

'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential

P. B. MARKO

Department of Marine Science, 12–7 Venable Hall, CB 3300, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, 27599–3300, USA

Abstract

In marine environments, many species have apparently colonized high latitude regions following the last glacial maximum (LGM) yet lack a life-history stage, such as a free-living larva, that is clearly capable of long-distance dispersal. Two hypotheses can explain the modern high latitude distributions of these marine taxa: (1) survival in northern refugia during the LGM or (2) rapid post-glacial dispersal by nonlarval stages. To distinguish these two scenarios, I characterized the genetic structure of two closely related northeastern Pacific gastropods that lack planktonic larvae but which have distributions extending more than 1000 km north of the southern limit of glaciers at the LGM. Despite having identical larval dispersal potential, these closely related species exhibit fundamentally different patterns of genetic structure. In *Nucella ostrina*, haplotype diversity among northern populations (British Columbia and Alaska) is low, no pattern of isolation by distance exists and a coalescent-based model of population growth indicates that during the LGM population size was reduced to less than 35% of its current size. In the congeneric and often sympatric *N. lamellosa*, northern populations harbour a diversity of ancient private haplotypes, significant evidence of isolation by distance exists and regional subdivision was found between northern (Alaska) and southern (southern British Columbia, Washington and Oregon) populations. Estimates of coalescent parameters indicate only a modest reduction in population size during the LGM and that northern and southern populations of *N. lamellosa* split ~50 Kyr before the LGM. The patterns are consistent with the hypothesis that *N. ostrina* recently reinvaded the northeastern Pacific but *N. lamellosa* survived the LGM in a northern refuge. A comparison of similar studies in this region indicates that depleted levels of genetic variation at high latitudes — evidence suggestive of recent colonization from a southern refuge — is more common among intertidal species that live relatively high on the shore, where exposure times to cold stress in air are longer than for species living lower on the shore. These data suggest that for some faunas, ecological differences between taxa may be more important than larval dispersal potential in determining species' long-term biogeographical responses to climate change.

Keywords: cytochrome oxidase, CO1, genetic break, glacial refugia, human coastal migration, *Nucella lamellosa*, *Nucella ostrina*, Pacific Northwest, phylogeography, Pleistocene, range expansion.

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Introduction

Frequent climate change during the late Pleistocene has led to the concept that many high latitude plant and animal

communities in Europe and North America are of very recent origin, having been established by northward range expansions from southern refugia following the end of the last glacial maximum (LGM) 14–20 Kyr ago (Blaise *et al.* 1990; Williams *et al.* 1998; Hewitt 1999, 2000). Evidence from fossils, pollen and other physical and biological residues indicate that many species have undergone dramatic

Correspondence: P. B. Marko. Fax: (919) 962 1254; E-mail: pmarko@unc.edu

latitudinal shifts in response to climate change (Huntly & Birks 1983; Bennett *et al.* 1991; Valentine & Jablonski 1993; Williams *et al.* 1998). For many of these species, latitudinal shifts occurred more rapidly than would be predicted based on direct estimates of dispersal capabilities, a phenomenon known as Reid's Paradox of rapid plant migration (Clark 1998; Clark *et al.* 1998).

For organisms that lack a fossil record, genetic data have also been used widely to infer changes in the geographical distributions of species (Hewitt 1999, 2001). Not surprisingly, where postglacial colonization of Europe and North America is supported by the geological record, numerous studies have shown that northern populations have reduced genetic diversity (Avise *et al.* 1987; Templeton 1998; Avise 2000; Hewitt 2001; but see Bernatchez & Wilson 1998; Tremblay & Schoen 1999; Petit *et al.* 2003). Low genetic diversity at relatively high latitudes, with a small number of alleles or haplotypes dominating disproportionately large areas, is a pattern most consistent with the hypothesis of a recent range extension from a southern refuge (Hewitt 1999).

In marine systems, comparatively little is known about the biogeographical responses of high latitude species to late Pleistocene glaciation because the palaeontological record from glacial periods is so poor. Although marine deposits from interglacials (i.e. high sea level stands) are rich with fossils and are easily accessible, the fossil record from glacials (i.e. low sea level stands) is largely inaccessible due to the subsequent Holocene rise in sea level (Valentine & Jablonski 1993). Nevertheless, the geographical distributions of species and their life-history characteristics indicate that analogues of Reid's Paradox also exist in the marine environment. For example, in the northeastern Pacific, despite the presence of coastal glaciers from the Aleutian Islands, Alaska to the Straits of Juan de Fuca, Washington only 14–20 Kyr ago (Fields *et al.* 1993; Sabin & Pisas 1996; Lyle *et al.* 2000; Herbert *et al.* 2001), benthic invertebrate and algal diversity is relatively high compared to other regions at the same latitude (Austin 1985; Scagel *et al.* 1993; Lambert 1994). More importantly, in contrast to the pattern observed at low latitudes — where species with planktonic larvae tend to have wider geographical distributions than species lacking planktonic larvae (Hansen 1978; Perron & Kohn 1985) — the geographical ranges of many high latitude species lacking planktonic larvae are enormous, often as broad as for species possessing planktonic larvae (Arnaud 1974; Cantera & Arnaud 1984; Highsmith 1985; Vermeij *et al.* 1990). In the case of the northeastern Pacific, if most cool temperate species were forced into refugia to the south of the Cordilleran ice sheet (i.e. south of Puget Sound, Washington) during the LGM, recolonization involved range extensions of more than 1000 km in less than 20 Kyr by a diversity of taxa lacking planktonic larvae (Vermeij *et al.* 1990). Most benthic marine invertebrates are sessile or slow-moving as adults, such

that adult dispersal cannot possibly explain these apparent range extensions. For example, based on locomotion rates and movement patterns for rocky-shore gastropods (Miller 1974; Palmer 1980; Papp & Duark 2001) a range extension of 1000 km by species lacking planktonic larvae could take millions of years.

In some cases, rare long-distance dispersal has been invoked to explain the broad geographical distributions of species at high latitudes that do not possess planktonic larvae (Johannesson 1988; Johannesson & Warmoes 1990; Vermeij *et al.* 1990). The dispersal potential of such species is enhanced probably by transport of egg cases and rafting of juveniles on macroalgal mats, because macroalgae is most abundant at high latitudes (Gerlach 1977; Zinsmeister & Emerson 1979; Deysher & Norton 1982; Highsmith 1985; Edgar 1987; Harold & Lisen 1989; Jokiel 1989; Helmuth *et al.* 1994; Kohn & Perron 1994; Worcester 1994; O'Foighil *et al.* 1999). Genetic data from a handful of studies are consistent with this hypothesis, indicating that postglacial range extensions have occurred in marine species that lack planktonic larvae (Marko 1998; Hellberg *et al.* 2001; Wares & Cunningham 2001). An alternative hypothesis that can also explain the high diversity of species with benthic development in the northeastern Pacific and elsewhere is that some species survived in northern refugia during the LGM (Wares & Cunningham 2001). For terrestrial communities, which have received more attention, a handful of phylogeographical studies have found significant genetic structuring of populations at high latitudes (Stewart & Lister 2001). This pattern, which is inconsistent with the hypothesis of postglacial range extension from a single southern refuge, suggests that postglacial recolonization of European and North American plant and animal communities may have been a more complex process than thought previously (Abbott *et al.* 1995; Stafford *et al.* 1999; Bilton *et al.* 1998; Cruzan & Templeton 2000; Willis *et al.* 2000; Hickerson & Ross 2001). The existence of so many marine species with benthic development at high latitudes could also reflect the historic importance of cryptic northern refugia in explaining how the modern geographical distributions of marine species have come to be (Stewart & Lister 2001).

To investigate the biogeographical history of broadly distributed intertidal marine species in the northeastern Pacific, I have gathered mtDNA sequences and characterized the population genetic structures of two congeneric intertidal gastropods, *N. lamellosa* and *N. ostrina*. Both species have northern range end points that are greater than 1000 km to the north of the southern limit of the Cordilleran ice sheet (Abbott 1974; Keen 1974; Kozloff 1983; Vermeij *et al.* 1990; O'Clair & O'Clair 1998) but both, like all members of the genus, lack planktonic larvae. In *Nucella*, fertilization is internal and females lay egg capsules attached to rocky intertidal surfaces; embryos complete development in 8–12 weeks and emerge from capsules as juvenile snails

(Strathmann 1987). Therefore, gene flow is likely to be limited, increasing the likelihood that the characteristic genetic signature of recent long-distance dispersal and colonization will not yet be eroded by subsequent migration (Avice 2000). *N. ostrina* was the subject of an earlier phylogeographical study, but only one population to the north of Washington State was analysed with either allozymes or mtDNA (Marko 1998). The population genetic structure of *N. lamellosa* has also been investigated previously with allozyme electrophoresis (Campbell 1978), which indicated consistent genetic differentiation between all populations sampled from California to Alaska but with no clear geographical pattern to the differentiation (and analyses were limited to calculation of genetic distances). I have therefore collected samples of both species and gathered mtDNA sequence data from the first subunit of cytochrome c oxidase to comparatively analyse patterns of genetic subdivision and diversity.

Materials and methods

Eight to 12 individual *N. ostrina* and *N. lamellosa* were each collected from 10 sites between Santa Cruz, California and Kodiak Island, Alaska between December 1997 and November 1998 (Fig. 1). Sites were selected close to those of Campbell (1978) so that analyses of mtDNA could be compared to earlier allozyme work.

Tissue samples were incubated overnight in 2× CTAB with proteinase-K and beta-mercaptoethanol. DNA was extracted twice with chloroform and isoamyl alcohol (24:1), precipitated with ethanol, washed, dried and resuspended in water. Partial cytochrome c oxidase-1 (CO1) sequences were amplified with the polymerase chain reaction using primers of Folmer *et al.* (1994) and *Taq* Extenders™ (Stratagene). Amplification products were generated by 35 repetitions of the following thermal cycler profile: 94 °C for 30 s; 48 °C for 30 s; 72 °C for 90 s. Products from these reactions were separated with gel electrophoresis, purified with spin columns (Qiagen), and sequenced in both directions on an autosequencer (Applied Biosystems). A 586-base pairs (bp) portion and a 607-bp portion of the CO1 gene were each sequenced from 89 and 84 individuals of *N. ostrina* and *N. lamellosa*, respectively.

Sequences were aligned easily due to an absence of insertions and deletions in coding DNA. For each data set an unrooted parsimony network was constructed with PAUP* version 4.0 (Swofford 2001) with heuristic searches, tree-bisection–reconnection branch swapping, and zero-length branches collapsed. I compared results from PAUP to those obtained using the statistical parsimony (Templeton *et al.* 1992) method in tcs version 1.13 (Clement *et al.* 2000), which also identifies the most probable ancestral haplotype in each network (Castelloe & Templeton 1994). To characterize the relationship between the number

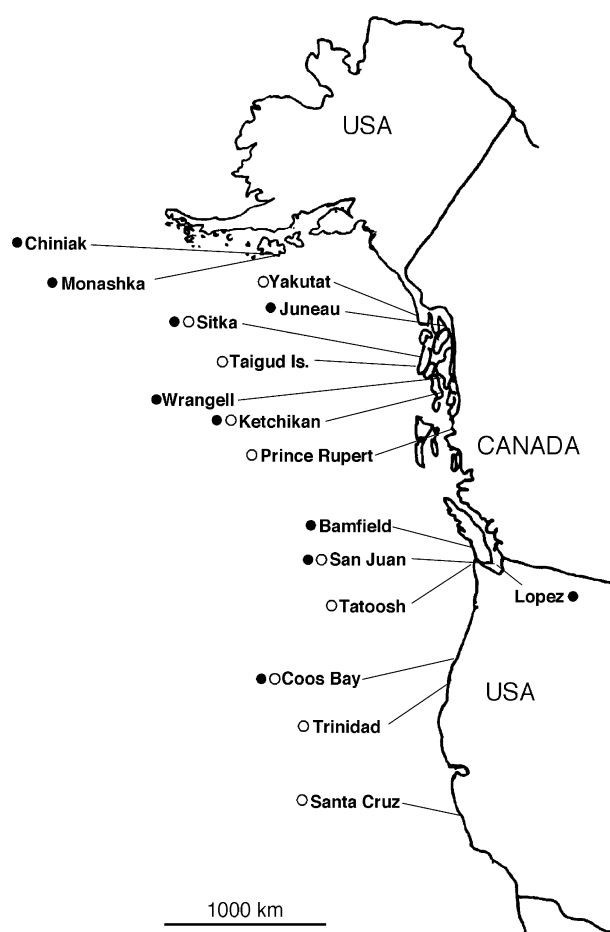


Fig. 1 Sample locations for *N. lamellosa* (closed circles) and *N. ostrina* (open circles) on the west coast of North America.

of segregating sites and nucleotide diversity, I calculated Tajima's (1989) *D* statistic for each species using ARLEQUIN version 2.001 (Schneider *et al.* 1997). For a large and constant population size under selective neutrality, estimates of θ based on either the number of segregating sites (polymorphism) or nucleotide diversity (the average number of nucleotide differences between haplotypes) should be equal. A significantly negative value of Tajima's *D* indicates an excess of low-frequency variants (the number of segregating sites > pairwise nucleotide differences), a signature of either a recent selective sweep or population expansion. Significance of the test statistic was determined by comparison to distributions generated from 10 000 random permutations of the original data.

Population genetic structure was characterized with an analysis of variance method (Excoffier *et al.* 1992) implemented in ARLEQUIN that partitions the total variance into components analogous to Wright's hierarchical *F*-statistics (Wright 1978). I conducted this analysis on both haplotype frequencies (i.e. conventional *F*-statistics) and sequence divergences (i.e. analysis of molecular variance or AMOVA).

For the AMOVAS, I used Kimura's two-parameter model (Kimura 1980) with rate heterogeneity among sites (K2P + G); the transition to transversion ratio and the gamma shape parameter were estimated separately with PAUP 4.0*. To examine each data set for evidence of isolation by distance (Slatkin 1993) I plotted pairwise values of F_{ST} against geographical distance between all sample sites. The strength and significance of the relationship between genetic differentiation and geographical distance was assessed with reduced major axis regression and Mantel tests using the program IBD (Bohonak 2002).

Lastly, I used a method implemented in the program FLUCTUATE (Kuhner *et al.* 1998) that uses Metropolis-Hastings sampling to generate joint estimates of the coalescent parameter θ ($2N\mu$ for the mtDNA genome, where N = the effective population size and μ = the substitution rate per site per generation) and an exponential growth parameter g (in units of μ^{-1}) for each species. These coalescent-based estimates of θ and g can be used to infer the relative effective population size at any time t (in generations) with the expression $N_t = \theta e^{(-g)t}$ (Kuhner *et al.* 1998). Because coalescent methods rely on the assumption of a constant molecular clock, I used a likelihood ratio test to assess the null hypothesis of a Poisson-distributed clock (Felsenstein 1981) for each species. I employed rates of substitution from mtDNA phylogenetic analyses of the genus *Nucella* in which substitution rates were inferred from calibration points in the fossil record (Collins *et al.* 1996; Marko *et al.* 2003).

Substitution rates per year were converted into substitution rates per generation based on the generation times of each species. At lower latitudes (Oregon and Washington), *N. ostrina* reach reproductive maturity in 1–2 years in the field (Moran & Emler 2001) whereas *N. lamellosa* typically matures in 3–4 years (Strathmann 1987). Assuming growth will be relatively slow during cooler climates and at higher latitudes, I used the upper-end estimates of 2 and 4 years for the generation times of *N. ostrina* and *N. lamellosa*, respectively. Co-estimation of θ and g was accomplished under the finite-sites model (HKY, Hasegawa *et al.* 1985) with 10 replicate runs using 20 short chains of 20 000 steps each and 10 long chains of 60 000, with a sampling increment of 200. The transition to transversion ratio and the rate categories for a discrete gamma distribution were first estimated with maximum likelihood in PAUP* 4.0.

Results

Sequence characteristics

A 607-bp portion of CO1 was sequenced from 89 individual *N. ostrina* and 586-bp of sequence was obtained for 84 individual *N. lamellosa*. For *N. ostrina*, only 11 nucleotide sites were polymorphic, 10 of which were parsimony informative;

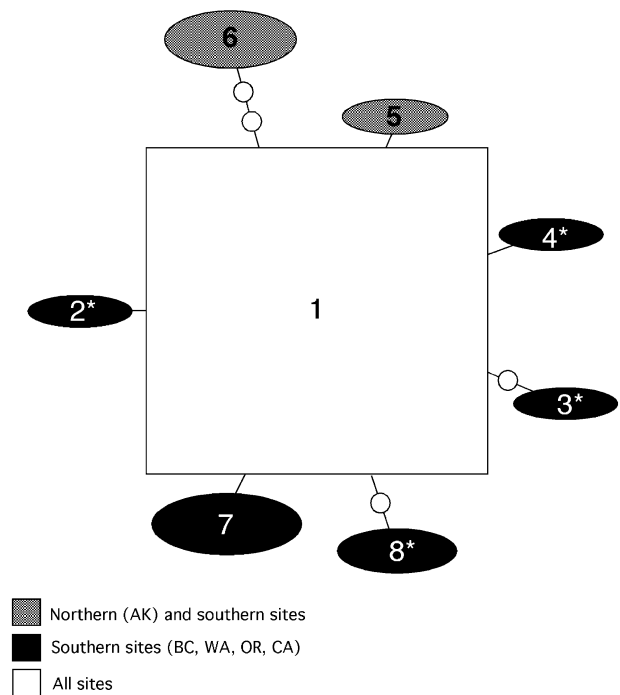


Fig. 2 Statistical parsimony cladogram of cytochrome oxidase-1 haplotypes (1–7) from *N. ostrina*. The size of each oval reflects the relative abundance of the haplotype in the data set, open circles indicate nucleotide substitutions between haplotypes and the ancestral haplotype is indicated by a rectangle. The geographical region where each haplotype was found is shown in the legend and private haplotypes are denoted with an asterisk. GenBank Accession nos for sequences: AY445323–AY445411.

a total of seven unique haplotypes were identified in *N. ostrina*. Given the same sampling effort, *N. lamellosa* exhibited more polymorphic sites and unique haplotypes: 58 sites were polymorphic, 16 were parsimony informative and 24 unique haplotypes were found. Nucleotide diversity among haplotypes in *N. lamellosa* (0.005, SD = 0.002) was greater than nucleotide diversity in *N. ostrina* (0.002, SD = 0.002). Across the entire geographical range of each species, Tajima's D was significantly negative for both *N. ostrina* ($D = -1.33$, $P = 0.0190$) and *N. lamellosa* ($D = -2.36$, $P < 0.000001$) indicating an excess of low frequency haplotypes in both species.

Phylogeographical patterns

Low haplotype diversity characterizes populations of *N. ostrina*. Haplotype no. 1, which occupies a central (ancestral) position in the unrooted haplotype network (Fig. 2), was by far the most common at all sites (Fig. 3). All other haplotypes differ from haplotype no. 1 by one to three changes, and most haplotypes occur at only one or two locations with the exception of haplotype no. 6, which was

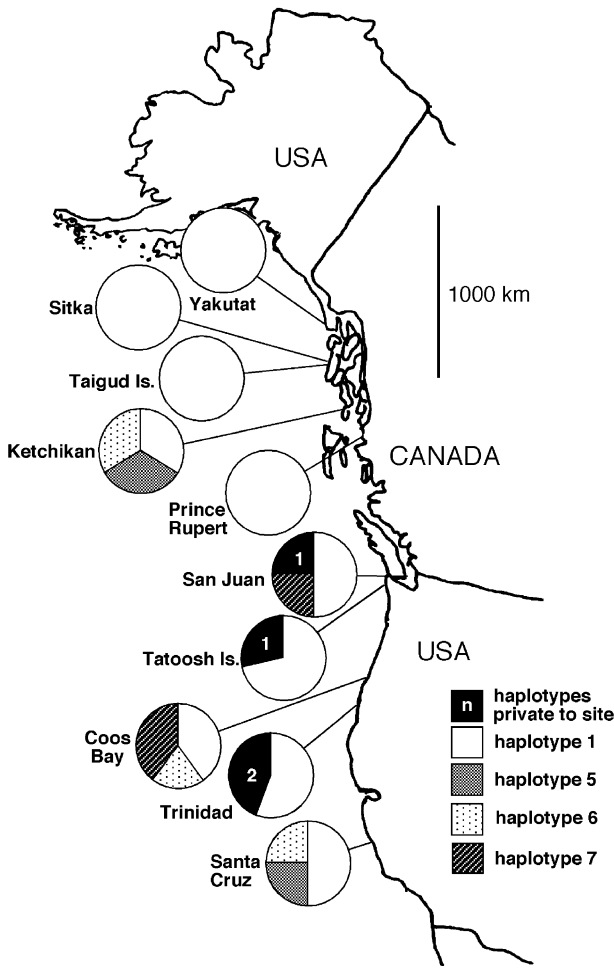


Fig. 3 Haplotype frequencies at each location for *N. ostrina*.

found at three sites (Fig. 3). Haplotypes restricted to one site (private haplotypes) were found at three locations, but all were found at places in the southern half of the species' geographical range (Fig. 3). Only haplotype no. 1 was found at four of the five northern sites (Yakutat, Sitka, Taigud Island, Ketchikan and Prince Rupert), and pairwise sequence divergences among these five northern samples was 0.11%, approximately half what was found (0.26%) among the five southern sites (San Juan, Tatoosh, Coos Bay, Trinidad and Santa Cruz).

In contrast, *N. lamellosa* shows greater nucleotide diversity across the same region, including several of the sites from which *N. ostrina* was sampled. Like *N. ostrina*, the most common haplotype in *N. lamellosa* (no. 6) sits at the centre of the unrooted network (Fig. 4); this haplotype was found at most sites, although somewhat less common among the four southernmost sites (Fig. 5). The most unusual feature of the haplotype distribution in *N. lamellosa* is the large number of private haplotypes found at northern sites (Fig. 5). For example, at each of Juneau, Sitka and Wrangell Island, most individuals possess private haplotypes. The population at Wrangell Island is most unusual in this respect: three-quarters of the individuals sampled possess one of six haplotypes found only at that site (Fig. 5). Many of the private haplotypes restricted to the northern portion of the geographical range of *N. lamellosa* are also several mutational steps away from the central haplotype in the network (Fig. 4), presumably relatively ancient in origin.

A second prominent feature of *N. lamellosa* is that the spatial distribution of haplotypes indicates the presence of a moderate disjunction in haplotype frequencies between northern British Columbia and southeast Alaska.

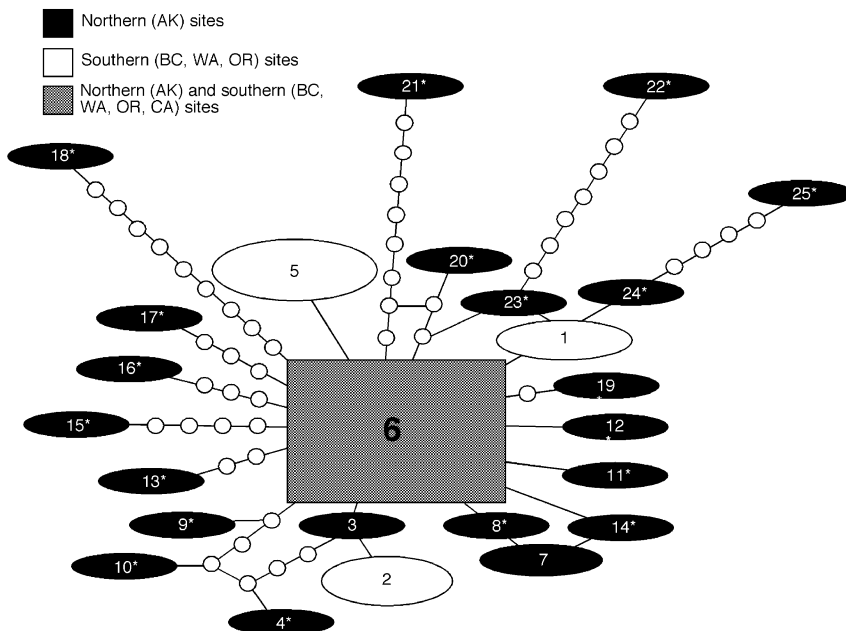


Fig. 4 Statistical parsimony cladogram of cytochrome oxidase-1 haplotypes (1–7) from *N. lamellosa*. The size of each oval reflects the relative abundance of the haplotype in the data set, open circles indicate nucleotide substitutions between haplotypes and the ancestral haplotype is indicated by a rectangle. The geographical region where each haplotype was found is shown in the legend and private haplotypes are denoted with an asterisk. GenBank Accession nos for sequences: AY445412–AY445495.

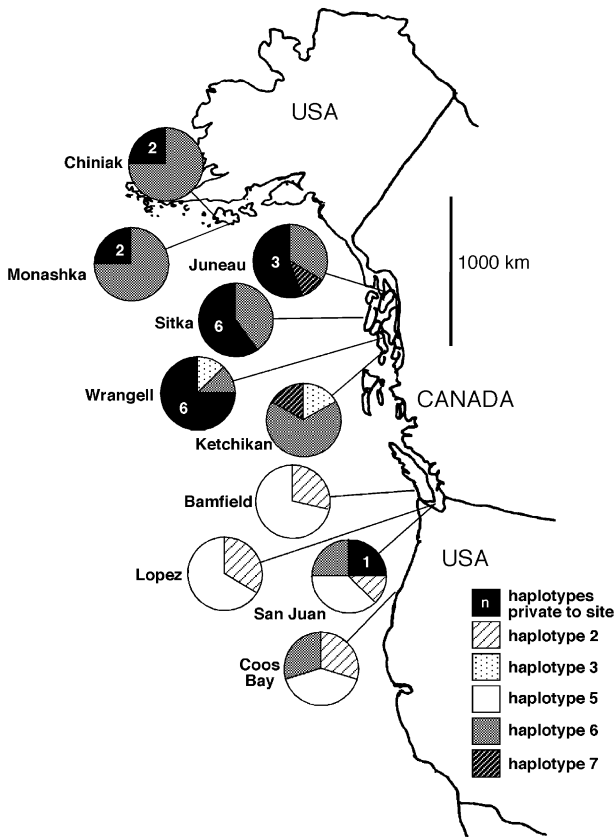


Fig. 5 Haplotype frequencies at each location for *N. lamellosa*.

Only the central haplotype in the network is shared between northern and southern sites; haplotypes no. 2 and no. 5 are both restricted to the four southern sites (Fig. 5) and haplotypes no. 3 and no. 7 were found only at the six northern sites (Fig. 5). In contrast with *N. ostrina*, pairwise sequence divergences among southern sites is small (0.03%); but sequence divergence among the six northern sites was more than four times as large (0.13%) as in the south.

Population subdivision

Across all sites, both species exhibit highly significant genetic subdivision, whether based on haplotype frequencies or sequence divergences (Table 1). Among sites for *N. lamellosa*, a Mantel test indicates a significant relationship ($P = 0.011$) between F_{ST} (i.e. ϕ_{ST}) and geographical distance among all 10 samples (Fig. 6a) indicating evidence of isolation by distance, with geographical distance explaining 30% of the variation in genetic differentiation for *N. lamellosa* ($r = 0.55$). In *N. ostrina*, no comparable relationship ($P = 0.28$) between geographical distance and genetic differentiation exists (Fig. 6b), with less than 1% of the variation in genetic differentiation explained by geographical distance ($r = 0.068$).

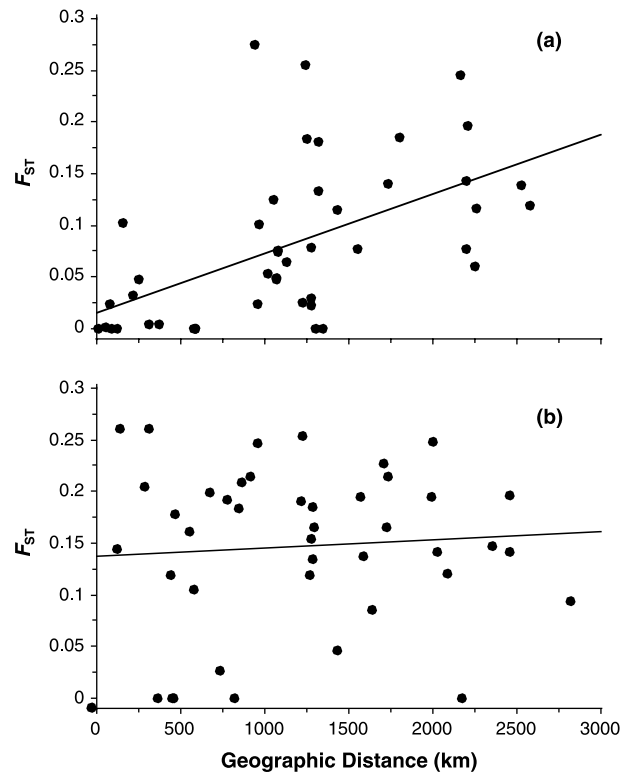


Fig. 6 Plot of pairwise estimates of F_{ST} (ϕ_{ST}) vs. geographical distance between samples of (a) *N. lamellosa* and (b) *N. ostrina* from the AMOVA analysis (see text); 95% confidence intervals for the slope (7.41×10^{-5} , 1.38×10^{-4}) and R^2 (0.127, 0.534) exclude zero for *N. lamellosa* but the slope (-1.36×10^{-4} , 1.43×10^{-4}) and R^2 (0, 0.138) for *N. ostrina* both encompass zero.

Based on the north–south disjunction in haplotype frequencies in *N. lamellosa*, I re-examined the mtDNA data for regional subdivision by splitting samples of *N. lamellosa* into a northern group (Chiniak, Monashka, Juneau, Sitka, Wrangell and Ketchikan) and a southern group (Bamfield, Lopez, San Juan and Coos Bay) of sites. Subdivision between these two groups is highly significant (see Table 2) for both haplotype frequencies ($\phi_{CT} = 0.198$) and sequence divergences ($\phi_{CT} = 0.103$). No significant subdivision was found within groups: only ~2% of the total variance in either haplotype frequencies or sequence divergences was attributed to variance among-samples within groups (Table 2).

Subdividing samples of *N. ostrina* into two groups along the same geographical lines results in similar results when F_{ST} is estimated from haplotype frequencies ($\phi_{CT} = 0.118$) except that variance in haplotype frequencies among samples within groups explained as much of the total variance as the variance between groups (~12%, Table 2). No significant subdivision between groups was detected when an AMOVA was conducted on sequence divergences; subdivision among sites within groups, however, remained significant for sequence divergences (Table 2).

Table 1 Population structure in *Nucella* based on AMOVA

Species	Source of variation	d.f.	Sum of squares	Variance component	% of variation	P-value
I. Haplotype frequencies						
(a) <i>Nucella lamellosa</i>	Among populations	9	7.88	0.061	14.49	< 0.000001
	Within populations	74	26.78	0.362	85.51	
(b) <i>Nucella ostrina</i>	Among populations	9	6.09	0.053	20.03	< 0.000001
	Within populations	79	16.68	0.211	79.97	
II. Sequence divergences						
(a) <i>Nucella lamellosa</i>	Among populations	9	21.90	0.109	6.72	< 0.000001
	Within populations	74	112.33	1.152	93.28	
(b) <i>Nucella ostrina</i>	Among populations	9	10.45	0.083	16.19	0.0001
	Within populations	79	33.99	0.430	83.81	

Table 2 Population structure in *Nucella* based on AMOVA and partitioning of populations into northern and southern groups

Species	Source of variation	d.f.	Sum of squares	Variance component	% of variation	P-value
I. Haplotype frequencies						
(a) <i>Nucella lamellosa</i>	Among groups	1	4.17	0.092	19.79	0.006
	Among populations within groups	8	3.71	0.012	2.63	0.122
	Within populations	74	26.78	0.362	77.58	
(b) <i>Nucella ostrina</i>	Among groups	1	1.98	0.033	11.82	0.032
	Among populations within groups	8	4.11	0.035	12.43	< 0.000001
	Within populations	79	16.68	0.211	75.74	
II. Sequence divergences						
(a) <i>Nucella lamellosa</i>	Among groups	1	7.84	0.156	10.28	0.005
	Among populations within groups	8	12.72	0.031	2.06	0.123
	Within populations	74	98.30	1.328	87.66	
(b) <i>Nucella ostrina</i>	Among groups	1	1.10	-0.003	-0.61	0.524
	Among populations within groups	8	9.36	0.085	16.57	0.0001
	Within populations	79	33.99	0.430	84.04	

Estimates of ancestral population size

Likelihood ratio tests indicate that a Poisson-distributed clock is rejected easily for complete CO1 sequences for both *N. lamellosa* and *N. ostrina* (not shown) under the model (HKY) implemented in FLUCTUATE. However, a clock could not be rejected for third codon positions for either *N. lamellosa* ($2\Delta \ln = 72.7$, d.f. = 82, $P > 0.1$) or *N. ostrina* ($2\Delta \ln = 33.7$, d.f. = 87, $P > 0.1$).

An mtDNA third codon position rate of 2.20×10^{-8} substitutions per site per year based on the divergence times of species of *Nucella* in the fossil record has been reported previously (Collins *et al.* 1996; Marko *et al.* 2003). This palaeontologically calibrated rate is approximately half that of a biogeographical-based rate reported elsewhere

for *Nucella* (Wares & Cunningham 2001), the latter having been derived from sequence divergences between Atlantic and Pacific *Nucella* lineages and the assumption that *Nucella* colonized the Atlantic Ocean at the peak of the trans-arctic migration 3.5 Mya. Application of the more recent biogeographical-based calibration to coalescent-based estimates of ancestral population size would yield greater inferred rates of population growth and therefore smaller estimates of ancestral population size for any given time in the past. Although such biogeographical events are used widely to calibrate molecular clocks, when tested independently with calibrations from the fossil record, biogeographical calibrations have been shown to yield inflated substitution rates (Hillis *et al.* 1996; Marko 2002). Therefore, I used the palaeontologically calibrated rate for

Table 3 Joint maximum likelihood estimates of θ ($2N\mu$, where N is the female effective population size μ is the per gene per generation DNA substitution rate) and g (the growth rate) for *N. lamellosa* and *N. ostrina* plus resulting inferred 95% confidence interval for relative population size at $t = 20\ 000$ year. Each estimate and its standard deviation represent a composite of 10 replicate analyses. Analyses based on third codon positions ($\mu = 2.20 \times 10^{-8}$ substitutions per site per year; see Methods)

Species	Samples	θ	g	Relative N 20 000 years ago
(a) <i>N. ostrina</i>	All	0.0098 \pm 0.0007	2924.1 \pm 250.5	0.22–0.34
(b) <i>N. lamellosa</i>	All	0.0981 \pm 0.0104	495.3 \pm 42.3	0.78–0.83
	Northern sites	0.7589 \pm 0.1254	1334.1 \pm 71.2	0.52–0.59
	Southern sites	0.0042 \pm 0.0015	–62.1 \pm 129.5	0.92–1.15

third-position data to estimate θ and g from the CO1 gene genealogy using the Metropolis–Hastings sampling method in FLUCTUATE.

For *N. ostrina*, the Metropolis–Hastings sampling estimate of g is positive, indicating recent population growth, and the 95% confidence interval (CI) for effective female population size 20 Kyr ago is 22–34% of the current population (Table 3a). In *N. lamellosa*, g is also positive, but θ is an order of magnitude larger than in *N. ostrina* (Table 3). The 95% CI for population size for *N. lamellosa* at the LGM (78–83% of the current female effective population size) was also significantly larger than *N. ostrina* (Table 3b). The Metropolis–Hastings sampling method implemented in FLUCTUATE, however, assumes exponential growth of a panmictic population. This assumption is violated for *N. ostrina* given the presence of significant structure among populations (Tables 1 and 2) and may make the coalescent process slower than expected than for a panmictic population. Because of significant north–south regional subdivision in *N. lamellosa* and an absence of subdivision within regions (Table 2) I re-estimated θ and g separately for northern (Alaska) and southern (southern British Columbia, Washington and Oregon) populations. For northern sites, g was also positive, yielding a 95% CI for population size at the LGM of 52–59% of the current size. For southern sites, g was nearly zero, indicating that the ancestral population size at the LGM was similar to the population size today (Table 3b). Although migration between northern and southern populations is expected to bias these estimates upwards, the effects of migration on ancestral population size estimates may be small because the only haplotype shared between northern and southern sites is the central (and putatively ancestral) haplotype in the parsimony network (Fig. 4).

Discussion

Numerous studies of population subdivision in benthic marine invertebrates have established that species with pelagic larvae typically exhibit less spatial genetic

structure than similar species with nonpelagic larvae (Berger 1973, 1977; Ament 1978; Ward 1990; McMillan *et al.* 1992; Duffy 1993; Hunt 1993; Hellberg 1995; Hoskin 1997; Arndt & Smith 1998; Ayre & Hughes 2000; Kyle & Boulding 2000). However, the extent to which larval mode dictates population genetic structure is not understood fully: some patterns of genetic subdivision in benthic marine species clearly do not meet expectations based on their apparent larval dispersal potential (for reviews see Burton 1983; Palumbi 1994). For *N. ostrina* and *N. lamellosa*, comparative study of mtDNA sequences demonstrates that closely related species with identical larval dispersal potential can exhibit fundamentally different patterns of population structure across the same geographical region. *N. ostrina* shows a pattern of low haplotype diversity (Figs 2 and 3), limited population substructuring in the northern half of its range (Table 2) and no relationship between geographical distance and genetic differentiation among sites across the species' entire geographical range (Fig. 6b). In contrast, *N. lamellosa* has a diversity of more deeply divergent private haplotypes at northern sites (Figs 4 and 5), exhibits a significant relationship between genetic differentiation and genetic distance among sites (Fig. 6a) and shows significant subdivision between northern (northern British Columbia and Alaska) and southern (southern British Columbia, Washington and Oregon) populations for both haplotype frequencies and sequence divergences (Table 2). Although haplotype frequencies from *N. ostrina* do show significant subdivision between northern and southern samples, no significant regional structure is detected if the small sequence divergences among haplotypes are considered in an AMOVA (Table 2).

Grossly dissimilar patterns of population structure between closely related species can have several potential explanations. Although it is difficult to distinguish the relative importance of contemporary and historical processes that together interact to produce a given pattern of population structure (Wakeley 2000; Nielsen & Wakeley 2001; Kalinowski 2002), high rates of contemporary (i.e. equilibrium) gene flow among populations of *N. ostrina* seems the

least likely explanation for limited genetic structure among northern populations and the complete absence of any relationship between genetic differentiation and geographical distance across the entire geographical range of *N. ostrina*. Although dispersal of species with non-planktonic larvae is expected to be enhanced nearer the poles due to rafting of egg cases and juveniles on macroalgae, it is highly doubtful — albeit difficult to reject outright — that populations of *N. ostrina* separated by hundreds of kilometres are homogenized by high rates of equilibrium gene flow in a species lacking planktonic larvae (see Collin 2001 for comparisons between larval modes at lower latitudes). An explanation for why equilibrium gene flow could be substantially higher in *N. ostrina* is not obvious, given that both *N. ostrina* and *N. lamellosa* typically live on the same shores and lay benthic egg capsules on similar substrates with no planktonic larval stage.

Two other processes can account more easily for all of the differences in mtDNA population structure and diversity observed between *N. ostrina* and *N. lamellosa*. First, a recent selective sweep is expected to produce a pattern of genetic homogeneity, such as among northern populations of *N. ostrina*. Although evidence for selective sweeps in mtDNA is rare, the lack of recombination in mtDNA means that positive selection on any nucleotide site may result in the fixation of the entire mtDNA genome, thereby eliminating all polymorphism (Maruyama & Birky 1991; William *et al.* 1995; Blier *et al.* 2001).

A second hypothesis that can explain a lack of haplotype diversity is that *N. ostrina* may have been forced partially or completely out of high latitudes during the LGM only to reexpand or reinvade in the last 20 Kyr. Under this scenario, relatively low mtDNA diversity and limited population structure in the northern half of the species' range reflects postglacial recolonization and subsequent rapid population growth, as suggested by the coalescent-based analysis that indicates more rapid population growth in *N. ostrina* than *N. lamellosa* since the LGM (Table 3). The extent to which populations of *N. ostrina* were driven to local extinction under this hypothesis is uncertain. Although most northern samples possess only a single haplotype, one locality (Ketchikan, Alaska) exhibits haplotype diversity on a par with southern populations (Fig. 3), suggesting the possibility that a refugium was maintained as far north as the southern end of southeast Alaska. A range expansion from a more southerly refuge is, however, supported by the fact that all haplotypes found at the five northern sites (including Ketchikan) are also found at southern sites but the converse is not true for all haplotypes found at southern sites, a pattern also consistent with the idea that all northern populations were colonized from the south (Wares & Cunningham 2001).

Although a survey of additional genetic markers is necessary to distinguish between the sweep and range

expansion hypotheses, a previous study of *N. ostrina* (Marko 1998) found that allozyme allele frequencies at Prince Rupert (northern British Columbia) were almost identical to those in Washington and Oregon, a pattern most consistent with recent recolonization of northern British Columbia and Alaska. British Columbia and Alaska populations of another gastropod (*Littorina sitkana*) also show depleted levels of mtDNA diversity (Kyle & Boulding 2000) and northern populations of a tide pool copepod (*Tigriopus californicus*) exhibit substantially reduced mtDNA diversity compared to southern populations (Edmands 2001). Other taxa living at lower latitudes also show evidence of post-Pleistocene range expansion. Reduced genetic diversity at high latitudes is therefore not unique to *N. ostrina*, which suggests a common demographic explanation involving postglacial recolonization for all three species.

In contrast to the patterns found in *N. ostrina*, regional subdivision between northern and southern sites, the existence of a diversity of relatively divergent private haplotypes at multiple northern sites, evidence of isolation by distance and only moderate reductions in population size during the LGM all indicate that *N. lamellosa* probably persisted throughout Alaska during the LGM. In a previous allozyme study, estimates of Nei's genetic identity (Campbell 1978; Table 2) revealed consistent divergence among all populations throughout the geographical range of *N. lamellosa*, with no apparent decrease in allelic diversity in northern British Columbia and Alaska. Because no *F*-statistic analogues were calculated, I reanalysed those data with ARLEQUIN for evidence of north-south genetic differentiation. The AMOVA on allozyme frequencies, like the analysis of mtDNA, also reveals significant subdivision between northern and southern populations ($\phi_{CT} = 0.056$, $P = 0.014$). Because correspondence between nuclear and mitochondrial markers is probably explained best by shared demographic history (Avice 2000), concordance of patterns with respect to both types of markers is explained most easily by relatively long-term persistence of *N. lamellosa* in northern British Columbia and Alaska.

Did genetic subdivision between northern and southern populations of *N. lamellosa* predate the LGM? Interpretations of patterns of population subdivision usually assume that either populations have been exchanging migrants at the same rate for an infinite period of time (i.e. calculation of Nm , the number of migrants exchanged each generation, directly from F_{ST}) or that populations were subdivided at some point in the past but have not exchanged any migrants since the split (i.e. calculation of divergence time from F_{ST} or other measures of population differentiation; see Wakeley 1996; Nielsen & Slatkin 2000; Nielsen & Wakeley 2001). A more realistic model with which to characterize the history of population subdivision must consider both the timing of population separation and subsequent migration. Therefore, to distinguish the relative contributions

of migration and isolation to regional north–south genetic subdivision in *N. lamellosa*, I used a Markov Chain Monte Carlo (MCMC) method implemented in the program MDIV (Nielsen & Wakeley 2001). MDIV generates joint posteriors for standard coalescent parameters, including divergence time ($T = t/N$, where N is the effective population size and t is generations) and gene flow ($M = 2Nm$ for mtDNA) between a pair of populations for a specified range of values of M and T . Using COI third positions, the same substitution rate derived from palaeontological calibration as in the FLUCTUATE analysis and a generation time of 4 years, the MCMC analysis indicates that northern and southern populations split 69 860 years ago ($M = 0.41$ since the split). A likelihood ratio test indicates that this inferred split is significantly older than 20 Kyr ago ($2\Delta \ln L = 4.98$, d.f. = 1, $P < 0.05$), indicating that subdivision between northern and southern populations of *N. lamellosa* predates the LGM.

This analysis, like the estimates of ancestral population size (Table 3) and all other coalescent-based inferences, relies fundamentally on the assumptions of neutrality and a molecular clock, but also requires reliable estimates of lineage-specific substitutions rates and generation times if coalescent-based estimates are converted into units of years. I used only third position data in coalescent analyses because they conform to expectations based on a Poisson-distributed clock and are also much less likely to cause amino acid substitutions, therefore being more likely to obey the assumption of neutrality. Use of third positions, however, may bias estimates of divergence due to the effects of saturation (Arbogast *et al.* 2002). Because the calibration point used in this study (~7.5 Mya, see Collins *et al.* 1996) is much older than the population-splitting dates of interest (tens of thousands of years), saturation between calibration taxa will lead to an overestimate of more recent divergences (because the rate of sequence evolution is underestimated at the calibration point). If the much larger biogeographical-based rate for *Nucella* mtDNA third positions (Wares & Cunningham 2001) is employed, the inferred MCMC divergence time between northern and southern populations is reduced to ~35 Kyr ago, a date not significantly different from 20 Kyr ago ($2\Delta \ln L = 1.85$, d.f. = 1, $P > 0.1$). Although the smaller palaeontologically calibrated rate for third positions and the assumption of 4 years/general are the best available estimates of these parameters (see Methods), population histories based on single loci also have very large variances (Kuhner *et al.* 1998; Fu & Li 1999). Use of additional independent loci is expected to decrease variance in coalescent time and therefore improve the accuracy of estimates of divergence times. Given this array of caveats, a definitive conclusion about rates of population growth and divergence times of high latitude *Nucella* populations requires independent corroboration from other loci.

Evidence of substantial genetic structure in northwest North America is not unprecedented. A growing list of examples indicates that numerous terrestrial and anadromous species expanded from northern refugia following deglaciation (e.g. Warner *et al.* 1982; Oglive & Roemer 1984; Ferguson 1987; O'Reilly *et al.* 1993; Byun *et al.* 1997; Soltis *et al.* 1997; Smith *et al.* 2001). Among the few studies of genetic structure in benthic marine organisms in this region, a strong mtDNA phylogeographical break has also been described in the sea cucumber *Cucumaria pseudocurata* (Arndt & Smith 1998) in the same region as the shift in haplotype frequencies observed in *N. lamellosa*. Substantial differences in allozyme and haplotype frequencies have also been reported between Alaska and British Columbia samples of the six-armed sea star *Leptasterias hexactis* (Kwast *et al.* 1990; Hrinkevich & Foltz 1996) and the gastropod *Littornia subrotundata* (Kyle & Boulding 2000). Similarly, no evidence of a range extension from a southern refuge was found in an intertidal fish (*Gobiosox maeandricus*) whose geographical range extends into southeast Alaska (Hickerson & Ross 2001).

Findings from glacial geology and palaeo-sea levels suggest that ice-free regions of coastline existed during the Late Wisconsinan maximum, and may have generated regional patterns of genetic subdivision in some species of *Nucella*, *Cucumaria*, *Leptasterias* and *Littorina*. Even though the outer coasts of British Columbia and Alaska have been described as covered by a continuous sheet of ice during the LGM (Prest 1969; Matthews 1979; Dyke & Prest 1987; Wright 1991), more recent analyses indicate that the majority of coastal glaciers were discrete lobes separated by areas of exposed land (Clague 1985; Luternauer *et al.* 1989; Josenhans *et al.* 1995; Mann & Hamilton 1995; Erlandson & Moss 1996; Barrie & Conway 1999; Mandryk *et al.* 2001), presumably allowing intertidal habitats to exist during glacial maxima. Interdigitation of ice and land may have disrupted species' ranges, leading to genetic differentiation. Present-day sea surface currents may also contribute to north–south subdivision, because the north–south divergence of the Alaska and California currents in northern British Columbia (Thompson 1981) corresponds roughly to the location of the mtDNA disjunctions in both *N. lamellosa* and *C. pseudocurata* (Arndt & Smith 1998). Recent palaeo-oceanographic studies, however, indicate that the California current probably collapsed during the LGM (Herbert *et al.* 2001). It is possible that this contemporary oceanographic barrier in northern British Columbia may act only to maintain differentiation that developed as a consequence of Pleistocene glaciation and range fragmentation.

Comparison of the population genetic structures of a diversity of intertidal species that have been studied in the northeastern Pacific reveals two distinct patterns. First, among the marine species for which significant north–south

genetic differentiation has been found, three (*N. lamellosa*, *C. pseudocurata* and *L. subrotundata*) lack planktonic larvae (Strathmann 1987). Strong patterns of geographical genetic structure in nonplanktonic species are not surprising, but evidence of substantial genetic differentiation at high latitudes in species with benthic development also indicates that survival of populations did not entail a steady supply of planktonic larvae from ice-free regions as has been suggested for transoceanic persistence of the north Atlantic intertidal fauna (Wares & Cunningham 2001). A second pattern that emerges is that among *N. lamellosa*, *L. subrotundata*, *C. pseudocurata*, *G. maeandricus* and *L. hexactis* – species that all exhibit significant regional population genetic structure – all but one (*L. subrotundata*) are found in the lower midlittoral zone (Zone 3 of Kozloff 1983) of the intertidal; some of these species, such as *N. lamellosa*, are also known to exist in subtidal populations (Kozloff 1983; O'Clair & O'Clair 1998). In contrast, northeastern Pacific species that show depleted levels of mtDNA variation among northern populations (*Littorina sitkana*, *N. ostrina* and *Tigriopus californicus*) are all obligate intertidal species of the upper midlittoral (Zone 2) and the supramidlittoral fringe (Zone 1). A χ^2 contingency table analysis for all eight species indicates that these qualitative differences in regional population genetic structure for high (Zones 2 and 1) and low (Zone 3) shore species are statistically significant ($\chi^2 = 4.8$, d.f. = 1, $P = 0.0285$). The potential importance of shore height in determining patterns of Pleistocene persistence is also supported by the phenomenon of submergence at high latitudes, where the northernmost populations of warm-water species are often restricted to subtidal habitats (Lindberg & Lipps 1996), presumably because of exposure to greater abiotic stress during low tides. Similar ecological-specific patterns of recolonization may exist at lower latitudes, but fewer studies are available for comparison. For example, a postglacial range extension from southern California across Point Conception into central California was characterized in the high-shore (Zone 2 of Kozloff 1983) gastropod *N. emarginata* (Marko 1998); a duplicate study of another Zone 2 gastropod also found a similar pattern of decreasing genetic variation with latitude (Hellberg *et al.* 2001).

Conclusion

Barring a selective mtDNA sweep in *N. ostrina*, analyses of population structure, geographical patterns of genetic diversity and coalescent-based estimates of population size and population divergence all indicate that the congeneric intertidal gastropods *N. ostrina* and *N. lamellosa* have had fundamentally different biogeographical histories in the northeastern Pacific despite possessing identical larval dispersal potential. Analyses of mtDNA sequences from populations of *N. lamellosa* are most compatible with the

hypothesis that most northern populations survived the last glacial maximum. In contrast, similar analyses of sequence data from *N. ostrina* indicate that most populations in the northern portion of this species' range are of more recent origin. Even for species that show no genetic variation at high latitude, a single northern refuge of very restricted size can never be ruled out with genetic data. Because of differences in the heights at which *N. ostrina* and *N. lamellosa* live on the shore, these data also suggest that high-latitude populations of the low-shore *N. lamellosa* may have persisted during the LGM as a consequence of shorter exposure to cold temperature stress in air during low tides. Six other studies of benthic marine invertebrate population structure in the same region are largely consistent with this hypothesis. If these patterns hold with the collection of additional data, they imply that ecological differences among species may have as much to do with the biogeographical responses of marine species to climate change as does larval dispersal mode. The general pattern that similar members of intertidal marine communities responded differently to glacial cycles is also consistent with what is known from the fossil record. Comparisons between the Recent and the Pleistocene faunas of the northeastern Pacific indicate that entire communities did not shift in concert in response to glacial cycles, but that many species responded individually, resulting in periodic changes in community composition during the Pleistocene (Valentine & Jablonski 1993; Lindberg & Lipps 1996; Graham *et al.* 1996).

Understanding the historical ecology of Pleistocene intertidal communities of the northeastern Pacific during the LGM also has a bearing on reconstruction of the ecological conditions that humans may have encountered as they colonized the New World from Asia, an event that occurred prior to 12.5 Kyr ago (Meltzer 1997; Mandryk *et al.* 2001). Identifying the first human presence in the Americas has always been tied to consideration of the geological and ecological preconditions for migration. The long-standing consensus that the first inhabitants of the Americas arrived on foot via an unglaciated ice-free corridor along the eastern edge of the Rocky Mountains has been challenged recently by palaeoecological analyses which indicate that the inland corridor region could not have sustained humans and was probably impassible (Mandryk *et al.* 2001). A coastal route may have presented more favourable conditions (Fladmark 1979), and early American archaeological sites typically show intensive use of marine resources (Keefer *et al.* 1998). Because *N. lamellosa* is a species characteristic of human Holocene shell middens in the northeastern Pacific (e.g. Wagner 1958), the results here provide evidence that marine taxa consumed by coastal people were present during the period when humans are thought to have first entered North America. Thus, in addition to a productive terrestrial coastal biota during the

late Pleistocene (Josenhans *et al.* 1997), marine intertidal communities may have provided a potential resource for early human migrants (Ward *et al.* 2003).

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