Molecular Ecology (2008) **17**, 3684–3696 doi: 10.1111/j.1365-294X.2008.03865.x

Solving cryptogenic histories using host and parasite molecular genetics: the resolution of *Littorina littorea*'s North American origin

APRIL M. H. BLAKESLEE, JAMES E. BYERS and MICHAEL P. LESSER
Department of Zoology, University of New Hampshire, 46 College Road, Durham, NH 03824, USA

Abstract

Even after decades of investigation using multiple sources of evidence, the natural histories of some species remain unclear (i.e. cryptogenic). A key example is *Littorina littorea*, the most abundant intertidal snail in northeastern North America. Native to Europe, the snail's ecological history in North America has been debated for over 100 years with no definitive resolution. To resolve its cryptogenic status, we used molecular genetics from a novel combination of the snail and a highly associated trematode parasite, *Cryptocotyle lingua*. Based on mitochondrial sequences of 370 *L. littorea* and 196 *C. lingua* individuals, our results demonstrate a significant reduction in genetic diversity in North America vs. Europe, North American haplotypes nested within European haplotypes, and mean divergence estimates of ~500 years ago from Europe for both host and parasite — thus supporting a recent introduction of both host and parasite to North America from Europe. Our study therefore resolves not only a specific cryptogenic history, but it also demonstrates the success of our approach generally and could be used in resolving difficult invasion histories worldwide.

Keywords: biogeography, *Cryptocotyle lingua*, cryptogenic, introduction, *Littorina littorea*, population divergence

Received 14 March 2008; revision accepted 18 June 2008

Introduction

In recent years, invasive species have become recognized as a major component of human-mediated impacts on natural systems. However, there remain a considerable number of species that cannot be demonstrably classified as native or non-native in many regions, and these species are referred to as cryptogenic (Carlton 1996; Ruiz *et al*. 2000). Especially in the case of conspicuous, abundant, or high-impact species, resolution of the ambiguous ecological history of cryptogenic species is a critical conservation goal. One cryptogenic species, whose history in North America has vexed scientists for 150 years (Ganong 1886; Clarke & Erskine 1961; Berger 1977; Reid 1996; Wares *et al*. 2002; Chapman *et al*. 2007, 2008; Wares & Blakeslee 2007; Cunningham 2008) is the European marine snail, *Littorina littorea* (common periwinkle) (Prosobranchia: Littorinidae; Linnaeus, 1758), a conspicuous rocky intertidal snail abundant on both North Atlantic coasts. Cryptogenic status often stems from incomplete or unknown historical knowledge of a species' presence in a location. Thus, the lingering ambiguity surrounding *L. littorea*'s status is particularly surprising given the vast amount of research over the past several decades that has been conducted on this species (e.g. Berger 1977; Wares *et al*. 2002). Considering its conspicuousness and dominance within intertidal regions in northeast North America and past use as a textbook case of invasion (e.g. Steneck & Carlton 2001), the resolution of *L. littorea*'s present-day cryptogenic status is a fundamental ecological question.

Correspondence: April M. H. Blakeslee, Marine Invasions Laboratory, Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037, USA. Fax: 443-482-2375; E-mail: blakesleea@si.edu

Equivocal evidence for Littorina littorea*'s North American Origin*

Littorina littorea's spread into the USA from Canada in the mid-1800s and its impacts upon marine biota in this region have been well documented (e.g. Carlton 1982; Brenchley & Carlton 1983; Lubchenco 1983; Bertness 1984; Yamada & Mansour 1987); yet uncertainty has remained regarding

the snail's status as native or non-native in North America. The two scenarios debated are whether the Canadian population that spread into the USA was a native glacial relic confined to the Canadian Maritimes until the mid-1800s, or whether it was an anthropogenically introduced population from Europe (Reid 1996). Although evidence has been provided by several researchers since 1886 and has included historical, archaeological, ecological, and molecular data (Ganong 1886; Clarke & Erskine 1961; Bird 1968; Berger 1977; Carlton 1982; Johannesson 1992; Reid 1996; Wares *et al*. 2002; Chapman *et al*. 2007, 2008; Wares & Blakeslee 2007; Cunningham 2008), its North American cryptogenic status remains unresolved because conclusions from these sources have been conflicting or equivocal (Johannesson 1992; Reid 1996; Chapman *et al*. 2007, 2008; Wares & Blakeslee 2007; Cunningham 2008).

The evidence related to *L. littorea*'s North American origin has been reviewed in several recent publications (especially Reid 1996; Blakeslee 2007; and Chapman *et al*. 2007, 2008); here, we will briefly summarize these findings. First, historical sources of evidence have included conflicting oral testimonies of its presence in the Canadian Maritimes prior to the 1850s (Verrill 1874; Ganong 1886; Reid 1996). In addition, prior to 1870 *L. littorea* was never described in any North American naturalist/field records, while less conspicuous snails, like *Littorina obtusata*, *Littorina saxatilis* and *Ilyanassa obsoleta*, were frequently found in published reports (e.g. Gould 1852; Binney 1863). Second, the archaeological evidence has also been equivocal. A handful of *L. littorea* specimens have been found in archaeological sites in maritime Canada; however, only a subset have been radiocarbon-dated and of these, none have predated early European and Norse visits to the maritime region (Chapman *et al*. 2007). Third, ecological sources of evidence/hypotheses for its North American presence have included the suggestion that *L. littorea* could have rafted on driftwood/seaweed for a natural invasion of North America from Europe (Bird 1968); however, *L. littorea* is not found in Iceland or Greenland (Johannesson 1988; Reid 1996), likely ruling out stepping-stone invasion across islands in the North Atlantic, and a direct crossing of drifting adults would be expected to end up south of the snail's current North American range (Kraeuter 1976). Moreover, *L. littorea* uses planktotrophic dispersal of larvae, and a direct crossing of larvae is believed highly improbable, if not impossible (Kraeuter 1976; Reid 1996). Further ecological evidence has involved the well-supported theory of enemy escape (Torchin *et al*. 2003), in which *L. littorea* was found to have a significantly lower trematode parasite species richness in *L. littorea* in North America compared to Europe—evidence which appears consistent with a recent introduction of the snail to North America (Blakeslee & Byers 2008). Lastly, a variety of molecular techniques have been used to understand *L. littorea*'s ecological history in North America, from allozyme analyses in the 1970s (Berger 1977; Morris 1979) to DNA sequencing (Wares *et al*. 2002) and amplified fragment length polymorphisms (AFLPs) (Wares & Blakeslee 2007) in recent times [this considerable evidence has been summarized and analysed extensively in both Chapman *et al*. (2007) and Blakeslee (2007)]. One study in particular (Wares *et al*. 2002), which included the greatest number of samples prior to our investigation, concluded that *L. littorea* was native to North America based on their DNA sequence data. However, recent studies (Chapman *et al*. 2007; Wares & Blakeslee 2007) have questioned the study's conclusions based on sample size issues, particularly regarding calculated divergence estimates and the assumption that unshared North American haplotypes represented endemism. Because no molecular study has yet been able to include enough information to conclusively answer the debate over *L. littorea*'s North American origin, several researchers have called for a larger molecular data set to definitively resolve *L. littorea*'s cryptogenic status in northeastern North America (Reid 1996; Wares & Blakeslee 2007; Chapman *et al*. 2008; Cunningham 2008).

Multiple lines of evidence to resolve L. littorea*'s cryptogenic status*

In our investigation, we set out to improve upon these prior limitations in the molecular work by heavily sampling the snail over a wide geographical range in both the European and North American populations. The mitochondrial data set we compiled is almost four times the size of the previously largest data set, and as we demonstrate here, our overall larger sample size allows us to produce robust estimates of population divergence. In addition, we recognize that due to the equivocal nature of *L. littorea*'s North American presence, multiple lines of evidence are necessary to fully resolve its cryptogenic history. In particular, we include here not only a molecular genetic analysis of the snail itself (Fig. 1) but additionally a broad molecular sampling of an associated, host-specific trematode parasite, *Cryptocotyle lingua* (Digenea: Heterophyidae; Creplin, 1825)—*L. littorea*'s most common parasite in both North America and Europe, and thus the most likely candidate for an associated introduction with the snail (Byers *et al*. 2008; Blakeslee & Byers 2008). Because parasites have recently become recognized as important tools/indicators in the understanding of host source populations (Criscione *et al*. 2006), we felt that the inclusion of such a corroborative data set along with evidence from the snail itself could help definitively resolve *L. littorea*'s cryptogenic status in North America. Finally, our complementary approach of host and parasite molecular genetics is important since our molecular data set for *L. littorea* includes one loci, and a recent AFLP data set (Wares & Blakeslee 2007) exploring multiple loci within the snail was unable to

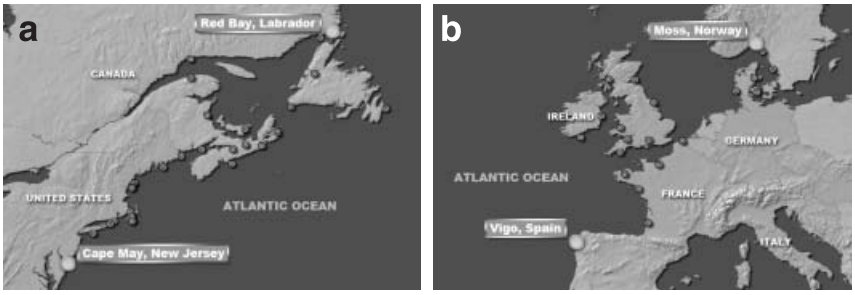

Fig. 1 North American (a) and European (b) collection sites for *Littorina littorea* snails and *Cryptocotyle lingua* trematodes. For *L. littorea*, there were a total of 29 North American sample sites, ranging from Red Bay, Labrador to Cape May, New Jersey, and 22 European sample sites, ranging from Moss, Norway to Vigo, Spain. *C. lingua* infections were found at a subset of *L. littorea* sites: there were 20 North American sample sites, ranging from Red Bay, Labrador to Point Judith, Rhode Island, and 16 European sample sites, ranging from Moss, Norway to Mindin, France. See Appendices I and II for site locations.

effectively provide any further resolution. Thus, novel information is truly needed to definitively resolve this century-old question.

In both *L. littorea* and *C. lingua*, we tested whether the snail and trematode exhibited molecular signatures expected of a recent founder event, which would include lower genetic diversity in North America vs. Europe, distributions of North American haplotypes nested within European haplotypes (Grosberg & Cunningham 2000), and short divergence time estimates between the North American and European populations because coalescent theory would predict a recent split between the two regions. However, if the alternative hypothesis were true — that *L. littorea* were native to North America having existed historically and through the most recent Ice Age in refugia in maritime Canada until its spread into the USA in the mid-1800s — we expected population divergence estimates to be prior to human contact with North America from Europe.

Materials and methods

Collections and molecular processing

Snails were collected from 29 North American (n = 183) and 22 European (n = 187) sites (Fig. 1; Appendix I). Snails were dissected to preserve foot tissue for DNA analyses and also to look for parasitism by *Cryptocotyle lingua*. Only uninfected snails (as a conservative precaution to avoid potential contamination issues) were used in host molecular analyses. *Cryptocotyle lingua* parasites were extracted from gonadal tissues of infected snails and preserved for molecular analyses. This trematode is easily distinguished from all other trematodes infecting *Littorina littorea* in that it is the only cercaria with two eyespots (James 1968). *Cryptocotyle lingua* parasites were found at 20 North American (n = 98) and 16 European (n = 98) sites (Fig. 1; Appendix II). All DNA was extracted using a standard CTAB protocol (France *et al*. 1996). In the snail, two sets of primers amplified cytochrome *b* (cyt *b*) and cytochrome oxidase I (COI) mitochondrial genes: cyt *b* (625 bp): *Primer1-F*, CCTTCCCGCACCTTCAAATC; *Primer4-R*, ATGAGAAATTTTCAGGGTC (Reid *et al*. 1996); COI (572 bp): *LLCOIAB-F*, CTCTCCTGGGAGATGACCAG; *LLCOIAB-R*: TTCTGGGTGACCGAAGAATC designed using prior sequence data (Williams & Reid 2004). Snail samples were amplified using an adapted polymerase chain reaction (PCR) protocol (Kyle & Boulding 1998) and subjected to 32 cycles of 95 °C for 30 s, 44 °C for 30 s, and 72 °C for 30 s. For the trematode, two sets of primers amplified two contiguous regions (1043 bp) of the COI mitochondrial gene: *COI2575F*: TTTTTTGGGCATCCTGAGGTTTAT; *COI3021R*: TAAAGAAAGAACATAATGAAAATG (Morgan & Blair 1998); *ABCOICLF*: TCTTTAGGATCATAAGCG; *ABCOICLR*: TAAACCCCCGTATCCAAACC designed using prior COI sequence data (Kane 1999). Trematode samples were amplified using an adapted PCR protocol (Huspeni 2000) subjected to 35 cycles of 94 °C for 30 s, 50.9 °C for 30 s, and 72 °C for 30 s. Following PCR, samples were cleaned-up for sequencing using a Qiaquick QIAGEN Kit. When samples were sequenced, each sample was run in both the forward and reverse directions and then later aligned in order to ensure haplotype identities were accurately assigned. Sequences were aligned using DNAStar Lasergene programs.

Statistical analyses

Phylogenetic relationships were analysed using PAUP* 4.0 (Swofford 2003). Phylogenetic trees were not only constructed using the full data set, but also using a truncated data set, where we excluded all third position sites (resulting in 798 total bp for *L. littorea* and 695 bp for *C. lingua*), which are the most variable sites in coding DNA because substitutions at these sites are often silent. We surmised that this latter approach would give us a conservative estimate of haplotype diversity in Europe vs. North America. Finally, in *L. littorea* we constructed a

phylogenetic tree of just North American individuals so we could compare Canadian vs. US sites. We performed this last analysis as a way to determine whether Canadian sites showed more diversity than US sites, which might be expected if *L. littorea* had existed in the Canadian Maritimes for thousands or hundreds of thousands of years before spreading into the USA ~150 years ago. For each of these phylogenetic analyses, the maximum likelihood root haplotype for each tree (designated by an asterisk) was determined using the program, TCS 1.21 (Clement *et al*. 2000).

Because haplotype diversity was high in our sampled populations, we used haplotype estimation curves to estimate haplotype diversity in each population and to quantify the effects of sampling effort on haplotype diversity. Specifically, we used ESTIMATES 8.0 (Colwell 2006) to calculate haplotype accumulation and haplotype estimation curves. ESTIMATES uses Monte Carlo resampling [through randomization of sample order over a number of replicates (e.g. 500)] to determine the mean accumulation of haplotypes (S_{obs}) as samples are added over the full data set, while also providing standard deviations and 95% confidence intervals for each data point (Gotelli & Colwell 2001). However, sample-based rarefaction curves may not capture the entire haplotype diversity within a population for a particular sampling effort, especially if these curves have not reached a stable asymptote (Gotelli & Colwell 2001). Thus, nonparametric estimators, such as Chao2, can be useful in predicting the eventual asymptote in haplotype diversity for a particular population and do so by including the effects of rare haplotypes on the total haplotype diversity (Gotelli & Colwell 2001). The Chao2 estimator has been found to be one of the most robust estimators (see Chao2 equation in Colwell 2006) when compared to empirical data from a variety of systems (Walther & Morand 1998; Foggo *et al*. 2003).

Finally, population divergence estimates were performed using the (isolation with migration) IM program (July 2006 version; Hey & Nielsen 2004, 2007). IM uses Markov chain Monte Carlo sampling and applies the isolation with migration model to genetic data taken from closely related species or populations of the same species. The program provides maximum-likelihood estimates of the time since two populations split (t) in terms of mutations, which can be converted to an estimate of the number of years since the populations diverged using the specific mutation rate for the gene in question. We performed 10 different runs/replicates of IM for each of the snail and trematode sequences using the following input parameters and 10 different random seeds: $q1 = 5000$, $m1 = m2 = 7$, $t = 2$, $b = 100\,000$. Divergence estimates were calculated using the following equation: $t = t/\mu$, where t is the total years of divergence time, t is the time parameter determined by IM from the sequence data, and μ is the gene substitution rate (Hey & Nielsen 2004). The substitution rate [3% per million years (Myr); ~1.8×10^{-5} for 1197 bp] that we used in calculating divergence estimates for *L. littorea* was determined from fossil record evidence of *Littorina* sp. (Reid *et al*. 1996; Wares & Cunningham 2001; and employed in Wares *et al*. 2002; Chapman *et al*. 2008; Cunningham 2008). However, we also included a range of mutation rates (2–4% per Myr) for comparison since a single mutation rate based on fossil evidence is still an estimate of substitution; thus, a range of mutation rates provide a more robust understanding of divergence time between the populations. Because trematodes do not preserve well in the fossil record, the best estimates of COI substitution rates for trematodes, like *C. lingua*, is a range between 2% and 4% per Myr (J.A.T. Morgan, personal communication): for 1043 bp, the rates used in calculating divergence estimates were therefore: 1.04×10^{-5} for 2% per Myr, 1.56×10^{-5} for 3% per Myr, and 2.09×10^{-5} for 4% per Myr.

Results

We found *Littorina littorea* to possess a total of 175 haplotypes (BLAST Accession nos EU875593–EU876332) from 370 total sequences ($n = 187$ in Europe and $n = 183$ in North America) over a total of 1197 base pairs (Fig. 2; Appendix I). Fifty-seven haplotypes were from North America, and 144 were from Europe (these numbers include shared haplotypes). Altogether, North America showed a significant reduction in genetic diversity compared to Europe ($\chi^2 = 37.7$, d.f. = 1, $P < 0.001$). The majority of European haplotypes in *L. littorea* were only observed once (89% were rare, 11% common), while in North America many more haplotypes were observed more than once (58% rare, 42% common). Furthermore, no clades were completely monophyletic for North American individuals (Fig. 2). To predict expected haplotype totals in each population, we performed haplotype estimation (Chao2) analyses and found the expected, maximum number of haplotypes in Europe to be 2456 (95% CI, 918–4115) compared to the 144 observed in Europe. In North America, 140 halotypes (95% CI, 89–273) were predicted compared to the 57 that were observed (Fig. 4); therefore, haplotype diversity in Europe was estimated by the Chao2 analysis to be 17.5 times greater than in North America.

For *Cryptocotyle lingua*, we found similar patterns to *L. littorea* in haplotype identities and frequencies: a total of 94 haplotypes (BLAST Accession nos EU876333–EU876528) were found from 196 sequences ($n = 98$ in both Europe and North America) over a total of 1043 base pairs (Fig. 3; Appendix II). Thirty-four haplotypes were North American and 75 were European (these numbers include shared haplotypes), and North America was significantly reduced in genetic diversity compared to Europe ($\chi^2 = 10.78$, d.f. = 1, $P < 0.001$). The majority of European haplotypes in *C. lingua* were only observed once (88% were rare, 12% common),

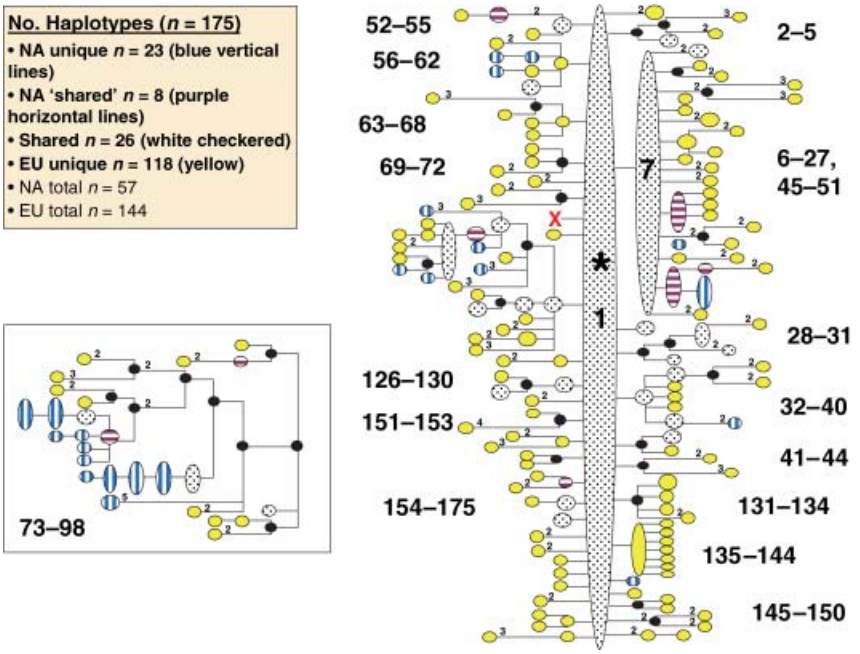

Fig. 2 Haplotype tree for *Littorina littorea.* Numbering represents haplotype identities within clades on the tree (see Appendix I). Haplotype bubbles are relatively sized based on haplotype frequencies and are coloured according to the following categories: unique to Europe (yellow), unique to North America (blue vertical lines), shared between populations (white chequered), and a fourth category (purple horizontal lines) for North American haplotypes basal to European haplotypes (and thus considered shared). The inset represents a clade that was too large for the scale of this diagram (see the 'X' for position on the overall tree). The haplotype with an asterisk represents the maximum likelihood root. The small numbers above lines connecting haplotype bubbles represent the number of mutations that have occurred since the prior haplotype to the haplotype in question. The bold numbers down each side of the figure refere to haplotype identities found in Appendix I.

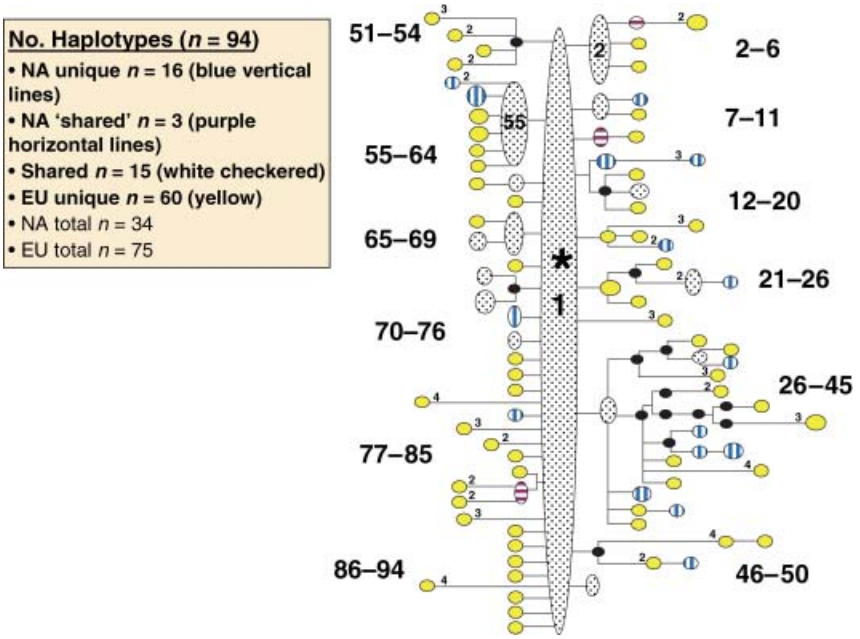

Fig. 3 Haplotype tree for *Cryptocotyle lingua.* Numbering represents haplotype identities within clades on the tree (see Appendix II). Haplotype bubbles are relatively sized based on haplotype frequencies and are coloured according to the following categories: unique to Europe (yellow), unique to North America (blue vertical lines), shared between populations (white chequered), and a fourth category (purple horizontal lines) for North American haplotypes basal to European haplotypes (and thus considered shared). The haplotype with an asterisk represents the maximum likelihood root. The small numbers above lines connecting haplotype bubbles represent the number of mutations that have occurred since the prior haplotype to the haplotype in question. The bold numbers down each side of the figure refere to haplotype identities found in Appendix I.

while in North America many more haplotypes were observed more than once (53% rare, 47% common). Again, no clades were completely monophyletic for North American individuals (Fig. 3). Haplotype estimates (Chao2) for the expected, maximum number of haplotypes were found to be 430 (95% CI, 293–656) in Europe vs. 64 (95% CI, 46–110) in North America (Fig. 4).

Phylogenetic comparisons within the North American region for *L. littorea* revealed diversity between the two populations that was essentially equal (Canada: 29 total haplotypes from 95 individuals, and USA: 28 total haplotypes from 88 individuals) and not significantly different ($\chi^2 = 0.2$, d.f. = 1, $P = 0.895$). Additionally, the number of unique Canadian haplotypes was nearly identical to the number of unique US haplotypes (19 and 18, respectively), and 10 haplotypes were shared between the two regions. Chao2 estimates of haplotype diversity were 82 (95% CI, 58–139) in Canada vs. 70 (95% CI, 60–117) in the USA, suggesting similar maximum estimates between the two regions.

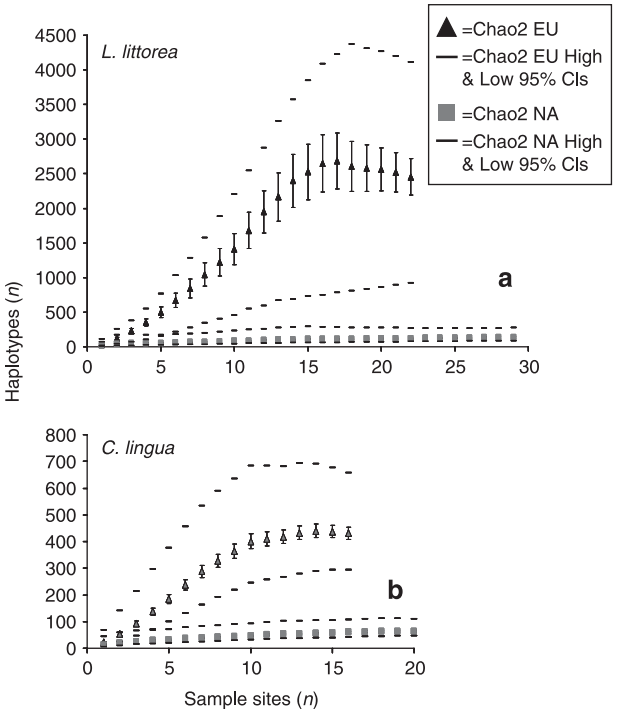

Fig. 4 Haplotype estimation curves for European (EU) vs. North American (NA) *Littorina littorea* (a) and *Cryptocotyle lingua* (b). The European Chao2 estimator suggests a maximum, expected number of haplotypes of ~2500 (with 95% CIs of ~920 and ~4110 haplotypes) for *L. littorea* and 430 (with 95% CIs of ~290 and ~650 haplotypes) for *C. lingua*, while the North American Chao2 estimator suggests a maximum, expected number of haplotypes of ~140 (with 95% CIs of ~90 and ~270 haplotypes) for *L. littorea* and 64 (with 95% CIs of ~45 and ~110 haplotypes) for *C. lingua*. These data demonstrate the much greater genetic diversity expected in Europe compared to North America, which strongly suggests that the 23 and 16 unique haplotypes found in North American *L. littorea* and *C. lingua*, respectively, are likely among the multiple haplotypes yet to be found in Europe (error bars are standard error).

Finally, divergence estimates from the snail and trematode sequence data for Europe and North America were calculated using the IM program (Hey & Nielsen 2004, 2007), which is well suited for asking questions about recent invasions since it does not include assumptions for equal effective population sizes between populations, which is known to have been problematic for prior divergence estimates using *L. littorea* sequence data (Chapman *et al*. 2007; Cunningham 2008). Over 10 replicate runs for *L. littorea*, we found mean (± SE) divergence estimates for the three mutation rates to be: 668 (± 132) years ago with 95% CIs between 518 (± 109) and 969 (± 205) years ago for the 2% per Myr mutation rate; 444 (± 88) years ago with 95% CIs between 344 (± 73) and 644 (± 137) years ago for the 3% per Myr mutation rate [the accepted mutation rate for littorine snails based upon molecular and fossil record evidence (Reid *et al*. 1996; Wares & Cunningham 2001; and employed in Wares *et al*. 2002; Cunningham 2008; Chapman *et al*. 2008)]; and 334 (± 66) years ago with 95% CIs between 259 (± 55) and 485 (± 102) years ago for the 4% per Myr mutation rate. Based on the uncertainty for mutation rates in trematodes, we also conducted IM over a range of mutation rates (2–4% per Myr) for *C. lingua*; all produced highly similar divergence estimates. We found the mean (± SE) divergence estimates to be: 460 (± 160) years ago with 95% CIs between 306 (± 123) and 690 (± 229) years ago for the 2% per Myr mutation rate; 306 (± 114) years ago with CIs between 204 (± 82) and 460 (± 153) years ago for the 3% per Myr mutation rate; and 230 (± 85) years ago with CIs between 153 (± 61) and 345 (± 115) years ago for the 4% per Myr mutation rate.

Discussion

Our results demonstrate several genetic signatures that strongly support a founder effect in North American *Littorina littorea* and its associated trematode *Cryptocotyle lingua*. First, both the snail and trematode showed significant reductions in overall genetic diversity in North America compared to Europe for both the complete data sets (Figs 2 and 3) and in a truncated data set where we excluded all third position sites, thus presenting a conservative estimate of genetic diversity in each population. In the latter data set, we found all but two North American *L. littorea* haplotypes and all but four *C. lingua* haplotypes to be nested within European haplotypes. Given the significant diversity in Europe, the fact that these two to four haplotypes were not nested within European haplotypes is likely a product of under-sampling in Europe and not North American endemism (see detailed discussion below regarding unshared haplotypes in North America).

Additionally, no clades for either the snail or the trematode were completely monophyletic for North American individuals (Figs 2 and 3). This demonstrates that not a single North American individual sequence or haplotype was completely independent from Europe, suggesting that further sampling should reveal shared status and thus nestedness of all North American genetic diversity within European diversity.

We found some seemingly unique haplotypes in North America (23 unique North American haplotypes in *L. littorea* and 16 in *C. lingua*). These findings are most likely the result of under-sampling (i.e. these unshared haplotypes went undetected in the native range) rather than representing endemism to North America. Our haplotype estimation curves support this interpretation because they predict European diversity in *L. littorea* to be over an order of magnitude greater than North American diversity, requiring significantly more sampling in Europe to reveal all predicted haplotypes (Fig. 4). Thus, the 23 unshared North

American haplotypes are likely among the 2000+ haplotypes that have yet to be discovered in Europe — a pattern consistent for the trematode as well. Due to practical limits on sampling effort, it is not uncommon even in investigations of definitively known species introductions to detect unique haplotypes in the founding populations that are not observed in samples from the source populations (Miura *et al*. 2006; Roman 2006; among others).

Divergence estimates also support a recent founding event for North American *L. littorea* and its associated trematode, *C. lingua*, since all estimates were within the time frame for human colonization of North America from Europe. *L. littorea*'s first reported sighting was in Pictou, Nova Scotia, settled by Europeans in the mid-1600s; however, Vikings are also believed to have visited maritime Canada as far back as ~1000 years ago (Spjeldnaes & Henningsmoen 1963). Even with a range of mutation rates, all of our estimates suggested a divergence between Europe and North America of less than 1000 years ago (when including 95% CI estimates) with mean estimates for the three mutation rates ranging between 334 years and 668 years ago for *L. littorea* and between 230 years and 460 years ago for *C. lingua*. We can have confidence that our greater sampling effort has produced robust divergence estimates for both the snail and trematode given our tight 95% CIs for all estimates [which were on the order of tens to hundreds of years different from the mean estimate as opposed to prior divergence estimates where CIs were sometimes thousands to tens of thousands of years different from the mean (e.g. Wares *et al*. 2002; Chapman *et al*. 2007, 2008; Cunningham 2008)]. Although, our molecular evidence cannot precisely pinpoint whether *L. littorea* arrived with very early (i.e. Vikings) or later (i.e. Pilgrims) Europeans, it is apparent that these divergence estimates are consistent with the time frame and mechanism for human-mediated transport from Europe within the last several centuries.

While glaciation could also result in genetic bottleneck signatures in North America, our evidence argues against a preglacial existence for *L. littorea* in North America. First, our IM divergence estimates between European and North American populations are many thousands of years later than the last glacial maxima (~20 000 years ago). Second, patterns for expansion following glacial refugia typically show low genetic diversity in the latitudes farthest from the source of the population expansion (Marko 2004). Our phylogenetic analysis of North American populations, which treated maritime Canada as a possible glacial refugial region (proposed as an alternative hypothesis to a recent introduction for the snail) compared against US populations found no difference in the amount of genetic diversity at either the regional level or at the site level, even when we compared Canadian sites vs. just the southernmost US sites. This pattern cannot simply be explained as the result of an under-sampling issue in Canada (as we have shown was an issue we accounted for in Europe) because Chao2 haplotype estimates predict only a handful more haplotypes (~12) in Canada compared to the USA. In contrast to this result for *L. littorea*, a Pacific North American marine snail, *Nucella lamellosa*, showed evidence for a northern latitude glacial refugia based on an AMOVA test, which revealed a significant amount of subdivision between northern and southern latitudes in the snail (Marko 2004). Using the same technique, we did not observe significant F_{ST} or F_{CT} values between the Canadian and US subpopulations (F_{ST}: 0.0145; $P = 0.20$; F_{CT}: −0.00038; $P = 0.37$), suggesting little subdivision between northern and southern latitudes for North American *L. littorea*. This lack of subdivision not only argues against a glacial refugia, but it also suggests that the genetic similarity between the US and Canadian populations could be due to *L. littorea*'s pelagic larval dispersal, or it may suggest multiple introductions of the snail – or a combination of both hypotheses. Furthermore, the suggestion that *L. littorea* existed in glacial refugia until changes in the environment allowed the snail to expand its range in the 1800s (Wares *et al*. 2002) is supported neither by historical evidence nor by the experience of other species exhibiting similar range expansions (Chapman *et al*. 2007). Moreover, this scenario would require that the most conspicuous, dominant intertidal snail lay essentially quiescent for at least 10 000 years. Finally, other marine rocky intertidal species, *Semibalanus balanoides* (acorn barnacle) and *Mytilus edulis* (blue mussel), with similar larval dispersing mechanisms to *L. littorea* and believed to have existed in refugia during the last glaciation (Wares & Cunningham 2001), were not confined to maritime Canada following glacial retreat (as was the suggestion for a native North American origin for *L. littorea*). Taken together, these results argue strongly against a glacial refugia theory in maritime Canada for North American *L. littorea*.

Therefore, the most parsimonious conclusion of our results is a recent introduction of *L. littorea* to North America from Europe — this conclusion is not only based upon molecular patterns consistent with a recent founder event in the snail but also due to the convergent patterns we found in *L. littorea*'s associated trematode, *C. lingua*. *L. littorea*'s recent introduction was likely human-mediated due to its close association with human means of transport (e.g. through rock ballast; Carlton 1982), which may have been the mechanism of introduction to the northwest Atlantic for other intertidal species in the 19th century, like *Carcinus maenas* (Roman & Darling 2007) and *Fucus serratus* (Coyer *et al*. 2006), or *L. littorea* could have been intentionally introduced as a food source (Packard 1870; Carlton 1982). Furthermore, the snail's absence from North Atlantic islands, such as Iceland and Greenland, which are believed to have aided in the natural, stepping-stone invasions of several marine intertidal species following the last glaciation

(Johannesson 1988; Ingolfsson 1992) is further evidence that the snail did not move naturally across the North Atlantic. Additionally, our molecular genetic data are consistent with recent ecological patterns in trematode parasite species richness for *L. littorea* (Blakeslee & Byers 2008), which was significantly reduced in North America vs. Europe for *L. littorea* but not for native congeners, *Littorina saxatilis* and *Littorina obtusata*. In this investigation, the trematode species richness displayed in *L. littorea* was consistent with the well-established theory of enemy escape, suggesting a recent invasion from Europe for *L. littorea*, while congeners *L. saxatilis* and *L. obtusata* showed patterns that were instead consistent with an older, natural (likely stepping-stone) invasion from Europe across the North Atlantic into northeast North America.

More generally, our results further highlight the value of parasites to help resolve cryptogenic histories. Our complimentary parasite data set not only demonstrates that the trematode was likely introduced with its snail host, but it was also particularly important for resolving *L. littorea*'s cryptogenic history. This is because without our convergent parasite genetic information, *L. littorea*'s status as native or non-native may have continued to remain equivocal. For example even a study using multiple loci (Wares & Blakeslee 2007) was unable to effectively provide clear evidence for or against a recent introduction (although in this AFLP study, no fixed differences were detected between the two populations, adding some support to a recent invasion for the snail). Overall, our corroborative parasite analysis provided conclusive, convergent data, showing that *C. lingua* possessed the same level of diversity reduction and short divergence estimates as its snail host.

Altogether, our study highlights how genetics, and specifically in the case of our study, parasite genetics, can aid in the resolution of questionable invasion histories. Without the convergent genetic information we provided, *L. littorea*'s cryptogenic status could continue to be debatable. Similarly, other studies have used molecular tools to discern invasion histories when natural history and ecological information have not been sufficient to fully understand a species' presence in a region. For example, global cryptic invasions of the European green crab, *Carcinus maenas*, were distinguished using mitochondrial DNA (Geller *et al*. 1997). Additionally, DNA sequencing tools were used to resolve the questionable invasion history of the nassariid gastropod, *Cyclope neritea*, in the northwest Iberian Peninsula (Couceiro *et al*. 2008). Thus, molecular techniques have been shown to be powerful tools for understanding and elucidating questionable invasion histories — and specifically, our investigation of *L. littorea* was not only able to resolve a specific cryptogenic history, but it also demonstrates the success of the approach we applied here for resolving cryptogenic histories even for those introductions occurring hundreds of years ago.

Acknowledgements

Our thanks to I. Altman, T. Backeljau, M. Blakeslee, D. Brazeau, R. Coleman, L. Collins, L. Curtis, J. Dijkstra, D. Ellisor, S. Fierce, A. Fowler, A. Freeman, G. Goldsmith, J. Grahame, B. Griffen, L. Harris, J. Hey, A. Houghton, R. Houghton, T. Huspeni, K. Johannesson, A. Kintner, T. Kocher, W-J. Lee, T. Maguire, J. Meyer, J. A. T. Morgan, L. Page, R. Poulsen, R. Ramsay, D. Reid, A. Rosenberg, V. Taibe, S. Teck, J. Wares, S. Williams, and we would like to especially thank Jim Carlton for his substantial help and advice. Support was provided by NSF (OCE-0503932), NH Sea Grant, USDA Hatch, CINEMAR, Sloan Foundation History of Marine Animal Populations (HMAP), the American Malacological Society, Sigma Xi, Lerner Gray, UNH Center for Marine Biology, UNH Zoology Department, UNH Graduate School, and the UNH Vice President for Research Discretionary Fund.

References

Berger EM (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*. *Biological Bulletin*, **153**, 255–264.

Bertness M (1984) Habitat and community modification by an introduced herbivorous snail. *Ecology*, **65**, 370–381.

Binney WG (1863) North American conchology previous to year 1860. Part I. American authors. *Smithsonian Miscellaneous Collections*, **5**, 1–650.

Bird JB (1968) *Littorina littorea*: occurrence in a northern Newfoundland beach terrace, predating Norse settlements. *Science*, **159**, 114.

Blakeslee AMH (2007) Native or invasive? The case history of the marine snail, *Littorina littorea*, in northeast North America. In: *Oceans Past: Management Insights from the History of Marine Animal Populations* (eds Starkey D, Holm P, Barnard M), pp. 7–24. Earthscan, London, UK.

Blakeslee AMH, Byers JE (2008) Using parasites to inform ecological history: comparisons among three congeneric marine snails in North America and Europe. *Ecology*, **89**, 1068–1078.

Brenchley G, Carlton J (1983) Competitive displacement of native mud snails by introduced periwinkles in the New England intertidal zone. *Biological Bulletin*, **165**, 543–558.

Byers JE, Blakeslee AMH, Linder E, Cooper A, Maguire T (2008) Controls of spatial variation in the prevalence of trematode parasites infecting a marine snail. *Ecology*, **89**, 439–451.

Carlton J (1982) The historical biogeography of *Littorina littorea* on the Atlantic coast of North America, and implications for the interpretation of the structure of New England intertidal communities. *Malacology Review*, **15**, 146.

Carlton J (1996) Biological invasions and cryptogenic species. *Ecology*, **77**, 1653–1655.

Chapman JW, Carlton JT, Bellinger MR, Blakeslee AMH (2007) Premature refutation of a human-mediated marine species introduction: the case history of the marine snail, *Littorina littorea*, in the Northwestern Atlantic. *Biological Invasions*, **9**, 737–750.

Chapman JW, Blakeslee AMH, Carlton JT, Bellinger MR (2008) Parsimony dictates a human introduction: on the use of genetic (and other) data to distinguish between the natural and human-mediated invasion of the European snail *Littorina littorea*. *North America. Biological Invasions*, **10**, 131–133.

Clarke AH, Erskine JS (1961) Pre-Columbian *Littorina littorea*. *Nova Scotia. Science*, **134**, 393–394.

Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.

Colwell RK (2006) *ESTIMATES, Statistical Estimation of Species Richness and Shared Species from Samples*. Available from http://purl.oclc.org/estimates.

Couceiro L, Míguez A, Ruiz JM, Barreiro R (2008) Introduced status of *Cyclope neritea* (Gastropoda, Nassariidae) in the NW Iberian Peninsula confirmed by mitochondrial sequence data. *Marine Ecology Progress Series*, **354**, 141–146.

Coyer JA, Hoarou G, Skage M, Stam WT, Olsen JL (2006) Origin of *Fucus serratus* (Heterokontophyta: Fucaceae) populations in Iceland and the Faroes: a microsatellite-based assessment. *European Journal of Phycology*, **41**, 235–246.

Criscione CD, Cooper B, Blouin M (2006) Parasite genotypes identify source populations of migratory fish more accurately than fish genotypes. *Ecology*, **87**, 823–826.

Cunningham C (2008) How to use genetic data to distinguish between natural and human-mediated introduction of *Littorina littorea* to North America. *Biological Invasions*, **10**, 1–6.

Foggo A, Rundle SD, Bilton DT (2003) The net result: evaluating species richness extrapolation techniques for littoral pond invertebrates. *Freshwater Biology*, **48**, 1756–1764.

France SC, Rosel PE, Agenbroad JE, Mullineaux LS, Kocher TD (1996) DNA sequence variation of mitochondrial large-subunit rRNA provides support for a two subclass organization of the Anthozoa (Cnidaria). *Molecular Marine Biology and Biotechnology*, **5**, 77–86.

Ganong WF (1886) Is *Littorina littorea* introduced or indigenous? *American Naturalist*, **21**, 931–940.

Geller JB, Walton ED, Grosholz ED, Ruiz GM (1997) Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. *Molecular Ecology*, **6**, 901–906.

Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379–391.

Gould AA (1852) *United States Exploring Expedition* (ed. Sherman C), Philadelphia, Pennsylvania.

Grosberg R, Cunningham CW (2000) Genetic structure in the sea from populations to communities. In: *Marine Community Ecology* (eds Bertness M, Gaines S, Hay M), pp. 61–84. Sinauer Associates, Sunderland, Massachusetts.

Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics*, **167**, 747–760.

Hey J, Nielsen R (2007) Available from http://lifesci.rutgers.edu/~heylab/HeylabSoftware.htm#IM.

Huspeni T (2000) *A molecular genetics analysis of host specificity, continental geography, and recruitment*. PhD Dissertation, University of California, Santa Barbara, California.

Ingolfsson A (1992) The origin of the rocky shore fauna of Iceland and the Canadian Maritimes. *Journal of Biogeography*, **19**, 705–712.

James B (1968) The distribution and keys of species in the family Littorinidae and of their digenean parasites, in the region of Dale, Pembrokeshire. *Field Studies*, **2**, 615–650.

Johannesson K (1988) The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? *Marine Biology*, **99**, 507–513.

Johannesson K (1992) Genetic variability and large scale differentiation in two species of littorinid gastropods with planktotrophic development, *Littorina littorea* (L.) and *Melarhaphe (Littorina) neritoides* (L.) (Prosobranchia: Littorinacea), with notes on a mass occurrence of *M. neritoides* in Sweden. *Biological Journal of the Linnaean Society*, **47**, 285–289.

Kane RA (1999) *NCBI Direct Sequence Submission*. Zoology, The Natural History Museum, London, UK.

Kraeuter J (1976) Offshore currents, larval transport, and establishment of southern populations of *Littorina littorea* Linne along the U. S. Atlantic Coast. *Thalassia Jugoslavia*, **10**, 159–170.

Kyle C, Boulding E (1998) Molecular genetic evidence for parallel evolution in a marine gastropod, *Littorina subrotundata*. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 303–308.

Lubchenco J (1983) *Littorina* and *Fucus*: effects of herbivores, substratum heterogeneity, and plant escapes during succession. *Ecology*, **64**, 1116–1123.

Marko PB (2004) 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. *Molecular Ecology*, **13**, 597–611.

Miura O, Torchin ME, Kuris AM, Hechinger RF, Chiba S (2006) Introduced cryptic species of parasites exhibit different invasion pathways. *Proceedings of the National Academy of Sciences, USA*, **103**, 19818–19823.

Morgan JAT, Blair D (1998) Relative merits of nuclear ribosomal internal transcribed spacers and mitochondrial CO1 and ND1 genes for distinguishing among *Echinostoma* species (Trematoda). *Parasitology*, **116**, 289–297.

Morris SR (1979) *Genetic variation in the genus* Littorina. PhD Thesis, University of Wales, Swansea, UK.

Packard AS (1870) Conchological notes. *American Naturalist*, **4**, 250.

Reid DG (1996) *Littorina littorea*. In: *Systematics and Evolution of Littorina* (ed. Reid D), pp. 95–120. The Ray Society, London.

Reid D, Rumbak E, Thomas R (1996) DNA, morphology and fossils: phylogeny and evolutionary rates of the gastropod genus *Littorina*. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **351**, 877–895.

Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2453–2459.

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution*, **22**, 454–464.

Ruiz G, Fofonoff P, Carlton J, Wonham M, Hines A (2000) Invasion of coastal marine communities in North America: apparent patterns, process, and biases. *Annual Review of Ecology and Systematics*, **31**, 481–531.

Spjeldnaes N, Henningsmoen KE (1963) *Littorina littorea*: an indicator of Norse settlement in North America? *Science*, **141**, 275–276.

Steneck R, Carlton J (2001) Human alterations of marine communities: students beware!. In: *Marine Community Ecology* (eds Bertness M, Gaines S, Hay M), pp. 445–468. Sinauer Associates, Sunderland, Massachusetts.

Swofford DL (2003) *PAUP*, Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4. Sinauer Associates, Sunderland, Massachusetts.

Torchin M, Lafferty K, Dobson A, McKenzie V, Kuris A (2003) Introduced species and their missing parasites. *Nature*, **421**, 628–630.

Verrill AE (1874) Results of dredging. *American Journal of Science*, **7**, 131–138.

Walther BA, Morand S (1998) Comparative performance of species richness estimation methods. *Parasitology*, **116**, 395–405.

Wares JP, Blakeslee AMH (2007) AFLP data provide poor resolution to the *Littorina littorea* puzzle. *Marine Biology Research*, **3**, 168–174.

Wares JP, Cunningham CW (2001) Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution*, **55**, 2455–2469.
Wares JP, Goldwater DS, Kong BY, Cunningham CW (2002) Refuting a controversial case of a human-mediated marine species introduction. *Ecology Letters*, **5**, 577–584.
Williams ST, Reid DG (2004) Speciation and diversity on tropical rocky shores: a global phylogeny of snails of the genus *Echinolittorina*. *Evolution*, **58**, 2227–2251.
Yamada S, Mansour R (1987) Growth inhibition of native *Littorina saxatilis* (Olivi) by introduced *L. littorea* (L.). *Journal of Experimental Marine Biology and Ecology*, **105**, 187–196.

April Blakeslee is interested in understanding marine invasions and their impacts, especially regarding effects on native biodiversity, parasite diversity, and population genetics. James Byers is interested in biological invasions and their impact on native communities; marine parasites and diseases; and ecosystem engineers. Michael Lesser is interested in physiological adaptation and acclimatization of marine organisms to environmental change and works in polar, temperate, and tropical ecosystems.

Appendix I

Littorina littorea haplotype frequency data. Haplotype identities are colored according to their status as unique to Europe (yellow), unique to North America (blue), and shared between populations (white). A fourth category (purple) represents North American haplotypes basal to European haplotypes and therefore considered shared. In Europe, there were 22 sample sites: Moss, NO (MO); Tjarno, SW (TJ); Varberg, SW (VA); Copenhagen, DE (CO); Nyborg, DE (NY); Ubdyhoj, DE (UB); Esbjerg, DE (ES); Ostende, BE (OS); Scheldt Estuary, NE (SC); Mindin, FR (MI); Roscoff, FR (RO); Trouville, FR (TR); Bay d'Arcachon, FR (BD); Vigo, Galicia, ES (GA); St Andrew's, UK (ST); Oban, UK (OB); Cardigan Bay, UK (CA); Plymouth, UK (PK), Robin Hood's Bay, UK (RH); Whitstable, UK (WH); Dublin, IR (DU); and Cork, IR (CK). There were 29 North American sites: Red Bay, Labrador (RB); Blanc Sablon, QC (BL); Flower's Cove, NL (FL); Bonne Bay, NL (BO); Searston, NL (SE); Portugal Cove, NL (PB); North Sydney, NS (NS); Mulgrave, NS (MU); Halifax, NS (HA); Truro, NS (TO); Pictou, NS (PI); St Peter's Harbor, PEI (SP); Pointe-Mitis, QC (PM); Iles de Mingan, QC (ID); Bay du Vin, NB (BV); St John, NB (SJ); Eastport, ME (EA); Acadia, ME (AC); Prout's Neck, ME (PN); Wells, ME (WE); York, ME (YK); Fort Stark, NH (FS); Plymouth, MA (PL); Buzzard's Bay, MA (BB); Sengakontacket Pond, MA (SP); Stonington Point, CT (SN); Crane's Neck, NY (CN); Montauk, NY (MT); and Cape May, NJ (CM). Country/state indicated as: NO, Norway; SW, Switzerland; DE, Denmark; BE, Belgium, NE, Netherlands; FR, France; ES, Spain; UK, United Kingdom; IR, Ireland; QC, Québec; NL, Newfoundland; NS, Nova Scotia; NB, New Brunswick; ME, Maine; NH, New Hampshire; MA, Massachusetts; CT, Connecticut; NY, New York; NJ New Jersey.

Hap	Europe																					North America																												Totals	Freq
	MO	TJ	VA	CO	NY	UB	ES	OS	SC	MI	RO	TR	BD	GA	ST	OB	CA	PK	RH	WH	DU	CK	RB	BL	FL	BO	SE	PC	NS	MU	HA	TO	PI	SP	PM	ID	BV	SJ	EA	AC	PN	WE	YK	FS	PL	BB	SP	SN	CN	MT	CM		
1	1	2	1				1	1	3	2	1	2	2		1	2							1			1				1	2		2		1		1			1	1	1					2	1		35	0,095
2				1								1																																						2	0,005
3						1																																1												2	0,005
4									1																																									1	0,003
5									1																																									1	0,003
6									1																																									2	0,005
7						1								1	1	1		1			1		1	1									1		2	1	4		1		1	1			1	2		2	2	1	27	0,073
8													1																																					1	0,003
9										1																																									1	0,003
10																		1																																	1	0,003
11						1																																													1	0,003
12							1																																												1	0,003
13															1																																				1	0,003
14							1																																												1	0,003
15										1				1																																					2	0,005
16																					1																															1	0,003
17							1																																												1	0,003
18															1																																				1	0,003
19																		1																																	1	0,003
20										1																																									1	0,003
21																														1			1											1			1	1		1	7	0,019
22																	1																																			1	0,003
23				1																																														1	0,003
24														1																																					1	0,003
25																		1																																	1	0,003
26																		1																																	1	0,003
27																																			1																1	0,003
28											1												1	2														1												5	0,014
29													1																																						1	0,003
30																						1														1														2	0,005
31														1																				1															2	0,005
32																				1																												1			2	0,005
33				1																																														1	0,003
34																			1																																1	0,003
35															1																																				3	0,008
36									1																																											1	0,003
37		1																																																	1	0,003
38															1																																				1	0,003
39											1												1																												2	0,005
40																																																		1	0,003
41											1																																								1	0,003
42																					1																															2	0,005
43																					1																															1	0,003
44								1																																											1	0,003
45	1																																																		1	0,003
46																	1																																		1	0,003
47																							1			1					1					1	1	1	2			1		1						10	0,027
48																																																			1	0,003
49																					1																															1	0,003
50																												1				1			2				2		1	1	1		1						9	0,024
51															1																															1						2	0,005
52					1																		1																							1						3	0,008
53											1																																										1	0,003
54																																																			1			2	0,005
55																		1																																		1	0,003
56																					1																															1	0,003
57																					1		1																							1					1	4	0,011
58										1																																										1	0,003
59																								1																												1	0,003
60																																																		1			1	0,003
61												1																																								1	0,003
62																										1																										1	0,003
63															1																																					1	0,003
64														1																																					1	0,003
65														1																																					1	0,003
66																		1																																		1	0,003
67												1																																							1	0,003
68												1																																								1	0,003
69							1																																												1	0,003
70														1																																					1	0,003

Appendix I *Continued*

Hap	Europe																					North America																							Totals	Freq
	MO	TJ	VA	CO	NY	UB	ES	OS	SC	MI	RO	TR	BD	GA	ST	OB	CA	PK	RH	WH	DU	CK	RB	BL	FL	BO	SE	PC	NS	MU	HA	TO	PI	SP	PM	ID	BV	SJ	EA	AC	PN	WE	YK	FS	PL	BB	SP	SN	CN	MT	CM		
89																							1			1		1				3		1					1				1	1	1						1	12	0.032
90																							1				1							1									1				1					5	0.014
91																								1																2						1						4	0.011
92																								1																													1	0.003
93			1																																																	1	0.003
94																						1																															1	0.003
95																1																																					1	0.003
96						1																																														1	0.003
97								1																																											1	0.003
98								1																																												2	0.005
99							1																																														1	0.003
100																			1																																		1	0.003
101												1																1																									2	0.005
102																						1																															1	0.003
103											1															1																											2	0.005
104											1																																										1	0.003
105									1																																											1	0.003
106						1																						1																									3	0.008
107																																	1																				1	0.003
108																									1																											1	0.003
109																									1	1																									1			3	0.008
110													1																																								1	0.003
111				1										1						1		1	1								1	1		1			1	1	1		3		1	1	1	1				1	21	0.057
112		1																																																		1	0.003
113													1																																							1	0.003
114											1																																									1	0.003
115																					1																															1	0.003
116																																		1																		1	0.003
117																																																				1	0.003
118																									1		1																									2	0.005
119												1																1																									2	0.005
120	1																				1																															2	0.005
121																1																																					1	0.003
122		1																																																		1	0.003
123												1																																									1	0.003
124																1																																					1	0.003
125																						1																															1	0.003
126																						1																															1	0.003
127																1																																					1	0.003
128		1																					1		1											1															4	0.011
129								1																																												1	2	0.005
130								1																																												1	0.003
131						1																																															1	0.003
132								1									1																																			2	0.005
133					1																																															1	0.003
134																		1																																			1	0.003
135																																																					1	0.003
136	1																																																				1	0.003
137	4	2		3	3	1						1																																									14	0.038
138	1																																																				1	0.003
139							1																																														1	0.003
140					1																																																1	0.003
141		1																																																			1	0.003
142	1																																																				1	0.003
143				1																																																1	0.003
144			1																																																	1	0.003
145							1																																														1	0.003
146						1																																															1	0.003
147	1																																																				1	0.003
148											1																																										1	0.003
149									1																																												1	0.003
150					1																																																1	0.003
151												1																																									1	0.003
152					1																																																1	0.003
153										1																																											1	0.003
154																		1														1																					2	0.005
155								1																																													1	0.003
156											1																																										1	0.003
157							1																																														1	0.003
158								1																																													1	0.003
159																1																																					1	0.003
160								1																																													1	0.003
161													1																																								1	0.003
162										1																																											1	0.003
163		1																																																		1	0.003
164																1																																					1	0.003
165													1																																								1	0.003
166										1																													2														3	0.008
167											1																																										1	0.003
168														1																																							1	0.003
169											1																																										1	0.003
170										1																																											1	0.003
171					1																																																1	0.003
172																					1																															1	0.003
173																																																					1	0.003
174																1																																					1	0.003
175													1																																								1	0.003
N	10	9	4	8	10	8	9	10	10	10	16	9	10	11	6	14	3	9	3	2	10	6	10	9	5	9	4	6	2	1	6	6	6	8	8	4	4	7	7	8	2	9	10	4	6	6	7	6	10	7	6	370	

Appendix II

Cryptocotyle lingua haplotype occurrence data by region and sample site. Haplotype identities are colour coded according to their status as unique to Europe (yellow), unique to North America (blue), and shared between populations (white). A fourth category (purple) represents North American haplotypes basal to European haplotypes and therefore considered shared. In Europe, there were 16 sample sites: Moss, NO (CLMO); Tjarno, SW (CLTJ); Varberg, SW (CLVA); Nyborg, DE (CLNY); Ubdyhoj, DE (CLUB); Esbjerg, DE (CLES); Copenhagen, DE (CLCO); Scheldt Estuary, NE (CLSC); Trouville, FR (CLTR); Mindin, FR (CLMI); St Andrew's, UK (CLST); Oban, UK (CLOB); Largs, Scotland (CLLR); Plymouth, UK (CLPK); Cork, IR (CLCK); and Dublin, IR (CLDU). There were 20 North American sample sites: Red Bay, Labrador (CLRB); Blanc Sablon, QC (CLBL); Flower's Cove, NL (CLFL); Bonne Bay, NL (CLBO); Searston, NL (CLSE); North Sydney, NS (CLNS); Mulgrave, NS (CLMU); Marie Joseph Park, NS (CLMJ); Halifax, NS (CLHA); Bay du Vin, NB (CLBV); Prout's Neck, ME (CLPN); Wells, ME (CLWE); York, ME (CLYK); Kittery, NH (CLKI); Fort Stark, NH (CLFS); Larus Ledge, Appledore Island, ME (CLLA); Gloucester, MA (CLGL); Plymouth, MA (CLPL); and Point Judith, RI (CLPJ). Country/state codes in addition to RI, Rhode Island, are as in Appendix I.

Hap	Europe																						North America																													Totals	Freq
	MO	TJ	VA	CO	NY	UB	ES	OS	SC	MI	RO	TR	BD	GA	ST	OB	CA	PK	RH	WH	DU	CK	RB	BL	FL	BO	SE	PC	NS	MU	HA	TO	PI	SP	PM	ID	BV	SJ	EA	AC	PN	WE	YK	FS	PL	BB	SP	SN	CN	MT	CM		
89																							1			1		1				3		1					1				1	1	1						1	12	0.032
90																							1				1							1									1				1					5	0.014
91																								1																2						1						4	0.011
92																								1																												1	0.003
93			1																																																	1	0.003
94																						1																														1	0.003
95																1																																				1	0.003
96						1																																														1	0.003
97								1																																												1	0.003
98								1																																										1		2	0.005
99							1																																													1	0.003
100																			1																																	1	0.003
101												1															1																								2	0.005
102																						1																														1	0.003
103											1															1																									2	0.005
104											1																																									1	0.003
105									1																																											1	0.003
106						1																					1																						1		3	0.008
107																																1																			1	0.003
108																									1																										1	0.003
109																									1	1																					1				3	0.008
110													1																																						1	0.003
111				1										1					1		1		1	1							1	1		1			1	1	1			3		1	1	1	1	1			1	21	0.057
112		1																																																		1	0.003
113													1																																						1	0.003
114											1																																									1	0.003
115																					1																															1	0.003
116																																	1																	1	0.003
117																																											1						1	0.003
118																									1		1																								2	0.005
119												1															1																								2	0.005
120	1																				1																															2	0.005
121																1																																				1	0.003
122		1																																																		1	0.003
123												1																																								1	0.003
124																1																																				1	0.003
125																						1																														1	0.003
126																						1																														1	0.003
127																1																																				1	0.003
128		1																					1		1											1																4	0.011
129								1																																											1	2	0.005
130								1																																												1	0.003
131						1																																														1	0.003
132								1									1																																			2	0.005
133					1																																															1	0.003
134																		1																																		1	0.003
135																																														1				1	0.003
136	1																																																		1	0.003
137	4	2		3	3	1						1																																							14	0.038
138				1																																																1	0.003
139							1																																													1	0.003
140					1																																															1	0.003
141		1																																																		1	0.003
142	1																																																		1	0.003
143				1																																																1	0.003
144			1																																																	1	0.003
145							1																																													1	0.003
146						1																																														1	0.003
147	1																																																		1	0.003
148											1																																									1	0.003
149									1																																											1	0.003
150					1																																															1	0.003
151												1																																								1	0.003
152					1																																															1	0.003
153										1																																										1	0.003
154																		1												1																					2	0.005
155								1																																												1	0.003
156											1																																									1	0.003
157							1																																													1	0.003
158								1																																												1	0.003
159																1																																				1	0.003
160								1																																												1	0.003
161													1																																						1	0.003
162										1																																										1	0.003
163		1																																																		1	0.003
164																1																																				1	0.003
165													1																																						1	0.003
166										1																								2																		3	0.008
167											1																																									1	0.003
168														1																																					1	0.003
169											1																																									1	0.003
170										1																																										1	0.003
171					1																																															1	0.003
172																				1																																1	0.003
173																																																1			1	0.003
174																1																																				1	0.003
175													1																																						1	0.003
N	10	9	4	8	10	8	9	10	10	10	16	9	10	11	6	14	3	9	3	2	10	6	10	9	5	9	4	6	2	1	6	6	6	8	8	4	4	7	7	8	2	9	10	4	6	6	7	6	10	7	6	370	