Population Genetics of the Hard Clam, *Mercenaria mercenaria*, at the Northern Limit of Its Range[1]

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Canadian populations of *Mercenaria* deserve recognition as stocks distinct from the larger population of the U.S. Atlantic coast. Although the hard clam occupies a virtually continuous range from Florida to Massachusetts, its distribution north of Cape Cod becomes disjunct. Here, we use protein electrophoresis to determine gene frequencies a seven polymorphic enzyme loci in clam populations from Maine, USA, and New Brunswick and Prince Edward Island, Canada, and compare our results with previously published data from Massachusetts. The fit to the Hardy–Weinberg expectation within populations was very good. The Maine population showed small but statistically significant divergence from its putative source population to the south at most loci, with an apparent loss of two rare alleles. Both Canadian populations showed larger levels of divergence, with the loss of 6–12 alleles and significant reductions in overall heterozygosity. The recognition of a St. Lawrence stock of hard clams at Prince Edward Island may have important implications for the fishery.

Les populations de *Mercenaria* du Canada devraient être reconnues comme étant des stocks distincts de la population plus importante de la côte atlantique des États-Unis. bien que la palourde américaine occupe une aire pratiquement continue depuis la Floride jusqu'au Massachusetts, au nord de Cape Cod, sa répartition devient discontinue. Dans la présente étude, nous avons utilisé l'électrophorèse des protéines pour déterminer la fréquence génétique sur sept loci à enzyme polymorphes chez les populations de palourdes américaines du Maine (É.-U.) ainsi que du Nouveau-Brunswick et de l'Île-du-Prince-Édouard (Canada); nous avons ensuite comparé nos résultats à des données du Massachusetts publiées antérieurement. Les résultats correspondaient très étroitement au modèle prévu en fonction de la loi de Hardy–Weinberg. La population du Maine présentait une divergence légère, mais importante sur le plan statistique, sur la plupart des loci, par rapport à sa population d'origine présumée située plus au sud, conjuguée à la perte apparente de deux allèles rares. Dans les deux populations canadiennes, outre la perte de 6 à 12 allèles et des diminutions dans la proportion globale d'hétérozygotes, le degré de divergence était plus élevé. La reconnaissance d'un stock de palourdes américaines du Saint-Laurent, à l'Île-du-Prince-Édouard, pourrait avoir une incidence considérable sur la pêche à ce mollusque.

Mercenaria mercenaria (L.), the commercial hard clam or quahog, is a common inhabitant of the U.S. Atlantic coast from Florida to Massachusetts (Menzel 1989). The dispersal capabilities of its larvae seem to be sufficient to maintain virtual panmixia over its entire range. Comparing *Mercenaria* populations from Massachusetts and Virginia, Dillon and Manzi (1987) detected a significant difference in allele frequency at only one of seven polymorphic enzyme loci. Similarly low levels of divergence have been reported among Virginia, South Carolina, and Florida populations (Dillon and Manzi 1989; Dillon 1992a).

Between Cape Cod and the Gulf of St. Lawrence (Fig. 1), however, *Mercenaria* populations have a patchy distribution. In Maine, they are reliably collected only in shallow estuaries warmed by the sun or by addition of freshwater. During the period 1939–67 only three substantial recruitment events occurred in the Gulf of Maine (1937, 1947, and 1952), all associated with unusually high sea temperatures (Dow 1973). Natural *Mercenaria* populations are even more rare on the eastern Coast of New Brunswick and Nova Scotia. Although Whiteaves (1901) quoted secondary reports of hard clams at St. Mary's Bay on the Nova Scotian side of the Bay of Fundy, and on the Atlantic coast of Nova Scotia, shellfish biologists with the Nova Scotia Department of Fisheries report no knowledge of natural *Mercenaria* populations in this region. No Atlantic or Bay of Fundy populations were mentioned by Bourne (1989) in his review of Canadian clam fisheries.

We surveyed the collections of five major museums: the U.S. National Museum in Washington, the Academy of Natural Sciences in Philadelphia, the American Museum of Natural History in New York (AMNH), the Museum of Comparative Zoology at Harvard (MCZ), and the Canadian Museum of Nature in Ottawa. Both the AMNH and MCZ have specimens of *Mercenaria* from the area of St. Andrews, New Brunswick. However, the museums contain no records of the species from the Bay of Fundy, and only a single sample (at the Canadian Museum) from the Atlantic coast of Nova Scotia near Halifax.

2574

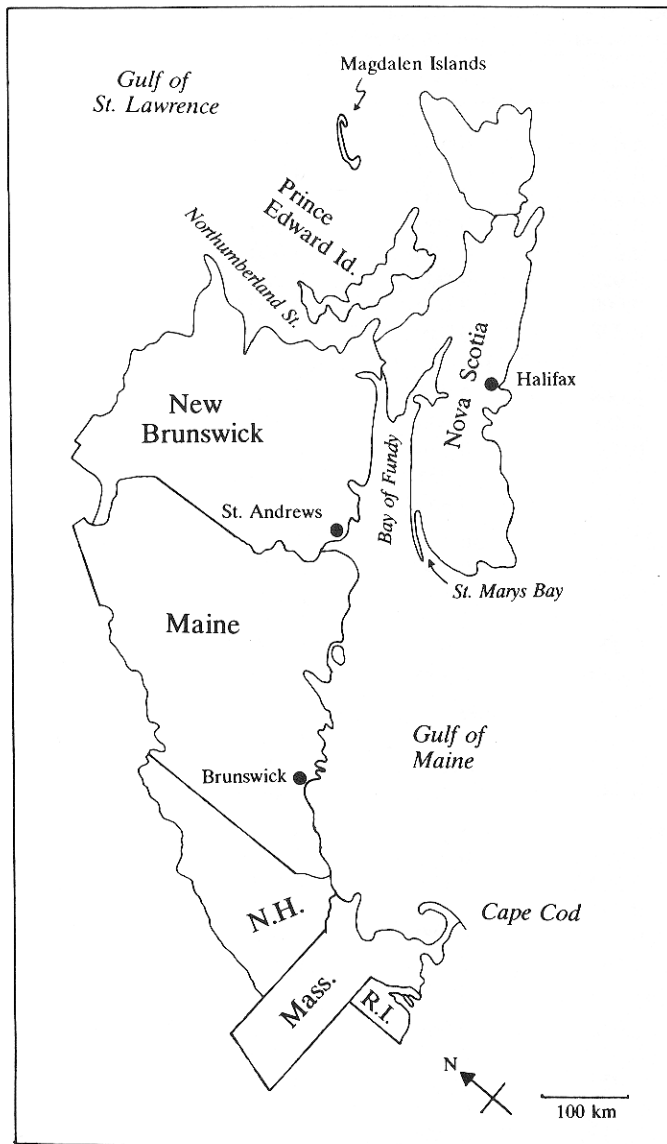*Can. J. Fish. Aquat. Sci., Vol. 49, 1992*

FIG. 1. United States and Canada at the northern limit of the range of *M. mercenaria*. R.I., Rhode Island; Mass., Massachusetts; N.H., New Hampshire. Populations of clams sampled were from Prince Edward Island and sites near St. Andrews, New Brunswick, and Brunswick, Maine.

Bathed by the warmer waters of the St. Lawrence River, *Mercenaria* populations again reach commercially harvestable levels in the Northumberland Strait on the northern coasts of New Brunswick and Nova Scotia, as well as Prince Edward Island (P.E.I.) (Bourne 1989). The northern limit of *Mercenaria*'s range is generally considered to be at the Magdelen Islands, in the Gulf of St. Lawrence. Given the virtual isolation of the St. Lawrence populations, it would seem possible that they constitute a stock genetically distinct from that inhabiting the Atlantic coast of the United States.

Protein electrophoresis has often proven to be a useful tool for the identification and characterization of fish stocks. A search of the Aquatic Sciences and Fisheries Abstracts for the years 1982–91 yielded 114 titles with both "stock identification" and "electrophoresis" among the descriptors. The topic has been thoroughly reviewed in the proceedings of several international meetings (Berst and Simon 1981; Kumpf 1987; Ryman and Utter 1987). Isozyme data do not seem to suggest

the existence of natural stocks of edible mussels (Koehn 1991), although population differentiation is occasionally noted in scallops (Beaumont 1991) and oysters (Fujio 1979; Buroker 1983).

To date, the only data bearing on the question of a northern *Mercenaria* stock are those gathered by Pesch (1972, 1974). His sample of 50 clams from P.E.I. showed gene frequencies strikingly different from those of Maine, Rhode Island, or South Carolina at two loci: one encoding lactate dehydrogenase and the other superoxide dismutase ("TO"). He noted much less divergence at two other, weakly polymorphic loci encoding malate dehydrogenase and an esterase. In this investigation, we used larger sample sizes and a larger number of loci to examine in greater detail the levels of genetic divergence at the northern extreme of *Mercenaria*'s natural range.

Materials and Methods

Populations of *M. mercenaria* sampled for this study were from an intertidal site near the city of Brunswick, Maine, from Sam Orr's Pond about 10 km east of St. Andrews in the province of New Brunswick, and from P.E.I. (Fig. 1). The environment of Sam Orr's Pond is especially unusual. The pond receives both a fairly constant freshwater input from a small stream and a tidal input of seawater from the Bay of Fundy. During the winter the freshwater freezes over the seawater, protecting the *Mercenaria* population beneath from more severe temperatures.

Samples were obtained by raking and hand-picking and include only the largest size classes available. Standard lengths of shells ranged from 8.5 to 11.5 cm in the Maine sample, from 5.4 to 10.0 cm in Sam Orr's Pond, and from 7.9 to 10.3 cm at P.E.I. Voucher specimens from all three populations have been deposited in the collections of both the Canadian Museum of Nature and the Academy of Natural Science of Philadelphia.

We compared allele frequencies at seven enzyme loci in these populations with data previously published on *Mercenaria* from Massachusetts (Dillon and Manzi 1987). The electrophoretic techniques employed here have been described previously (Dillon 1982, 1985). We examined isozyme variation in 6-phosphogluconate dehydrogenase (*Pgd*), glucose-6-phosphate isomerase (*Gpi*), superoxide dismutase (*Sod*) two phosphoglucomutase loci (*PgmF* and *PgmS*), mannose-6-phosphate isomerase (*Mpi*), and leucine aminopeptidase (*Lap*). Multiple buffer systems were used to resolve several of these loci. The first two loci were examined using an aminopropylmorpholine pH 6 buffer, the second, third, and fourth loci were resolved using the Poulik discontinuous buffer system, and for the last four loci, a Tris – citric Acid pH 6 buffer was used. A detailed description of all our electrophoretic equipment, materials, and methods is given in Dillon (1992b). Mendelian inheritance of isozyme phenotype at five of these loci, *Pgd*, *Gpi*, *PgmF*, *PgmS*, and *Lap*, has been verified by Adamkewicz et al. (1984).

We used BIOSYS-1 (Swofford and Selander 1981) to calculate gene frequencies, heterozygosities, Wright's F-statistics (Wright 1978), and Nei's unbiased genetic distance (Nei 1978). Two separate analyses of population differentiation using F-statistics were performed. In the more conventional analysis, mean F_{is}, F_{it}, and F_{st} over seven loci were calculated within and among the four populations: Massachusetts, Maine, Sam Orr's Pond, and P.E.I. We also performed a hierarchical analysis, grouping the two U.S. populations and comparing this group with the two separate Canadian populations. Mean F-statistics calculated in this fashion were labelled F_{sh} (population

TABLE 1. Samples sizes (N), allele frequencies, and observed heterozygosities (H) at seven loci in four samples of *M. mercenaria* (data on the Massachusetts population taken from Dillon and Manzi 1987).

Locus	Allele	Massachusetts	Maine	Orr's	P.E.I.
Gpi	N	79	123	156	199
	110	0.006	0.004	0.010	0.005
	105	0.019	0.004	0.000	0.018
	100	0.848	0.882	0.990	0.952
	90	0.019	0.024	0.000	0.000
	80	0.006	0.057	0.000	0.000
	70	0.063	0.028	0.000	0.025
	60	0.038	0.000	0.000	0.000
	H	0.278	0.228	0.019	0.095
Mpi	N	65	122	131	183
	108	0.038	0.008	0.008	0.046
	105	0.307	0.488	0.676	0.533
	100	0.354	0.430	0.286	0.235
	95	0.281	0.074	0.031	0.186
	H	0.600	0.533	0.450	0.617
Lap	N	77	123	156	191
	104	0.084	0.093	0.000	0.131
	100	0.448	0.411	0.580	0.542
	96	0.448	0.476	0.375	0.319
	94	0.019	0.020	0.045	0.008
	H	0.623	0.650	0.494	0.613
Sod	N	78	123	109	179
	100	0.692	0.533	0.339	0.338
	90	0.308	0.467	0.661	0.662
	H	0.410	0.447	0.422	0.486
Pgd	N	78	123	118	180
	110	0.006	0.053	0.000	0.050
	100	0.647	0.553	0.606	0.625
	90	0.346	0.382	0.394	0.325
	80	0.000	0.012	0.000	0.000
	H	0.602	0.642	0.398	0.489
PgmS	N	78	122	118	185
	103	0.019	0.016	0.064	0.019
	100	0.731	0.693	0.818	0.773
	97	0.027	0.053	0.119	0.065
	92	0.154	0.123	0.000	0.059
	87	0.071	0.115	0.000	0.084
	H	0.449	0.484	0.322	0.384
PgmF	N	77	122	149	198
	105	0.040	0.000	0.000	0.000
	103	0.104	0.102	0.483	0.008
	100	0.857	0.877	0.517	0.992
	97	0.000	0.020	0.000	0.000
	H	0.260	0.230	0.376	0.015

to "hierarchy"), F_{ht}, and F_{st}. The fit to Hardy–Weinberg equilibrium within each population was tested using goodness-of-fit χ^2 tests, combining rare genotypic classes as necessary. The overall heterozygosity of each population was compared with all others by means of χ^2 contingency tests, corrected for continuity, totalling the number of heterozygous genotypes observed over all individuals and all loci.

Populations were compared by their gene frequencies at individual loci using χ^2 contingency tests, corrected for continuity in 2×2 cases, again combining rare alleles as required. Exact binomial tests were used to assess the significance of the apparent absence or low frequency of individual alleles from particular populations. For example, if the frequency of allele *PgmF* *105* in Massachusetts is fairly estimated at 0.040, the chance that its absence from 122 Maine clams is due to sampling error would be $(1 - 0.040)^{244} = 5 \times 10^{-5}$. In applying this method, we take 0.040 as a parameter, rather than the estimate that it is. Thus the probability obtained (5×10^{-5} in this example) is also only an estimate, but nonetheless often highly suggestive of significance.

Results

Gene frequencies at seven enzyme loci from the three northern populations are given in Table 1, along with previously published results from Massachusetts. Fits to Hardy–Weinberg equilibrium within populations were generally very good. Since $3 \times 7 = 21$ loci were newly examined for this study, Bonferroni correction would suggest a value of χ^2 nominally significant at the $p = 0.05/21 = 0.0024$ level to reject Hardy–

2576

Can. J. Fish. Aquat. Sci., Vol. 49, 1992

TABLE 2. Above the diagonal are values of χ^2 comparing overall observed heterozygosities between all pairs of four northern *Mercenaria* populations. Values greater than 3.84 are significant at or above the 0.05 level. Below the diagonal are Nei's (1978) unbiased genetic distances.

	Massachusetts	Maine	Orr's	P.E.I.
Massachusetts	—	0.00	15.6	8.33
Maine	0.012	—	20.0	11.1
Sam Orr's Pond	0.085	0.066	—	2.26
P.E.I.	0.042	0.026	0.066	—

Weinberg (Rice 1989). No value of χ^2 this extreme was in fact observed. Mean heterozygosities over all seven loci, by direct count, were 0.460, 0.459, 0.354, and 0.386 for Massachusetts, Maine, Sam Orr's Pond, and P.E.I. respectively. These figures agree well with their respective Hardy–Weinberg expected heterozygosities: 0.442, 0.450, 0.393, and 0.380. Table 2 shows that the two U.S. populations did not differ significantly in overall observed heterozygosity, nor did the two Canadian populations. The two Canadian populations were, however, both significantly less heterozygous than the two U.S. populations.

Although not striking, most of the allele frequency differences between Massachusetts and Maine *Mercenaria* populations (Table 1) are significant at the 0.05 level. Chi-square contingency tests showed significant differences between the two U.S. populations at the *Mpi* and *Sod* loci. The unexpectedly high frequency of *Pgd 110* in Maine was significant by an exact test, as were the absences of *Gpi 60* and *PgmF 105*. Note, however, that *PgmF 97* and *Pgd 80* were present in Maine but apparently missed in Massachusetts. (These alleles have been observed further south, and their absences from the smaller Massachusetts sample are not significant). Thus, there was no net difference between the Massachusetts and Maine populations of *Mercenaria* in number of alleles observed.

The two Canadian populations were significantly different from the two U.S. populations in allele frequencies at all loci examined. Interestingly, the U.S. populations were more similar to the P.E.I. population than to the geographically closer Sam Orr's Pond population. The Maine and Massachusetts populations were the most genetically similar pair of populations, followed by the Maine/P.E.I. pair (Table 2). In addition to missing the two alelles previously noted absent from Massachusetts and the two absent from Maine, the P.E.I. population was significantly missing two other GPI alleles, *Gpi 90* at 0.024 in Maine and *Gpi 80* at 0.057. The Sam Orr's Pond population was missing these six alleles as well as six others: *Lap 104, Pgd 110, PgmS 92, PgmS 87, Gpi 70,* and *Gpi 105*, all but the last significant, using Maine frequencies as expected. Table 1 shows several other striking genetic features of the Canadian populations, including the reversal in relative frequencies of the two *Sod* alleles and the very high frequency of *PgmF 103* in Sam Orr's Pond.

The value of F_{is} (Table 3) differed from 0.0 only by rounding error, as may be expected when genotype frequencies match Hardy–Weinberg expectation within populations. Then, $F_{st} = F_{it}$, and all the gene diversity is attributable to differentiation between populations. The observation that F_{ht} accounts for most of the total F_{st} confirms that divergence is due primarily to differentiation by the two Canadian populations from the larger U.S. population. In Wright's island model, a value of $F_{st} = 0.05$ implies that Nm, the average number of individuals moving among populations, equals 4.75 (Slatkin and Barton 1989). This suggests that although these populations are distinct, gene flow has been strong enough to prevent substantial differentiation among them due to genetic drift.

TABLE 3. Wright's F-statistics (averaged over seven loci) measuring the amount of differentiation between individuals (i), population (s), hierarchy (h, defined in the text), and total (t).

Comparison	Coefficient
F_{is}	-0.001
F_{st}	0.050
F_{it}	0.049
F_{sh}	0.013
F_{ht}	0.034
F_{st}	0.047

Discussion

Although commercially important sets of hard clams occur infrequently in Maine, local densities may be quite great. Dow and Wallace (1965) reported densities as high as 25 000 clams·ft^{-2} (270 000 clams·m^{-2}) shortly after the 1947 set. We speculate, therefore, that the northern migration of *M. mercenaria* may have occurred episodically, i.e. that exceptional years of reproduction and dispersal may have been followed by longer periods of high mortality in all but a few isolated refugia. The apparent loss of rare alleles from all three clam populations newly examined here is compelling evidence of such bottlenecking.

Ihssen et al. (1981) have defined a fisheries stock as "an intraspecific group of randomly mating individuals with temporal or spatial integrity". None of the populations examined here could be considered randomly mating with any other. But although significant, the genetic differences apparent between the Maine and Massachusetts populations are of the order of magnitude we have previously ascribed to isolation by distance (Dillon and Manzi 1987, 1989). We can identify no obvious barrier to gene flow between Massachusetts and Maine. Thus, in the case of the Maine population, the evidence for "temporal or spatial integrity" is not strong.

By contrast, the divergence of the Sam Orr's Pond and P.E.I. populations, both from more southern populations and from each other, is much more striking. Our results confirm and expand upon those of Pesch (1974), suggesting both that Canadian populations of *Mercenaria* are genetically distinct from their putative source population to the south and that they show reduced variation. Taken together with the observed distribution of *Mercenaria* in Canada, the data of Tables 1 and 2 suggest strongly that these be considered distinct stocks.

The Sam Orr's Pond population is small and not important commercially, although it supports occasional recreational use. Its origin and age are unknown; *Mercenaria* have been taken from the pond as long as anyone can remember. Our findings here are interesting as a confirmation of population genetics theory, which predicts that small populations may initially be characterized by reduced allelic diversity (Leberg 1992).

Can. J. Fish. Aquat. Sci., Vol. 49, 1992

2577

The recognition that the *Mercenaria* of the Gulf of St. Lawrence and the Northumberland Strait comprise a distinct stock has management implications for the Canadian hard clam fishery. Bourne (1989) reported that Canadian landings of *Mercenaria* between 1951 and 1981 have fluctuated over an order of magnitude, from 2040 to under 200 t annually. He attributed this variability to localized distribution of stocks, environmental degradation, and overexploitation. It is clear that the unusually slow growth rates observed in Canadian *Mercenaria* (6–7 yr to market size) contribute to the danger of overfishing, as does the correspondence between optimum harvest size and age at first reproduction. The evidence presented here that little annual immigration from the vast U.S. population is to be expected may be cause for additional concern.

There have also been a number of experiments with hard clam mariculture, both on P.E.I. and on the northern coast of New Brunswick (Bourne 1989). Given the relatively low genetic variability of the St. Lawrence stock, some effort at outbreeding with U.S. stock, along the lines pursued by Manzi et al. (1991), may be desirable.

Acknowledgements

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2578

Can. J. Fish. Aquat. Sci., Vol. 49, 1992