the current study, regional differences in molecular and quantitative characters were only partially consistent with expectations based on glaciation. Microsatellite data did show the expected reduction in variation both within and between northern populations. Quantitative trait data, however, revealed a reduction in variation within, but not between, northern populations. The different pattern for molecular and quantitative traits might be explained by differences in mutation rates, because mutation rates for quantitative characters are typically several orders of magnitude higher than those for molecular characters (e.g., Pfrender et

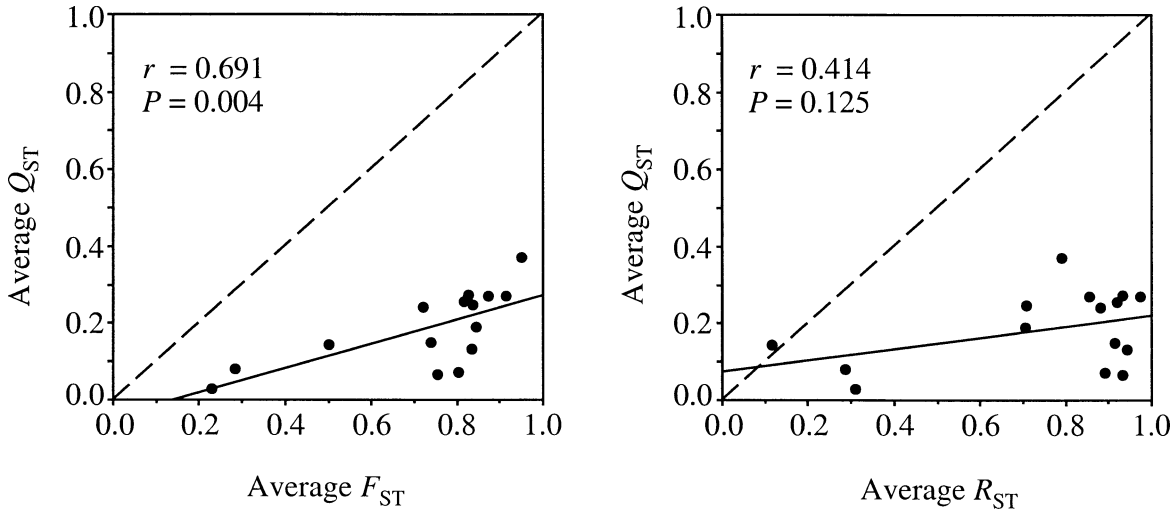

FIG. 2. Correlation between average estimates of subdivision for microsatellite loci (F_{ST}, R_{ST}) and quantitative traits (Q_{ST}) for all possible population pairs. Dashed line equals 1:1.

al. 2000). That is, the time since colonization may have been sufficient to allow mutational differences between populations to reach an equilibrium point for quantitative traits but not for microsatellites. It should be noted, however, that mutation rates for both quantitative characters and microsatellite loci are unknown for this particular species.

These overall regional patterns conceal considerable variation among populations within regions. While northern populations showed an overall decrease in both heritability (in laboratory-reared progeny) and heterozygosity, a population by population comparison reveals no significant correlation between these two factors. This is consistent with an increasing body of data suggesting these measures are frequently discordant (Pfrender et al. 2000; Reed and Frankham 2001). There are several reasons for this lack of correspondence, including differential selection and mutation rates. In the case of *Tigriopus*, an additional important factor may be the conversion of nonadditive variation underlying quantitative traits to additive variation under conditions of isolation and drift (Goodnight 1988; Fernandez et al. 1995; Meffert 2000; Lopez-Fanjul et al. 2002). These copepods live in supralittoral splash pools that are prone to frequent evaporation and rehydration (Dybdahl 1994; Burton 1997). Such bottlenecks could contribute to the decoupling of molecular and quantitative variation.

Unlike the situation within populations, there was some evidence for a correlation between quantitative and molecular divergence between populations (significant for F_{ST} but not for R_{ST}). Of course, the correlation between estimates of between population divergence can be disrupted by the same forces that decouple diversity indices within populations (e.g., mutation, selection, nonadditive variation). However, estimates of population subdivision appear to be somewhat more robust to these forces (e.g., Pfrender et al. 2000). Our finding of correlations between populations but not within populations parallels the patterns found for Q_{ST} versus F_{ST} in two species of *Daphnia* (Lynch et al.1999; Morgan et al. 2001) and in the annual plant *Senecio vulgaris* (Steinger et al. 2002).

The finding of somewhat different patterns of molecular subdivision for parameters F_{ST} versus R_{ST} is intriguing given that studies of microsatellite versus quantitative trait variation have typically used only F_{ST} (e.g., Lynch et al. 1999; Merilä and Crnokrak 2001; McKay and Latta 2002; but see also Palo et al. 2003). There is certainly no consensus on which parameter is most accurate for estimating microsatellite variation (Wang et al. 2001). The best metric depends in part on the mechanism of mutation: an infinite-alleles model favors F_{ST} (e.g., Neff et al. 1999), while a stepwise mutation model favors R_{ST} (e.g., Zhu et al. 2000). The ideal metric also depends on the balance between mutation and drift. When mutation predominates, the differences in number of repeats may be critical, whereas when drift predominates, alternative alleles evolve relatively independently of their mutational origin. In one example of human evolution where drift is thought to be important (Pérez-Lezaun et al. 1997), patterns found using F_{ST} were consistent with those from other sources (genetic and archeological), whereas those found using R_{ST} were substantially different. In the case of *T. californicus*, both genetic drift and nonstepwise mutation could influence the pattern of quantitative subdivision correlating with F_{ST} but not R_{ST}.

As studies with joint estimates of F_{ST} and Q_{ST} accumulate, there is an emerging consensus that quantitative subdivision typically exceeds molecular subdivision (Lynch et al. 1999; Merilä and Crnokrak 2001; McKay and Latta 2002). This suggests that quantitative subdivision is being driven by divergent selection in local environments. In this study, we find the opposite pattern (F_{ST} = 0.83, Q_{ST} = 0.30), suggesting that quantitative characters may be subject to either stabilizing or fluctuating selection. Further insight into the forces driving population divergence can be gained by looking at variation in Q_{ST} among individual characters. Because the ability of a character to respond to directional selection is positively related to heritability (Falconer and Mackay 1996), divergent selection in different locations should promote a correspondence between Q_{ST} and h^2 as has been shown in other cases (e.g., Yang et al. 1996; Lynch et al. 1999). Instead,

we find no correlation between these two measures for the 22 quantitative characters ($r = 0.309$, ns). This provides further evidence for constraints on the evolution of quantitative characters.

Morphological stasis appears to be particularly common in copepods. Studies of a number of copepod genera have found morphological stasis in the face of substantial molecular divergence (McKinnon et al. 1992; Bucklin et al. 1995; Rocha-Olivares et al. 2001). Lee and Frost (2002) were the first to formally measure genetic subdivision for molecular and quantitative characters in a copepod species (*Eurytemora affinis*). Using F_{ST} derived from allozyme data and Q_{ST} derived from the morphology of secondary sex characters, Lee and Frost found a pattern of excess molecular subdivision ($F_{ST} = 0.617$, $Q_{ST} = 0.162$) quite similar to the present study. The authors considered several factors that could promote this pattern, including weak diversifying sexual selection and constraints due to stabilizing or fluctuating selection. It should be noted that morphological stasis does not necessarily imply a lack of adaptive evolution. Considerable geographic variation in temperature and salinity tolerance has been reported in the *E. affinis* species complex (e.g., Lee 1999). Similarly, allopatric populations of *T. californicus* show divergence in osmoregulatory adaptation (e.g., Burton and Feldman 1983; Burton 1990).

Our study found depressed subdivision for general morphological characters as well as a few characters that might be subject to sexual selection such as antennule width and male clasper width. In addition, we found particularly reduced subdivision for life-history characters. These patterns could be caused by stabilizing selection in which similar phenotypes for this particular set of characters are favored in different geographic locations. Fluctuating selection could also explain the observed pattern because reversals in the direction of selection could retard quantitative trait divergence by promoting canalization (Kawecki 2000). This seems a particularly attractive hypothesis for species like *T. californicus* inhabiting environments subject to frequent changes in temperature, salinity, and other variables. A third possibility is that the excess molecular subdivision may be due to accelerated molecular evolution rather than decelerated quantitative evolution. Recent work shows accelerated rates of molecular evolution in halophilic crustaceans relative to their freshwater allies (Hebert et al. 2002). Because variation in ionic strength can impact the structure of both DNA and proteins, this acceleration might be caused by reduced fidelity of DNA replication in hypersaline environments. Although we have no rate data for *T. californicus*, the extensive molecular divergence between many geographically proximal populations suggests the possibility of elevated rates of molecular evolution (Edmands 2001). At the extreme, populations just 10 km apart have greater than 17% mitochondrial DNA divergence (Burton 1998), which translates into approximately 12 million years of isolation according to a standard molecular clock (Knowlton and Weigt 1998). It seems highly improbable that neighboring populations could have remained isolated for millions of years in the face of substantial and repeated changes in sea level.

The level of concordance among various measures of genetic diversity has important implications for conservation biology. Our results add to the growing body of data cautioning against the use of molecular metrics of variation within populations, such as heterozygosity, as a surrogate for the ability of a population to respond to environmental change. Our findings do, however, lend limited support to the practice of using molecular markers to diagnose evolutionarily significant units worthy of enhanced conservation priority (Waples 1991; Crandall 2000). Yet, while molecular markers may provide a conservative estimate of adaptive divergence in many cases, results such as ours and those of Lee and Frost (2002) show that in some taxa quantitative trait divergence is less than that predicted from presumably neutral molecular markers. In such cases, molecules would provide an inflated estimate of adaptive differences.

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Literature Cited

Armbruster, P., W. E. Bradshaw, and C. M. Holzapfel. 1998. Effects of postglacial range expansion on allozyme and quantitative genetic variation of the pitcher plant mosquito, *Wyeomyia smithii*. Evolution 52:1697–1704.

Arnett, A. E., and N. J. Gotelli. 1999. Geographic variation in life-history traits of the ant lion, *Myrmeleon immaculatus*: evolutionary implications of Bergmann's rule. Evolution 53: 1180–1188.

Ashton, K. G. 2002. Do amphibians follow Bergmann's rule? Can. J. Zoology 80:708–716.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proc. Royal Soc. Lond. B. 265:457–463.

Brisoe, D. A., J. M. Malpica, A. Robertson, G. J. Smith, R. Frankham, R. G. Banks, and J. S. F. Barker. 1992. Rapid loss of genetic variation in large captive populations of *Drosophila* flies: implications for the genetic management of captive populations. Conserv. Biol. 6:416–425.

Bucklin, A., B. W. Frost, and T. D. Kocher. 1995. Molecular systematics of six *Calanus* and three *Metridia* species (Calanoida: Copepoda). Mar. Biol. 121:655–664.

Burton, R. S. 1985. Mating system of the intertidal copepod *Tigriopus californicus*. Mar. Biol. 86:247–252.

———. 1990. Hybrid breakdown in physiological response: a mechanistic approach. Evolution 44:1806–1813.

———. 1997. Genetic evidence for long-term persistence of marine invertebrate populations in an ephemeral environment. Evolution 51:993–998.

———. 1998. Intraspecific phylogeography across the Point Conception biogeographic boundary. Evolution 52:734–745.

Burton, R. S., and M. W. Feldman. 1983. Physiological effects of an allozyme polymorphism: glutamate-pyruvate transaminase and response to hyperosmotic stress in the copepod *Tigriopus californicus*. Biochem. Genet. 21:239–251.

Burton, R. S., and B.-N. Lee. 1994. Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. Proc. Nat. Acad. Sci. USA 91:5197–5201.

Burton, R. S., and S. G. Swisher. 1984. Population structure of the

intertidal copepod *Tigriopus californicus*: as revealed by field manipulation of allele frequencies. Oecologia 65:108–111.
Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace, and R. K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends Ecol. Evol. 15:290–295.
Crnokrak, P., and J. Merilä. 2002. Genetic population divergence: markers and traits. Trends Ecol. Evol. 17:501.
Dybdahl, M. F. 1994. Extinction, recolonization and the genetic structure of tidepool copepod populations. Evol. Ecol. 8: 113–124.
Egloff, D. A. 1967. Ecological aspects of sex ratio and reproduction in experimental and field populations of the marine copepod *Tigriopus californicus*. Ph.D. diss., Stanford University, Stanford, CA.
Edmands, S. 2001. Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. Mol. Ecol. 10:1743–1750.
Falconer, D. S., and T. F. C. McKay. 1996. Introduction to quantitative genetics. 4th ed. Longman, Harlow, U.K.
Fernandez, A. M., M. A. Toro, and C. Lopez-Fanjul. 1995. The effect of inbreeding on the redistribution of genetic variance of fecundity and viability in *Tribolium castaneum*. Heredity 75: 376–381.
Goodman, S. J. 1997. Rst Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Mol. Ecol. 6: 881–885.
Goodnight, C. J. 1988. Epistasis and the effect of founder events on additive genetic variance. Evolution 42:441–454.
Goudet, J. 1995. FSTAT (vers. 1.2), a computer program to calculate F-statistics. J. Heredity 86:485–486.
Hebert, P. D. N., E. A. Remigio, J. K. Colbourne, D. J. Taylor, and C. C. Wilson. 2002. Accelerated molecular evolution in halophilic crustaceans. Evolution 56:909–926.
Hendry, A. P. 2002. $Q_{ST} > = \neq < F_{ST}$? Trends Ecol. Evol. 17: 502.
Hewitt, G. 1996. Some genetic consequences of ice ages and their role in divergence and speciation. Biol. J. Linn. Soc. 58: 247–276.
Kawecki, T. J. 2000. The evolution of genetic canalization under fluctuating selection. Evolution 54:1–12.
Knowlton, N., and L. A. Weight. 1998. New dates and new rates of divergence across the Isthmus of Panama. Proc. R. Soc. Lond. B. 265:2257–2263.
Latta, R. G., and J. K. McKay. 2002. Genetic population divergence: markers and traits. Trends Ecol. Evol. 17:501–502.
Lee, C. E. 1999. Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. Evolution 53:1423–1434.
Lee, C. E., and B. W. Frost. 2002. Morphological stasis in the *Eurytemora affinis* complex (Copepods: Temoridae). Hydrobiologia 480:111–128.
Lopez-Fanjul, C., A. Fernandez, and M. Toro. 2002. The effect of epistasis on the excess of the additive and nonadditive variances after population bottlenecks. Evolution 56:865–876.
Lynch, M., M. Pfrender, K. Spitze, N. Lehman, J. Hicks, D. Allen, L. Latta, M. Ottone, F. Bogue, and J. Colbourne. 1999. The quantitative and molecular genetic architecture of a subdivided species. Evolution 53:100–110.
Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.
Machida, R., M. U. Miya, M. Nishida, and S. Nishida. 2002. Complete mitochondrial DNA sequence of *Tigriopus japonicus* (Crustacea: Copepoda). Mar. Biotechnol. 4:406–417.
McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. Trends Ecol. Evol. 17:285–291.
McKinnon, A. D., W. J. Kimmerer, and J. A. H. Benzie. 1992. Sympatric sibling species within the genus *Acartia* (Copepoda: Calanoida): a case study from Westernport and Port Philips Bays Australia. J. Crust. Biol. 12:239–259.
Meffert, L. M. 2000. The evolutionary potential of morphology and mating behavior: the role of epistasis in bottlenecked populations. Pp. 177–193 *in* J. B. Wolf, E. D. Brodie, and M. J. Wade, eds. Epistasis and the evolutionary process. Oxford Univ. Press, New York.
Merilä, J., and P. Crnokrak. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. J. Evol. Biol. 14: 892–903.
Morgan, K. K., J. Hicks, K. Spitze, L. Latta, M. E. Pfrender, C. S. Weaver, M. Ottone, and M. Lynch. 2001. Patterns of genetic architecture for life-history traits and molecular markers in a subdivided species. Evolution 55:1753–1761.
Neff, B. D., P. Fu, and M. R. Gross. 1999. Microsatellite evolution in sunfish (Centrarchidae). Can. J. Fish. Aquat. Sci. 56: 1198–1205.
Palo, J. U., R. B. O'Hara, A. T. Laugen, A. Laurila, C. R. Primmer, and J. Merilä 2003. Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative genetic data. Mol. Ecol. 12:1963–1978.
Pérez-Lezaun, A., F. Calafell, E. Mateu, D. Comas, R. Ruiz-Pacheco, and J. Bertranpetit. 1997. Microsatellite variation and the differentiation of modern humans. Hum. Genet. 99:1–7.
Pfrender, M. E., K. Spitze, J. Hicks, K. Morgan, L. Latta, and M. Lynch. 2000. Lack of concordance between genetic diversity estimates at the molecular and quantitative-trait levels. Cons. Genet. 1:263–269.
Reed, D. H., and R. Frankham. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103.
Reid, D. G. 1990. Trans-arctic migration and speciation induced by climatic change: the biogeography of *Littorina*. Bull. Mar. Sci. 47:35–49.
Rocha-Olivares, A., J. W. Fleeger, and D. W. Foltz. 2001. Decoupling of molecular and morphological evolution in deep lineages of a meiobenthic harpacticoid copepod. Mol. Biol. Evol. 18(6): 1088–1102.
Rogers, S. M., V. Gagnon, and L. Bernatchez. 2002. Genetically based phenotype-environment association for swimming behavior in lake whitefish ecotypes (*Coregonis clupeaformis* Mitchill). Evolution 56:2322–2329.
Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin: a software for population genetics data analysis. Ver. 2.000. Genetics and Biometry Lab, Dept. of Anthropology, Univ. of Geneva.
Spicer, J. I., and K. J. Gaston. 1999. Amphipod gigantism dictated by oxygen availability? Ecol. Lett. 2:397–401.
Spitze, K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. Genetics 135:367–374.
Steinger, T., P. Haldimann, K. A. Leiss, and H. Muller-Scharer. 2002. Does natural selection promote population divergence? A comparative analysis of population structure using amplified fragment length polymorphism markers and quantitative traits. Mol. Ecol. 11:2583–2590.
Storz, J. F. 2002. Contrasting patterns of evolution in quantitative traits and neutral DNA markers: analysis of clinal variation. Mol. Ecol. 11:2537–2551.
Wang, R., L. Zheng, Y. T. Touré, T. Dandekar, and F. C. Kafatos. 2001. When genetic distance matters: measuring genetic differentiation at microsatellite loci in whole genome scans of recent and incipient mosquito species. Proc. Natl. Acad. Sci., USA. 98: 10769–10774.
Waples, R. S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of ''species'' under the Endangered Species Act. Mar. Fish. Rev. 53:11–22.
Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.
Whitlock, M. C. 1999. Neutral additive genetic variance in a metapopulation. Genet. Res. 24:215–221.
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.
Zhu, T., D. C. Queller, and J. E. Strassmann. 2000. A phylogenetic perspective on sequence evolution in microsatellite loci. J. Molec. Evol. 50:324–338.

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