

PHYLOGEOGRAPHY AND HISTORICAL ECOLOGY OF THE NORTH ATLANTIC INTERTIDAL

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Abstract.—Recent glaciation covered the full extent of rocky intertidal habitat along the coasts of New England and the Canadian Maritimes. To test whether this glaciation in fact caused wholesale extinction of obligate rocky intertidal invertebrates, and thus required a recolonization from Europe, we compared American and European populations using allelic diversity and techniques adapted from coalescent theory. Mitochondrial DNA sequences were collected from ampho-Atlantic populations of three cold-temperate obligate rocky intertidal species (a barnacle, *Semibalanus balanoides*, and two gastropods, *Nucella lapillus* and *Littorina obtusata*) and three cold-temperate habitat generalist species (a seastar, *Asterias rubens*; a mussel, *Mytilus edulis*, and an isopod, *Idotea balthica*). For many of these species we were able to estimate the lineage-specific mutation rate based on trans-Arctic divergences between Pacific and Atlantic taxa. These data indicate that some obligate rocky intertidal taxa have colonized New England from European populations. However, the patterns of persistence in North America indicate that other life-history traits, including mechanisms of dispersal, may be more important for surviving dramatic environmental and climatic change.

Key words.—Coalescent, glaciation, North Atlantic, phylogeography, range expansion.

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A primary goal of historical ecology is to determine how communities and biological interactions develop (Ricklefs and Schluter 1993; Stone et al. 1996). Interactions among species may vary geographically based on the ecological context, and such variation may arise in very recent species associations (Vermeij 1992; Travis 1996). For example, the isopod *Idotea balthica* is a more dominant herbivore and is found in a broader range of algal and seagrass habitats (J. P. Wares, pers. obs.) in New England populations than in European populations. In European populations, this species tends to be ecologically marginalized through competition with congeneric grazers (Franke and Janke 1998). What ecological variables play a determinant role in producing this variation in community composition and interactions? Only by knowing both the historical and ecological contexts of these interactions can we decide (Endler 1982; Brooks and McLennan 1991; Vermeij 1992), and studies of comparative phylogeography represent a solid approach to this problem.

In North Atlantic intertidal marine communities, the last glacial maximum (around 20,000 years ago; Barash et al. 1992; Holder et al. 1999) is thought to have been especially difficult for obligate rocky intertidal species on the North American coast. Many such species may have been forced from their typical habitat by Pleistocene glaciation, because there is currently little hard substrate beyond the southernmost extent of the glaciers at Long Island Sound (Knott and Hoskins 1968; Ingólfsson 1992; Riggs et al. 1996). If glaciation caused localized extinction in New England rocky intertidal species, then much of the extant rocky intertidal community may have recently immigrated from Europe, where hard substrata were more available and Pleistocene climatic changes were not as severe (Vermeij 1991; van Oppen et al. 1995). Distributional evidence suggests this is a likely scenario because the list of obligate rocky intertidal

species in North America is a subset of the same group of species found in Iceland, which in turn represents a subset of these species found in northern Europe (Ingólfsson 1992). Although many marine species may have been able to shift or contract their geographic range into southern refugia along the North American coast, species requiring hard substrata may not have been able to find suitable habitat.

Analysis of data from multiple species with varied life-history traits (including larval dispersal, trophic level, and ecological traits such as substrate requirements) allows us to focus on species or groups of species that did not survive the most recent glacial maximum in North America. Populations of these species in New England should be recognizable based on two basic genetic patterns. First, recently founded populations are expected to represent a subset of the genetic diversity in the source population. This leads to the prediction that recently founded populations should have a significantly lower genetic diversity and have a high frequency of alleles that are identical to or descended from alleles in the founding population (Hewitt 1996, 2000; Austerlitz et al. 1997; Johnson et al. 2000; Zink et al. 2000). Second, recently founded populations should show the genetic signature of a rapidly expanding population (e.g., Nee et al. 1995; Kuhner et al. 1998), in part because they are unlikely to have reached an equilibrium between allelic diversity (mutation) and demographic changes (genetic drift).

In this paper, we use genetic markers to describe the post-glacial reassembly of the intertidal marine community in New England and the Canadian Maritimes. We will focus on the hypothesis that substratum requirements have played a major role in North Atlantic historical biogeography. Because there are recognizable patterns that we expect in recently founded populations, we test these predictions using both standard population genetic measures as well as recently developed coalescent methods. Coalescent methods (for review, see Hudson 1990) are expected to more accurately estimate these patterns than methods using pairwise sequence comparisons

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TABLE 1. Species collected for this study, with geographic range and collection sites indicated. Dispersal type for each species is summarized in the first column and described further in the Discussion. Sister groups used to ensure proper identification and for estimate of μ are also listed. Collection sites include Beaufort, NC (NC); Beavertail State Park, RI (RI); Woods Hole, MA (WH); Darling Marine Center, Damariscotta, ME (M); Antigonish, Nova Scotia (NS); St. John's, Newfoundland (W); Reykjavik, Iceland (Ic); Trondheim, Norway (No); Galway, Ireland (Ir); and Roscoff, France (Fr). Species considered to be obligate rocky intertidal taxa are marked with an asterisk. Outgroup species used for mutation rate calibration are marked with the symbol ‡.

Species (<i>n</i>) dispersal type	Geographic range	Collection sites	Congeners identified	Data collected (Genbank accession)
<i>Asterias rubens</i> (48) Echinodermata: Asteroidea broad planktonic dispersal	N. Europe, Iceland; N. America to Long Island Sound, submerged to mid-Atlantic	M, NS, W, Ic, No, Ir, Fr	‡ <i>A. amurensis</i> (Pacific) <i>A. forbesii</i> (Atlantic, N. American endemic)	627 bp AF240022–240081
<i>Idotea balthica</i> (34) Arthropoda: Isopoda low dispersal, brooder	Mediterranean, W. Europe; N. America from Cape Hatteras north	NC, IR, M, NS, W, Ic, No, Ir, Fr	<i>I. metallica</i> , <i>I. emarginata</i> , <i>I. granulosa</i> (Atlantic)	516 bp AF241889–241935
<i>Mytilus edulis</i> (91) Mollusca: Bivalvia broad planktonic dispersal	Mediterranean, W. Europe; N. America from Cap Hatteras north	NC, RI, M, NS, W, Ic, No, Ir, Fr	<i>M. galloprovincialis</i> (Atlantic, included) ‡ <i>M. trossulus</i> (Pacific, Atlantic populations)	463 bp AF241936–142035
<i>Littorina obtusata</i> * (44) Mollusca: Gastropoda low dispersal, crawl-away	N. Europe, Iceland; N. America to Long Island Sound	RI, M, NS, W, Ic, No, Ir, Fr	<i>L. fabalis</i> (Atlantic) ‡ <i>L. saxatilis</i> (Atlantic)	537 bp AF242067–242117
<i>Semibalanus balanoides</i> * (69) Arthropoda: Cirripedia broad planktonic dispersal	N. Europe, Iceland; N. America to Long Island Sound; Alaska	RI, M, NS, W, Ic, No, Ir, Fr	<i>S. cariosus</i> (Pacific) ‡ <i>S. balanoides</i> (Alaskan populations)	615 bp AF242660–242728
<i>Nucella lapillus</i> * (61) Mollusca: Gastropoda low dispersal, crawl-away	N. Europe, Iceland; N. America to Long Island Sound	RI, M, NS, W, Ic, No, Ir, Fr	‡ <i>N. freyceneti</i> (Pacific)	589 bp AF242118–242178

(e.g., Slatkin and Hudson 1991; Rogers and Harpending 1992), which do not make full use of the genealogical structure of the data (Felsenstein 1992). Furthermore, because we were able to estimate lineage-specific mutation rates for the species in this study, we use these coalescent methods to compare the population sizes and ages of North American and European populations. Although there are uncertainties in using such estimates, they nevertheless allow us to establish a quantitative, as well as qualitative, test of the hypothesis that New England rocky intertidal populations were founded since the most recent glaciation.

Here we apply these methods to a series of empirical datasets that allow us to compare the idiosyncratic history of each species with expectations based on the coalescent model. We have studied mitochondrial DNA (mtDNA) sequences from amphi-Atlantic populations of three cold-temperate obligate rocky intertidal species (a barnacle, *Semibalanus balanoides*, and two gastropods, *Nucella lapillus* and *Littorina obtusata*) and three cold-temperate habitat generalist species (a seastar, *Asterias rubens*; an isopod, *I. balthica*; and the blue mussel, *Mytilus edulis*). These data indicate that some obligate rocky intertidal taxa have colonized New England from European populations; however, the patterns of persistence in North America indicate that other life-history traits, including mechanisms of dispersal, may be more important for surviving dramatic environmental and climatic change.

MATERIALS AND METHODS

Specimen Collection and Preservation

Specimens were collected from intertidal sites listed in Table 1, and tissues were then placed in 95% ethanol or a DMSO-based buffer (Seutin et al. 1991) immediately. With two exceptions, species were identified based on morpho-

logical characters listed in Hayward and Ryland (1995) and Gosner (1978).

For *M. edulis*, the phenomenon of doubly uniparental mitochondrial inheritance (reviewed in Quesada et al. 1996) required that female lineages be identified by comparison to identified male and female lineages in Hoeh et al. (1996); only females were incorporated into our analyses. *Mytilus edulis* were distinguished from the European endemic species *M. galloprovincialis* using both the nuclear *Glu-5'* polymerase chain reaction (PCR) marker (Rawson et al. 1996) and comparisons with sequence data from the mitochondrial cytochrome oxidase III locus (Quesada et al. 1995). Individuals identified as *M. galloprovincialis* were not excluded if their mitochondrial type was that of *M. edulis*.

Littorina obtusata is difficult to distinguish morphologically from its congener *L. fabalis*. We identified a clade of *L. fabalis* individuals with our cytochrome oxidase I (COI) data and confirmed their identity by comparing sequence data from the cytochrome oxidase *b* gene to published *L. fabalis* sequences (Reid et al. 1996); these individuals were removed from further analysis.

DNA Extraction and Amplification

Tissues were phenol-extracted and amplified with the PCR protocol listed in Wares (2001a) and the mitochondrial COI gene primers LCO1490/HCO2198 (Folmer et al. 1994). For some taxa, species-specific primer pairs were developed from initial sequence data (available from authors). All individuals were sequenced from both directions using PCR primers. Sequences were obtained in both directions and edited using Sequencher 3.0 (Genecodes Corp., Ann Arbor, MI). No indels were found, but missing or ambiguous end regions were trimmed so that all individuals in a species had the same sequence length.

Phylogenetic Analysis

Parsimony networks were developed for each species using the methods of Templeton et al. (1992). The network for each species was compared with the topology of the maximum-likelihood (ML) phylogeny to ensure concordance; the best-fit ML model (Goldman 1993; Cunningham et al. 1998) for each dataset was determined using ModelTest (Posada and Crandall 1998). Searches were performed with stepwise addition (simple addition sequence) and TBR branch swapping with zero-length branches collapsed.

Isolation by Distance and Haplotype Diversity

Pairwise estimates of Nm (where N is the effective population size and m is the rate of migration) between sampled locations for each species were calculated based on an island model using Slatkin's linearized F_{ST} -values (Slatkin 1991, 1995) in the program Arlequin 2.001 (Schneider et al. 1997), along with AMOVA tests (Excoffier et al. 1992) for each species. Geographic distances (km) between locations were calculated using the great circle distance between each site. A Mantel test (1000 permutations using Mantel 2.0; available via www.sci.qut.edu.au/NRS/mantel.htm) compared the correlation between $\log(Nm)$ and $\log(\text{km})$ distance matrices to the null hypothesis that they are not linearly related (Slatkin 1993; Hellberg 1994). Additionally, haplotype diversity (H , Nei 1987, eq. 8.4) and its sampling variance were calculated for each species and/or continental population using Arlequin vers. 2.001.

Estimation of Ancestral Population Parameters

The full geographic range of each species was divided into North American and European/Icelandic regions. Estimates of Θ ($= 2N\mu$, where μ is the mutation rate for mtDNA) were made for each region as well as the North Atlantic as a whole. The ML estimate of the parameters Θ and g (the exponential growth parameter in units of μ^{-1}) were made using Fluctuate (Kuhner et al. 1998). Seeds for all analyses were generated randomly. The appropriate transition:transversion ratio (estimated by maximum likelihood in PAUP* 4.0; Swofford 1998) was input for each dataset. Analyses were repeated five times per species and/or population to ensure stability of parameter estimates; the mean values are reported. Final analysis of each dataset employed 10 short Monte Carlo chains of 4000 steps each and five long chains of length 20,000, with a sampling increment of 20. Fluctuate generated a random topology for initial searching. These estimates of exponential growth are used to generate the historical size of each population:

$$N_t = \Theta e^{-(g\mu)t}, \quad (1)$$

where N_t is the effective population size at any time t in the past (Kuhner et al. 1998).

RESULTS

Phylogenetic Analysis

Haplotype networks representing the complete datasets for all six species are shown in Figure 1. Because outgroup root-

ing is not reliable for intraspecific genealogies (Castelloe and Templeton 1994), the most likely root haplotypes are indicated on each network. For datasets that are compatible with the infinite-sites model (Watterson 1975), the likelihood of the genealogy given each possible root haplotype was calculated using Genetree (Griffiths and Tavaré 1994). The data for *M. edulis* violate this model, so the heuristic method of Castelloe and Templeton (1994) was used. This method also obtained the same haplotype for each of the other species as the ML methods described above.

Isolation by Distance and Haplotype Diversity

There were no patterns of isolation by distance in these North Atlantic species that were more than marginally significant (J. P. Wares, unpubl. data). In each case, the signal of population structure could be traced to the European populations; trans-Atlantic isolation by distance does not seem to be an important mechanism. Haplotype diversities (H) of American and European populations are shown in Table 2. In all cases but *M. edulis*, North American populations showed significantly lower haplotype diversity than European populations. Complete AMOVA results indicating how genetic variation is partitioned in each species are reported in Appendix 2. Only in two species (*A. rubens* and *L. obtusata*) was there any indication of subdivision between North American and European populations. In *Littorina* there is a distinct clade in the European population (Fig. 1) that contributes to this statistical partitioning, whereas in *Asterias* all American haplotypes are shared with Europe, suggesting that the AMOVA result is artifactual (see genealogical description below). This result is likely an artifact of pairwise F_{ST} analysis generated by the significant difference in genetic diversity between these two populations (see Whitlock and McCauley 1999).

Lineage-Specific Estimates of Mutation Rate

Fossil evidence indicates that many of the lineages in this study invaded the North Atlantic from the Pacific during the trans-Arctic interchange 3.5 million years ago (Worley and Franz 1983; Vermeij 1991; Collins et al. 1996; Reid et al. 1996). Although earlier estimates have been made for the opening of the Bering Strait (Marincovich and Gladenkov 1999), the first fossil evidence of large-scale invasion from the Pacific was at 3.5 million years ago (Vermeij 1991). Because this invasion took place just before the onset of Northern Hemisphere cooling and glaciation (Berggren and Hollister 1974), the cold-temperate species in this study were able to invade the North Atlantic, but have had little opportunity for recent gene flow across the Arctic. For each species (except *I. balthica*), we identified the Pacific sister taxon, and used Atlantic/Pacific sequence divergence as the basis for our lineage-specific mutation rates, with an estimated date of divergence of 3.5 million years ago.

To account for polymorphism in ancestral species lineages, the ML estimate of the internal branch length separating the sister taxa/populations from the Pacific and Atlantic was used to estimate the appropriate amount of divergence per site (see Edwards and Beerli 2000). This measure represents the net nucleotide divergence, d (Nei and Li 1979), and allows a

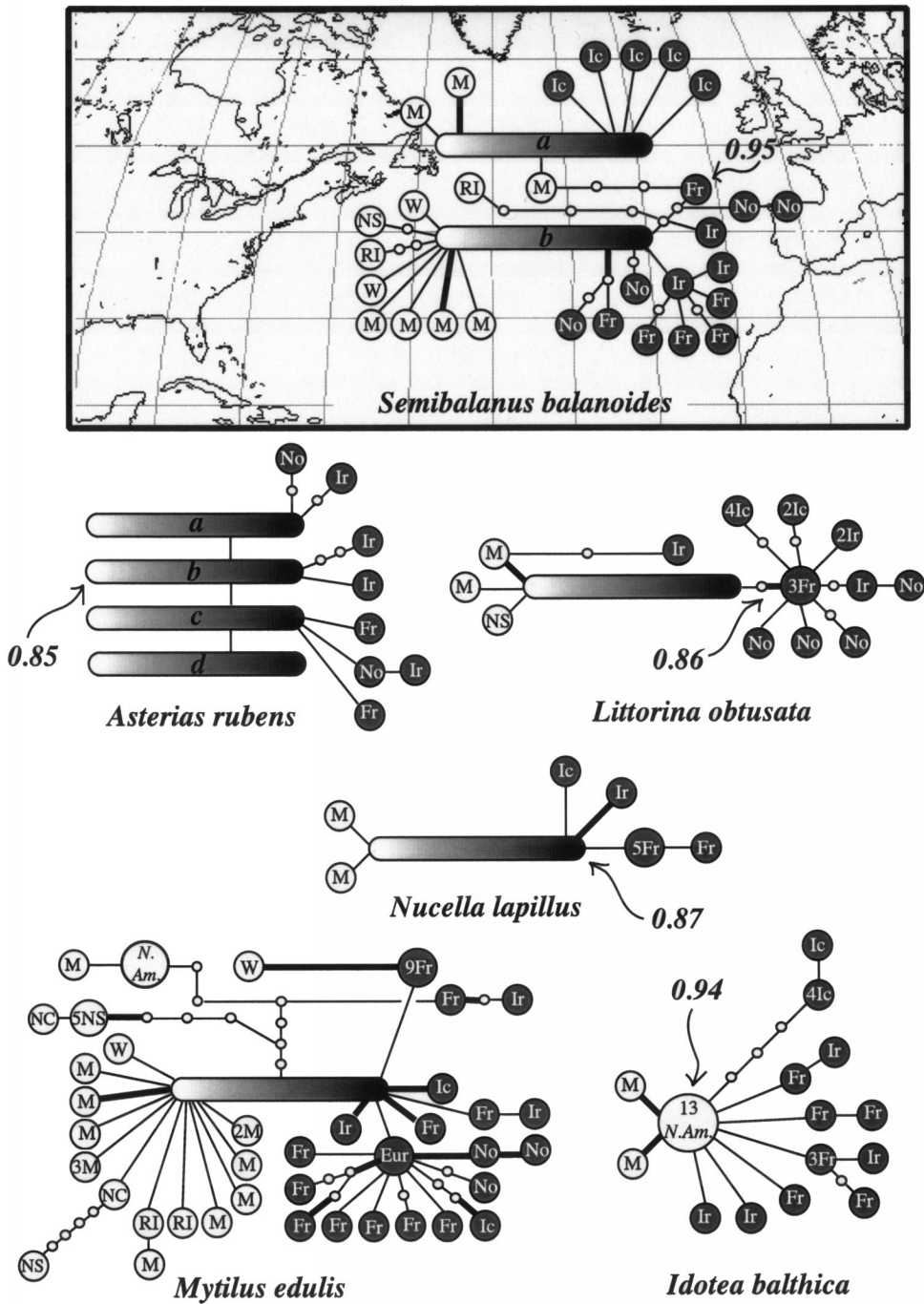


FIG. 1. Parsimony networks for each species in this study. Dark circles represent alleles found in Europe, and white circles represent alleles found in North America. Each branch represents a single nucleotide substitution, with missing intermediates shown as small empty circles. Bold lines indicate first/second position substitutions. Shared alleles are shown as half-shaded ovals. Sample locations are designated as in Table 1. The maximum-likelihood root presented (arrows) is always at least an order of magnitude greater likelihood than other possible root alleles; in *Mytilus* the maximum-likelihood root could not be calculated using an infinite-sites model. The Castelleo and Templeton (1994) method indicates that the root probability of the shared allele combined with the alleles marked "9Fr," "Eur," and "N.Am." is 0.662. Additional information on allele sampling and composition of shared alleles for each species is available in Appendix 1.

TABLE 2. Comparisons of haplotype diversity in North American and European populations. Haplotype diversity (H) and sampling variance were calculated for each species in Arlequin 2.001 (Schneider et al. 1997) using equation (8.4) in Nei (1987). In each species except for *Mytilus edulis*, haplotype diversity is significantly higher ($P < 0.05$) in European populations than in North American populations.

Species	North American (H)	Europe (H)
<i>Asterias rubens</i>	0.597 \pm 0.063	0.893 \pm 0.038
<i>Semibalanus balanoides</i>	0.771 \pm 0.052	0.936 \pm 0.033
North American clades separated by maximum-likelihood root	0.275 \pm 0.015; 0.662 \pm 0.116	
<i>Mytilus edulis</i>	0.893 \pm 0.032	0.923 \pm 0.025
<i>Littorina obtusata</i>	0.186 \pm 0.110	0.933 \pm 0.025
<i>Nucella lapillus</i>	0.145 \pm 0.089	0.434 \pm 0.101
<i>Idotea balthica</i>	0.257 \pm 0.142	0.942 \pm 0.038

calculation of the mutation rate as $\mu = (1/2)d/(3.5 \times 10^6$ years), using the trans-Arctic divergence estimate discussed above. These ML estimates were calculated using the F84 model (separate rates for transitions and transversions), which is the only model applied in Fluctuate. Only the *M. edulis* data rejected the assumption of a molecular clock (χ^2 distributed with $[n - 2]$ degrees of freedom, $P < 0.05$; Goldman 1993).

Estimates of μ were based on divergence between Atlantic populations and the Pacific sister groups. For two species, direct estimates of Atlantic/Pacific divergence were not available. For *L. obtusata*, we were not able to obtain specimens of the Pacific outgroup *L. sitkana* (Reid et al. 1996). Our estimate of μ in *Littorina* was based on comparisons between *L. obtusata*, *L. saxatilis*, and *L. sitkana* for cytochrome *b* (Reid et al. 1996) and our COI divergence between *L. obtusata* and *L. saxatilis*. A direct estimate for *I. balthica* is not available because the trans-Arctic history and interspecific phylogeny of this genus are both unknown. Subsequent analysis of *I. balthica* used the mean estimate of μ from other species in this study, a rate very similar to other crustacean rate estimates for this fragment of COI, as shown in Table 3.

We chose to restrict our estimates of mutation rate to third positions for two reasons. First, substitutions at the third position are much less likely to cause amino-acid substitutions and are more likely to obey the assumption of neutrality required by our coalescent methods; even within-species

comparisons often contain more nonneutral polymorphisms than would be expected under the neutral model (Hasegawa et al. 1998). Second, to include all three codon positions, an estimate of among-site rate variation is necessary. Because our Pacific and Atlantic divergences are very recent, we believe that the appropriate parameter for the gamma distribution cannot be accurately estimated based on the low sequence variation in these samples. Using the discrete gamma distribution, the mean substitution rate across site classes must be one (Yang 1994); yet in these datasets one class of substitutions is always dramatically more frequent than others, meaning its rate is proportional to the number of classes chosen for the discrete gamma. In fact, Pacific and Atlantic divergences differed by up to an order of magnitude depending on how many discrete rate categories were estimated for the gamma distribution (C. Cunningham, pers. obs.). Mutation rates based on third positions are presented in Table 3.

Estimates of Population Growth

The parameters Θ and g were jointly estimated for third position datasets for all six species using Fluctuate (Table 4; Kuhner et al. 1998) under two conditions: an unconstrained exponential growth parameter and an assumption of constant N ($g = 0$). Allowing Fluctuate to estimate Θ and g jointly produced estimates of N that were two to three times larger than when Θ was estimated with no growth. These jointly estimated parameters were used to generate plots of the change in relative size of N over time. For example, the time at which the population was half its current size is given as $\ln(0.5)/\mu g$. Table 4 presents confidence intervals indicating the size of each population 20,000 years ago relative to its current size. The plots of relative N through time are given in Figure 2. For the barnacle *S. balanoides*, the genealogical structure and root likelihood suggested that the analysis should be performed assuming multiple colonization events (see below). If population size is assumed constant, each species except for *M. edulis* has significantly lower estimates of Θ and H in North American populations than in European populations (Tables 2, 4). The approximate age of each population is presented in the Results and Discussion as the age at which N drops below 1% of the current estimated size.

Genealogical Descriptions for Each Species

Asterias rubens

The North American population of this seastar contained no unique μg alleles relative to the European population, which

TABLE 3. Estimated mutation rates for species in this study (substitutions per site per generation). Estimates are based on the internal branch length separating populations of ingroup and outgroup taxa (see Materials and Methods) using the best-fit model (F84) for third position characters only. Estimates of third-position mutation rates were also made using datasets for species separated by the Isthmus of Panama, including the crab genus *Sesarma* (*S. rhizophorae* vs. transisthmian sister group; Schubart et al. 1998), the shrimp *Alpheus* (*A. chacei* vs. *A. penultimum*; Knowlton and Weigt 1998), and the barnacle *Euraphia* (Wares 2001a).

Species	Mutation rate (SE)
<i>Asterias rubens</i>	4.84×10^{-8} (8.77×10^{-9})
<i>Mytilus edulis</i>	9.51×10^{-8} (1.96×10^{-8})
<i>Littorina obtusata</i>	2.49×10^{-8} (1.3×10^{-8})
<i>Nucella lapillus</i>	4.43×10^{-8} (1.07×10^{-8})
<i>Semibalanus balanoides</i>	2.76×10^{-8} (6.32×10^{-9})
<i>Idotea balthica</i>	3.6×10^{-8} (see text)
<i>Sesarma</i> spp. (Panama)	2.1×10^{-8}
<i>Alpheus</i> spp. (Panama)	1.9×10^{-8}
<i>Euraphia</i> spp. (Panama)	3.8×10^{-8}

TABLE 4. Estimates of Θ (the compound parameter representing the effective population size and substitution rate) and g (the exponential growth parameter) for species in this study using Fluctuate (Kuhner et al. 1998). Each species is divided into North American and European populations to calculate the range of projected ages for each region. The Fluctuate estimates (the composite of five replicate analyses) indicate the maximum-likelihood estimate of the joint probability of Θ and g . The estimates of N during the most recent glacial maximum (20,000 years ago) relative to present-day estimates of N are based on g and μ . The standard deviation is presented after each estimator. Estimated parameters for *Idotea* (*) based on results using all codon positions, as the North American population is invariant at third position. However, the maximum-likelihood estimate of g is maximal using either all codon positions or a dataset simulated using the same mutational parameters as the *Idotea* third position data, and this estimate is concordant with high estimates of g in other species with very low North American allelic diversity (*Littorina*, *Nucella*).

Species	Population	Θ (no growth)	Θ (jointly estimated)	g	Time to 1% relative N	Relative N 20,000 years ago
<i>Asterias rubens</i>	North America	0.00429	0.0056 \pm 0.002	875.3 \pm 437.4	104,800 (59,500–253,900)	0.17–0.99
	Europe	0.2663	0.4190 \pm 0.301	962.1 \pm 314.2	95,400 (61,200–171,600)	0.22–0.72
<i>Idotea balthica</i>	North America	0.00398*	0.3404 \pm 0.328*	10,000*	13,400	2.5 $\times 10^{-3}$ –0.011
	Europe	0.05004	0.1883 \pm 0.012	278.3 \pm 18.6	482,700	0.81–0.85
<i>Mytilus edulis</i>	North America	0.02965	0.1259 \pm 0.045	109.4 \pm 49.4	1.84 $\times 10^6$ (1.12–3.89 $\times 10^6$)	0.91–0.99
	Europe	0.03264	0.3180 \pm 0.0953	356.6 \pm 55.3	5.66 $\times 10^5$ (4.32–7.74 $\times 10^5$)	0.81–0.89
<i>Littorina obtusata</i>	North America	0.00248	0.0134 \pm 0.0115	10,000 \pm (1,946)	18,500 (11,047–38,389)	6.8 $\times 10^{-3}$ –6.8 $\times 10^{-3}$
	Europe	0.03137	0.1170 \pm 0.0228	493.6 \pm 39.3	3.74 $\times 10^5$ (2.47–6.80 $\times 10^5$)	0.75–0.82
<i>Semibalanus balanoides</i>	North America	n/a	0.0325 \pm 0.0023	30.8 \pm 5.9	5.14 $\times 10^6$ (3.54–8.14 $\times 10^6$)	0.98–0.99
	South clade	0.00309	71.531 \pm 39.23	2326.3 \pm 460.2	68,000 (46,685–108,401)	0.15–0.44
<i>Nucella lapillus</i>	North clade	0.02341	0.4456 \pm 0.5994	10,000 \pm (1,138)	15,800 (11,679–22,826)	2.9 $\times 10^{-3}$ –2.9 $\times 10^{-3}$
	Europe	0.06347	0.1826 \pm 0.0173	176.0 \pm 26.3	8.99 $\times 10^5$ (6.42 $\times 10^5$ –1.35 $\times 10^6$)	0.87–0.93
<i>Nucella lapillus</i>	North America	0.00434	0.0966 \pm 0.0749	10,000 \pm (1,161)	10,400 (7479–15,496)	1.4 $\times 10^{-3}$ –1.4 $\times 10^{-3}$
	Europe	0.00534	0.5379 \pm 0.5342	4391.4 \pm 396.7	23,600 (17,400–34,300)	0.01–0.04

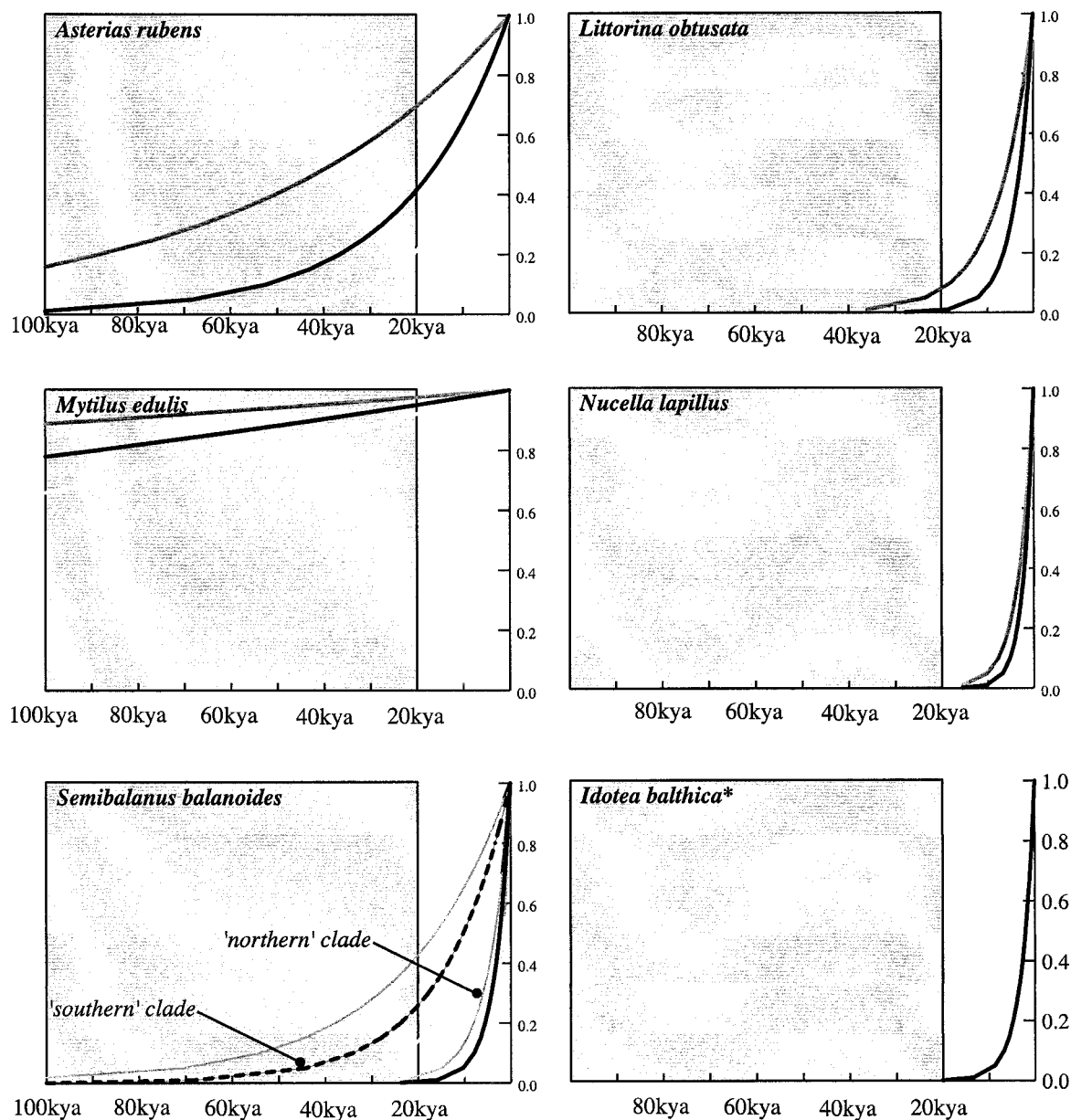


FIG. 2. Patterns of growth in each species (with 95% confidence intervals) as reconstructed from estimates of the parameter g estimated jointly with Θ using Fluctuate (Kuhner et al. 1998). The vertical axis in each graph represents ancestral N_e relative to current population size; horizontal axis is time, measured in thousands of years. The most recent glacial maximum is indicated at 20,000 years on each chart. Populations that appear to have less than 1% of current N at 20,000 years (see Table 4) are likely to be recent colonists. The reconstruction of *Idotea* is based on the same growth parameter being obtained for both analysis of full dataset and third position only with random substitutions. It is also the same growth parameter obtained for *Nucella* and *Littorina* with nearly identical North American allelic patterns. The pattern shown for *Asterias* is illustrative of the effect of ancestral polymorphism on these reconstructions, as it is similar to the pattern obtained for European populations (data not shown) despite significantly lower haplotype diversity in North America (see Discussion).

is consistent with a recent founding event from Europe (Fig. 1). This is supported by a significantly lower haplotype diversity in North America (Table 2). However, parameter estimates (Θ and g) are very similar in North American and European populations, and the North American population appears to date back beyond the recent glacial maximum (Table 4, Fig. 2). Such a contradiction indicates that this analysis was confounded by ancestral polymorphism. When a population is founded by more than one haplotype, ancestral

polymorphism from the founding population will inflate the estimated size and age of the newly founded population. This issue is further described in the Discussion.

Semibalanus balanoides

With very large diversity in Europe and two shared haplotypes with North America, the genealogical pattern in *S. balanoides* appears consistent with multiple colonization

events from Europe to North America (Fig. 1). When all North American individuals are included, our Fluctuate analysis suggests an unrealistic time of origin for North American populations, an age that actually predates the trans-Arctic interchange (Table 4). As with *A. rubens*, this result can be easily explained by ancestral polymorphism, because the two major clades in North America (Fig. 1) are distantly related to one another. Each clade shares a common haplotype with European populations and taken individually, each clade is significantly less diverse than the European population (Table 2). This hypothesis of multiple colonization events is supported by the separation of the two North American clades by the ML root in Europe (Fig. 1). Separate analysis of each North American clade indicates that, whereas one of these events may be quite recent (time to most recent common ancestor [TMRCA] = 3.3×10^4 years, population less than 1% current size less than 20,000 years ago), the other clade appears to predate the last glacial maximum (Table 4), a hypothesis also suggested by Brown et al. (2001).

Mytilus edulis

The mussel *M. edulis* appears to have long histories on both coasts (Table 4), with a tremendous amount of endemic diversity present in each region. The only shared allele between North America and Europe was also identified as the root haplotype; note that the presence of high allelic diversity in North America cannot be easily explained by a very large, recent founding population because there is only a single shared haplotype. Inspection of the network in Figure 1 shows several unique clades to each continent, including two North American lineages that are distantly related to an isolated European clade and the shared North American–European haplotype. This pattern of missing intermediate haplotypes may indicate incomplete lineage sorting or gene flow from North America to Europe. When these genetically isolated lineages are removed, our conclusion of a long history for *M. edulis* in North America is not affected; similarly, exclusion of the haplotypes collected from *M. galloprovincialis* individuals (see Materials and Methods) did not affect our conclusion of a long history in Europe.

Littorina obtusata and *Nucella lapillus*

Littorina obtusata and *N. lapillus* share remarkably similar patterns in the North Atlantic (Fig. 1). Both have a high-frequency haplotype in North America that is shared with Europe, and each species has two unique North American haplotypes. Both have significantly lower haplotype diversity in North America than in Europe and both have population ages that are almost identical; these results are consistent with postglacial colonization from Europe. For both species, translation of the ML estimates of *g* indicate that the North American populations of these species were less than 1% of the current size during the most recent glacial maximum (Table 4, Fig. 2). The apparent migration event from North America to Europe by *L. obtusata* (Fig. 1) seems to be the result of a homoplastic amino-acid substitution that unites a North American and European haplotype.

Idotea balthica

The isopod *I. balthica* has significantly lower diversity in North America than in Europe (Tables 2, 4). Unlike all other species in this study, there are no shared haplotypes between North America and Europe. Moreover, Genetree identifies the common North American haplotype as the root of the network and, therefore, the oldest allele in the North Atlantic. Because all seven European lineages are descended from the root haplotype, it is parsimonious to conclude that this haplotype was once present in the European population. Our failure to find it in our European sample can be explained by its loss due to lineage sorting or simply by our limited sampling effort. The very large diversity of the European population confirms a long history in Europe. We conclude that this pattern is consistent with a recent colonization of North America from Europe. The alternative hypothesis—that the root allele originated in North America—would require either seven independent invasions of Europe or seven unique mutation events following a single invasion, followed by extinction of the invading haplotype(s). These hypotheses have been statistically rejected (J. P. Wares, unpubl. data).

Although *I. balthica* has three haplotypes in North America, the two singleton haplotypes are characterized by first/second-position substitutions and are therefore not present in our third position datasets. This means that at third positions, the North American population is monotypic, making an estimate of age from third positions by Fluctuate impossible. Dating the age of the North American population of *I. balthica* is also complicated because it is the only species for which we lack lineage-specific estimates of mutation rate. That being said, the allelic diversity of the North American population is comparable to other species whose ages appear to date since the last glacial maximum (*L. obtusata* and *N. lapillus*; Table 4, Fig. 2), and analysis of either the full dataset or a third position dataset with two randomly introduced substitutions generates a maximal estimate of population growth and is reflected in the curve drawn in Figure 2.

DISCUSSION

Glaciation and North Atlantic Populations

Our central focus has been to test the hypothesis that glacial events had a greater impact on the rocky intertidal of North America than in Europe, and species requiring rocky substrata would be more likely to go extinct in North American populations. This hypothesis makes two specific predictions. First, all species that currently have an amphi-Atlantic distribution will show a genetic signature of a long population history in Europe. Second, some or all amphi-Atlantic species will show a genetic signature of postglacial range expansion from Europe to North America. By distinguishing between species with North American populations that did or did not survive the last glacial maximum, we can begin to understand the organismal characteristics necessary to survive this extreme climatic event.

Of these two predictions, it is easiest to show that all European populations of amphi-Atlantic species survived the last glacial maximum. An older population is more likely to be at an equilibrium level of genetic diversity and is char-

acterized by high allelic diversity with many unique haplotypes. All of our European populations fit this prediction, and in each case except for *N. lapillus*, the estimated age of the European population appears to be much older than the last glacial maximum (Table 4). In the case of *Nucella*, high haplotype diversity (Table 2) and the fact that French populations are distinguished from other European populations by a fixed substitution (Fig. 1) substantiate the claim for the old age of European populations. Additionally, there are high levels of both allozyme and karyotypic polymorphism in Europe (Day et al. 1993; Collins et al. 1996). This evidence of geographic subdivision in European *N. lapillus* populations is evidence of an older population than is estimated using coalescent methods.

The prediction that some or all amphi-Atlantic species will have recently colonized North America from Europe is more difficult to test definitively, but makes the following three predictions: (1) haplotype diversity will be significantly lower in North American populations; (2) the estimated age (based on the time to the most recent common ancestor) of North American populations will postdate the last glacial maximum; and (3) all North American haplotypes will either be shared with Europe or be descended from European haplotypes that have participated in range expansion.

Of our six species, five show evidence of colonization from Europe, including the barnacle *S. balanoides*, the gastropods *N. lapillus* and *L. obtusata*, the seastar *A. rubens*, and the isopod *I. balthica* (see Results). Of these, all but *S. balanoides* show patterns consistent with postglacial colonization from Europe (Fig. 2, Table 4). One of two American clades of *S. balanoides* appears to have survived the last glacial maximum, a result supported by an independent study of this species (Brown et al. 2001). Although the actual times since colonization of some of these species, such as *Asterias*, are confounded by ancestral polymorphism or cannot be explicitly determined using the coalescent methods we employed (i.e., *Idotea*), each dataset is consistent with postglacial range expansion from Europe. This conclusion follows the data showing consistently lower allelic diversity in North America and the signal of population growth obtained from coalescent analysis of each species' gene tree.

Several methods of inference were used in this study because it is difficult to accurately test hypotheses about the age of recently founded populations due to ancestral polymorphism. Ancestral polymorphism is known to confound estimates of population divergence time in many typical phylogeographic studies (Nielsen et al. 1998; Edwards and Beerli 2000; Hewitt 2000). Estimating the time to the most recent common ancestor in a population will be biased when the founding population itself was genetically diverse, as in studies such as this one where populations from North America and Europe are not divergent but are largely composed of the same common alleles. We have taken ancestral polymorphism into account by considering how alternative colonization histories could affect our estimates. In *S. balanoides*, it seems apparent that there are two independent colonization events to be analyzed separately. However, dating the invasion of species such as *A. rubens* is still complicated because it is unclear how much diversity in North America is due to multiple events or to founding population diversity.

We realize that an alternative hypothesis exists that is consistent with some, but not all, of our observations. If North American populations did not go extinct, but existed in a glacial refugium of restricted size, we could expect significantly lower North American haplotype diversity. Our estimates of recent population age from our Fluctuate analyses do not rule out this hypothesis, because Fluctuate was only used to generate a growth curve that suggests the time to the most recent common ancestor of the alleles in a population (the *minimum* population age, assuming no effects due to ancestral polymorphism).

Our best chance of distinguishing the refugium bottleneck and colonization hypothesis comes from our repeated observation that North American haplotypes are either shared with or descend from European haplotypes (Fig. 1). The repeated observation of descent from European haplotypes is unlikely under the refugium bottleneck hypothesis, and is specifically predicted by the colonization hypothesis. Given the length of time that hypothetical North American refugia and European refugia would have been separated due to unfavorable climatic conditions during glacial maxima, simulations indicate that the likelihood of observing shared alleles between these populations today is vanishingly small if gene flow was not maintained (Johnson et al. 2000; J. P. Wares, unpubl. ms.). Simply put, separate refugia in North America and Europe would tend to generate greater divergence among alleles from each population.

Even within our data, there is a good example of the difference expected between the North American refugium and recolonization hypotheses. All *I. balthica* individuals collected from Iceland form a clade with low haplotype diversity, and this population shares no alleles with other Atlantic populations. The Icelandic clade has three fixed substitutions relative to all other North Atlantic haplotypes. Applying a Nei and Li (1979) correction for the polymorphism extant in the ancestral population, the divergence between Iceland and the rest of Europe is greater than 200,000 years ago (conservatively based on the most rapid estimate of μ , Table 3). This is consistent with the hypothesis that southwestern Iceland was unglaciated during the last glacial maximum (see Holder et al. 1999) and that *I. balthica* survived in this refugium. Other species, including the ocean quahog *Arctica islandica*, also show evidence of high endemic diversity in Iceland (Dahlgren et al. 2000), supporting a refugium hypothesis over predictions of recent gene flow.

Of the species that do not appear to have survived the last glacial maximum, both *N. lapillus* and *L. obtusata* have been found as fossil remains on the North American coast prior to Pleistocene glaciation (Vermeij 1991), and archaeological evidence places these species again in New England prior to human colonization from Europe (Jenkins et al. 1997). A literal reading of the fossil record in these cases could be misleading, because our data strongly suggest that they have not been continually present in North America and yet they have not been introduced by humans either. Although the possibility of historical introduction by humans should always be considered (Carlton and Geller 1993), the amount of unique genetic diversity in North American populations of each of these species (see Hewitt 2000; Johnson et al. 2000; J. P. Wares, D. Goldwater, and C. W. Cunningham,

unpubl. ms.) and the fact that they are easily recognized macroinvertebrates suggests human-mediated introduction is unlikely.

Persistence and Range Expansion: Associations with Life-History Characteristics

What characteristics allowed species to survive the last glacial maximum in North America? We have presented strong evidence that a population of the obligate rocky intertidal barnacle *S. balanoides* survived the most recent glacial maximum (see also Brown et al. 2001), and other obligate rocky intertidal species in this study were apparently distributed through New England long before those rocky shores were defined by glacial scouring (Pratt and Schlee 1969; Vermeij 1991). It may be more appropriate to assume that there has long been suitable substrata for a variety of species in this region, and glacial maxima simply increased the amount of rocky shore available north of Cape Cod. Some obligate rocky intertidal species were able to find suitable habitat during glacial maxima, whereas others were not. Therefore, substrate requirements do not adequately explain the patterns of persistence and range expansion seen in these species.

Persistence and colonization may require a mechanism of dispersal that allows species to find suitable habitat quickly during environmental change. Modes of dispersal are probably not indicative of colonization ability (Johannesson 1988); *L. obtusata* and *N. lapillus*, two species that appear to have recently colonized North America, have crawl-away larvae or very short planktonic dispersal (Crothers 1985; Reid et al. 1996), suggesting that lack of a pelagic phase does not prevent trans-Atlantic colonization. However, dispersal modes may explain the persistence of taxa in North America through glacial maxima. The two species with the strongest signatures of preglacial persistence in North America, *M. edulis* and *S. balanoides*, each have long-dispersing planktotrophic larvae (Gosling 1992; Holm and Bourget 1994).

To the list of glacial survivors in North America we can add several other temperate species. Although *A. rubens* has recently colonized North America from Europe, its sister species *A. forbesi* is endemic to North America, has planktotrophic larvae (Clark and Downey 1992), and clearly survived glacial maxima (Worley and Franz 1983; Wares 2001c). Our data show that whereas most haplotypes of *I. balthica* share a recent ancestor with European colonists, two endemic North American cryptic species have also been identified (Wares 2001b). *Idotea* broods its offspring but is frequently found on algal rafts that play a large role in its dispersal (Locke and Corey 1989; Ingólfsson 1995), suggesting again that dispersal may allow persistence through climatic changes. Several other North American endemic species with broadly dispersing larvae are reciprocally monophyletic with respect to sister species in Europe (e.g., the hermit crab, *Pagurus acadianus*, Cunningham et al. 1992; C. W. Cunningham, unpubl. data; and the lobster, *Homarus americanus*, Kit and Kornfield 1998), suggesting long-term persistence in North America.

These results suggest that persistence through glacial maxima in North America may be facilitated by a capability for increased dispersal, whether by pelagic larvae or by other

mechanisms (i.e., algal rafting in isopods). This might be explained by opportunistic colonization of ephemeral habitats during glaciation. The hypothesis that high dispersal ability may help species resist extinction was proposed by Jablonski and Lutz (1983) and Valentine and Jablonski (1986) based on paleontological findings. These fossil data illustrate that planktonic species tend to survive longer in the fossil record and survive major extinction events with a higher frequency than expected. This life-history character may be crucial to understanding the patterns of diversity found in regions that were previously altered by glaciation or other climatic and environmental changes. However, it is also of great interest to community ecologists to recognize the differences among species in terms of community assembly and colonization dynamics. Although planktonic larval dispersal may play a role in the long-term survival of species populations, it is also clear that other ecological and physiological dynamics play a role in this system.

Methodological Considerations

Inference of population histories has become a popular and controversial enterprise. In this paper, we focus on estimating rates of population growth using coalescent methods (Fluctuate, Kuhner et al. 1998). Here we focus on three aspects of our approach that deserve attention: our reliance on mtDNA, our estimation of taxon-specific substitution rates, and the idiosyncrasies of coalescent methods for estimating ancestral population parameters.

Corroboration from additional loci

It is well known that estimates of population histories based on a single locus will have high variances (Kuhner et al. 1998; Wollenberg and Avise 1998; Fu and Li 1999). Although mtDNA is especially useful for inferring population history, with its advantages of a high mutation rate, lack of recombination, and small effective population size (Avise et al. 1987), we must take care to recognize its shortcomings. In this study, we are mainly concerned with identifying species that survived the last glacial maximum in North America. How might our mtDNA data be misleading in this regard? If mtDNA shows a signature of long population history on both coasts, this is unlikely to be contradicted by the addition of more loci. This is confirmed for *M. edulis*, which has a second locus available in its paternally inherited mtDNA. In fact, the male mtDNA shows even stronger evidence than the female mtDNA for long histories on both coasts. Based on limited sampling, Quesada et al. (1998) found reciprocal monophyly between Europe and North America in the male mtDNA, a result that is confirmed by additional sampling in our laboratory (C. Riginos, pers. comm.).

However, our inference of recent immigration from Europe for several species can only be regarded as tentative without additional loci. What we interpret as a recent colonization from Europe might simply reflect a recent introgression of European haplotypes into a long-standing North American population. If this were the case, nuclear loci should show evidence of long divergence between Europe and North America. We tested this hypothesis with preliminary studies using sequence data from the ribosomal internal transcribed

spacer (ITS) region for the seastar *A. rubens* (Wares 2001c), the barnacle *S. balanoides* and the gastropod *N. lapillus* (J. P. Wares, unpubl. data). For *Asterias* we sampled the Pacific outgroup and both Atlantic species (*A. amurensis*, *A. forbesi*, and *A. rubens*). Based on the Pacific and Atlantic divergences, the ITS showed a mutation rate that was comparable to the COI third position rate ($\mu = 3.6 \times 10^{-8}$), but found no substitutions at all between five North American and five European *A. rubens* individuals. This is entirely consistent with the recent colonization event from Europe inferred from the mtDNA data and inconsistent with a hypothesis of long-term residence on both coasts. Very limited ITS analyses in the other two species also indicated no divergence between European and North American populations.

Estimating mutation rates

As with any estimation of mutation rate, our estimates must be considered provisional. A potential source of error is our use of a date of 3.5 million years ago for the divergence between North Pacific and North Atlantic cold-temperate populations (see Materials and Methods). There have been reports of the Bering Strait opening as early as 5 million years ago (Collins et al. 1996; Marincovich and Gladenkov 1999), although the major fossil evidence of invasion did not appear until 3.5 million years ago (Vermeij 1991). Using the older date would increase our population age estimates by about 40%, which does not dramatically change our interpretations of postglacial histories.

It is encouraging that our estimates of mtDNA mutation rate are similar to those in other taxa. Our estimates for COI third positions range from $\mu = 2.5\text{--}4.8 \times 10^{-8}$ (Table 2), excluding *Mytilus*, which is known to have an elevated mutation rate (Hoeh et al. 1996). These are within the range noted for COI third position data in other taxa estimated from either side of the Isthmus of Panama using our methodology (Table 3). Finally, our estimate of mutation rate in *Littorina* using the date of the Bering Strait opening (3.5 million years ago; $\mu = 2.5 \times 10^{-8}$) is close to the estimate obtained if we use a fossil date ($\mu = 1.9 \times 10^{-8}$), based on fossil-data estimates of divergence between *L. obtusata* and *L. saxatilis* (2.0 million years ago, Reid et al. 1996).

Coalescent methods and coalescent problems

Our inferences are also clearly affected by methods of estimating Θ and the exponential growth parameter g . When an estimate of the growth parameter g is incorporated, estimates of Θ are much higher than under a model of constant population size. Thus, although the use of an exponential growth model may or may not be biologically accurate (see Kuhner et al. 1998), it is again conservative in that our estimates of historical population size assuming growth are larger than when we do not.

However, this highlights potentially serious biases that arise during joint estimation of parameters from genealogical data (Wakeley 2000). There appears to be a strong bias in the estimation of growth parameters that may cause overestimation of population size and age; this bias may be an inherent property of estimating growth from genealogical data (Kuhner et al. 1998). Thus, strong covariation among

parameters estimated from genealogical data makes definitive estimates of historical demographics elusive (Markovtsova et al. 2000).

The strongest confirmation of the methods we advocate is their success when applied to other empirical systems. Hellberg et al. (2001) propose that the gastropod *Acanthinucella spirata* expanded its range north of Point Conception in California since the last glacial maximum. As with our two gastropods with crawling larvae (*N. lapillus* and *L. obtusata*), newly colonized northern populations are predominantly composed of a common haplotype shared with the south, with several unique haplotypes descended from the putative founding haplotype. When we applied our approach using Fluctuate to *A. spirata* third positions using our mutation rate calculated for *N. lapillus*, the population growth curve strongly suggested that northern populations were founded in the last 20,000 years (C.W. Cunningham, unpubl. data). In general, it appears that estimates of population age are affected more by the problem of ancestral polymorphism (i.e., our *Asterias* data) than the problem of coestimation of parameters such as Θ and g .

Finally, it should be noted that although nested clade analysis (reviewed in Templeton 1998) is potentially useful in such studies, it can only infer range expansion when the ranges of younger allelic lineages are more broadly distributed than older lineages. In our datasets, in which range expansion is too recent to be associated with much genetic diversity, there is not enough allelic or geographic resolution to make a statistical inference using this method. For our taxa, the putatively invading haplotypes are the most deeply nested haplotype in the European (putative source) population (Fig. 1). This is to be expected, because deeply nested ancestral haplotypes are often the most common (Castelloe and Templeton 1994), therefore they have a higher probability of participating in long-distance dispersal events.

Conclusions: Historical Biogeography and its Role in Comparative Ecology

Reconstruction of historical change in a complex intertidal community is perhaps inseparable from the idiosyncrasies introduced by the population genetics and life-history characteristics of the component species (Ricklefs and Schluter 1993; Cunningham and Collins 1998). In many ways, it is this variation among species in their response to climatic change and community disruption that is most interesting for the study of historical ecology and community reassembly. Despite some broad similarities among groups of species in this study, there are also patterns of community history and composition that cannot be fully explained in terms of the traits discussed here.

For example, the two species of *Asterias* in North America are distinguished not only by historical patterns, but also by physiological and geographic range differences (Worley and Franz 1983), and there are hypotheses remaining to be tested about the historical associations among endemic North American lineages of *Idotea* and their ecological milieu (Wares 2001b). Although there are apparent relationships between modes of larval dispersal and genetic patterns in the North Atlantic, comparative work in two polychaete species (*Nean-*

thes virens and *Hediste diversicolor*) also points to adaptation to estuarine environments as another mechanism that could isolate populations and prevent trans-Atlantic migration events (S. Breton, P. Blier, F. Dufresne, and G. Desrosiers, unpubl. ms.).

Nevertheless all of these species have experienced the same history of climatic change and this common cause should lead to some common patterns. In the North Atlantic, Pleistocene glaciation not only scoured the rocky intertidal of New England, but also caused sea level to drop up to 200 m; this probably exposed suitable habitat for organisms displaced by glaciers and accompanying climatic change (Emery and Garrison 1967; Cronin 1988; Riggs et al. 1996), although the true shoreline and its composition is as yet unknown for glacial periods (Pielou 1991). Here we show, albeit with only a handful of species, that mechanisms of dispersal in intertidal organisms may be an important factor in the survival of North American populations because these proposed offshore reefs and mounts may have been widely scattered and only temporarily available.

Some important patterns are described with these data that do not directly test the proposed hypotheses of this study but that illustrate the utility of comparative phylogeography to studies of the genetics and ecology of North Atlantic invertebrates. The growing body of literature on the population genetics of species such as *M. edulis* and *N. lapillus* indicate that there are many elements of mitochondrial heredity and patterns of gene flow that may only be resolved by a better understanding of the underlying life-history traits, an understanding gained through comparisons with other species such as in this study. Additional ecological experimentation, within the context of these phylogeographic patterns, may be able to discern the historical influences on the diversity of this community. It is likely to be difficult to tease apart all the influences, as studies show that predicting even simple community responses to climatic change are quite complex (Bertness et al. 1999; Sullivan et al. 2000).

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APPENDIX 1

Populations Represented in Common or Shared Alleles in Figure 1 Asterias rubens

- Haplotype A: individuals from Maine ($n = 8$), Nova Scotia ($n = 2$), Newfoundland ($n = 2$), Iceland ($n = 1$), Norway ($n = 2$), and Ireland ($n = 1$)
- Haplotype B: individuals from Maine ($n = 2$), Nova Scotia ($n = 4$), Newfoundland ($n = 3$), Iceland ($n = 1$), Ireland ($n = 2$), and France ($n = 2$)
- Haplotype C: individuals from Maine ($n = 1$), Norway ($n = 3$), Ireland ($n = 2$), and France ($n = 1$)
- Haplotype D: individuals from Maine ($n = 1$) and Ireland ($n = 1$)

Idotea balthica

North American common haplotype represents 13 individuals: Maine ($n = 8$), Rhode Island ($n = 1$), and Nova Scotia ($n = 5$).

Mytilus edulis

- Haplotype NA: individuals from North Carolina ($n = 1$), Maine ($n = 5$), and Nova Scotia ($n = 1$)
- Haplotype A: individuals from North Carolina ($n = 3$), Rhode Island ($n = 3$), Maine ($n = 6$), Iceland ($n = 3$), Norway ($n = 1$), and France ($n = 2$)
- Haplotype Eur: individuals from Iceland ($n = 2$), Norway ($n = 1$), Ireland ($n = 1$), and France ($n = 3$)
- Haplotype 9Fr: individuals sampled at Roscoff, France

Littorina obtusata

Shared Haplotype: individuals from Maine ($n = 7$), Nova Scotia ($n = 8$), Newfoundland ($n = 4$), Iceland ($n = 3$), and Norway ($n = 1$)

Nucella lapillus

Shared Haplotype: individuals from Maine ($n = 14$), Nova Scotia ($n = 7$), Rhode Island ($n = 3$), Iceland ($n = 8$), Norway ($n = 7$), Ireland ($n = 7$), and Newfoundland ($n = 5$)

Semibalanus balanoides

- Haplotype A: individuals from Maine ($n = 4$), Nova Scotia ($n = 5$), Rhode Island ($n = 2$), Iceland ($n = 7$), and Newfoundland ($n = 1$)
- Haplotype B: individuals from Rhode Island ($n = 2$), Maine ($n = 7$), Nova Scotia ($n = 2$), Newfoundland ($n = 2$), and Iceland ($n = 4$)

APPENDIX 2

AMOVA results for each species being compared.

Species	Source of variation	(df)	Percentage of variation	Fixation indices
<i>Asterias rubens</i>	North America vs. Europe	(1)	9.11	$F_{CT} = 0.091^*$
	Within continental groups	(5)	1.23	$F_{SC} = 0.013$
	Within populations	(41)	89.66	$F_{ST} = 0.103$
<i>Mytilus edulis</i>	North America vs. Europe	(1)	6.16	$F_{CT} = 0.062$
	Within continental groups	(8)	21.90	$F_{SC} = 0.233^*$
	Within populations	(78)	71.94	$F_{ST} = 0.281^*$
<i>Idotea balthica</i>	North America vs. Europe	(1)	-14.37	$F_{CT} = -0.144$
	Within continental groups	(3)	66.91	$F_{SC} = 0.585^*$
	Within populations	(28)	47.47	$F_{ST} = 0.525^*$
<i>Nucella lapillus</i>	North America vs. Europe	(1)	-11.77	$F_{CT} = -0.117$
	Within continental groups	(5)	65.11	$F_{SC} = 0.582^*$
	Within populations	(51)	46.66	$F_{ST} = 0.533^*$
<i>Littorina obtusata</i>	North America vs. Europe	(1)	41.89	$F_{CT} = 0.418^*$
	Within continental groups	(5)	3.87	$F_{SC} = 0.066$
	Within populations	(38)	54.24	$F_{ST} = 0.457^*$
<i>Semibalanus balanoides</i>	Pacific vs. North America vs. Europe	(2)	60.23	$F_{CT} = 0.602$
	Within continental groups	(6)	6.84	$F_{SC} = 0.172^*$
	Within populations	(59)	32.93	$F_{ST} = 0.671^*$
	(Atlantic clades only)			
	Between Atlantic clades	(1)	73.22	$F_{CT} = 0.732$
	Within Atlantic clades	(2)	6.06	$F_{SC} = 0.226^*$
	Within populations	(60)	20.72	$F_{ST} = 0.792^*$

* $P < 0.05$.