

How to Use Genetic Data to Distinguish Between Natural and Human-Mediated Introduction of *Littorina littorea* to North America

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Received: 26 August 2005 / Accepted: 22 September 2005 / Published online: 17 August 2007
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Abstract The rapid range southward expansion of the periwinkle *Littorina littorea* from the Canadian maritimes has fueled a long-running debate over whether this species was introduced to North America by human activity. A reappraisal of the mitochondrial DNA sequence evidence finds considerable endemic allelic diversity in the American population. The degree of endemic genetic diversity is higher than expected from human-mediated colonization, but not so much to suggest that it survived the last glacial maximum in America. Coalescent estimates of population divergence agree that colonization of America preceded European contact. A reappraisal of the ITS nuclear sequence data finds extensive recombination. Taking this recombination into account strengthens the genetic case against human-mediated introduction. Finally, a reappraisal of conflicting allozyme studies from the 1970's supports a claim of limited divergence between American and European populations. This is consistent with post-glacial colonization, but the allozyme data cannot distinguish between natural or human-mediated colonization. Taken as a whole, the DNA sequence data supports the many sub-fossil reports of an American

L. littorea population in the Canadian maritimes that preceded even the first visits by the Vikings.

Keywords Haplotypes diversity · Invasive Species · ITS · Mitochondrial DNA

Introduction

The debate over whether the periwinkle *Littorina littorea* was introduced by European settlement to North America has raged for over 150 years (Ganong 1886, 1887; Clarke 1963, 1971, Clarke & Erskine 1961; Bird 1968; Berger 1977; Vermeij 1982, Reid 1996, Wares *et al.* 2002). All sides agree that prior to the mid-19th century, all records of this gastropod were in the Canadian maritimes. Since 1870, the range of *L. littorea* has expanded dramatically as far south as Delaware, becoming the most common intertidal gastropod in the New England region.

The crux of the argument has been whether the Canadian population of *L. littorea* first noted in 1841 was introduced by humans, or was brought over by natural means before the first European exploration. The argument for human-mediated introduction is based on the very fact of *L. littorea*'s dramatic range expansion. If it was here all along, why did it wait until the 19th century to expand its range to the south?

By the 1970's, there were multiple lines of evidence that *L. littorea* was present throughout the Canadian maritimes before European contact.

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L. littorea was found in both New Brunswick and Nova Scotian Micmac shell middens estimated to be >13 kya (Clarke and Erskine 1961, Clarke 1971, Reid 1996). A harder theory to dismiss held that pre-Columbian Viking ships brought *L. littorea* to North America. In a devastating plot-twist, Bird (1968) found *L. littorea* shells in undisturbed post-glacial deposits several feet *underneath* the foundations of Viking dwellings in Newfoundland. Moreover, *L. littorea* has been absent from *both* Greenland and Iceland – the most likely embarkation points for Viking expeditions – for at least 100 kya (Leifsdóttir and Símonarson, 2002).

In the 1970's, two allozyme studies came to drastically different conclusions when comparing American and European populations of *L. littorea*. While Berger (1977) found entirely different alleles at 5 polymorphic loci between Cape Cod and Roscoff, Morris (1979) found a Maine population to be nearly identical to several populations in Wales. Unfortunately, Morris never published his work, leaving his work to be cited anecdotally in the literature.

Although the 1980's brought no new empirical data, Carlton's (1992) review of molluscan invasions asserted that *L. littorea* was introduced by human-mediated activity. While he admits that *L. littorea* was "...prehistorically present in the northwestern Atlantic [it] was returned to North America before 1840 either intentionally (released by European settlers in eastern Canada to establish a periwinkle fishery) or accidentally (with ballast stones)." (Carlton 1992).

During the late 20th century, *L. littorea* became one of the most frequently cited examples of a human introduction (Bertness 1999). If Carlton (1992) is correct, the *L. littorea* populations that left their mark in Micmac middens and deep below Viking dwellings must have undergone extinction and human-mediated recolonization in few hundred 500 years. As we noted, this kind seemingly implausible theory of geologically instantaneous extinction-recolonization would have dramatic implications for ecological theory and beyond.

In 2002, I was senior author of the first empirical study in a quarter century bearing on *L. littorea*'s widely perceived status as an introduced species (Wares et al. 2002). In this volume, Chapman *et al* (2007) criticize (sometimes rightly) the methodology

we used in our study. At the same time, they seem to move away from Carlton's (1992) hypothesis of very recent extinction-recolonization, but resurrect the old idea that Vikings were responsible for the original introduction event.

Before discussing the *L. littorea* data, I will illustrate some of the genetic patterns found in other New England marine invertebrates. These examples illustrate the strengths and weaknesses of genetic approaches to the ages of recently diverged populations.

Distinguishing between natural colonization and human-mediated introduction

The great strength of comparative phylogeography comes from the ability to identify similar genetic patterns in species from the same biota. Several examples of indigenous American species whose American populations appear to have been founded from Europe include *L. littorea*'s congener *Littorina obtusata*, the snail *Nucella lapillus*, the starfish *Asterias rubens*, and the barnacle *Semibalanus balanoides* (Wares and Cunningham 2001). In each case, the COI mitochondrial DNA sequence data show American haplotypes nested within European haplotypes, with American effective population sizes significantly smaller than Europe (Wares and Cunningham, 2001). In each species there are shared haplotypes between North American and Europe, as expected from relatively recent colonization events.

The American populations of both snails — *L. obtusata* and *N. lapillus* — show genetic patterns consistent with colonization *after* the last glacial maximum ≈ 20 kya, but *before* contact with European civilization. As expected from very recent colonization, most American haplotypes are also found in Europe. Also as expected from the rapid rates of mitochondrial substitution, a few endemic American haplotypes have arisen in the past 20 kya, all descended from founding haplotypes from Europe (3 in *L. obtusata*, and 2 in *N. lapillus*; Wares and Cunningham, 2001). The American population of the indigenous starfish *Asterias rubens* also shows American haplotypes shared with Europe. Unlike the snails however, *A. rubens* had *no* new endemic American haplotypes. While this may reflect inadequate sampling in North America, the lack of American endemic haplotypes in

A. rubens is precisely the pattern expected from human-mediated introduction.

The American population of the barnacle *S. balanoides* also shows American haplotypes deeply nested within European populations. Unlike the previous three species, there are many endemic American haplotypes – an even dozen – with only 2 shared with Europe (Wares and Cunningham, 2001). This is many more new haplotypes than we expect to arise in only 20 kya, suggesting that the American population of *S. balanoides* arrived before the last glacial maximum (Wares and Cunningham, 2001).

Three points from this discussion are relevant to the *L. littorea* data.

- Many taxa in the New England/Canadian maritimes region were founded by “natural” colonization from Europe; after the last glaciation maximum ≈ 20 kya, but *before* the first human expeditions from Europe.
- Immediately after a colonization event, all haplotypes are shared with the founder population
- Each of the founding haplotypes can come from anywhere in the genealogy of the founding population. This means that founding haplotypes are not necessarily closely related to one another. This confounds some measures of effective population size (but not the measure of haplotype diversity). It also presents a challenge to methods measuring the amount of population divergence.
- Given time, mutation will increase the number of endemic haplotypes in the founder population.

The mitochondrial evidence

In a sample of 57 American individuals from Newfoundland to Cape Cod, Wares et al (2002) American population of *L. littorea* found 11 mitochondrial haplotypes, 5 of which are shared with European populations, and 6 endemic to North America. As expected if the European population was much older, 60 individuals collected from Norway to France had considerably more genetic diversity, with 37 haplotypes. As with *Semibalanus balanoides* (see above), the founding American haplotypes come from different parts of the genealogy of the founding population (Wares and Cunningham 2001). This can present a challenge to estimates of population divergence, as will be shown below.

On the one hand, a majority of endemic American haplotypes is expected from a relatively old American population. On the other, there is so much genetic diversity in Europe that the apparently “American” haplotypes may have simply been missed in our small European sample. Both Wares et al. (2002) and Chapman et al. (2007) recognized this possibility, but it can only be resolved by more extensive sampling. Further sampling by our laboratory (11 American and 4 European sequences) has revealed one more endemic American haplotype, but more European sampling is clearly necessary.

Comparing coalescent methods for estimating the timing of population divergence

The MDIV program was the first coalescent method allowing developments in simultaneous estimates of migration and the timing of population divergence (Nielsen and Wakeley 2001). This method found evidence of migration between Europe and North America *L. littorea*, but found that European and American populations diverged before the possibility of human-mediated contact. A non-coalescent method that assumes no migration gave similar ages of divergence between American and European *L. littorea* (Table 1, also see Wares et al. 2002).

Chapman et al. (2007) correctly note that *L. littorea* violates MDIV’s assumption of equal population sizes, which has the potential to inflate estimates of population divergence. They reanalyze our data using a new coalescent method (IM: Nielsen and Hey 2004) and find estimates of population divergence that are orders of magnitude lower than MDIV (Table 1).

While IM does allow the consideration of unequal population sizes, this is not the only difference between IM and MDIV, IM finds much older divergences between American and European populations than MDIV, even when IM is set to emulate MDIV’s assumptions of equal population size and migration (Table 1).

The nuclear evidence: allozymes

With the mitochondrial evidence in dispute, it is critical to resolve the old conflict between the allozyme studies of Berger (1977) and the

Table 1 Comparing mtDNA estimates of divergence between populations of *L. littorea* from all 3 codon positions in 991 bp of combined COI (424 bp) and Cytb (527 bp).

	Estimated Age (kya)	Min. Age (95%) (kya)
<i>America vs. Europe</i>		
Nei/Li ^a	23	11
MDIV ^b (=M and = θ)	30	22
IM ^c (=M and = θ)	133	129
IM ^c (\neq M and \neq θ)	55	16

Results are the same when 11 new sequences are added (11 American and 4 European). The assumed rate of evolution for all 3 positions is 1.5×10^{-8} (1.5×10^{-5} for the whole). This estimate is based Atlantic/Pacific comparisons of *Littorina* as described in Wares and Cunningham (2001)

^a Using JC distances in DNAsp (Rozas and Rozas 1999)

^b Using the HKY model in MDIV (Nielsen and Wakeley 2001). MDIV always assumes equal θ and M, 10 million chains with multiple runs, max age

^c Using the HKY model in IM (Nielsen and Hey 2004) with runs ranging from 10 to 100 million chains. These results are based on a version of IM (built on 7/30/06) that fixed Major bug in the HKY model reported by Hey in 11/7/05. Conservative priors were set: Q=50 m=50 f=2. To emulate MDIV, M and θ were set to a single value by invoking the option -j45)

unpublished thesis of Morris (1979). With the kind assistance of R. Hughes, I obtained copies of the relevant tables in Morris (1979).

Fortunately, the two studies analyzed 5 of the same loci, including 3 loci that Berger (1977) reported to share no alleles in common (PGI, PGM1, AP3). For all 3 of these loci, the American populations sampled by Berger (1977) and Morris (1979) had almost exactly the relative allele frequencies, as did the Welsh populations sampled by Morris (1979). This comparison strongly suggests that something was amiss in Berger's (1977) allozyme study from France.

The nuclear data: ITS sequences

Wares et al (2002) analyzed sequences from the nuclear internal-transcribed spacer region. We used MDIV to analyze a small sample of ITS data, with 12 American and 19 European alleles. This analysis found ages consistent with the mtDNA data, but this difference was not significant. My reanalysis of the

ITS data found rampant evidence of recombination, violating the assumptions of both MDIV and IM.

In cases of recombination, different blocks of sequence have experienced distinct histories. These individual blocks can be analyzed by IM if they are treated as distinct loci (Nielsen and Hey 2004). Using SITES program (Wakeley and Hey 1996) I identified between 6 and 8 recombining blocks in the ITS data. Under a variety of analyzes and assumptions, all the 6 and 8 block analyses found minimum ages of divergence significantly greater than 20 kya (results not shown).

Discussion

In response to the critique by Chapman et al. (2007), I have reassessed each of the three lines of genetic evidence that Wares et al. (2002) used to argue against human-mediated introduction of *L. littorea* to North America: mtDNA sequences, nuclear DNA sequences, and the old allozyme study of Berger (1977). Of these, I found the allozyme data to be the simplest to dismiss. Comparison with the unpublished thesis of Morris (1979) clearly shows that Berger's report of multiple fixed differences between North American and European *L. littorea* was simply wrong. In contrast my reanalysis of the ITS data – taking recombination into account – actually strengthens the case for an older divergence between American and analysis of the nuclear ITS DNA sequences.

The evidence from the mitochondrial DNA is less conclusive than we believed in 2002. Using an earlier, flawed version of IM, Chapman et al. (2007) found divergences between Europe and America on the order of hundreds of years. Hey (pers. com.) has since fixed a bug in the HKY version of IM, and new estimates of divergence rule out post-European contact colonization of America (Table 1). On the other hand, the minimum estimates do not rule out natural colonization of America after the last glacial maximum (but before European contact: Table 1).

Future Directions for Genetic Research

Taken as a whole, the genetic data suggest an American population no older than the last glacial maximum 20 kya. While I once proposed that *L. littorea* may have survived through the last ice age in a Canadian refugium (Wares et al. 2002), my rejection of Berger's

(1977) report of many fixed allozyme differences makes this increasingly unlikely.

Further research must focus on genetic markers that have a large degree of genetic variation. While the ITS data is sufficiently variable, it has many undesirable qualities, such as concerted evolution, that make it difficult to interpret according to population genetic theory. Although single-copy nuclear loci should be investigated, they may not a sufficiently high mutation rate for new alleles to arise in only 20 kya.

This leads us back to greater sampling of the mtDNA. More geographical sampling in Europe is clearly indicated, although no amount of sampling can ever *prove* the negative observation of the absence of American haplotypes in Europe. Longer mtDNA sequences may reveal differences between some of the 5 “shared” haplotypes between America and Europe. Conversely, if extremely long (such as whole mtDNA sequences) fail to show differences in any of the shared haplotypes, the probability of an extremely recent, human-mediated introduction increases.

Parsimony Weighs Against Human-Mediated Introduction of L. littorea

Chapman et al (2007) conclude that human-mediated introduction of *L. littorea* is the most parsimonious hypothesis. While I have reassessed the genetic evidence – and even strengthened Chapman et al’s case by discrediting Beger’s (1977) allozyme data – parsimony clearly weighs against human-mediated introduction of *L. littorea*.

First and foremost, *all* reports of *L. littorea* prior to 1840 are in the Canadian maritimes (including the only American fossil *L. littorea*: Wagner, 1977). While Chapman et al. (2007) continue to suggest the possibility of introduction by Vikings, the shells beneath Viking dwellings rules this out.

If all pre-Columbian *L. littorea* are in the Canadian maritimes, and if the first living collections are also in the Canadian maritimes, it is simplest conclude that these populations are genealogically continuous. In the end, those who support human introduction rely on a single argument – how else to explain the rapid southward spread of *L. littorea* since the mid-1800s? This is indeed difficult to explain (but not impossible, see Clarke, 1971 and Reid 1996). The alternative of

extinction of American *L. littorea* since 1300, followed by reintroduction by humans pre-1840 (Brenchley and Carlton 1983), is also hard to believe, and is certainly not parsimonious.

The existing genetic data shows considerable endemic genetic diversity in American *L. littorea*, consistent with a hypothesis of genealogical continuity with the sub-fossil samples of *L. littorea* throughout the Canadian maritimes. Pending the collection of more genetic data, the evidence continues to support the hypothesis that extant American populations of *L. littorea* were established long before European exploration.

References

- Brenchley GA, Carlton JT (1983). Competitive displacement of native mud snails by introduced periwinkles in the New England intertidal zone: *Bio. Bull.* 165:543–558
- 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
- Bird JB (1968) *Littorina littorea*: occurrence in a northern Newfoundland beach terrace, predating Norse settlements. *Science* 159:114
- Carlton JT (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. *Malacological Review* 15:146
- Carlton JT (1992) Introduced marine and estuarine mollusks of North America: an end-of-the- 20th-century perspective. *Journal of Shellfish Research* 11:489–505
- Chapman JW, JT Carlton, MR Bellinger, AMH Blakeslee (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*
- Clarke AH (1963) *Littorina littorea* as an indicator of Norse settlements. *Science* 142:1022
- Clarke AH (1971) *Littorina littorea*, native or introduced. *The Biologist* 53:160–162
- Clarke AH, Erskine JS (1961) Pre-Columbian *Littorina littorea* in Nova Scotia, *Science* 134:393
- Ganong WF (1886) Is *Littorina littorea* introduced or indigenous. *American Naturalist* 20:931–940
- 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
- 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* 47:285–299
- Leifsdóttir OE, Símonarson LA (2002) The mesogastropod *Littorina littorea* (Linné, 1758) in Iceland: palaeobiogeography and migration. *Cainozoic Research* 1:2–12
- Morris SR (1979) Genetic variation in the genus *Littorina*. Ph.D., University College of Swansea, Wales, UK
- Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences* 76:5269–5273
- Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics* 158:885
- Reid DG (1996) Systematics and evolution of *Littorina*. The Ray Society, The Dorset Press, Dorchester, Dorset, England, pp 463
- Rozas J., Rozas R (1999) DnaSP, Version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* 15:174–175
- Vermeij GJ (1982) Environmental change and the evolutionary history of the periwinkle *Littorina littorea* in North America. *Evolution* 36:561–580
- Wares JP, Goldwater S, Kong BY, Cunningham CW (2002) Refuting a controversial case of a human-mediated marine species introduction. *Ecology Letters* 5:577–884
- Wagner FJE (1977) Palaeoecology of marine Pleistocene Mollusca, Nova Scotia. *Canadian Journal of Earth Science* 14:1305
- Wares JP, Cunningham CW (2001) Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution* 55:2455–2469