

CONTRASTING QUATERNARY HISTORIES IN AN ECOLOGICALLY DIVERGENT SISTER PAIR OF LOW-DISPERSING INTERTIDAL FISH (*XIPHISTER*) REVEALED BY MULTILOCUS DNA ANALYSIS

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Abstract.—Recurrent glacial advances have shaped community histories across the planet. While biogeographic responses to glaciations likely varied with latitude, the consequences for temperate marine communities histories are less clear. By coalescent analyses of multiloci DNA sequence data (mitochondrial DNA control region, α -enolase intron, and α -tropomyosin intron) collected from a low-dispersing sister pair of rocky intertidal fishes commonly found from southeastern Alaska to California (*Xiphister atropurpureus* and *X. mucosus*), we uncover two very different responses to historical glaciations. A variety of methods that include a simulation analysis, coestimates of migration and divergence times, and estimates of minimum ages of populations sampled up and down the North American Pacific coast all strongly revealed a history of range persistence in *X. atropurpureus* and extreme range contraction and expansion from a southern refugium in *X. mucosus*. Furthermore, these conclusions are not sensitive to the independent estimates of the DNA substitution rates we obtain. While gene flow and dispersal are low in both species, the widely different histories are rather likely to have arisen from ecological differences such as diet breadth, generation time, and habitat specificity.

Key words.—Coalescence, phylogeography, Pleistocene, refugia, rocky intertidal.

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The periodic glacial epochs of the Quaternary were prolonged and occupied nearly 80% of the last 2 million years (Lambeck et al. 2002). The composition of Northern Hemisphere temperate communities during warmer interglacial periods such as the current Holocene era is widely thought to be the result of range expansion and colonization from southern glacial refugia, leading to directional selection favoring generalists with higher dispersal ability and lower overall taxonomic diversity than tropical regions (Valentine and Jablonski 1993; Hewitt 1999; Dynesius and Jansson 2000). While these glaciations have often resulted in genetic signatures of low population divergence and gradients in genetic diversity in temperate North American and European taxa (Avice 2000; Hewitt 2000; Lessa et al. 2003), some genetic studies are increasingly suggesting that many temperate communities consist of taxa that retained the geographical extent of their ranges in cryptic multiple refugia (Stewart and Lister 2001; Jacobs et al. 2004).

The community composition of temperate intertidal areas such as the northeastern Pacific is likely to be the result of both historical processes such as these cyclical glaciations as well as contemporary oceanographic processes and changes (Horn and Allen 1978; Roy et al. 1995; Lindberg and Lipps 1996; Roy et al. 2001; Hohenlohe 2004). The craggy and complex coastline of the northeastern Pacific intertidal contains a highly diversified biota, yet it has experienced coastal sea ice, lower sea surface temperatures (SST), shifts in major current patterns (Sabin and Pisias 1996; Lyle et al. 2000; Herbert et al. 2001; Pisias et al. 2001), and sea levels falling as much as 150 m (Pielou 1991; Williams et al. 1998; Rahmsdorf 2002). While the periodic glaciations have led to increasing rocky substrate and consequently more habitat for

this community during warmer periods (Jacobs et al. 2004), these recurrent glacial advances call into question ecological biogeographic studies that neglect historical processes that occur on Pleistocene time scales (Wiens and Donoghue 2004). However, before history can be incorporated into studying the biogeography of this region, species range stability and the consequent history of ecological associations must be tested with historical data. While much work has been done using fossils, the higher sea level following the last glacial period has submerged the glacial intertidal in many areas (Valentine and Jablonski 1993; Roy et al. 1995, 2001), making phylogeographic data an attractive tool for historical biogeographic reconstruction.

To this end, phylogeographic studies of this region have focused on testing two general hypotheses of Pleistocene range stability that are suggested from geological and ecological data: (1) the southern refugium hypothesis; and (2) the range persistence hypothesis. While incomplete coastal glaciation would suggest persistence (Pielou 1991), winter-time SST were much lower during these glacial periods (Pisias et al. 2001), such that southern retraction is more likely if SST strongly determines range (Hubbs 1948, 1960; Horn and Allen 1978). If history is determined by species-specific ecological characteristics like dispersal (Bohonak 1999), intertidal depth (Marko 2004), or habitat specificity (Wares and Cunningham 2001), then we might expect a mixture of histories across codistributed taxa. If one splits the results of previous phylogeography studies of this region into southern refugium and range persistence histories, five taxa fall into each of the two respective histories: *Cucumaria miniata*, *Nucella ostrina*, *Pollicipes polymerus*, *Strongylocentrotus franciscanus*, and *Tigriopus californicus* as taxa with southern refugium histories (Van Syoc 1994; Arndt and Smith 1998; Burton 1998; Marko 1998; Edmands 2001) and *Balanus glandula*, *Cucumaria pseudocurata*, *Gobbiosox maeandricus*,

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Littorina scutulata, and *Nucella lamellosa* as taxa with range persistence histories (Arndt and Smith 1998; Kyle and Boulding 2000; Hickerson and Ross 2001; Marko 2004; Sotka et al. 2004).

In this study we collect multiloci DNA sequence data in a sister pair of coexisting rocky intertidal fish, the black prickleback and rock prickleback (*Xiphister atropurpureus* and *X. mucosus*, respectively), members of the family Stichaeidae and order Perciformes (Stoddard 1985; Yatsu 1986). Both species have similar dispersal capabilities and nearly identical ranges that presently span the rocky intertidal from California to southeastern Alaska, half of which was glaciated during at least the last two glacial maxima (LGMs; Yokoyama et al. 2001; Mecklenburg et al. 2002).

While similarities between *X. atropurpureus* and *X. mucosus* include nearly identical ranges, breeding habitats, and dispersal that is actively resisted during larval and adult stages (Hart 1973; Wourms and Evans 1974; Marliave 1986; Mecklenburg et al. 2002), there are several ecological differences that could have influenced gene flow and the probability of range persistence during the Pleistocene. Characteristic ecological differences distinguishing *X. mucosus* from *X. atropurpureus* include a more specialized herbivorous diet that seasonally varies, larger adult size, (Barton 1982; Horn et al. 1982; Horn 1983), and more than twice the age at sexual maturity in *X. mucosus* (Fitch and Lavenberg 1975). Another key difference is that *X. mucosus* is more restricted to open coastline locations (not within bays or inlets), whereas *X. atropurpureus* is found in both open coastline locations and areas within bays and inlets that are exposed enough to receive a sufficient amount of wave action (Hart 1973; Eschmeyer et al. 1983).

To distinguish between the southern refugium and range persistence hypotheses, we collected DNA sequence data from two nuclear loci (introns from α -enolase and α -tropomyosin) and one mitochondrial DNA (mtDNA) locus (control region). Fortunately, the two tested histories (southern refugium and range persistence) are predicted to have two very different phylogeographic signatures that are testable with coalescent models used to estimate population divergence times and minimum population ages. As criteria for rejecting the southern refugium hypothesis, we use divergence time estimates between population pairs that span the glacial margin (Fig. 1) that are an order of magnitude greater than 19,000 years, the time of the LGM (Anderson 1968; Blaise et al. 1990; Sabin and Piasias 1996). Likewise, rejecting the southern refugium hypothesis includes minimum population age estimates greater than 190,000 years for populations north of the glacial margin (Fig. 1).

While the methods used here are based on a variety of models that are all far simpler than the actual biogeographic histories, robust conclusions can be obtained from strong concordance among methods. For example, we used models that ignore subdivision (Saillard et al. 2000), migration (Nei and Li 1979), divergence time (Beerli and Felsenstein 2001), and models that account for both divergence time and migration at the expense of ignoring other parameters (Nielsen and Wakeley 2001). However, we investigate the validity of oversimplification in some of these models by simulation (Excoffier et al. 2000). While we do not specifically focus

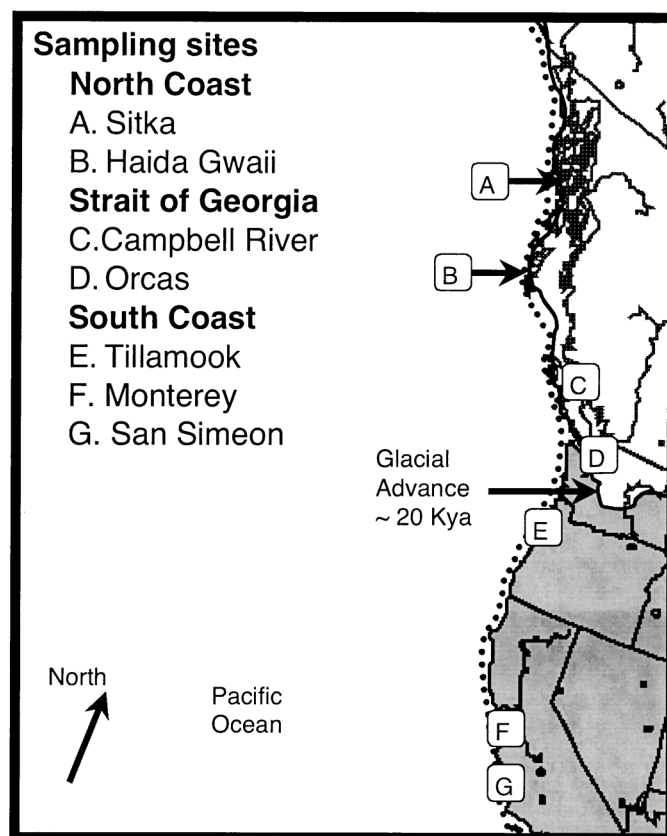


FIG. 1. Sample sites of *Xiphister atropurpureus* and *X. mucosus*. The dotted line depicts the approximate coastline at the last glacial maximum (LGM), and the shaded region depicts the approximate extent of glaciations at the LGM. *Xiphister atropurpureus* was found and collected in sites A through F, whereas *X. mucosus* was found and collected from sites A, C, E, and G.

on migration estimates, we argue that this parameter must be considered in the context of other parameters describing population subdivision and age because presence of migration decreases the ability to discriminate between older population ages (Nielsen and Slatkin 2000; Nielsen and Wakeley 2001; Kalinowski 2002a).

MATERIALS AND METHODS

Collections and Geographic Sampling

Specimens of *X. mucosus* and *X. atropurpureus* were collected from populations throughout their ranges from southeastern Alaska to central California in the summer of 1999 and 2001. The sampling localities of these species were divided into three regions: (1) south coast; (2) Strait of Georgia; and (3) north coast (Fig. 1). The south coast region corresponds to areas proximal to the putative southern refugium that was spared from glaciation at the LGM, and includes sampling localities in Tillamook, Oregon; Monterey, California; and San Simeon, California. The Strait of Georgia is an inland waterway that was glaciated at the LGM (Fig. 1) between Vancouver Island and mainland British Columbia and includes sampling localities in Campbell River, British Columbia, and Orcas Island, Washington. The north coast

TABLE 1. Sample sizes, number of alleles, number of unique alleles (alleles found only once in sample), and haplotype diversities of the sampled populations of *Xiphister atropurpureus* and *X. mucosus*. Haplotype diversities (H) were calculated using equation (8.4) in Nei (1987). The results in this table are based on the all of the DNA polymorphism data, regardless of possible intralocus recombination.

Species, Gene	No. alleles	No. unique alleles	Sample size	Population	H	
<i>X. atropurpureus</i>	control region	40	28	10	Sitka, AK	0.8211
				11	Haida Gwaii, BC	0.8657
				12	Campbell River, BC	0.6958
				13	Orcas, WA	0.8988
				13	Tillamook, OR	0.8248
	α -enolase	18	10	10	Monterey, CA	0.9473
				12	Sitka, AK	0.8116
				16	Haida Gwaii, BC	0.8871
				12	Campbell River, BC	0.8261
				14	Orcas, WA	0.6878
				6	Tillamook, OR	0.6667
	α -tropomyosin	24	10	12	Monterey, CA	0.7826
				14	Sitka, AK	0.8783
				8	Haida Gwaii, BC	0.8000
				8	Campbell River, BC	0.8667
10				Orcas, WA	0.6105	
12				Tillamook, OR	0.5072	
8				Monterey, CA	0.9000	
<i>X. mucosus</i>	control region	6	1	15	Sitka, AK	0.3310
				9	Orcas, WA	0.0000
				11	Tillamook, OR	0.0000
				7	San Simeon, CA	0.7912
				7	San Simeon, CA	0.7912
	α -enolase	3	1	12	Sitka, AK	0.0000
				12	Orcas, WA	0.0000
				18	Tillamook, OR	0.0000
				14	San Simeon, CA	0.5185
	α -tropomyosin	4	1	14	Sitka, AK	0.4233
				14	Orcas, WA	0.0000
				14	Tillamook, OR	0.5079
				12	San Simeon, CA	0.7246

region corresponds to areas even further north of the glacial margin (Fig. 1), and includes localities in Sitka, Alaska, and the Queen Charlotte Islands (Haida Gwaii), British Columbia. Fin clips were taken from individuals and were stored in 100% ethanol. Sample sizes are indicated in Table 1.

To estimate the rates of DNA substitution, we used data from six species of *Pholis*, an intertidal fish genus that is closely related to *Xiphister* and is distributed on both sides of the Arctic Ocean (Robins and Ray 1986; Mecklenburg et al. 2002). The specimens of *P. gunnellus* were collected from three localities on the North American coast (Rockport, MA; Bar Harbor, ME; and Rocky Harbor, NF) and four localities on the European coast (Iceland, Ireland, Scotland, and southern Norway) in the fall of 2002. We collected specimens of the other *Pholis* species from various localities in the north-eastern and northwestern Pacific Ocean during the summer of 2002. *Pholis picta*, *P. nebulosa*, and *P. crassispina* were collected from various localities on the coast of Hokkaido, Japan. *Pholis ornata* and *P. laeta*, were collected from Friday Harbor, Washington.

DNA Sequence Markers

DNA sequence data were collected from three loci (419–457 bp 5' end portion of the mtDNA control region, 420 bp α -enolase intron, and 495 bp α -tropomyosin intron). The in-

tron sequences include 13–20 bp of flanking exon sequences. Sample sizes from each locus are given on Table 1. DNA was extracted using a Qiagen (Valencia, CA) DNA extraction kit following the manufacturer's protocol. After samples were incubated for 12 h in 0.18 ml of lysis buffer and 0.02 ml proteinase K, DNA was purified using the silica gel spin column provided in the kit. Under the specified salt and pH conditions, DNA was retained within the silica matrix.

The control-region fragment was amplified from both species using A-PRO 5'-TTCCACCTCTAACT CCCAAAGCT AG and 3'-TATGCTTTAGTTAAGGCTACG as primers (Lee et al. 1995). The fragment containing the α -enolase intron VIII was amplified using CCAGGCACCCAGTCTA CCTG as the 5' primer and TGGACTTCAAATCCCCCGA TGATCCCAGC as the 3' primer (Friesen 1997). The fragment containing the α -tropomyosin intron V was amplified using GAGTTGGATCGCGCTCAGGAGCG as the 5' primer and CGGTCAGCCTCCTCAGCAATGTGCTT as the 3' primer (Friesen et al. 1999). Polymerase chain reaction (PCR) amplifications of the control-region fragment were carried out in 50- μ l reactions: 50 mM Tris-HCl(pH 9.0), 20 mM ammonium sulfate, 1.5 mM MgCl₂, 250 mM each dNTP, 500 nM each primer, 0.1–1.0 μ g of genomic DNA, and 0.5 unit of Taq DNA polymerase. PCR conditions for the amplification of α -enolase intron and the α -tropomyosin intron were

similar except for being 2.5 mM MgCl₂ in the case of α -enolase intron and 1.25 mM DMSO in the case of the α -tropomyosin intron. Specific thermocycling parameters were optimized and varied depending on species and gene amplified. Reactions without genomic DNA were included in every amplification series to screen for possible foreign-DNA contamination. Each amplified product was extracted using a QIAquick gel extraction kit (Qiagen) following the manufacturer's guidelines. The extracted double-stranded DNA was cycle-sequenced by the dideoxy chain termination method using an ABI Prism dye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA). Excess dye terminators were removed by ethanol precipitation. Single-stranded dideoxy chain terminated products were sequenced with an automated sequencer in both directions (Applied Biosystems, 373A). To discern both nuclear alleles from heterozygous individuals observed to have more than one equally intense multiple peak in direct sequences of PCR products (both directions), products were TA cloned (Invitrogen, Carlsbad, CA) and sequenced. Multiple clones were analyzed (both directions) until the phase of polymorphisms was discerned.

The DNA sequences of *X. atropurpureus* were submitted to GenBank using the following accession numbers: mtDNA control region, AY554422–AY554490; α -enolase, AY554704–AY554776; and α -tropomyosin, AY554906–AY554965. The DNA sequences of *X. mucosus* were submitted to GenBank using the following accession numbers: mtDNA control region, AY554565–AY554606; α -enolase, AY554607–AY554692; and α -tropomyosin, AY554839–AY554898. The DNA sequences of *P. gunnellus* were submitted to GenBank using the following accession numbers: mtDNA control region, AY554491–AY554549; α -enolase, AY554704–AY554776; and α -tropomyosin, AY554906–AY554965. The mtDNA control region sequences for the five North Pacific *Pholis* species were submitted to GenBank using the following accession numbers: *P. ornata*, AY554555; *P. laeta*, AY554556; *P. picta*, AY554553; *P. nebulosa*, AY554554; and *P. crassispina*, AY554550–AY554552. The α -enolase sequences for the five north Pacific *Pholis* species were submitted to GenBank using the following accession numbers: *P. ornata*, AY554695; *P. laeta*, AY554696; *P. picta*, AY554698; *P. nebulosa*, AY554697; and *P. crassispina*, AY554699–AY554703. The α -tropomyosin sequences for the five North Pacific *Pholis* species were submitted to GenBank using the following accession numbers: *P. ornata*, AY554902; *P. laeta*, AY554901; *P. picta*, AY554900; *P. nebulosa*, AY554903; and *P. crassispina*, AY554899.

Genetic Diversity and Selective Neutrality

A measure of genetic diversity of each sampled population was calculated using Nei's haplotype diversity (Nei 1987). Tajima's *D* (Tajima 1989) statistic was applied to test the null hypothesis of selective neutrality for each locus within each species. Significance was tested by comparisons to null distributions constructed from 10,000 random Monte Carlo permutations of the original data matrix using program Arlequin 2.001 (Schneider et al. 1997). All calculations using

Arlequin used a model of DNA substitution that best approximated the best-fit model determined by MODELTEST.

Recombination

Population genetic models used for the inference of population history usually assume that the genealogical history of each locus is bifurcating, such that intralocus recombination is negligible. Therefore, we identified potential cases of recombination in our intron loci so that only blocks of nonrecombining sets of linked nucleotides were used for genealogical analyses. Because recurrent mutations are likely to be rare in the two intron loci used in this study (approximately 10-fold lower DNA substitution rates than the mtDNA control region rate), we used Hudson's four-gamete test of recombination to identify likely blocks of nonrecombining sequence (Hudson 1985). As a way to identify such blocks, we employed SITES (Hey and Wakeley 1997) and such blocks were confirmed to be consistent with no recombination if they yielded a consistency index of 1.0 when the data was used to reconstruct a tree by way of maximum parsimony using PAUP (ver. 4.0 b10; Swofford 2000).

Phylogenetic Analysis

For both species, parsimony trees were constructed from a parsimony analysis (PAUP, v 4.0 b10; Swofford 2000). Each pool of most parsimonious trees was compared to a maximum likelihood phylogeny (Swofford 2000) constructed using the best-fit model of evolution (Goldman 1993b; Cunningham et al. 1998). The best-fit model of DNA sequence evolution for each locus was determined using MODELTEST (Posada and Crandall 1998). Heuristic searches were performed with stepwise addition and TBR branch swapping with zero-length branches collapsed. Sequences were aligned by eye. For the two intron loci, analyses were conducted on the nonrecombinant blocks identified by SITES and Hudson's four-gamete test (Hudson 1985).

Rates of DNA Substitution

Parameter estimates used to distinguish alternate population histories often rely on assumptions about the rates of DNA substitution. These parameters include divergence times, historical effective population sizes, and minimum population ages. The rates of substitution for the three loci were estimated from three measurements of corrected genetic divergence between five Pacific and one Atlantic representative of the intertidal fish genus *Pholis*, an intertidal genus that is closely related to *X. atropurpureus* and *X. mucosus* (Stepien et al. 1997). As with many other temperate marine taxa, the Atlantic species (*P. gunnellus*) is nested within several Pacific congeners (Yatsu 1985), suggesting its ancestor migrated from the Pacific during the trans-Arctic interchange 2.4–3.5 million years ago (Durham and MacNeil 1967; Vermeij 1991). North Atlantic *Pholis* (*P. gunnellus*) is found on both the American and European coastlines, and the samples from each region (26 and 33 individuals from each) were compared independently to each of the five potential North Pacific sister taxa (*P. ornata*, *P. laeta*, *P. picta*, *P. nebulosa*, and *P. crassispina*). Subsequently, the mean was used for the

overall estimate. To correct for ancestral polymorphism, we used the larger intraspecific samples of *P. gunnellus*. Specifically, because large within species samples (more than five individuals) were only available from this species, the mean number of pairwise distances between individuals within the North American and European coastlines were used to correct for ancestral polymorphism using Nei and Li's corrected divergence (Nei and Li 1979). We estimated the rates based on maximum likelihood genetic distances to correct for multiple hits using the best-fit model of DNA substitution (Goldman 1993a; Cunningham et al. 1998) as determined by MODELTEST (Posada and Crandall 1998). Because a 3.5 million years date for the opening of the Bering Strait is a maximum age for divergence between Pacific and Atlantic *Pholis*, our estimate can therefore be viewed as a minimum estimate of substitution rates. Assuming a single molecular clock is hazardous, however, and therefore we always ask whether applying a two-fold higher rate would change our conclusions.

Isolation by Distance

The observed relationship between geographical and genetic distance was compared to equilibrium expectations (Slatkin 1993). We used the program Arlequin 2.001 (Schneider et al. 1997) to calculate pairwise estimates of Nm (N , effective population size; and m , per generation probability of migration), which were based on pairwise Φ_{ST} . To determine significance, the correlation between $\log(Nm)$ and $\log(\text{km})$ matrices was compared to 1000 randomly permuted matrices using a Mantel test. Additionally, as an overall gauge of population subdivision, we calculated Φ_{ST} based on each entire sample using Arlequin. For the intron loci, these estimates were based on the nonrecombinant blocks identified by SITES and Hudson's (1985) four-gamete test. All calculations using Arlequin used a model of DNA substitution that best approximated the best-fit model determined by MODELTEST.

Estimates of Migration Rates and Divergence Times

We used divergence time estimates to test the southern refugium hypothesis using a coalescent model that simultaneously estimates migration and divergence time (MDIV; Nielsen and Wakeley 2001) and represents an explicitly non-equilibrium approach. As a comparison, we also obtained migration estimates based on two equilibrium approaches; one based on a coalescent model in which population divergence times are assumed to be infinite (MIGRATE; Beerli and Felsenstein 2001) and one based on pairwise Φ_{ST} values calculated using Arlequin 2.001.

While MIGRATE assumes infinite divergence time among all population, MDIV estimates this divergence time in addition to migration between a pair of populations using a Markov chain Monte Carlo (MCMC) algorithm. Migration rate and divergence time are parameters that covary and are difficult to distinguish using traditional methods (Wakeley 1996; Kalinowski 2002a); Nielsen and Wakeley's (2001) MDIV method, however, uses a Bayesian framework to estimate both parameters by calculating the posterior probabilities given the genealogy (Nielsen and Wakeley 2001) un-

der a finite sites model of sequence evolution (Hasegawa et al. 1985). The product of the posterior densities from the three loci were used to obtain multilocus estimates of migration among the adjacent pairs of populations. We constructed 95% confidence intervals around the maximum posterior estimate of migration with likelihood ratio tests (Nielsen and Wakeley 2001), in which the test statistic is assumed to follow a χ^2 distribution with one degree of freedom. To assure that MDIV converged on consistent estimates of the parameter values, multiple Markov chains that used different starting parameters and genealogies were run. Each Markov chain used 5×10^6 steps and a burn-in time of 2×10^5 steps.

For the divergence time parameter we used uninformative (flat) priors bounded by zero and a biologically plausible value (e.g., 2 million years). We compared MDIV estimates of divergence time with migration to estimates that are based on a model in which migration is assumed to be zero (Nei and Li 1979). We considered divergence time estimates greater than 190,000 years between adjacent populations spanning the glacial margin (between the Tillamook and Orcas samples; Fig. 1) as criterion for rejecting the southern refugium hypothesis. Although this is an order of magnitude older than the LGM, this requirement is robust to molecular-clock assumptions. In both types of divergence time estimates, the analysis of the intron data was only based on the data from the nonrecombinant blocks. For the intron loci, all migration rate and divergence time estimates were based on the nonrecombinant blocks identified by SITES and Hudson's (1985) four-gamete test.

Ages of Geographically Restricted Clades

As another means of testing the southern refugium hypothesis, we used subsets of the genealogical control region data to estimate the minimum ages of populations or geographic regions using an approach borrowed from the human genetics literature (Saillard et al. 2000). In this case, if estimates of populations north of the glacial margin (Fig. 1) are older than the LGM, we rejected the southern refugium hypothesis. This method involves identifying groups of related alleles that are restricted to a geographical area and use the mutational genealogies of these alleles to estimate the minimum ages of these geographical areas. For example, if an allele and most of its descendants are restricted to a particular area (geographically restricted clades), then it is likely those alleles arose in that area (Satta and Takahata 2004), making the age of that clade an approximation of that locality's minimum age. This approach is most effective if there are multiple geographically restricted clades in the dataset. We considered clades using two criteria: (1) if an ancestral allele and all of its descendants are indicated as a monophyletic clade in a strict consensus parsimony tree; and (2) if all but one of these alleles are restricted to a population or a region of interest. In this case, an allele was regarded to be ancestral if it was closest to the root determined using the heuristic method implemented in the program TCS (Castelloe and Templeton 1994; Clement et al. 2000). The area of interest might be a single collection locality or might be a larger area such as all populations north of the glacial margin. We

estimated the ages of the geographically restricted clades using two coalescent-based methods.

Following Saillard et al. (2000), we estimated the time to the most recent common ancestor of each set of geographically restricted alleles from the average number of mutations between the ancestral and descendant alleles given an assumed mutation rate. Saillard's estimate assumes a starlike genealogy and constructs confidence intervals for this age estimate from the mutational variance and by using the degree to which the genealogy deviates from a star phylogeny. Age estimates of populations north of the glacial margin (Fig. 1) that are older than the LGM were considered rejections of the southern refugium hypothesis.

Simulation Analysis

The highly stochastic processes underlying phylogeographic data can make comparing alternate population histories hazardous without conducting a simulation analysis. As a heuristic guide to hypothesis testing, we therefore conducted a simulation-based power analysis of divergence time estimates that ignore migration (Nei and Li 1979) using empirically derived coestimates of migration and divergence from *X. atropurpureus* (MDIV; Nielsen and Wakeley 2001). Similar to Knowles (2001). We specifically estimate the minimum divergence time for rejecting the southern refugium history in this species and the statistical power for this test, defined as the probability of a test statistic rejecting the southern refugium history given that the estimated population history is the true history. To this end, we employ Nei and Li's (1979) corrected nucleotide divergence as a test statistic and focus on the divergence time estimate between Orcas and Tillamook, the adjacent pair of *X. atropurpureus* populations that span the putative glacial margin at the LGM (Fig. 1). Using SIMCOAL (Excoffier et al. 2000), we generated computer simulations that incorporate the stochastic processes of the coalescence, DNA mutation, and migration. This simulates replicate genealogies on which the test statistic is calculated. This was done for two specific histories: a constrained southern refugium history and an estimated history using the empirical parameter estimates from *X. atropurpureus*. Nei and Li's corrected divergence was obtained from these simulated datasets by ARLEQUIN (Schneider et al 1997).

With sample sizes identical to those of *X. atropurpureus*, a six-population history was simulated 1000 times under each of these two population histories. Under the estimated history, samples were simulated using divergence times corresponding to the empirical MDIV divergence time estimates obtained from adjacent *X. atropurpureus* population pairs (Fig. 2, see also Table 3). Going backward in time, adjacent populations representing our samples would sequentially merge according to these divergence time estimates and merged ancestral populations would further merge with respect to these estimates (Fig. 2). Samples would be simulated identically under the southern refugium history, with the exception of the divergence times being proportionally rescaled such that divergence times involving comparisons north of or spanning the glacial margin are less than 19,000 years ago. Migration rates between adjacent populations and effective

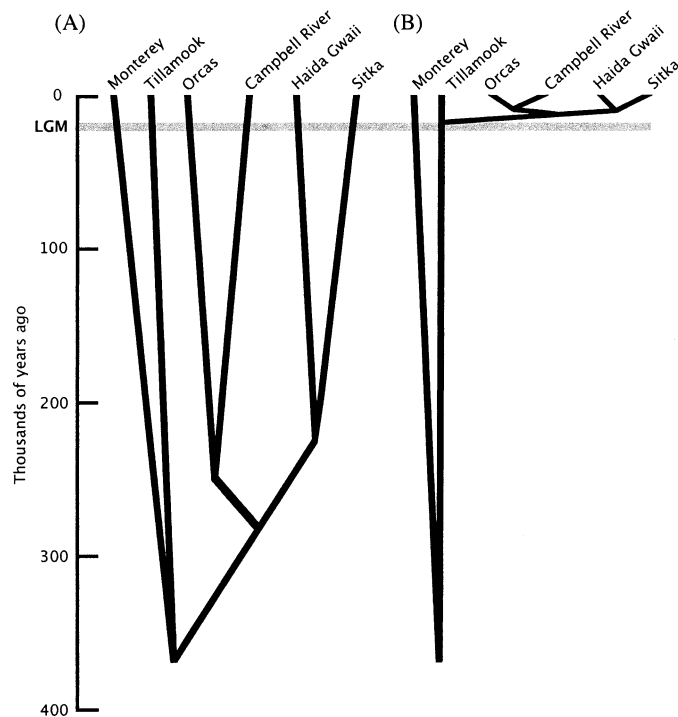


FIG. 2. Graphical representations of two hypothesized population histories simulated in SIMCOAL. (A) Population history with migration rates and divergence times based on MDIV estimates between adjacent populations of *Xiphister atropurpureus*. (B) Southern refugium history in which simulations used the identical parameter values that were used in (A), with the exception of divergence times being proportionally rescaled such that divergences that span or are north of the glacial margin are less than 19,000 years ago. Models (A) and (B) used effective population size estimates from *X. atropurpureus* using MIGRATE. In the case of the population trichotomy, the population ancestral to populations Orcas through Sitka are each founded by 100 individuals from the ancestral Monterey population, whereas the remaining divergence events involve 100 individuals from a southern ancestral population colonizing a new northern population. These graphical depictions are based on the mitochondrial DNA empirical estimates (three-loci and intron graphical depictions not shown).

population sizes were constrained to be the empirical estimates obtained from *X. atropurpureus* using the MDIV and MIGRATE estimates, respectively (assuming a stepping stone model in the latter case). Although ideally the test statistic would be obtained from the same method as the divergence time estimate used for constraining the two histories (MDIV), Nei and Li's estimate facilitates rapid calculation from the simulated datasets and is expected to have a downward bias given migration (Kalinowski 2002a), such that it is conservative with respect to rejecting the southern refugium hypothesis. Divergence/founding events involved 100 individuals from the ancestral population colonizing the new population followed by instantaneous growth to its current size, such that southern populations were always ancestral to a northern population at the time of population divergence. In the case of a population trichotomy, the two most northern populations are each founded by 100 individuals from the southernmost population (the ancestral population). The effective size of every ancestral population is the mean of its two daughter populations, and migration rates

TABLE 2. (A) Overall Φ_{ST} ; (B) levels of significance of the Mantel tests comparing the correlation between pairwise Φ_{ST} and geographic distance matrixes of the sampling localities to the null hypothesis that they are not correlated; (C) Tajima's D statistic (1989). Statistical significance (indicated by an asterisk) was tested using 10,000 Monte Carlo permutations of the dataset.

Locus	Mitochondrial DNA control region	α -enolase	α -tropomyosin
(A) Overall Φ_{ST}			
<i>Xiphister atropurpureus</i>	0.417 ($P < 0.001^*$)	0.136 ($P < 0.001^*$)	0.041 ($P = 0.007^*$)
<i>X. mucosus</i>	0.551 ($P < 0.001^*$)	0.202 ($P < 0.001^*$)	0.163 ($P = 0.001^*$)
(B) Mantel tests			
<i>X. atropurpureus</i>	$P = 0.678$	$P = 0.893$	$P = 0.773$
<i>X. mucosus</i>	$P = 0.091$	$P = 0.335$	$P = 0.811$
(C) Tajima's D			
<i>X. atropurpureus</i>	-0.26 ($P = 0.42$)	-0.25 ($P = 0.42$)	0.38 ($P = 0.35$)
<i>X. mucosus</i>	-1.60 ($P = 0.05^*$)	-1.105 ($P = 0.14$)	0.59 ($P = 0.09$)

between ancestral populations are the rates between the ancestral population and its geographically closest daughter populations. Simulating both histories involved using the DNA substitution rates estimated from the trans-Arctic *Pholis* data. Intralocus rate heterogeneity was also built into the simulations, and we used the parameters estimated from the best-fit model of sequence evolution estimated from the empirical data using MODELTEST (Posada and Crandall 1998). This simulation analysis was conducted from the mtDNA data, the intron data, and the combined three-loci data. In all cases, the empirically derived parameter constraints used the respective combined parameter estimates from *X. atropurpureus*. In the case of the intron and three-loci simulated data, Nei and Li's corrected divergence time was calculated as the mean across loci.

We first obtained the distributions for our test statistic simulated 1000 times under both histories (Fig. 2) and verified their monotonicity. Because the monotonicity of these distributions implies monotonicity of the corresponding power functions, statistical power is essentially the probability of the test statistic rejecting the null model (constrained history) given that the alternative model (estimated history) is the true model at a certain α -level (Casella and Berger 1990). Because divergence time will be close to the end of parameter space (zero) under the constrained southern refugium history, we chose the highest 5% of the 1000 values of the test statistic simulated under the constrained southern refugium history ($\alpha = 0.05$; one-tailed test) as the empirical cutoff point for rejecting the southern refugium history. Although using the adjacent divergence times to constrain our histories stems from the linear arrangement of intertidal populations, other possible histories are conceivable, but are beyond the scope of this analysis.

RESULTS

Genetic Diversity and Selective Neutrality

Overall, there was a huge difference in genetic diversity between the two species. At the control region, haplotype diversity averaged 0.84 (SD = 0.08) in *X. atropurpureus*, whereas it averaged just 0.28 (SD = 0.37) in *X. mucosus*. These patterns of haplotype diversity at the intron loci were much the same, with *X. atropurpureus* averaging 0.78 (SD = 0.08) and *X. mucosus* averaging 0.16 (SD = 0.24). No

relationship between haplotype diversity and latitude was found in the three loci collected from *X. atropurpureus* (Table 1). In fact, for the two introns, the mean haplotype diversity was lower south of the glacial margin than north of the glacial margin (0.72 vs. 0.80 for enolase, and 0.70 vs. 0.79 for tropomyosin). In contrast, a south-to-north decreasing gradient in haplotype diversity was found in *X. mucosus* at all three loci, with the mean haplotype diversity south of the glacial margin consistently higher than north of the margin (0.40 vs. 0.16 for control region, 0.26 vs. 0.0 for enolase, and 0.62 vs. 0.21 for tropomyosin). Tajima's (1989) D statistic was applied to test the null hypothesis of selective neutrality for each locus within each species and indicated only one significant deviation from the neutral model (Table 2). The exception was the mtDNA control region in *X. mucosus*, where there was a significantly negative value. Although this pattern is expected when there is a selective sweep at a closely linked locus (or the locus itself), a significantly negative value is also expected when there is substantial population growth, which is consistent with the hypothesis of a northward expansion from a southern glacial refugium. However, because there was not a significantly negative Tajima's D -value in the two intron loci of *X. mucosus*, we can not entirely rule out a selection explanation in favor of a demographic expansion explanation.

Recombination

SITES and the four-gamete test of recombination identified two likely blocks of nonrecombining sequence (Hudson 1985) within both the α -enolase and α -tropomyosin loci collected from *X. atropurpureus* (215 and 141 contiguous base pairs respectively; see Appendix, available online only at <http://dx.doi.org/10.1554/04-126.1.s1>). From *X. mucosus*, however, recombination was not detected in either intron loci. The variable sites and potential nonrecombinant blocks used for further analyses are shown in the online appendix.

Phylogenetic Analysis

Maximum likelihood phylogenies were estimated based on best-fit models of evolution for the three loci from *X. atropurpureus* and *X. mucosus*. Although there are strong differences between the two species with respect to diversity levels and genealogical depths, the genealogical patterns within species were not inconsistent. Although this is expected from

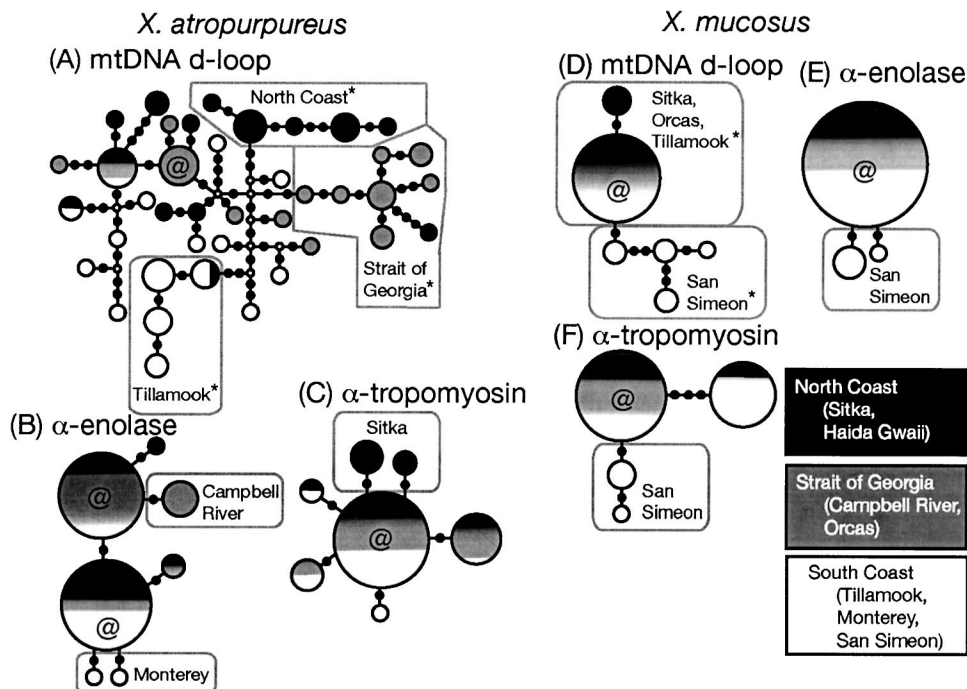


FIG. 3. Parsimony trees depicting genealogical relationships of three loci (mitochondrial DNA control region, α -enolase intron, and α -tropomyosin intron) collected from *Xiphister atropurpureus* (A, B, C) and *X. mucosus* (D, E, F). The trees were constructed from PAUP*4.0 b10 (Swofford 2000); for the control region, the tree was selected from a pool of most parsimonious trees based on the topology of the maximum likelihood phylogeny obtained under the best-fit model of evolution using MODELTEST. The alleles are depicted as circles and the size of each allele is proportional to its frequency. The shadings are based on three regions from which the samples were collected; black (north coast: Sitka, Haida Gwaii), gray (Strait of Georgia: Orcas, Campbell River), and white (south coast: Tillamook, Monterey, and San Simeon). The unobserved alleles are depicted at internal nodes using the smallest open circles. The black dots between the alleles are inferred mutations separating the different alleles. Alleles that are found in multiple regions are depicted with multiple shadings. The intron trees are based on the inferred nonrecombinant blocks (online Appendix). Each @ symbol refers to the most probable ancestral allele determined by the methods of Castelleo and Templeton (1994) as implemented in TCS (Clement et al. 2000) and two symbols within a tree indicates two roots with equal probability. The sets of control region alleles used for estimating the minimum ages of populations are regions indicated by asterisk, and these sets of alleles were revealed to be monophyletic in strict consensus parsimony trees. Clades were considered geographically restricted if all but one allele was found in a population or region of interest.

neutral loci that share a common history, the expected interloci variation in mutation rates and effective population sizes resulted in substantially less phylogeographic resolution at the intron loci. There was a single base insertion/deletion in both *X. atropurpureus* and *X. mucosus* at the tropomyosin locus. For all three loci, *X. atropurpureus* has many more alleles than *X. mucosus* (Fig. 3, Appendix online) and many of the *X. atropurpureus* alleles are geographically restricted, as compared to *X. mucosus* (Appendix online). Finally, the patterns of geographic restriction are congruent among loci, although less resolved for the introns. For example, in *X. mucosus*, an entire control region clade is restricted to the southernmost locality (San Simeon), while this geographic restriction is manifested in enolase and tropomyosin with the presence of only two geographically restricted alleles that are both restricted to San Simeon, the southernmost sampled population (Fig. 3).

Rates of DNA Substitution

By obtaining the mean estimate of corrected (multiple hits and ancestral polymorphism) nucleotide divergence across independent comparisons between the two *P. gunnellus* samples (North America and Europe) and the five potential North

Pacific sister taxa (*P. ornata*, *P. laeta*, *P. picta*, *P. nebulosa*, and *P. crassispina*), we obtained the three substitution rate estimates (Nei and Li 1979). For the control region, this yielded a rate of 0.85% million years, and for α -enolase and α -tropomyosin this yielded rates of 0.17% and 0.18% per million years respectively (one-half the commonly used divergence rate).

Isolation by Distance

None of the species or loci showed the significant correlation between pairwise Φ_{ST} and geographic distance that is expected at equilibrium under a stepping stone model (Table 2). The overall Φ_{ST} -values in *X. atropurpureus* were significant at all three loci with the exception of the α -tropomyosin locus (Table 2), while all three loci collected from *X. mucosus* suggested restricted gene flow between San Simeon (the most southerly population) and other sampled populations.

Estimates of Migration and Divergence Times

In *X. atropurpureus*, the divergence time estimates between adjacent pairs of populations using the control region data ranged from 91,000 to 485,000 years, while in *X. mucosus*

TABLE 3. Divergence time estimates among adjacent populations of *Xiphister atropurpureus* and *X. mucosus* based on a model without migration (Nei and Li 1979) and with migration (bold values; MDIV; Nielsen and Wakeley 2001). Multilocus divergence time estimates are the mean of three estimates from the three collected loci. The italic values are migration rate estimates using MDIV. These migration estimates are based on the marginal posterior probability densities from mitochondrial DNA data or multiplied over the three loci, and the values in parentheses are confidence intervals determined by likelihood ratio tests. (A) *X. atropurpureus*; (B) *X. mucosus*.

	Divergence time Nei and Li (1979)		Divergence time MDIV		Migration MDIV	
	mtDNA	multilocus	mtDNA	multilocus	mtDNA	multilocus
(A) <i>X. atropurpureus</i>						
Sitka	91K	68K	221K	129K	<i>0.60 (0.00–3.78)</i>	<i>0.40 (0.04–1.64)</i>
Haida Gwaii	95K	249K	281K	121K	<i>0.22 (0.00–1.32)</i>	<i>0.29 (0.05–0.98)</i>
Campbell River	241K	297K	254K	92K	<i>0.22 (0.00–1.32)</i>	<i>0.29 (0.05–0.98)</i>
Orcas	485K	211K	370K	129K	<i>0.06 (0.00–0.90)</i>	<i>0.20 (0.00–0.62)</i>
Tillamook	281K	78K	370K	137K	<i>0.08 (0.00–2.10)</i>	<i>0.40 (0.00–1.95)</i>
Monterey						
(B) <i>X. mucosus</i>						
Sitka	3.7K	1.4K	8K	2.9K	<i>0.09 (0.0–23.16)</i>	<i>0.42 (0.23–11.26)</i>
Orcas	0	1.1K	0	0.3K	<i>n/a</i>	<i>n/a</i>
Tillamook	187K	71K	150K	86K	<i>0.04 (0.00–0.52)</i>	<i>0.15 (0.00–0.26)</i>
San Simeon						

these ranged from zero to 187,000 years (using the estimated rates of DNA substitution; see Table 3). The only high value in *X. mucosus* (187,000 years) is attributed to a comparison between San Simeon and Tillamook, which are both south of the putative glacial advance. The divergence time estimates averaged across the three loci ranged from 68,000 to 297,000 years in *X. atropurpureus*, while it ranged from 1000 to 71,000 years in *X. mucosus* (Table 3).

As would be expected from the covariance between the two parameters, divergence time estimates were inversely proportional to migration rate estimates (Table 3). The MDIV-based migration estimates were all less than 1.0, and therefore consistent with limited dispersal in both species. The MDIV and MIGRATE based migration estimates differed to varying degrees, with the latter estimates sometimes yielding values greater than 10,000 migrants per generation (not shown). The MDIV and Nei and Li (1979) estimates of divergence times among adjacent pairs of populations were generally concordant, with the former often corresponding to older divergence times, an expected result, given that estimates based on no-migration models will usually be lower when there is some migration (Nielsen and Wakeley 2001; Kalinowski 2002a).

Estimates of Minimum Population Ages

Striking differences were found in the ages of geographically restricted clades in both *X. atropurpureus* and *X. mu-*

cosus (see Table 4, Fig. 3). Clade age estimates using the estimator of Saillard et al. (2000) reflected minimum population ages across the range of *X. atropurpureus* that were an order of magnitude greater than the LGM. This included clades restricted to the recently deglaciated inland waterway of the Strait of Georgia, the Northern reaches of this species' range (Sitka and Haida Gwaii), and a third clade restricted to the Oregon coast, whereas the estimates from *X. mucosus* cannot reject a southern refugium history. Indeed, only the age of the southernmost San Simeon population in *X. mucosus* was estimated to be much greater than the LGM (see Table 4). The other three *X. mucosus* populations encompass a wide area spanning the LGM margin (Tillamook, Orcas, and Sitka) and consist of two alleles estimated to be only 22,212 years old ($\pm 22,212$).

Simulation Analysis

Simulating under the southern refugium history and calculating Nei and Li's (1979) corrected divergence time between populations (Fig. 2B) revealed the approximate critical values for this test statistic to reject the southern refugium hypothesis to be 50,000, 100,000, and 100,000 years ago for the control region, two introns, and all three loci, respectively (one-tailed test; $\alpha = 0.05$; Fig. 4). Given these critical values for the divergence time estimate between the *X. atropurpureus* Tillamook and Orcas population samples, the southern refugium hypotheses could be rejected from mtDNA and

TABLE 4. Estimates and confidence intervals for the ages of geographically restricted clades using the methods of Saillard et al. (2000). Analyses were conducted on geographically restricted clades to calculate lower-bound estimates of population ages.

Mitochondrial DNA control region		
Species	Location of geographically restricted clade	Age of geographically restricted clade
<i>Xiphister mucosus</i>	1. San Simeon	185,096 \pm 111,058
	2. Tillamook, Orcas, and Sitka	22,212 \pm 22,212
<i>X. atropurpureus</i>	1. North Coast (Haida Gwaii, Sitka)	413,280 \pm 158,272
	2. Strait of Georgia (Orcas, Campbell River)	300,567 \pm 106,267
	3. Tillamook	400,756 \pm 169,879

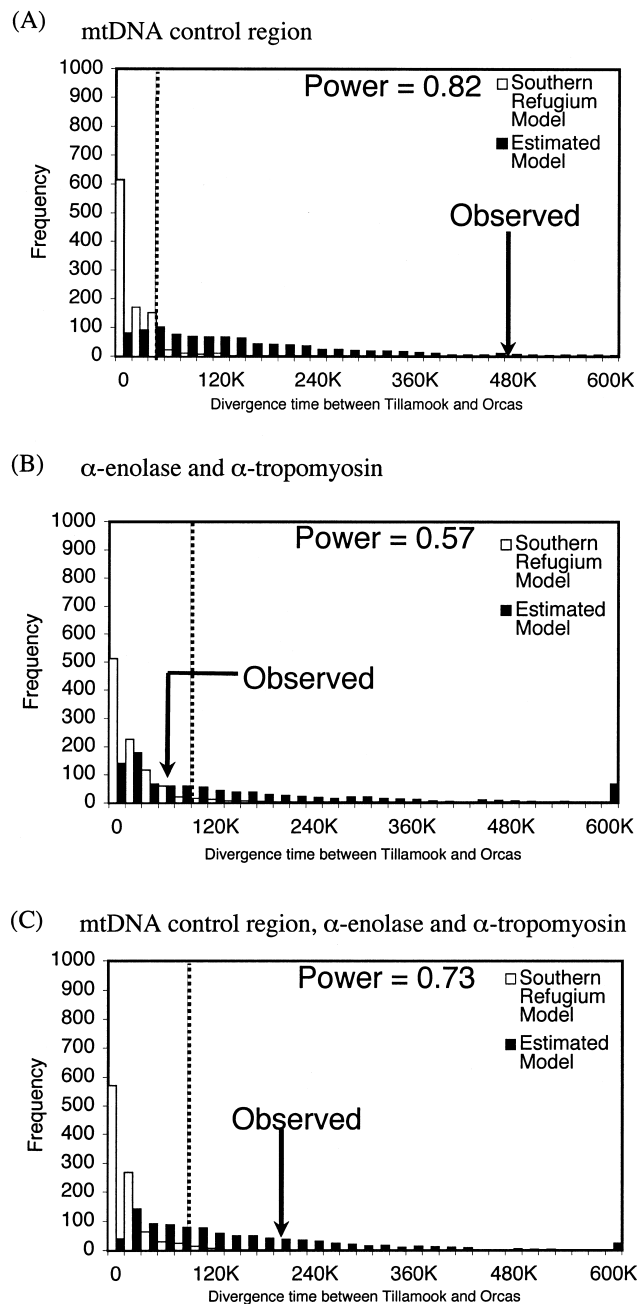


FIG. 4. Frequency distributions of divergence times based on the assumed rate and Nei and Li's (1979) corrected nucleotide divergence calculated between the simulated Tillamook and Orcas samples (Fig. 2) from simulated 1000 datasets that were each produced from SIMCOAL (Excoffier et al. 2000) under the two models depicted in Figure 2A (black bars) and Figure 2B (white bars). Single mitochondrial control region (A), two loci (α -enolase and α -tropomyosin, B) and three loci datasets (C) were each simulated 1000 times under both histories. In the latter two cases, Nei and Li's (1979) corrected nucleotide divergence was averaged across loci. Each dashed line delineates the upper 5% of the 1000 simulated values of the test statistic ($\alpha = 0.05$) simulated under the southern refugium history (Fig. 2B). Power is the probability of correctly rejecting the southern refugium history (white bars; Fig. 2B) given that data arose under a history estimated from *Xiphister atropurpureus* (black bars; Fig. 2A).

combined estimates, but not from estimates obtained from the intron data alone (Fig. 4).

This discrepancy was consistent with the power analysis. After determining that the simulated distributions under the two hypotheses formed a stochastically monotonic family of distributions (Fig. 4), and thereby corresponding to monotonic power functions (Casella and Berger 1990), statistical power was found to be highest when mtDNA estimates were used alone (0.82) and substantially less when using the mean of the two introns (0.57) or the mean across all three loci (0.73; Fig. 4).

DISCUSSION

While it has been argued that the Pleistocene glacial advances were a destructive force to the diversity of temperate taxa (Hewitt 2000; Jansson and Dynesius 2002; Jansson 2003), studies such as this are counter to this generality. However, invoking Pleistocene glaciations as clear determinants for allopatric divergence is difficult for benthic and intertidal systems because marine barriers to gene flow are less obvious and more ephemeral (Palumbi 1994). Using paleontological data to make historical inferences is also obscured because the rising sea level stands following the last glacial period have submerged the Pleistocene intertidal in many areas (Valentine and Jablonski 1993). Despite these difficulties, there are good reasons to suspect that the Pleistocene glaciations have had a more varied and less destructive affect on taxa in the temperate intertidal than their terrestrial counterparts. Temperate marine communities such as the northeastern Pacific intertidal have elevated levels of diversity for some groups (Scagel et al. 1993; Lambert 1994), despite the proximity of substantial coastal glaciation only 19,000 years ago (Anderson 1968; Blaise et al. 1990; Sabin and Pisiyas 1996). Also, low dispersal marine taxa are often distributed over huge areas in the temperate latitudes (Perron and Kohn 1985; Vermeij et al. 1990), suggesting that these high-latitude, low-dispersing taxa with large ranges either persisted and diverged in cryptic refugia or expanded from southern refugium by rare colonizations (Gerlach 1977; Vermeij et al. 1990; Ingolfsson 1992, 1995).

The geographic patterns of genetic diversity, coestimates of migration and divergence times, and minimum population age estimates all suggest that the late Pleistocene population history of *X. atropurpureus* entailed persistence in multiple fragmented refugia distributed north and south of the glacial advance during the last and perhaps several previous glacial peaks, whereas *X. mucosus* was forced into areas south of the glacial advance during the LGM. This conclusion is consistent across loci and is not heavily dependent on the particular molecular-clock calibration we use. The low migration estimates in both species are consistent with the high site fidelity and low observed larval dispersal found in these two species (Wourms and Evans 1974; Marliave 1986) and further strengthen our ability to make inferences about population age (Nielsen and Slatkin 2000; Nielsen and Wakeley 2001; Kalinowski 2002a). While the geographic sampling was weaker in *X. mucosus* than *X. atropurpureus*, sampling localities of *X. mucosus* spanned their range by including southeastern Alaska, the Strait of Georgia, and central California

(Fig. 1). Although sample sizes were relatively low for both species, sample sizes of five to 10 individuals per population often contain much of the per locus information and therefore can detect the genetic signature of long-term persistence given that migration is low and sampled populations are widely distributed (Tajima 1983; Wakeley et al. 2001).

Mitochondrial control region clades that are now geographically restricted to different areas south and north of the late Pleistocene ice sheets are estimated to be at least an order of magnitude older than the LGM in *X. atropurpureus* (Table 4). On the other hand, only one geographically restricted control region clade in *X. mucosus* is estimated to be greater than an order of magnitude older than the LGM, and this control-region clade was restricted to San Simeon, a population far south of the glacial advance (Fig. 1). The rest of the *X. mucosus* control-region sample is found north and south of the glacial margin (Tillamook, Orcas, and Sitka) and is nearly fixed for one ancestral allele, with Sitka containing three individuals that are divergent from this widespread allele by a single mutation (Fig. 3). Although this two-allele clade is estimated to be slightly older than the LGM (22,212 years old; see Table 4), inferring a specific region of origin is tenuous because of its widespread geographic distribution (Satta and Takahata 2004). Furthermore, the overall lower genetic diversity and shallower genealogical depth in all three loci in *X. mucosus* does not support a range persistence history.

Although this study suggests that winter SSTs do not necessarily determine the northern boundaries for northeastern Pacific intertidal organisms, there are probably many intertidal and marine species whose northern limits strongly follow winter SSTs and ocean mass formation (Hubbs 1948, 1960; Valentine and Jablonski 1993; Roy et al. 1995). However, it is most likely that the probability of persisting in the face of coastal glaciation depends on a suite of factors such that the extreme differences in population histories are the result of both biotic and abiotic factors. While a reduction of genetic diversity and divergence can arise after a selective sweep occurs on a tightly linked locus (Maruyama and Birky 1991; Galtier et al. 2000), the consistency between the three loci in *X. mucosus* strengthens the case for historical range contraction being ecologically determined.

Phylogeographic Methodology

Recent population expansion from a glacial refugium into deglaciated areas often result in genetic diversity gradients, with newer populations having a subset of the higher allelic diversity found in older source populations (Hewitt 1996). However, exceptions to this rule can easily emerge if multiple isolated refugia are the source of colonists (Whitlock and McCauley 1990; Kolbe et al. 2004) or if recent bottlenecks occurred in the old populations (Lessa et al. 2003). Nevertheless, patterns of genealogical depth that are manifested in estimates of population divergence and minimum population age can discern whether such misleading exceptions have occurred. Indeed, differential population persistence in the face of climate change can contribute directly to patterns of genetic divergence within a species because two regions simply cannot accumulate significant genetic divergence if one

of them repeatedly goes extinct during glacial advances (Pannell 2003).

In this study we conservatively use various methods to distinguish alternate phylogeographic hypotheses (i.e., population divergence and minimum population age), yet several issues emerge from this approach that are relevant to many other phylogeographic studies. One important issue is that different methods often yield disparate estimates for parameters such as migration rates, divergence times, and population ages. For instance, estimating migration using a model that assumes finite population ages (MDIV) is likely to be more appropriate for taxa subjected to Pleistocene glaciations than methods based on a model that assumes populations with infinite ages (MIGRATE). Another important caveat is that the models we use to represent the two alternate population histories are likely to be far simpler than the actual population histories. Either history certainly consisted of multiple disjointed populations with idiosyncratic migration regimes and many unsampled populations rather than the simpler models we consider. However, our simulations at least demonstrate that Nei and Li's (1979) simple two-population/no-migration model used to estimate divergence time can distinguish these histories when there is low migration and more than two populations. Yet, the set of conditions we considered are dwarfed by the myriad possible histories, all of which could be consistent with our two general hypotheses. Our guide is then to use various methods and be conservative by requiring genetic divergence to be much greater than the LGM between populations spanning the glacial margin to reject the southern refugium history.

A possibly counter-intuitive finding from our simulation analysis is the diminishing statistical power achieved by collecting two intron loci in addition to a single locus mtDNA dataset. While this is only demonstrated with respect to Nei and Li's (1979) net divergence, a summary statistic with high variance (Takahata and Nei 1985), the superior statistical power of mtDNA used alone is not entirely unexpected. This is because the mitochondrial control region has an effective population size that is one-quarter that of intron loci, and hence substantially smaller variance associated with the coalescent process. Furthermore, the 10-fold higher mutation rate results in more fixed differences to accumulate among populations, thereby deflating this statistic when using intron loci. While we show that using the three loci resulted in lower power than one mtDNA locus, it is expected that collecting additional nuclear loci would eventually improve the statistical power of Nei and Li's summary statistic (Maddison 1997; Kuhner et al. 1998; Edwards and Beerli 2000; Arbogast et al. 2002; Kalinowski 2002b). However, collecting the intron loci did benefit this study because the consistent patterns of genealogical diversity across all three loci strengthened our rejection of a range persistence history in *X. mucosus*.

Paleo-Sea Surface Temperatures and Coastal Glaciations

Although key ecological differences could be important historic biogeographic determinants and therefore relevant to questions of range stability, allopatric speciation potential, and range delineation (Alee 1931; Rosenzweig 1995; Dynesius and Jansson 2000; Hubbell 2001; Roy et al. 2001;

Bascompte et al. 2002; Melian and Bascompte 2002) the probability of historical persistence is the result of interplay between ecological factors and extrinsic abiotic forces (Wares and Cunningham 2001; Wares 2002; Jacobs et al. 2004). Extrinsic abiotic forces influencing the probability of persistence and divergence include changes in SST gradients (Horn and Allen 1978), glacial margins and sea level changes (Roy et al. 1995; Lindberg and Lipps 1996), and historical changes in ocean currents (Hohenlohe 2004).

While it is suggested that *X. atropurpureus* persisted throughout its present range, this history is not necessarily supported by nongenetic evidence. For instance, the observed high level of endemic genetic diversity found within Strait of Georgia populations of *X. atropurpureus* (Orcas and Campbell River) is counter to evidence suggesting that the Strait of Georgia was completely glaciated at the LGM (Clague 1983; Pielou 1991; Easterbrook 1992). The control-region alleles found in the *X. atropurpureus* Orcas population make up a clade that is almost exclusively found in this inland waterway (with the exception of one allele found on the outer coast). If we estimate the minimum population age of this clade with Saillard et al.'s (2000) method, an age of greater than 200,000 years is implied. A similar pattern from control region samples of the rocky intertidal northern clingfish, *Gobiosox maeandricus* (Hickerson and Ross 2001) raises the likelihood that much of the rocky intertidal community in the Strait of Georgia is much older than the LGM and hence experienced substantial isolation. How could these old populations persist in the glaciated Strait of Georgia throughout the glacial maximum?

Perhaps this apparent anomaly is better understood in the context of deme turnover within a set of disconnected rocky populations found along the jagged coastline of the northeastern Pacific. Given that periodic deme extinction was outpaced by colonization in the set of demes on the outer coast, genealogical depth could have persisted and accumulated from before and during the LGM (Pannell 2003). With the rising sea level and the consequent processes of intertidal deme extinction and colonization, this array of populations would have gradually moved into the inland fjords of the Puget Sound and Strait of Georgia after deglaciation. Given that sampled demes greatly outnumber unsampled demes, low migration, and deme colonization rates kept up with deme extinction rates, this historically mobile array of demes would have resulted in a retention of genealogical depth and population divergence times older than LGM (Pannell and Charlesworth 2000; Wakeley and Aliacar 2001). Indeed, it would lead to the ancient population divergence found between Strait of Georgia and outer coastal populations of *X. atropurpureus* and *G. maeandricus*.

High genetic diversity and ancient divergence times also reach far north of the Strait of Georgia in *X. atropurpureus*, countering the hypothesis that changes in species limits are strictly determined by late winter SST gradients (Hubbs 1948, 1960). Paleopollen and microfossil analyses suggest that late winter SST on northeastern Pacific coastline were as much as 2–5°C colder during the LGM than today (Sabin and Pisas 1996; Lyle et al. 2000; Herbert et al. 2001; Pisas et al. 2001). The winter SST gradient that coincides with the northern limit of *X. atropurpureus* and *X. mucosus* is presently along the

Alaskan coastline, approximately 200 to 300 km west of the Sitka population sample. At the LGM, however, this SST gradient is estimated to have been as far south as northern Vancouver Island, making it unlikely that northern range limits are wholly determined by these winter SST gradients (McIntyre 1981). Indeed, studies of distributional limits of northeastern Pacific coastal fishes and mollusks suggest that range delineations are not likely to be determined by a single abiotic factor (Valentine 1966; Horn and Allen 1978).

Perhaps the distribution of coastal sea ice at the LGM better predicts northern range limits. While the LGM ice sheets are thought to have completely covered the Strait of Georgia, parts of the outer coast north of Vancouver Island (Blaise et al. 1990), and the shoreline on the western coast of Vancouver Island (Anderson 1968), parts of the coastline north of Vancouver Island are thought to have been free of ice at the LGM (Pielou 1991). This includes portions of northwestern Vancouver Island (Clague 1983), the Haida Gwaii archipelago (Warner et al. 1982; Josenhans et al. 1995; Barrie and Conway 1999), Queen Charlotte Sound (Blaise et al. 1990), and southeastern Alaska (Mann and Hamilton 1995; Heaton et al. 1996). Although the extent of the ice sheet cover on the northern coastline is far from certain, elevated levels of terrestrial endemism (Oglive and Roemer 1984; Ferguson 1987; Cowen 1989; Brodo 1995) and paleobotanical evidence (Warner et al. 1982) has suggested that some areas north of the Strait of Georgia harbored a substantial coastal community at the LGM.

However, incongruent biogeographic, paleoecological, and phylogeographic patterns in the northeastern Pacific raise the possibility that persistent survival in these northern coastal refugia was not universal but variable across species such that coastal communities do not geographically shift as a unit (Hubbs 1948, 1960; Fitch 1967; Horn and Allen 1978). Indeed, analysis of fossil pollen data suggest that plant communities dramatically changed north of Vancouver Island at the LGM (Heusser 2000). Likewise, molecular phylogeographic studies of several northeastern Pacific terrestrial and anadromous taxa have shown both contrasting evidence of northern refugia (Byun et al. 1997; Soltis et al. 1997; Conroy and Cook 2000; Clarke et al. 2001; Cook et al. 2001; Ritland et al. 2001; Smith et al. 2001) and southward range contraction/expansion (Lance and Cook 1998; Stone et al. 2002). While range shifts and extinction are likely related to abiotic factors (Jansson 2003), it is most plausible that the interplay between ecological, demographic, and abiotic factors plays a deterministic role in northern range limits. Therefore, historical incongruence among coexisting species is likely to be the result of biotic factors such as trophic level, demographic parameters, specialization, and area requirements (Alee 1931; Rosenzweig 1995; Dynesius and Jansson 2000; Hubbell 2001; Bascompte et al. 2002; Melian and Bascompte 2002).

Both *X. atropurpureus* and *X. mucosus* have immobile eggs and actively resist dispersal at both juvenile and adult stages (Wourms and Evans 1974; Marliave 1986), whereas their differences are likely to have affected the disparate population histories. Characteristic ecological differences distinguishing *X. mucosus* from *X. atropurpureus* include a more specialized herbivorous diet that seasonally varies, larger adult size, (Barton 1982; Horn et al. 1982; Horn 1983), more

than twice the generation time (Fitch and Lavenberg 1975), and greater habitat specificity in *X. mucosus* (Mecklenburg et al. 2002). Regarding differences in diet, adult *X. mucosus* primarily eat annual red and green algae in the warmer months and perennial algae in the winter, whereas *X. atropurpureus* has a more general diet (Horn et al. 1982), making northern persistence more tenuous for *X. mucosus* if the array of available annual algae species shifted south of the glacial advance at the LGM. Although the older age at sexual maturity in *X. mucosus* could have contributed to northern extinction because populations would have had lower net reproductive rates and hence more difficult recoveries from local population crashes (Fenchel 1974; Bascombe et al. 2002), this explanation is more tenable for the 20- to 30-year generation times thought to have contributed to the extinctions of large animals after the LGM rather than the approximately three-year generation time difference in the *Xiphister* pair (Martin and Klein 1984; Alroy 2001). Regarding habitat specialization, *X. mucosus* populations are generally restricted to open coastlines, whereas *X. atropurpureus* populations are found on both open coastal areas as well as exposed intertidal areas within the many bays and inlets that are common along the complex northeastern Pacific coastline (Eschmeyer et al. 1983; Mecklenburg et al. 2002). This key difference would have resulted in fewer potential refugia for northern *X. mucosus* populations to persist, therefore making the species more susceptible to local extinction during glacial peaks.

Historical Ecology of the Northeastern Pacific Rocky Intertidal

Do previous phylogeographic studies of northeastern Pacific intertidal taxa also show a relationship between population persistence and ecological characteristics such as age at sexual maturity, trophic level, or habitat specificity? To investigate, we reviewed phylogeographic studies of northeastern Pacific intertidal taxa that are largely restricted to the Oregonian marine province (southeastern Alaska to Cape Conception, CA) and thereby presently span both sides of the Late Pleistocene glacial margin. We further restrict this review to studies that actually sample populations throughout each taxa's geographic range using molecular genealogical data such as mtDNA. If we roughly dichotomize these taxa into range contraction and range persistence taxa from their genealogical data (including this study), six taxa fall into each of these two respective histories. These include *C. miniata*, *N. ostrina*, *P. polymerus*, *S. franciscanus*, *T. californicus*, and *X. mucosus* as taxa with range contraction histories (Van Syoc 1994; Arndt and Smith 1998; Burton 1998; Marko 1998; Edmonds 2001), whereas *B. glandula*, *C. pseudocurata*, *G. maeandricus*, *L. scutulata*, *N. lamellosa*, and *X. atropurpureus* are taxa with range persistence histories (Arndt and Smith 1998; Kyle and Boulding 2000; Hickerson and Ross 2001; Marko 2004; Sotka et al. 2004).

Of these studies, only one other species (*N. lamellosa*) is thought to have an age of sexual maturity greater than two years; yet, unlike *X. mucosus*, this snail has a strong genetic signature of persistence in multiple fragmented refugia (Marko 2004). Regarding diet, three of the six range-contraction

taxa could be considered to have specialist diets, whereas only one of the six range-persistence taxa could be considered a diet specialist. In one case, a predator (*N. ostrina*) and prey species (*P. polymerus*) both have range contraction patterns, suggesting that climate-driven range shifts can be reinforced by ecological associations.

In a study of temperate Atlantic intertidal taxa subjected to severe glaciation at the LGM, high dispersal was found to be associated with trans-Atlantic persistence (Wares and Cunningham 2001). However, northeastern Pacific taxa appear to have the opposite pattern, with only two range-persistence species having high-dispersing planktonic larvae (*B. glandula* and *L. scutulata*), and three of six range-contraction taxa with high-dispersing larvae. However, an important caveat regarding species having high-dispersing life history is that this trait can actually obscure or erase the genetic pattern of divergence expected under a range-persistence history (Nielsen and Slatkin 2000; Nielsen and Wakeley 2001; Kalinowski 2002a). In this case, high gene flow in these three southern refugium species could have resulted in falsely not rejecting a range contraction history.

Vertical distribution could play an important role in how an intertidal species range responds to glaciation (Marko 2004). Indeed, four of the six range-contraction taxa are distributed in the upper intertidal or high-splash zone of the rocky intertidal, whereas *Littorina scutulata* is the only range-persistence species found this high up. On the other hand, four of six of the range-persistence taxa are restricted to the middle intertidal zone, an area that would be less exposed to the colder air temperatures occurring during the LGM. However, the two *Xiphister* species in this study do not markedly differ in their vertical depth, suggesting that other ecological determinants were more important.

The ecological trait in which the pair of *Xiphister* species differ the most, aside from diet, is habitat specificity. Indeed, *X. mucosus* is restricted to open coastlines whereas *X. atropurpureus* is commonly found in open coastline areas as well as within bays and inlets provided that there is enough wave action, algae, and rocky substrate (Eschmeyer et al. 1983; M. Hickerson, pers. obs.). For example, *X. mucosus* was often absent from populations in which *X. atropurpureus* was found, such as Campbell River. This difference would be manifested in a marked difference in the number of demes, which is entirely consistent with the disparate genetic signatures found in these two species. This is because the number of demes is proportional to genealogical depth given subdivision in a species with many unsampled demes (Wakeley and Aliacar 2001; Pannell 2003). Furthermore, the fewer demes in *X. mucosus* would make this species more vulnerable to extinction across the northern half of its present range from coastal glaciations. However, this sort of relationship between habitat specificity and biogeographic history is not found in the other reviewed temperate intertidal taxa.

Further evaluating the relative roles that ecology and climate change had on the community history of the northeastern Pacific intertidal will require reconstructing the ecological history by genetically sampling over broad taxonomic breadth that includes taxa with known contemporary trophic interactions (Paine 1974). In such a study, phylogeographic analysis using statistical methodology based on appropriate

models can show how historical interactions could either have been stable or temporally and geographically shifted (Van Valkenburg 1995). While such broad taxonomic sampling might seem temporally and economically infeasible, the development of DNA chip technology might allow such a survey to be tenable in the foreseeable future. However, these taxonomically broad sampling schemes will likely rely on single-locus genetic sampling (mtDNA), such that statistically testing alternate biogeographical histories will remain a challenge given the enormous parameter space implicit in any particular biogeographic history (Knowles 2001; Knowles and Maddison 2002). Therefore infusion of paleoecological data will further enable researchers to reconstruct the histories of these ecological interactions and determine the variables that correlate with species range limits over time so that the hypothesized influence of climate change on taxonomic diversity can be tested (Cruzan and Templeton 2000; Hugall et al. 2002).

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