

REPORT

## Refuting a controversial case of a human-mediated marine species introduction

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### Abstract

Human activities have strongly impacted natural communities through the introduction of non-native species in historical times. A frequently cited marine example is *Littorina littorea*, a common intertidal gastropod that was first reported in North America in 1840. The seemingly sudden appearance and rapid geographical spread of this species southward from Nova Scotia has led many researchers to consider *L. littorea* a human-mediated species introduction. This is despite allozyme and subfossil evidence that the 'European periwinkle' was in North America long before 1840. Our mitochondrial and nuclear DNA sequence data confirm that *L. littorea* has been in continuous residence in North America for at least 8000 years. It appears most likely that ecological interactions, rather than oceanographic or climatic forces, maintained the limited geographical distribution of *L. littorea* prior to the 19th century.

### Keywords

*Littorina littorea*, introduced species, coalescent, cytochrome oxidase I, internal transcribed spacer.

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### INTRODUCTION

The introduction and spread of non-indigenous species is a dramatic environmental problem (see Grosholz 2002 for review). From ostensibly beneficial introductions of agricultural species or genetic strains, to accidental introductions of species that change the competitive and trophic interactions in a community, humans have in many cases permanently altered natural communities and caused the extirpation of native species. One species that has long puzzled biologists in terms of its origins and its ability to dramatically change the dynamics of intertidal populations is the periwinkle *Littorina littorea*. The debate regarding the origins of *Littorina littorea* in North America has lasted well over 100 years (Ganong 1886; Clarke & Erskine 1961; Bird 1968; Berger 1977; Vermeij 1978; reviewed in Reid 1996). Arguments in favour of human introduction rely on the unquestioned evidence of its rapid spread southward (e.g. first reported in Nova Scotia in 1840; first appearances at Saco, Maine in 1873; abundant at Cape Cod by 1875; presently as far south as Delaware; Ganong 1886, Bertness 1999). At the same time, there is strong archaeological evidence showing that *L. littorea* was present in North America long before any European settlements. This includes: findings of *L. littorea* in ancient Micmac camps in

Nova Scotia that have been radiocarbon-dated to 1300 AD (Clarke & Erskine 1961); two shells found below the surface of a raised marine terrace in Newfoundland, on which Norse settlements were constructed around 1000 AD (Bird 1968); and a single Nova Scotian specimen that has been dated to the Mid-Wisconsin age (about 40 kya; Wagner 1977).

Due to the dramatic ecological effects that accompanied the southward spread of this important herbivore since 1840 (Lubchenco 1983; Bertness 1984; Behrens Yamada & Mansour 1987; Steneck & Carlton 2001), the question of whether this snail was historically introduced has enormous implications (Carlton & Geller 1993). The undisputed presence of *L. littorea* in America hundreds or thousands of years ago could be explained as extinction with subsequent recolonization or introduction on a remarkably short time-scale. Even an apparently continuous fossil record cannot definitively rule out the possibility of extinction and rapid recolonization (Wares & Cunningham 2001). Because significant genetic divergence between populations requires the passage of time, genetic data may be used to show whether American and European populations have diverged due to continuous residence on both coasts of the North Atlantic (Hewitt 1996; Johnson *et al.* 2000; Wares & Cunningham 2001). Although a

pioneering allozyme study from 1977 showed fixed differences at five of 12 allozyme loci between populations in France and New England (Berger 1977), the 'European periwinkle' is still widely regarded as an introduced species (Bertness 1984; Behrens Yamada & Mansour 1987; Carlton 1992; Steneck & Carlton 2001; Grosholz 2002). This is in part due to continued debate regarding the allozyme data of Berger (1977), and in a thorough review of this problem in Reid (1996) it was suggested that further genetic evidence should be obtained.

To resolve this issue, we have collected sequence data from two mitochondrial genes (cytochrome *b* and cytochrome oxidase I) and one nuclear locus (ribosomal internal transcribed spacer) from individuals collected in a number of European and North American populations. The increased resolution of these data relative to allozymes not only allows us to discriminate between populations that are genetically distinct from those that have a very recent shared history, but provides temporal information about the relationship between these populations. Coalescent theory (Kingman 1982; Hudson 1990) describes a very elegant relationship between genetic variation in a population and the demographic history of that population, including parameters such as the genetic effective population size ( $N_e$ ) and the age of the alleles being considered. Here we apply methods based on coalescent theory to examine the hypothesis that American populations of *L. littorea* have only been founded in the past few hundred years.

## METHODS

All DNA fragments were obtained using standard methods and published primer sequences (Reid *et al.* 1996; Wares & Cunningham 2001; Wares 2001a). For each statistical analysis using coalescent theory, estimates of the substitution rate ( $\mu$ ) at each locus were obtained from the literature. These estimates are derived from comparisons of 3rd codon position data from the cytochrome oxidase I (COI) and cytochrome *b* (cyt *b*) genes (the full data sets were also analysed with similar results) among Atlantic *Littorina* species and their Pacific sister taxa (Reid *et al.* 1996; Wares & Cunningham 2001) for the mitochondrial genes (infinite alleles  $\mu = 5.18 \times 10^{-5}$ ). Across a broad range of taxa, the nuclear ribosomal internal transcribed spacer region (ITS) appears to evolve at a rate of approximately 1–1.4% per million years (Wares 2001a); this full range was used in temporal estimates based on this nuclear locus.

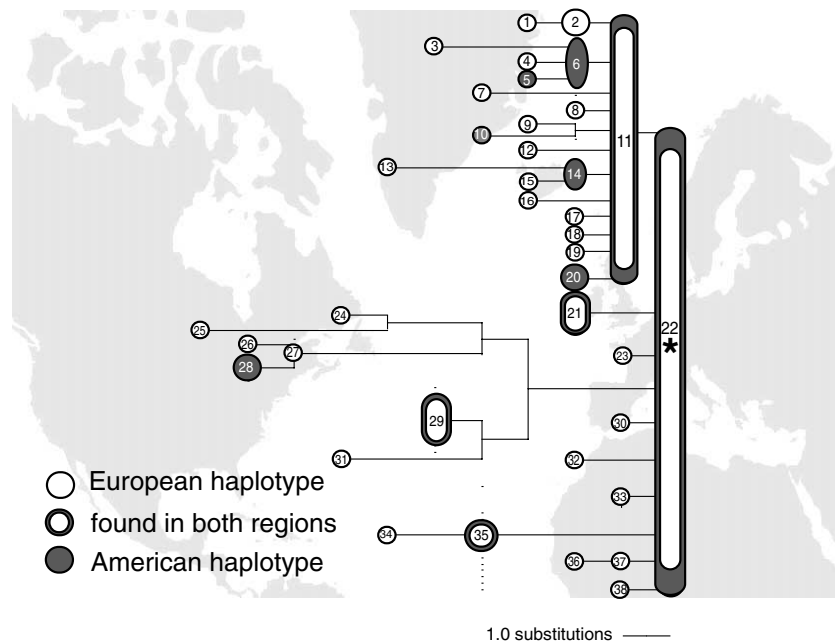
The finite-sites version of MDIV (Nielsen & Wakeley 2001) was used to perform the coalescent analysis of these data; five initial runs were applied to ensure the stability of the posterior estimate, each time sampling  $2 \times 10^6$  trees after discarding the first  $5 \times 10^5$  samples. The final run

sampled  $10 \times 10^6$  genealogies from a finite surface ( $T < 2.0$  in coalescent units,  $M < 7.0$ ). Divergence estimates presented here are calculated from the MDIV output based on a per-locus rate estimated from 3rd position sites. Log-likelihood tests were used with the likelihood surfaces generated by MDIV to test the hypotheses that American and European populations have diverged only in the past 2000 or 10 000 years. A simpler estimate of population divergence that accounts for the coalescent history of ancestral populations is Nei & Li's (1979) measure of net pairwise divergence ( $d_A$ ). This statistic was calculated, along with estimates of sampling error, using DNAsp version 3.53 (Rozas & Rozas 1999). The time of divergence between two populations is then calculated as  $t = d_A/2\mu$  (infinite sites  $\mu$ ).

Power analysis of these data was performed to determine the null expected distribution of unique American alleles for the temporal hypotheses being investigated. This involved a simple beta-binomial sampling distribution, constructed using two beta-distributed prior distributions of the genetic identity of the European and American populations: one to reflect the non-molecular evidence supporting a recent introduction of *L. littorea* ( $\alpha = 1.0$ ,  $\beta = \sqrt{(\alpha/2)}$ ), and another to reflect the non-molecular evidence supporting an ancient North American population of this snail ( $\alpha = 4.0$ ). The binomial distribution  $B(N, p)$  was used to calculate the likelihood of observing the sequence data for both mitochondrial and nuclear genes given a European sample size  $N$  and the probability  $p$  of any allele sampled in North America being identical to one sampled in Europe. It was assumed that this probability ( $p$ ) is equal to the expected genetic identity,  $I = e^{-2\mu t}$  (Nei 1987). Inability to reject the human introduction hypothesis with this test, based on the upper 95% confidence interval of this distribution, could indicate inadequate population sampling.

## RESULTS

We surveyed 992 bp of two mitochondrial genes (cytochrome *b*, cytochrome oxidase I; GenBank accession numbers AF494545-AF494736) from four European (Denmark, France, Ireland, Norway;  $n = 60$ ) and five American (Cape Cod, Maine, Newfoundland, New Brunswick, Nova Scotia;  $n = 57$ ) populations across the entire range of *L. littorea* (see Appendix for details on sampled populations). In contrast to the predictions of a human-mediated introduction, we find that of 11 haplotypes found in North America, six are endemic to North America, and only five are shared with Europe (Fig. 1). We also surveyed 864 bp of the ribosomal internal transcribed spacer (ITS; GenBank accession numbers AF494737-AF494759) regions from 12 divergent alleles from 8 individuals in North



**Figure 1** Sequence data from two mitochondrial genes (cytochrome oxidase I and cytochrome *b*) strongly argue against a human introduction of *L. littorea*. Illustrated is a parsimony network indicating the relationship of mitochondrial haplotypes (combined data) found in North America, Europe, or both locations (Appendix; vertical axis of each represented haplotype does not directly reflect frequency). Of 11 haplotypes found in North America, six are endemic to the region. An introduction recent enough to have been caused by human activity is not expected to acquire this much endemic diversity (see power analysis). The star indicates the maximum-likelihood root haplotype for this network, and the network is illustrated as European-ancestral because of the known ages of *L. littorea* in Europe being much greater than those in North America. Although not illustrated, data from the nuclear ITS region also strongly support a deeper history of *L. littorea* in North America; of eight North American individuals screened, none share an allele with individuals from Europe ( $n = 10$ ).

America and 11 divergent alleles from 10 individuals in Europe (alleles from the same individual that were each other's closest relatives were excluded from the analysis). No shared alleles between North America and Europe were found for the ITS.

The likelihood surfaces generated by MDIV indicate that the formation of American and European populations of *L. littorea* from an ancestral population is likely to be quite old. Log-likelihood tests strongly reject the possibility that these populations have diverged in the past 2000 years ( $P < 0.001$ ) or in the past 10 000 years ( $P < 0.01$ ). The maximum-likelihood estimate of this divergence time is roughly 23.2 kya, with the most recent date in the 95% credible set being about 16 100 years ago. The minimum age of divergence obtained using Nei and Li's estimator ( $d_A = 0.00201 \pm 0.00116$ ) is about 8100 years ago. Using a range of published mutation rates for the ITS region (see Wares 2001a), the same pairwise divergence measure estimates the divergence between America and Europe to be 18–26 kya, though unlike the mitochondrial data, the confidence intervals do not exclude zero.

Additionally, the power analysis using a beta-binomial sampling test is able to reject ( $P < 0.05$ ) the founding of the

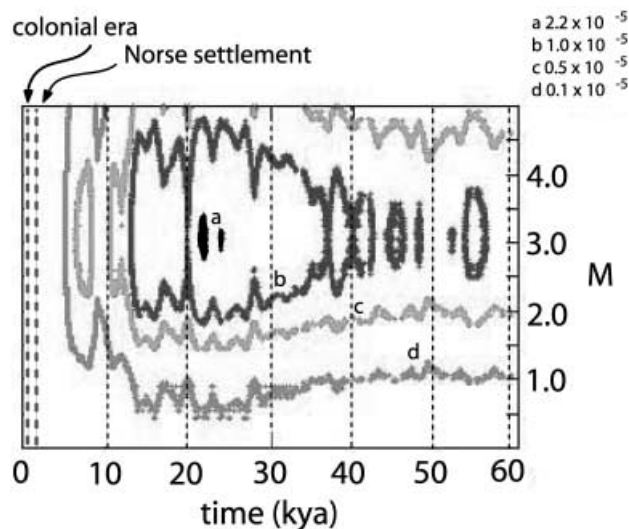
American population at any time in the past 1200 years (which includes Viking expeditions). This conclusion holds for both loci, irrespective of the prior distribution used, and is at least a crude measure of our ability to reject the human introduction hypothesis on the basis of endemic genetic diversity.

## DISCUSSION

When a species expands its geographical range by long-distance dispersal, newly founded populations will represent a strict subset of the genetic diversity present in the source population (Hewitt 1996; Austerlitz *et al.* 1997; Johnson *et al.* 2000). Therefore, if *L. littorea* has been in America for less than 200 years, we expect every allele sampled in America to be present in European populations. In contrast to the predictions of a human-mediated introduction, we find that of 11 haplotypes found in North America, six are endemic to North America, and only five are shared with Europe (Fig. 1). No shared alleles between North America and Europe were found for the ITS.

The minimum age of the divergence between American and European populations can be quantified for non-recombining loci using a recently developed method based

on coalescent theory that jointly estimates the likelihood surfaces for migration rates between two populations and the divergence time of these populations (Nielsen & Wakeley 2001). Using published mitochondrial mutation rates (Reid *et al.* 1996; Wares & Cunningham 2001) for *Littorina*, this method dated the founding of the American population at about 23.2 kya, with the most recent date in the 95% credible set being about 16 100 years ago. These values are based on the numerical output from our analysis; an alternative view (Fig. 2) shows the maximum-likelihood surface, with likelihood contours around 25 kya that are over 20 times greater than the likelihood values representing 4–5 kya. Similarly, a widely used statistic based on pairwise differences among populations (Nei & Li 1979) gives a minimum age of divergence of 8.1 kya. The estimates based on pairwise sequence divergence alone tend to be lower than those generated by coestimation of the divergence time and the pairwise migration parameter. Indeed, the estimated levels of migration across the Atlantic in *L. littorea* are relatively high, suggesting that some recent or ongoing colonization via larval dispersal is possible. While strong levels of migration may limit our statistical ability to estimate the time of divergence between two populations (Wakeley 2000), the number of unique American haplotypes is still greater than would be expected given a human introduction and the cumulative likelihood (based on MDIV) for a divergence time (i.e. time of formation of a second



**Figure 2** A representative likelihood surface describing the likely time of separation and levels of migration ( $M$ ) between European and North American populations of *L. littorea*. Estimates of divergence time in the text are based on point estimates in the output from MDIV; the likelihood contours on this surface (a–d, see legend for likelihood values) indicate that divergence dates of less than 5000 years ago are more than 20 times less likely than the maximum likelihood estimate.

population, allowing for ongoing migration) of less than 2000 years between the European and American populations is zero.

Although based on a much smaller sample size, similar conclusions are obtained from our nuclear ITS data. Using a range of published mutation rates for this gene (Wares 2001a), the same pairwise divergence measure estimates the divergence between American and Europe to be 18–26 kya, though unlike the mitochondrial data, the confidence intervals do not exclude zero. Together with the large trans-Atlantic divergence observed by Berger (1977) with 12 allozyme loci (5 fixed differences and a Nei's  $D$  of  $> 4.0$ ), results from the nuclear genome are consistent with the mitochondrial data in rejecting a human-mediated introduction from Europe. A simple power analysis of both data sets indicates that the amount of endemic genetic diversity in North America is greater than would be expected if the population had been founded in only the past few hundred years. Finally, it is worth noting that the levels of endemic diversity in American populations of *L. littorea* are much higher than for two other gastropods (*L. obtusata* and *Nucella lapillus*) that are believed to have recolonized North America since the last glacial maximum (Ingólfsson 1992; Wares & Cunningham 2001).

With the concordance of multiple loci, including allozymes and both mitochondrial and nuclear sequence data, we can statistically reject the possibility that populations of *L. littorea* arrived in North America due to human movements. This leaves to be explained first why *L. littorea* was confined to the Canadian maritimes, and secondly, why it suddenly began to spread southwards during the 19th century. Although this species clearly does not represent an historical introduction via human activities, the North American population is nevertheless likely to be derived from Europe many thousands of years ago (Fig. 1; Vermeij 1982; Ingólfsson 1992). *L. littorea* is not currently found in intermediate regions of the North Atlantic such as Iceland or Greenland, though it occurred in Iceland during post-glacial warm periods (Vermeij 1978; Reid 1996). Several plausible hypotheses have been offered for its arrival in North America, either via the drift of planktonic larvae (Krauter 1974; Reid 1996) or through founding populations arriving on driftwood (Clarke & Erskine 1961; Bird 1968).

Despite the most recent glacial maximum (about 18 kya) covering most of New England and the Canadian maritimes with ice, there is growing genetic evidence for an unglaciated refugium in the maritimes (Holder *et al.* 1999; Wares 2002; Young *et al.* 2002). This genetic evidence is further supported by the discovery of a temperate molluscan assemblage in Nova Scotia around 33–40 kya (Wagner 1977). This assemblage includes *L. littorea* and other temperate species including the mussel *Mytilus edulis*. The

existence of this temperate assemblage during the height of glacial activity has fuelled speculation that warm water currents may have supported a temperate assemblage in close proximity to a continental ice sheet (reviewed in Wagner 1977, p. 1315). *L. littorea* may have survived in this northern refugium, but not further south, and its relative scarcity in North American Pleistocene and Holocene sediments is also reflected in the lack of *L. littorea* fossils in many collections (e.g. Philadelphia Academy of Sciences, G. Rosenberg, personal communication), though other littorinids are evident in the Atlantic fossil record (Vermeij 1991) and there is an abundance of *L. littorea* in the European fossil record (Reid 1996).

The study of invasive and human-introduced species is gaining attention and is an important element of studying the impact of human dynamics on natural communities (Carlton 1992; Carlton & Geller 1993; Ruiz *et al.* 2000; Steneck & Carlton 2001; Grosholz 2002). While the rapid spread of *L. littorea* in New England is cause for further ecological and evolutionary research regarding the dynamics of community change, it is clear that natural forces have been responsible for the arrival of the 'European periwinkle' in North America. What remains of great interest is to determine the forces that were responsible for maintaining this species in only a small portion of its current geographical range, as human activity may still be responsible for the ultimate spread of *L. littorea*. It has been suggested, for example, that the sudden southward spread of *L. littorea* during the 19th century is due to increased commerce along the New England coast. Prevailing currents may have prevented the spread of larvae from the Nova Scotian region for thousands of years; 19th century shipping routes then assisted the colonization of this species to other ports (Clarke & Erskine 1961). Climatic fluctuations in the late 19th century could also be responsible for the sudden spread of this species (Clarke 1971).

A second class of hypotheses suggest that changes in ecological interactions played a role in the rapid expansion of *L. littorea*. Human impacts on New England intertidal communities in the 19th century range from species introductions to altered fishing practices, events that could reduce the effects of competitor or predatory species on *L. littorea* (Vermeij 1982, 1993) or that provided additional food sources (e.g. the introduction of *Fucus serratus*; Robinson 1903; Edelman *et al.* 1971–73). Given these hypothetical changes in trophic dynamics, *L. littorea* could have suddenly expanded its geographical range and overall population size; such rapid population expansions appear to be more common in marine systems than with terrestrial species (Grosholz 1996, 2002). Patterns of meso-scale ocean currents are unlikely to have been stable enough to prevent the spread of this planktonically dispersing species for

thousands of years; many extant currents have only formed in the past 6000–7000 years, and tidal currents as well as storm currents have been increasing throughout the Canadian Maritimes and Bay of Fundy for the past 6000 years (Syvitski 1996). As palaeontological evidence strongly suggests a disparity in the historical population size of *L. littorea* between European and American populations (see above), the rapid expansion of this species was probably stimulated by ecological changes that provided *L. littorea* with greater resources over a greater geographical area.

The expansion of the 'European periwinkle' is well documented because this gastropod is morphologically distinct from any other species in the New England intertidal community. However, it is well established (Holder *et al.* 1999; Wares 2002; Young *et al.* 2002) that other taxa were also preserved during glacial maxima in unglaciated refugia along the Canadian coast. The influence of northern unglaciated refugia on the diversity of the modern biota may be very strong (Stewart & Lister 2001). If particular changes in ecological parameters allowed the natural expansion and invasion of *L. littorea*, including reduced species diversity in the New England community (Vermeij 1989; Stachowicz *et al.* 1999) or some of the changes described above, we may expect that a genetic signal for range expansion would be an idiosyncratic pattern found in few species.

On the other hand, if a long-standing barrier to dispersal was somehow breached by human activity (Clarke & Erskine 1961) or climatic fluctuations permitted the expansion of temperate populations from warm waters in the north (e.g. Gulf of St Lawrence; Clarke 1971), then we may see such genetic signatures in a variety of northern refugial species through population genetic comparisons throughout New England for species known to have persisted in multiple refugia (e.g. *Idotea balthica*; Wares 2001b). The effect of this expansion on the entire community is then spread across a number of refugial lineages (see Grosholz 2002) and may involve coevolutionary interactions that have yet to be described. The testing of this hypothesis awaits the collection and thorough analysis of genetic data from a broad number of taxa in this region.

The preservation of native biota is clearly an important goal of the academic and conservation community (Ruiz *et al.* 2000; Steneck & Carlton 2001). Detailed genetic analysis is often the only means for distinguishing between endemic and exogenously introduced species in studies of community assembly and dynamics. A continued effort to correlate the history of populations – and genetic information within these populations – with the histories of human activity, geological and environmental change, and those of interacting species, will improve our ability to predict further change in natural communities.

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## REFERENCES

- Austerlitz, F., Jung-Muller, B., Godelle, B. & Gouyon, P.-H. (1997). Evolution of coalescence times, genetic diversity and structure during colonization. *Theor. Pop. Bio.*, 51, 148–164.
- Behrens Yamada, S. & Mansour, R.A. (1987). Growth inhibition of native *Littorina saxatilis olivi* by introduced *Littorina littorea* L. *J. Exp. Mar. Biol. Ecol.*, 105, 187.
- Berger, E. (1977). Gene-enzyme variation in three sympatric species of *Littorina*. II. The Roscoff population, with a note on the origin of North American *L. Littorea*. *Biol. Bull.*, 153, 255.
- Bertness, M.D. (1984). Habitat and community modification by an introduced herbivorous snail. *Ecology*, 65, 370.
- Bertness, M.D. (1999). *The Ecology of Atlantic Shorelines*. Sinauer Associates, Sunderland, Massachusetts.
- Bird, J.B. (1968). *Littorina littorea*: occurrence in a northern Newfoundland beach terrace, predating Norse settlements. *Science*, 159, 114.
- Carlton, J.T. (1992). Introduced marine and estuarine mollusks of North America: an end-of-the-20th-century perspective. *J. Shellfish Res.*, 11, 489.
- Carlton, J.T. & Geller, J.B. (1993). Ecological roulette: the global transport of nonindigenous marine organisms. *Science*, 261, 78.
- Clarke, A.H. (1971). *Littorina littorea*, native or introduced? *The Biologist*, 53, 160–162.
- Clarke, A.H. & Erskine, J.S. (1961). Pre-Columbian *Littorina littorea* in Nova Scotia. *Science*, 134, 393.
- Edelstein et al. (1971–73). Investigations of the marine algae of Nova Scotia. X. Distribution of *Fucus serratus* L. and some other species of *Fucus* in the maritime provinces. *Proc. Nova Scotia Inst. of Science*, 27, 33–42.
- Ganong, W.F. (1886). Is *Littorina littorea* introduced or indigenous? *Am. Naturalist*, 20, 931.
- Grosholz, E. (1996). Contrasting rates of spread for introduced species in terrestrial and marine systems. *Ecology*, 77, 1680–1686.
- Grosholz, E. (2002). Ecological and evolutionary consequences of coastal invasions. *TREE*, 17, 22–27.
- Hewitt, G.M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.*, 58, 247.
- Holder, K., Montgomerie, R. & Friesen, V.L. (1999). A test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). *Evolution*, 53, 1936.
- Hudson, R.R. (1990). Gene genealogies and the coalescent process. *Oxf. Surv. Evol. Biol.*, 7, 1–40.
- Ingólfsson, A. (1992). The origin of the rocky shore fauna of Iceland and the Canadian maritimes. *J. Biogeogr.*, 19, 705.
- Johnson, K.P., Adler, F.R. & Cherry, J.L. (2000). Genetic and phylogenetic consequences of island biogeography. *Evolution*, 54, 387.
- Kingman, J.F.C. (1982). The coalescent. *Stoch. Proc. Appl.*, 13, 235–248.
- Kraeuter, J.N. (1974). Offshore currents, larval transport, and establishment of southern populations of *Littorina littorea* Linné along the U.S. Atlantic coast. *Thalassia Jugoslavica*, 10, 159–170.
- Lubchenco, J. (1983). *Littorina* and *Fucus*: effects of herbivores, substratum heterogeneity, and plant escapes during succession. *Ecology*, 64, 1116–1123.
- Nei, M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Nei, M. & Li, W.-H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA*, 76, 5269.
- Nielsen, R. & Wakeley, J. (2001). Distinguishing migration from isolation: a Markov Chain Monte Carlo approach. *Genetics*, 158, 885.
- Reid, D.G. (1996). *Systematics and evolution of Littorina*. Ray Society, London.
- Reid, D.G., Rumbak, E. & Thomas, R.H. (1996). DNA, morphology, and fossils: phylogeny and evolutionary rates of the gastropod genus *Littorina*. *Phil. Trans. R. Soc. Lond. B*, 351, 877.
- Robinson, C.B. (1903). The distribution of *Fucus serratus* in America. *Torreya*, 3, 132–134.
- Rozas, J. & Rozas, R. (1999). DnaSP, Version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics*, 15, 174–175.
- Ruiz, G.M., Fofonoff, P.W., Carlton, J.T., Wonham, M.J. & Hines, A.H. (2000). Invasion of coastal marine communities in North America. Apparent patterns, processes and biases. *Annu. Rev. Ecol. Syst.*, 31, 481.
- Stachowicz, J.J., Whitlatch, R.B. & Osman, R.W. (1999). Species diversity and invasion resistance in a marine ecosystem. *Science*, 286, 1577–1579.
- Steneck, R.S. & Carlton, J.T. (2001). Human alterations of marine communities: students beware. In: *Marine Community Ecology*, eds Bertness, M.D., Gaines, S.D. & Hay, M.E.. Sinauer Associates, Sunderland, Massachusetts, pp. 445–468.
- Stewart, J.R. & Lister, A.M. (2001). Cryptic northern refugia and the origins of the modern biota. *TREE*, 16, 608–612.
- Syvitski, J.P.M., Lewis, C.F.M. & Piper D.J.W. (1996). Palaeoceanographic information derived from acoustic surveys of glaciated continental margins: examples from eastern Canada. In: *Late Quaternary Palaeoceanography of the North Atlantic Margins*, eds Andrews, J.T., Austin, W.E.N., Bergsten, H. & Jennings, A.E. Geological Society Special Publication 111. Geological Society, Boulder, CO, 51–76.
- Vermeij, G.J. (1978). *Biogeography and Adaptation: Patterns of Marine Life*. Harvard University Press, Cambridge, Massachusetts.
- Vermeij, G.J. (1982). Environmental change and the evolutionary history of the periwinkle (*Littorina littorea*) in North America. *Evolution*, 36, 561.
- Vermeij, G.J. (1989). Invasion and extinction: the last three million years of North Sea pelecypod history. *Cons. Biol.*, 3, 274–281.

- Vermeij, G.J. (1991). Anatomy of an invasion: the trans-Arctic interchange. *Paleobiology*, 17, 281–307.
- Vermeij, G.J. (1993). Biogeography of recently extinct marine species: implications for conservation. *Conservation Biol.*, 7, 391–397.
- Wagner, F.J.E. (1977). Palaeoecology of marine Pleistocene Mollusca, Nova Scotia. *Can. J. Earth Sci.*, 14, 1305.
- Wakeley, J. (2000). The effects of subdivision on the genetic divergence of populations and species. *Evolution*, 54, 1092–1101.
- Wares, J.P. (2001a). Biogeography of *Asterias*: North Atlantic climate change and speciation. *Biol. Bull.*, 201, 95–103.
- Wares, J.P. (2001b). Intraspecific variation and geographic isolation in *Idotea balthica* (Isopoda: Valvifera). *J. Crust. Biol.*, 21, 1007–1013.
- Wares, J.P. (2002). Community genetics in the northwestern Atlantic intertidal. *Mol. Ecol.*, 11, 1131–1144.
- Wares, J.P. & Cunningham, C.W. (2001). Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution*, 55, 2455.
- Young, A.M.C., Torres, J.E., Mack & Cunningham, C.W. (2002). Morphological and genetic evidence for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit crab *Pagurus longicarpus*. *Mar. Biol.*, 140, 1059–1066.

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## APPENDIX

Populations in which each mitochondrial haplotype (Fig. 1) occurred. Haplotypes found only in North American populations are indicated in bold. Populations are designated by the following abbreviations: FR (Roscoff, France); IR (Galway, Ireland); NO (Trondheim, Norway); DE (Espergerde, Denmark); DK (Karrebaeksminde, Denmark); NW (St Johns, Newfoundland, Canada); NSC (Cranberry Cove, Nova Scotia, Canada); NSB (Blomidon, Nova Scotia, Canada); NBM (Murray Beach, New Brunswick, Canada); ME (Damariscotta, Maine, USA); CC (Cape Cod, Massachusetts, USA)

Haplotype	FR	IR	NO	DE	DK	NW	NSC	NSB	NBM	ME	CC
1	–	–	1	–	–	–	–	–	–	–	–
2	1	–	12	3	2	–	–	–	–	–	–
3	1	–	–	–	–	–	–	–	–	–	–
4	1	–	–	–	–	–	–	–	–	–	–
<b>5</b>	–	–	–	–	–	<b>3</b>	–	–	–	–	–
<b>6</b>	–	–	–	–	–	–	<b>1</b>	–	–	–	–
7	1	–	–	–	–	–	–	–	–	–	–
8	1	–	–	–	–	–	–	–	–	–	–
9	2	–	–	–	–	–	–	–	–	–	–
<b>10</b>	–	–	–	–	–	–	–	–	–	<b>1</b>	–
11	2	2	–	1	1	–	–	–	–	–	–
12	1	–	–	–	–	–	–	–	–	–	–
13	1	–	–	–	–	–	–	–	–	–	–
<b>14</b>	–	–	–	–	–	<b>2</b>	<b>1</b>	–	–	–	–
15	1	–	–	–	–	–	–	–	–	–	–
16	1	–	–	–	–	–	–	–	–	–	–
17	1	–	–	–	–	–	–	–	–	–	–
18	–	–	1	–	–	–	–	–	–	–	–
19	–	1	–	–	–	–	–	–	–	–	–
<b>20</b>	–	–	–	–	–	<b>1</b>	<b>1</b>	–	–	<b>1</b>	<b>1</b>
21	1	–	–	–	–	1	1	–	–	1	1
22	1	1	2	–	–	3	–	3	1	1	–
23	1	–	–	–	–	–	–	–	–	–	–
24	1	–	–	–	–	–	–	–	–	–	–
25	1	–	–	–	–	–	–	–	–	–	–
26	–	1	–	–	–	–	–	–	–	–	–
27	–	–	1	–	–	–	–	–	–	–	–
<b>28</b>	–	–	–	–	–	<b>2</b>	–	–	<b>1</b>	<b>3</b>	–
29	1	–	–	–	–	2	1	2	1	2	1
30	–	1	–	–	–	–	–	–	–	–	–
31	–	1	–	–	–	–	–	–	–	–	–
32	1	–	–	–	–	–	–	–	–	–	–
33	2	–	–	–	–	–	–	–	–	–	–
34	1	–	–	–	–	–	–	–	–	–	–
35	–	1	–	–	–	6	3	2	1	3	1
36	1	–	–	–	–	–	–	–	–	–	–
37	–	–	2	–	–	–	–	–	–	–	–
38	1	–	–	–	–	–	–	–	–	–	–