Supporting Information

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SI Methods

Shipping Records. Records were collected from the following primary sources covering the period 1773 to 1861: (*i*) Impost & Excise Documents (Pictou records survive from 1814, CBI records from 1823) and Collector of Colonial Duties' Documents in the Public Archives of Nova Scotia (1) and (*ii*) weekly "Shipping Intelligence" columns in period newspapers: *The Nova Scotian* (1824–1827, 1834–1837), *The Colonial Patriot* (12/7/27–1833), *The Pictou Observer* (1838–1840), *The Mechanic and Farmer* (1842–1843) and *The Eastern Chronicle* (October 4, 1843–1861). The primary sources were also compared with earlier compilations of ships carrying emigrants to Pictou and Cape Breton Island (CBI) in several secondary sources (1–4). Records from 1773–1815 rely on genealogical information because Pictou Customs' records do not exist prior to 1814 in the Public Archives of Nova Scotia (PANS, Halifax), and *The Nova Scotian* begins in 1824. The data reported for 1773–1815 underestimate both Scottish and English trading ships during at least 1800~1808, based upon contemporary reports (i.e., refs 5 and 6). We spot-checked some of the genealogical records for accuracy at the National Archives of Scotland (NAS, Table S3). *The Galway Vindicator* (Ireland) was checked from 1841–1854 to confirm the absence of any ships sailing from Galway for Pictou and to determine Galway shipping patterns.

Shipping records for Pictou (e.g., "Shipping Intelligence") often give the explicit origin (Great Britain or North America) for places that have identical names in the 2 regions (e.g., Belfast, PEI vs Belfast (Maine), USA vs Belfast, United Kingdom; Liverpool, GB vs Liverpool, NS; Bristol, GB vs Bristol (Maine), USA). In cases where this was not given, whether a ship came to Pictou from a North American or European port was determined by a combination of other data including: (*i*) length of voyage (a few days vs. 3–7 weeks), (*ii*) type of cargo (i.e., matching that of ships from the European location vs. the North American location), and (*iii*) a ship that other data showed to be a ship that regularly sailed between the European location and Pictou (i.e., same captain, etc.) when one record for that ship did not explicitly list "GB" or "NS". In a small number of cases, a ship is believed, without certainty, to have originated from the European port, and is marked "?" when listed in Table S3.

Population Sampling and Genetic Analysis of *Fucus serratus*. Three Nova Scotian populations of *F. serratus* [Pictou, Inverness (CBI), Caplin Cove (CBI)] were compared with European populations studied by Coyer et al. (7) and new sites (Table S6) in Scotland and Ireland [Scotland: Peterhead (adjacent to Aberdeen), Cromarty, Ullapool/Loch Broom, Fort William, Greenock; Ireland: Sligo, Galway]. DNA was extracted from silica-stored samples, amplified, and genotyped with 7 microsatellite primers following previous protocols (7). Standard population genetic statistics were estimated and tested by using the GENETIX 4.02 program and mean number of alleles$^{-\text{locus}}$ was normalized to a sample size of 30 (equal to the smallest sample at Pictou, Caplin Cove, Greenock) by using the GENCLONE 1.0 (β version) (8) program and a resampling of 1,000. Pairwise distances and the NJ tree were calculated by using the program GENETIX 4.02 [Belkir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996-2004) Genetix 4.05. http://genetix.univ-montp2.fr/genetix/. Accessed April 20, 2009] and PHYLIP 3.5 [Felsenstein J (1994) PHYLIP (Phylogeny inference package) (available at http://evolution.genetics.washington.edu/phylip.html). Assignment tests were performed by using GENECLASS2 (9). Assignment scores reflect the relative strength of the log-likelihood value associated with a given reference population. Because we were interested in a historical introduction, as opposed to detection of first-generation migrants, we used a group (rather than individual) assignment, thereby integrating the various multilocus genotypes and reducing the bias associated with an unknown time of introduction. Results equivalent to the Bayesian analysis were obtained with an allelic frequency method and a distance-based method (10, 11).

Population Sampling and Genetic Analysis of *Littorina littorea*. Snails were collected (Table S6) in Pictou (2005, $n = 30$), Scotland (2006, same new sites listed for *F. serratus*, $n = 84$) and Ireland (2008; Sligo, Galway, Limerick; $n = 28$) and preserved in 95% ethanol. DNA extractions and genetic analyses followed published protocols (12) for cytochrome *b* amplification and sequencing. A total of 30 European locations were compared with our Pictou and other Nova Scotian sites (Dataset S1, using additional data from ref. 12, GenBank accession nos. EU875593–EU875963; haplotypes deposited from this study with GenBank accession nos. FJ750983–FJ751157). Because of the extremely high diversity of haplotypes found throughout the species' range, only shared haplotypes between Pictou/North America and the European source region were compared in our haplotype probability analysis, and sample sizes were adjusted using 2 Monte Carlo sample standardization procedures. First, the lowest individual sampling value (LISV) of shared haplotypes in a European region was used to calculate standardized shared haplotypes between European regions and (*i*) Pictou (LISV = 35) or (*ii*) all of Nova Scotia (LISV = 36). Second, we calculated the expected total number of shared haplotypes between a European region and (*i*) Pictou or (*ii*) Nova Scotia using the Chao2 estimator (13), which not only standardizes for unequal sample size but also accounts for the relative difficulty in detecting shared haplotypes amid the total diversity of a region. Chao2 analysis accomplishes this latter feature by estimating the total expected number of haplotypes that could be shared between a particular European region and Pictou or Nova Scotia that may have been missed in our sampling of the populations (12, 13).

Estimating the Time of the Introductions. The distribution of *F. serratus* in North America was updated by surveying the Canadian shore (Québec south from the Gaspé Peninsula, New Brunswick, Nova Scotia, and Prince Edward Island) between 2005 and 2007 at ≈75-km intervals, with 0.5-km resolution near borders of *F. serratus* populations. Expansion rates were based on the 2007 distribution in comparison with historical records of secondary introductions considered to originate from the primary introduction (e.g., ref. 14).

Expansion rates for *L. littorea* cannot be estimated directly. Instead, population divergence estimates for Nova Scotian *L. littorea* were compared to 30 European populations by using the Isolation with Migration (IMa) program (April 21, 2008 version; see ref. 15, available from http://lifesci.rutgers.edu/~heylab/HeylabSoftware.htm; accessed April 20, 2009) with 10 replicate runs/treatment.

1. Campey LH (2004) *After the Hector. The Scottish Pioneers of Nova Scotia and Cape Breton 1773–1852* (Natural Heritage Books, Toronto).
2. Martell JS (1942) *Immigration to and Emigration from Nova Scotia 1815–1838* (The Public Archives of Nova Scotia, Halifax).
3. Johnston AA (1960) *A History of the Catholic Church in Eastern Nova Scotia* (St. Francis Xavier Univ Press, Antigonish, Nova Scotia) Vol 1, 1611–1827.
4. Dobson D (1998) *Ships from Scotland to America, 1628–1828* (Genealogical Publishing Co, Baltimore).
5. White PCT, ed (1969) *Lord Selkirk's Diary, 1803–1804* (Greenwood Press, New York).
6. Johnson RS (1986) *Forests of Nova Scotia* (De of Lands and Forests, Halifax, Nova Scotia), p 64).
7. Coyer JA, Peters AF, Stam WT, Olsen JL (2003) Post-Ice Age recolonization and differentiation of *Fucus serratus* L. (Fucaceae: Phaeophyta) populations in Northern Europe. *Mol Ecol* 12:1817–1829.
8. Arnaud-Haond S, Belkhir K (2007) Genclone: A computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Mol Ecol Notes* 7:15–17.
9. Piry S, et al. (2004) GeneClass2: A software for genetic assignment and first-generation migrant detection. *J Heredity* 95:536–539.
10. Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Mol Ecol* 4:347–354.
11. Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: Models and estimation procedures. *Am J Hum Genet* 19:233–257.
12. Blakeslee AMH, Byers JE, Lesser MP (2008) Solving cryptogenic histories using host and parasite molecular genetics: The resolution of *Littorina littorea*'s North American origin. *Mol Ecol* 17:3684–3696.
13. Colwell RK (2005) Statistical estimation of species richness and shared species from samples. v 7.5. Available at http://purl.oclc.org/estimates (accessed April 20, 2009).
14. Edelstein T, Greenwell M, Bird CJ, McLachlan J (1971) -73) Investigations of the marine algae of Nova Scotia. X. Distribution of *Fucus serratus* L. and some other species of *Fucus* in the Maritime Provinces. *Proc N S Inst Sci* 27:33–42.
15. Hey J, Nielsen R (2007) Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proc Natl Acad Sci USA* 104:2785–2790.

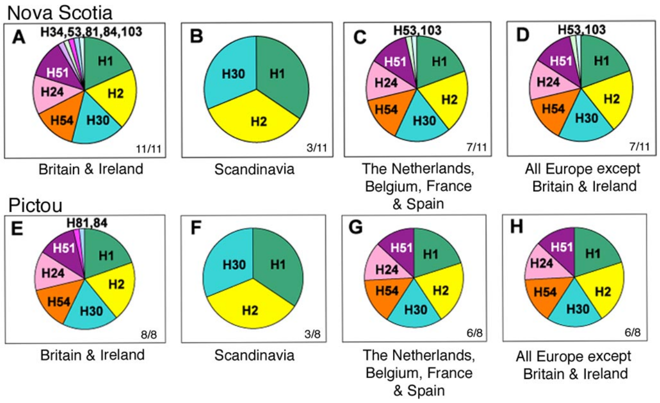

Fig. S1. Pie charts showing proportional frequencies of shared cytochrome *b* haplotypes in *Littorina littorea* in Nova Scotia (*A*–*D*) or Pictou (*E*–*H*) that are shared with various regions of Europe. Detailed data for each shared haplotype (e.g., H51) are given in Dataset S1. The number of shared haplotypes for each regional comparison from a total of 11 shared haplotypes (Europe and Nova Scotia) or 8 shared haplotypes (Pictou and Europe) is given in each box at the lower right. Britain/Ireland (England, Ireland, Scotland, Wales); Scandinavia (Denmark, Norway, Sweden); midcontinental Europe (Belgium, France, Spain, The Netherlands).

Other Supporting Information Files

Dataset S1 (PDF)
Dataset S2 (PDF)
Table S1 (PDF)
Table S2 (PDF)
Table S3 (PDF)
Table S4 (PDF)
Table S5 (PDF)
Table S6 (PDF)