Florida as a Biogeographic Boundary: Evidence from the Population Genetics of *Littorina irrorata*[1]

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ABSTRACT

The marsh periwinkle, *Littorina irrorata* (Say, 1822), ranges from New York to Texas with an apparently large disjunction around southern Florida. We examined gene frequencies at eight polymorphic enzyme loci in populations from Virginia, South Carolina, Atlantic Florida, Gulf Florida, and Louisiana. The within-population deviation from Hardy-Weinberg expectation was small at all loci examined (F_{IS}=0.049). Small but significant gene frequency differences among populations were detected at four loci, indicating some isolation by distance. Hierarchical gene diversity analysis suggested, however, that very little of the population divergence present (F_{ST}=0.033) is attributable to a division between coasts (F_{CR}=0.004). Nei genetic distances calculated between pairs of sites sharing the same coast were comparable to such distances between Atlantic and Gulf pairs. Thus we find no evidence that the Florida peninsula constitutes a significant barrier to *L. irrorata*, in spite of the apparently extensive gap in its range. We offer three hypotheses, not mutually exclusive: that the barrier is not real, that it was broached in the not-too-distant past, or that balancing selection may be ongoing to hold polymorphisms constant at multiple enzyme-encoding loci simultaneously.

Key words: Gastropoda, electrophoresis, allozymes, range disjunction, balancing selection.

INTRODUCTION

Shallow, protected regions along the southern Atlantic coast of the United States and the northern coast of the Gulf of Mexico are characterized by temperate climate, depositional environment, and the dominant salt marsh cordgrass, *Spartina*. The molluscan faunas of these two regions are described as "Carolinian" (Rehder, 1954; Coomans, 1962), sharing over 60% of their shallow water gastropod species (query to database of Rosenberg, 1993). However, striking climatic and geological variation along the Florida peninsula seems to impose a biogeographic boundary between these regions (Briggs, 1974). Due to tropical conditions, mangroves replace salt marshes in protected bays and estuaries around 27°– 29° N latitude (Kangas & Lugo, 1990). This transition occurs between Cedar Key and Tampa Bay on the Gulf coast of Florida, and between St. Augustine and Cape Canaveral on the Atlantic coast. Coralline sands become the dominant sediment type along south Florida coasts, replacing terrigenous silt and mud.

Scheltema (1989) surveyed the ranges of 88 mesogastropod and neogastropod species, dividing the western Atlantic coast into eight regions from Arctic Canada to Brazil. He reported that 58 of 72 species inhabiting his "region IV" (Beaufort, North Carolina to Miami, Florida) also occurred in his "region V" (Gulf of Mexico). However, 18 of the 58 shared species did not occur in Scheltema's "region VI", encompassing the southern tip of Florida and the Greater Antilles. For at least these 18 species (including such common species as *Fasciolaria hunteria*, *Polinices duplicatus*, and *Littorina irrorata*) Florida would seem to constitute a potential barrier. This general distributional pattern extends beyond the nearshore molluscan fauna to include many other elements of the flora and fauna of the southeastern United States.

Recent molecular techniques have commonly detected substantial genetic differentiation between animal populations of the southern Atlantic coast and those of the northern Gulf of Mexico. Most of the 19 such species for which mtDNA surveys have been completed, including horseshoe crabs, toadfish, black sea bass, diamondback terrapins, and seaside sparrows, show distinct differentiation associated with the Florida peninsula (Avise, 1992). To this list could be added the coastal North American tiger beetle, *Cicindela dorsalis*, where mitochondrial DNA sequence data were used to assign four subspecies to either an Atlantic coastline lineage or a Gulf of Mexico coastline lineage (Vogler & DeSalle, 1993). Significant divergence at enzyme-encoding loci has been detected between Atlantic and Gulf populations of such diverse taxa as the sea anemone, *Bunodosoma cavernata* (McCommas, 1982), and the marsh crab *Sesarma reticulatum* (Felder & Staton, 1994).

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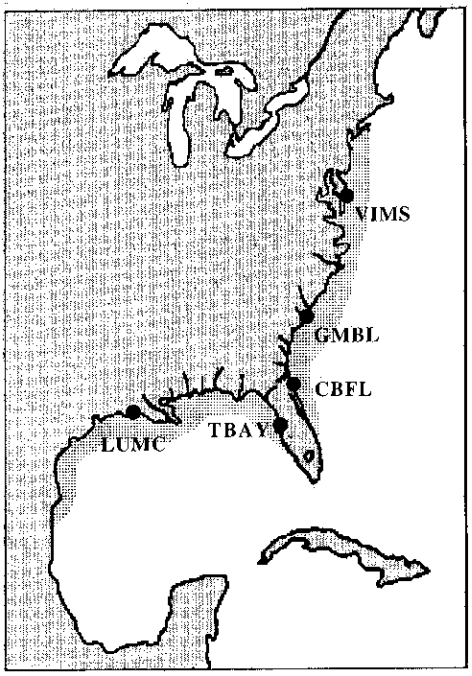

Figure 1. The range of *Littorina irrorata* (shaded), showing sample sites for the present study.

No Florida disjunction is apparent in the range of the American oyster, *Crassostrea virginica*, nor was any divergence in allozyme frequencies detected by Buroker (1983) in his survey of 19 populations from Massachusetts to Texas. Thus the report of substantial mtDNA divergence between Atlantic and Gulf oyster populations by Reeb and Avise (1990) was greeted with unusual interest. Karl and Avise's (1992) re-examination of the issue using restriction fragment length polymorphisms in anonymous single-copy nuclear genes confirmed the mtDNA results, suggesting that Florida does, in fact, constitute a barrier to the dispersal of oysters. Karl and Avise went on to propose that the similarity in allozyme frequencies reported by Buroker might result from balancing selection at multiple enzyme-encoding loci. The importance of natural selection to the preservation of enzyme polymorphism has been a central question in evolutionary biology for over 25 years (Lewontin, 1991). Thus if the findings of Karl and Avise can be generalized beyond oysters, there will be implications for our understanding of evolution as a whole.

The purpose of this study is to document divergence in allozyme frequency between Atlantic and Gulf populations of an heretofore genetically unsurveyed intertidal mollusk, the marsh periwinkle *Littorina* (or *Littoraria*) *irrorata* (Say, 1822). The snail is primarily an inhabitant of salt marshes dominated by the cord grass *Spartina alterniflora*, leaving the marsh surface with the incoming tide to climb the vegetation. It ranges from Jamaica Bay, Long Island, New York (Jacobson, 1965) to Port Isabel, Texas (Bequaert, 1943), with a disjunction around southern Florida (Figure 1). As such it would seem an excellent candidate for an attempt to confirm the Karl and Avise phenomenon.

Littorina has been the object of considerable population genetics research worldwide, with much effort directed toward questions of systematics (Ward & Warwick, 1980; Maestro *et al.*, 1991; Boulding *et al.*, 1993; Zaslavskaya *et al.*, 1992). Other workers have prospected for environmental clines (Newkirk & Doyle, 1979; Janson & Ward, 1984; Johannesson *et al.*, 1993), founder effects (Janson, 1987), or correlates of heterozygosity (Noy *et al.*, 1987; Foltz *et al.*, 1993). However, the only previous examination of *L. irrorata* allozymes, prior to this report, was that of Berliner (1981) in a Virginia salt marsh. He found no significant difference in heterozygosity between young and old size classes, but noted a lower value of heterozygosity in snails of median age. Berliner's study did not extend beyond his single population.

MATERIALS AND METHODS

Approximately 50–60 *L. irrorata* per site were collected from three sites on the Atlantic coast of the southeast United States and two from the Gulf of Mexico: the Virginia Institute of Marine Science Laboratory (VIMS) at Wachapreague, Virginia, the Grice Marine Biological Laboratory (GMBL) at Charleston, South Carolina, Crescent Beach, Florida (CBFL), Tampa Bay, Florida (TBAY), and the Louisiana University Marine Consortium Laboratory (LUMC) at Cocodrie, Louisiana (Figure 1). All sites except TBAY were typical salt marshes dominated by the salt marsh cord grass, *S. alterniflora*. The Tampa Bay site was a small patchy area of *S. alterniflora* growing on sand, rather than mud. At all sites, the snails were found on the stalks of the salt marsh cord grass or on the substrate at the base of the stalks, generally in the mid-marsh to high marsh areas. The snails were transported to Charleston alive, where tissues were frozen at −60°C in 150–300 μl of 0.05 M Tris Tissue buffer pH 7.5 (Dayan, 1994).

Horizontal protein electrophoresis was conducted using methods and equipment previously described (Dillon, 1985; 1992; Dayan, 1994). The 12% starch gels were a 1:1 mixture (by volume) of Electrostarch (Otto Hillar, Madison, WI) and Sigma starch (Sigma Chemical, St. Louis, MO). We initially compared the zymograms of individuals from GMBL and LUMC using 19 enzyme stains and 9 buffer systems. Results were poor or uninterpretable for six enzymes and appeared invariable for seven others. Ultimately we were able to resolve allozymes interpretable as the products of codominant Mendelian alleles at eight loci (encoding six enzymes) using

Table 1. Allele frequencies at eight enzyme-encoding loci in five populations of *Littorina irrorata*. (n) = Sample size.

Locus	Allele	Population				
		VIMS	GMBL	CBFL	TBAY	LUMC
Est1	A	0.571	0.689	0.638	0.556	0.507
	B	0.429	0.292	0.362	0.444	0.485
	C	0.000	0.019	0.000	0.000	0.007
(n)		56	53	58	62	67
Est2	A	0.984	0.989	0.992	0.968	1.000
	B	0.016	0.011	0.008	0.032	0.000
(n)		61	44	62	62	63
Pgm	A	0.648	0.441	0.490	0.411	0.490
	B	0.270	0.480	0.288	0.218	0.127
	C	0.082	0.078	0.202	0.371	0.343
	D	0.000	0.000	0.019	0.000	0.039
(n)		61	51	52	62	51
Mpi	A	0.992	0.952	0.992	0.968	0.971
	B	0.008	0.048	0.008	0.032	0.029
(n)		63	83	62	62	70
Isdh1	A	0.934	0.965	0.877	0.966	0.918
	B	0.057	0.035	0.098	0.017	0.061
	C	0.008	0.000	0.025	0.017	0.020
(n)		61	57	61	59	49
Isdh2	A	0.992	0.990	1.000	1.000	0.990
	B	0.008	0.010	0.000	0.000	0.010
(n)		60	50	62	62	49
Sdh	A	0.814	0.788	0.856	0.805	0.698
	B	0.161	0.205	0.136	0.161	0.250
	C	0.025	0.008	0.008	0.034	0.052
(n)		59	66	59	59	48
Lap	A	0.917	0.700	0.900	0.860	0.862
	B	0.075	0.255	0.067	0.061	0.078
	C	0.008	0.036	0.025	0.061	0.043
	D	0.000	0.009	0.008	0.018	0.017
(n)		60	55	60	57	58

three buffer systems. The AP6 buffer (Clayton & Tretiak, 1972) was used to resolve esterases (*Est*, two loci), and phosphoglucomutase (*Pgm*). The WW1 (Ward & Warwick, 1980) buffer was also used to resolve *Pgm*, as well as isocitrate dehydrogenase (*Isdh*, two loci), and sorbitol dehydrogenase (*Sdh*). The TC6 buffer (Dillon, 1985) was used to resolve leucine aminopeptidase (*Lap*) and mannose-phosphate isomerase (*Mpi*). Working with *L. saxatilis*, Ward *et al.* (1986; 1991) have confirmed Mendelian inheritance at all these loci except *Est* and *Sdh*.

Data analysis was by Biosys-1 (Release 1.7, Swofford & Selander, 1981) unless otherwise specified. We tested the fits to Hardy-Weinberg expectation for each locus at each population using goodness of fit χ^2 statistics, combining rare genotypic classes as necessary. We then performed two separate gene diversity analyses using Wright's (1978) F-statistics. In the more conventional analysis, the total deviation from Hardy-Weinberg expectation over all loci (F_{IT}) was divided into a component within populations (F_{IS}) and a component between the five populations (F_{ST}). We also performed a hierarchical analysis (Dillon & Manzi, 1992; Dillon & Wethington, 1995), grouping the three Atlantic and two Gulf populations to determine the proportion of gene diversity attributable to coast. Mean F-statistics calculated in this way we labeled F_{SC} (between populations within coasts), F_{CB} (between coasts) and F_{SB}. Note that F_{SB} is expected to be less than F_{ST}, since that proportion of the variance between populations between coasts remains unattributed in the hierarchical analysis.

Table 2. Wright's (1978) F-statistics, averaged over eight loci, measuring gene diversity attributable to individuals (*I*), populations (*S*), and coast (*C*). Values from the hierarchical analysis of coastal variance are set under F_{ST}, the variance attributable to population structure.

Comparison	Coefficient
F_{IS}	0.049
F_{ST}	0.033
F_{SC}	0.020
F_{CB}	0.004
F_{SB}	0.024
F_{IT}	0.080

For each locus at which n alleles were identified, divergence among populations was tested with a $5 \times n$ χ^2 contingency test. To avoid the necessity of combining or eliminating rare alleles for this analysis, we estimated the significance of our values of χ^2 using the Monte Carlo approach of Roff and Bentzen (1989). Unbiased genetic identity and distance between all pairs of populations was calculated using the method of Nei (1978).

RESULTS

Gene frequencies at eight enzyme-encoding loci from five populations of *Littorina irrorata* are shown in Table 1. The fits to Hardy-Weinberg expectation within populations were very close in most cases. Goodness-of-fit values of χ^2 nominally significant at the 0.05 level were obtained only at *Pgm* in VIMS, and at *Isdh* in TBAY, well within expectation for type I error. The mean value of F_{IS} over all loci, measuring deviation from Hardy-Weinberg within-sites over the entire study, was small (F_{IS}=0.049, Table 2).

The mean value of F_{ST} = 0.033, measuring deviation from Hardy-Weinberg expectation between sites, was lower than the deviation within-sites. However, the data of Table 1 reflect significant divergence among the five populations at four loci. The value of χ^2 testing homogeneity in *Pgm* allele frequencies was 103.6 ($p<0.001$), with notably high frequencies of *Pgm-A* in VIMS, *Pgm-B* in GMBL, and *Pgm-C* in the three most southern populations. The GMBL population was distinguished by a significantly high frequency of *Lap-B* (overall χ^2=46.4, $p<0.001$), the LUMC population by high *Sdh-B* (overall χ^2=21.7, p=0.034), and the CBFL population

Table 3. Nei's (1978) unbiased genetic identity (above diagonal) and distance (below diagonal) between all pairs of five *L. irrorata* populations.

Populations	VIMS	GMBL	CBFL	TBAY	LUMC
VIMS	—	0.986	0.998	0.990	0.991
GMBL	0.014	—	0.990	0.981	0.974
CBFL	0.002	0.011	—	0.996	0.993
TBAY	0.010	0.019	0.004	—	0.999
LUMC	0.009	0.026	0.007	0.001	—

by high *Isdh1-B* (overall χ^2=19.6, p=0.047). Differences at the remaining four loci were not significant.

The contribution of the coastal level to the hierarchical gene diversity analysis was negligible. Table 2 shows that F_{CB}, the deviation from Hardy-Weinberg expectation between coasts, was 0.004. This was lower than the variance between populations within coasts. The values of Nei's unbiased similarity and distance among all pairs of sites are shown in Table 3. The four within-coast values of Nei's distance ranged from 0.001 to 0.014, only slightly less than the range for the six values between coasts (0.004 to 0.026).

DISCUSSION

The data of Table 1 constitute substantial evidence of low-level genetic divergence among populations of *Littorina irrorata* separated by distances on the order of hundreds of kilometers. A general relationship between interpopulation divergence, mode of embryonic development, and dispersal capability has often been noted in marine mollusks (Burton & Feldman, 1981; Scheltema, 1989; Yamada, 1989). *Littorina irrorata* spawn at high tide, females releasing eggs just beneath the water level (Gallagher & Reid, 1974). Egg capsules are planktonic, but slightly negatively buoyant in calm seawater (Bingham, 1972). They hatch into swimming veliger larvae after one to two days of further development, but the time to settlement is unknown. Settlement occurs on *Spartina* shoots (Boothe, 1969), where juveniles remain hidden in curled blades until they reach about 5 mm (Stiven & Hunter, 1976; Crist & Banta, 1983). Berger (1973) compared population divergence in three North American *Littorina* species, *L. littorea* (pelagic larval development), *L. obtusata* (juveniles hatch from gelatinous egg masses), and *L. saxatilis* (ovoviviparous). The levels of genetic differentiation we report here in *L. irrorata* are, as might be expected, comparable to those of *L. littorea* and much less than those of *L. obtusata* or *L. saxatilis*.

Such interpopulation genetic divergence as we have identified does not, however, seem to reflect a barrier to dispersal around the Florida peninsula. Table 1 shows no allele unique to either coast; even the five rarest alleles (*Est2-B*, *Isdh2-B*, *Lap-C*, *Lap-D*, and *Mpi-B*) were found in both Atlantic and Gulf populations. Nor did our hierarchical gene diversity analysis or our inspection of interpopulation genetic distances suggest any evidence of a barrier to gene flow corresponding to the Florida peninsula. The divergence between Atlantic and Gulf populations of *L. irrorata* is indistinguishable from differences among populations sharing the same coast. We offer three (not mutually-exclusive) hypotheses for this unexpected result: no-barrier, past-dispersal, and balancing selection.

It is possible that, in spite of the climate shift in southern Florida and the disappearance of salt marsh habitat, no barrier currently exists between Atlantic and Gulf populations of *L. irrorata*. We are unaware of any collections of adult snails south of Ft. Pierce, Florida. But it is possible that sparse populations do exist on mangroves or concrete bulkheads along the remainder of the southern Florida coast. Long distance dispersal of larvae or juveniles (perhaps on dead *Spartina* rack) is also possible. Only a few days might be required to transport larvae on the Gulf Stream from the west coast of Florida into the eastern Atlantic, although onshore currents would still be necessary to carry larvae back into estuaries. It is difficult, however, to imagine how such a passive dispersal mechanism might be effective for *Littorina* larvae and not for toadfish, black sea bass, horseshoe crabs, sea anemones, or oyster larvae, all of which do exhibit genetic divergence between coasts.

A second possible explanation for the absence of divergence between Atlantic and Gulf *L. littorina* populations would invoke higher levels of gene flow in the not-too-distant past. The Suwannee Straits (or Okeefenokee Trough) most recently connected the two coasts of northern Florida during the Pliocene epoch (Avise, 1992). Fossil *L. irrorata* found in North Carolina, South Carolina, and Florida have been dated from the upper Miocene and Pliocene, while fossils found in Louisiana and Texas are Pleistocene in age (Bequaert, 1943). Faunal exchange between Atlantic and Gulf may also have occurred in the Pleistocene epoch, although it is difficult to predict under what environmental conditions. *L. irrorata* could certainly have extended its range southward during glacial periods, but lowering sea levels would have elongated the Florida peninsula. The distance between Atlantic and Gulf shortened as interglacial seas rose, but the snail may have been driven north by the advance of tropical conditions. It would appear, however, that the past-dispersal hypothesis is no better than the no-barrier hypothesis in accounting for the difference between *Littorina* and those (many) species that do show intercoastal divergence.

The third hypothesis would recall Karl and Avise's (1992) work with oysters. Although allozyme frequencies were homogeneous, Karl and Avise were able to establish that a barrier has existed between Atlantic and Gulf oyster populations using mtDNA and anonymous nuclear DNA markers. They concluded that balancing selection may be holding Atlantic and Gulf oyster populations undifferentiated in their enzyme polymorphism in the absence of gene flow, and that caution should be used

regarding the assumption of neutrality for allozyme markers.

The present data are insufficient to distinguish among these three hypotheses. Further, more detailed surveys of *L. irrorata*'s range in south Florida would be helpful, along with an expansion of isozyme studies if necessary. Some examination of the larval behavior and development time of *L. irrorata* would clarify the likelihood of long distance gene flow. And further surveys of genetic divergence among Atlantic and Gulf samples of *L. irrorata* using mtDNA or nuclear DNA markers are strongly indicated.

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