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THE BIOGEOGRAPHY OF SEA ISLANDS: CLUES FROM THE POPULATION GENETICS OF THE FRESHWATER SNAIL, *PHYSA HETEROSTROPHA*

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Abstract.—Although entirely surrounded by estuarine waters, the sea islands of South Carolina are of dubious biogeographic integrity. Pleistocene sea level fluctuations have periodically inundated these regions and left them de-watered inland. Samples from 10 populations of the freshwater pulmonate snail, *Physa heterostropha,* inhabiting six land masses isolated by putative estuarine barriers in the Charleston area were used for allozyme electrophoresis to estimate gene frequencies at 10 polymorphic loci. Although intrapopulation and interpopulation gene diversity was extensive, none of the variance could be attributed to island or land mass by hierarchical analysis of *F* statistics. The matrix of genetic distances among all sites was significantly correlated with the matrix of linear geographic distances, even though estuaries dissect the study area. Nearest neighbors separated by estuaries tended to be more similar genetically than nearest neighbors sharing a land mass. These data on the population genetics of freshwater snails seem to hold no evidence of biogeographic boundaries among sea islands. Dispersal by birds is a plausible explanation for the observed overall correlation between genetic and geographic distance, but vicariance or natural selection hypotheses cannot be excluded. [Islands; biogeography; isozymes; allozymes; Pulmonata.]

The island concept has been of central importance to the study of biogeography since the 19th century formalization of the discipline. Difficulties may arise, however, when what comprises an island to the organism(s) of study does not correspond to the island concept held by the researcher. For example, the Atlantic coast of South Carolina and Georgia is dissected into scores of land masses entirely surrounded by marshes, estuaries, and tidal creeks, known physiographically as sea islands (Zeigler, 1959). Sea islands in the vicinity of Charleston, South Carolina, include James Island (approximately 10 × 12 km) and Johns Island (12 × 18 km). Sea islands directly fronting the ocean tend to be smaller and more elongate, e.g., Kiawah Island (3 × 15 km) south of Charleston. These are more generally described as barrier islands (Hoyt, 1967). Because distances of isolation from the mainland are not large in any case, it is not clear that sea islands such as these effectively constitute biogeographic islands.

Biogeographers generally find substantially fewer species of plants, birds, reptiles, and amphibians on sea islands than are reported from the mainland (Gaddy, 1982). For example, Gibbons and Coker (1978) reported only 30 species of reptiles and amphibians on Kiawah Island (none endemic), of nearly 100 species known to occur on the coastal mainland of South Carolina. Gibbons and Harrison (1981) found only 15 species on Capers Island, 40 km north. These authors attributed much of this reduction in diversity to harsher island environments (drought, saltwater flooding) but felt that the absence of some species might reflect barriers to their dispersal. Species richness is, however, sensitive to the size of the area surveyed. It is not entirely clear that the herpetofauna of these islands is any poorer than would be expected from a similarly sized slice of the adjacent mainland.

The biogeographic integrity of sea islands becomes especially uncertain when examined from a historical perspective. Sea levels were much higher during interglacial periods prior to 100,000 years be-

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fore the present. Hoyt and Hails (1967) identified six major Pleistocene shorelines in Georgia, ranging in elevation from 30 m to 1.5 m. The coast has been many kilometers inland from its present position during these earlier stages, and current sea island regions were inundated. Because barrier islands are sometimes viewed as "moving" before an advancing sea level (Pilkey, 1990), at least some elements of their biota doubtless survived. The sea generally fell during the Wisconsin glaciation 100,000–20,000 years ago, to 20–40 m below its current level (Wellner et al., 1993). For organisms perhaps more adapted to cooler and drier climates, movement would have been unimpeded during this time through the regions we currently identify as sea islands. Climate has warmed and sea level risen in the last 10,000 years. Against this general background, evidence has been gathered recently of striking but short-term warm cycles during the Wisconsin glacial period (Kerr, 1993). Although controversial, it has been suggested that the Pleistocene shore observable 1.5 m above present sea level may have been formed as recently as 23,000–34,000 years ago (Finkelstein and Kearney, 1988). The safest conclusion concerning the past climate and topography of the sea island region of the southeastern United States is that conditions have been much different from those prevailing today.

Because movement may have been unimpeded through the sea island regions as recently as 10,000 years ago, a biogeographer might wish to select a study organism of especially low vagility. Snail populations have often been used as biogeographic markers for studies of this sort (Vagvolgi, 1975; Solem and Van Bruggen, 1984). Among the most common and widespread snails in the vicinity of Charleston is the freshwater pulmonate *Physa heterostropha* (Say). The ability to breathe atmospheric oxygen allows pulmonates such as *Physa* often to thrive in stagnant and perhaps even ephemeral ponds and ditches. Today, this snail inhabits hundreds of such habitats dotting the Charleston area. Because its range currently extends to Nova Scotia and Ontario (Burch and Tottenham, 1980), *P. heterostropha* would not have been eliminated here by Pleistocene cooling. Although populations of *P. heterostropha* inhabit slightly brackish water, this snail does not long survive salinities >5 ppt.

Physa has a variety of attributes that may be interpreted as adaptations for dispersal. Adults are simultaneously hermaphroditic, as are pulmonate snails generally. Their ability to colonize new habitats is doubtless facilitated by their large sperm storage capabilities and ability to self-fertilize (Wethington and Dillon, 1991, 1993). Population growth rates are potentially great. Our laboratory observations suggest that a generation may be completed in less than 10 weeks, with individual fecundities ranging to several thousand eggs per adult.

As seems to be the case with *Physa* generally (Buth and Suloway, 1983; Liu, 1993), Charleston-area populations show substantial levels of isozyme polymorphism. Estimates of genetic diversity and divergence over multiple enzyme loci have, under the assumption of selective neutrality, long been used as indirect measures of gene flow among populations of freshwater snails (Mulvey and Vrijenhoek, 1982; Dillon, 1984, 1988; Bandoni et al., 1990). One approach involves the calculation of *F* statistics apportioning the deviation from Hardy–Weinberg expectation into within-population (F_{IS}) and between-population (F_{ST}) components (Wright, 1978). The average number of migrants among a group of populations may be estimated from F_{ST} (Slatkin and Barton, 1989). Because *F* statistics may be calculated in hierarchy, that portion of F_{ST} due to an island level may be compared with the portion of variation at the more traditional levels of individual, population, and total. A second approach might involve an analysis of genetic distances by geographically based correlation methods (Sokal and Oden, 1978). If the estuaries dissecting our study area indeed constitute significant barriers to dispersal, the matrix of genetic distances between all

pairs of populations would not be expected to correlate closely with linear geographic distance. Neighboring populations sharing a land mass would be expected to show significantly smaller genetic distances than neighbors isolated by an estuarine barrier. However, if estuaries do not constitute barriers, the reverse should be true. A significant correlation would be expected between genetic and overall geographic distance, but no significant difference should appear from a comparison of neighbors within a land mass with neighbors separated by estuaries.

In this work, we estimated gene frequencies at 10 polymorphic enzyme loci in 10 populations of *Physa heterostropha* from six isolated land masses in the vicinity of Charleston. We compared the proportions of the total genetic diversity within populations, between populations within land mass, and across all land masses using hierarchical F statistics. We then calculated genetic distances among all pairs of populations and examined their correlation with linear geographic distance. The 10 populations were located such that their minimally connected graph, based on geographic distance, includes four segments of equal length within land masses and five segments between land masses. In the final analysis, we compared these two subsets of paired populations to see whether coastal islands constitute significant barriers to *Physa*.

METHODS

Snails were collected from May 1990 to May 1991 from 10 sites located in Charleston County, South Carolina (Appendix; Fig. 1); sample sizes typically were 28–70 individuals/site. Whole-animal homogenate was subjected to horizontal starch gel electrophoresis using techniques previously described (Dillon, 1985, 1992). The 14% gels were physical mixtures of three parts Sigma starch for electrophoresis (S-4501; Sigma Chemical Co., St. Louis, MO) and one part Electrostarch (Otto Hiller, Madison, WI). Four buffer systems were used to resolve allozymes at 10 apparent loci. The products of most loci were examined using multiple buffers to lessen the likelihood that hidden allozyme variation might be missed. Phosphoglucomutase (two loci: *PGM-1, PGM-2*) was resolved using both Tris citrate, pH 6 (TC6), and Tris citrate, pH 6.8 (TC6.8), buffers. Glucose phosphate isomerase (GPI) and isocitrate dehydrogenase (ISDH) were both examined using TC6.8 and aminopropylmorpholine, pH 6 (AP6), buffers. A Tris-EDTA-borate, pH 8, buffer (TEB8), as well as AP6, were used to resolve 6-phosphogluconate dehydrogenase (6PGDH) and leucine aminopeptidase (LAP). TC6 alone was used to examine aconitase (ACON), and TEB8 alone was used for esterases (three loci: *EST-2, EST-3, EST-6*). (The recipe for TC6.8 was published by Mulvey and Vrijenhoek [1981] and those for all remaining buffers and stains were published by Dillon [1992].) Inheritance is Mendelian at *LAP, EST-2, EST-3,* and *EST-6* in *P. heterostropha* (Dillon and Wethington, 1994). Mendelian inheritance has been verified at *ACON, GPI, PGM,* and *6PGDH* loci in the planorbid pulmonate *Biomphalaria* (Mulvey and Vrijenhoek, 1984; Mulvey et al., 1988).

Gene frequencies, genic diversity analyses, and genetic distances were calculated using Biosys-1 (release 1.7; Swofford and Selander, 1981). We first estimated mean F_{IS}, F_{IT}, and F_{ST} across all 10 loci among the 10 populations using standard methods (Nei, 1977; Wright, 1978). Then the 10 populations were formed into six island groups (Fig. 1), and F_{ST} was subdivided into two components. We designated F_{LT} (lands to total) as that portion of the reduction in heterozygosity among populations due to the island structure of the study area and F_{SL} (subpopulations to lands) as that portion among populations not attributable to island structure:

$$(1 - F_{SL})(1 - F_{LT}) = (1 - F_{ST}).$$

To examine spatial correlations, we felt that our measure of genetic distance should be Pythagorean in Euclidean hyperspace. Among metrics of this sort, Wright (1978) preferred the arc and chord genetic distances of Cavalli-Sforza and Edwards (1967). Thus, we calculated the 10 $\times$

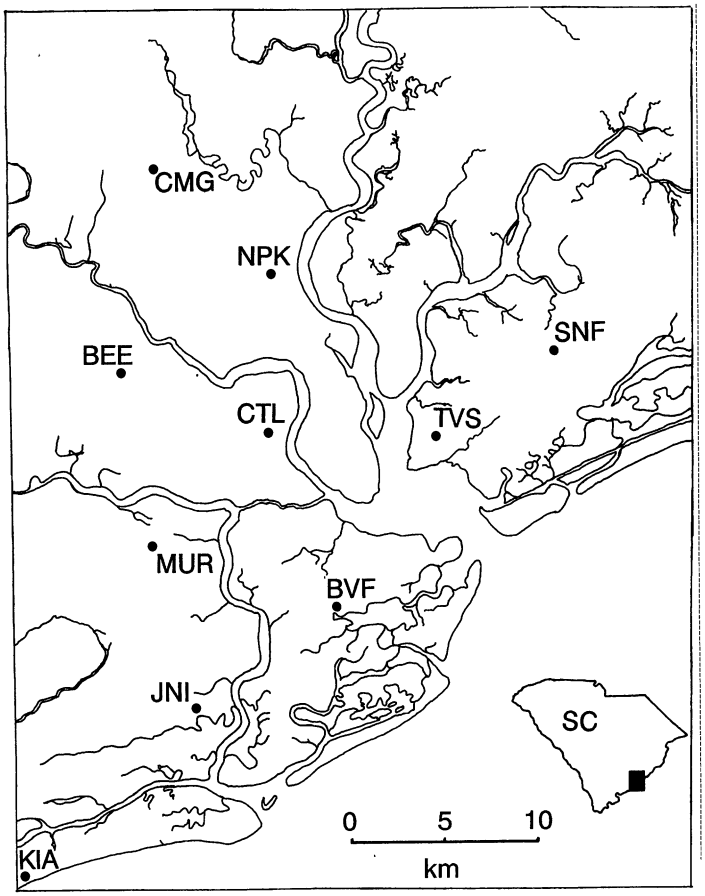

FIGURE 1. Map of the study area, Charleston County, South Carolina, showing collection sites for the 10 populations of *Physa heterostropha*. Abbreviations for sites are given in the Appendix.

10 symmetric matrix of linear (chord) Cavalli-Sforza and Edwards genetic distances among all pairs of populations and then used the nonparametric methods of Dietz (1983) to estimate their correlation with the matrix of geographic distances among all pairs of populations, measured linearly. The FORTRAN program (supplied by E. J. Dietz) calculates three statistics (Spearman, Mantel, and Kendall) and estimates their significance by comparing each to values obtained in 2,000 random permutations.

An $N \times N$ symmetric matrix contains only $N - 1$ entries that may be accorded statistical independence. Nine values were selected by calculating the minimally connected graph (or minimum-length nondirectional tree; Sneath and Sokal, 1973) over the matrix of geographic distances using the method of Prim (1957). Then the Cavalli-Sforza and Edwards chord distances corresponding to the five pairs of nearest neighbors separated by water (Fig. 2) were compared with the chord distances between the four pairs of nearest neighbors connected over land. We used a Mann–Whitney *U*-test for the one-tailed hypothesis that the genetic distances between pairs in the former set should be greater than those between pairs in the latter set.

RESULTS

Gene frequencies in all 10 populations are given in Table 1. Fits to Hardy–Weinberg expectation within populations were

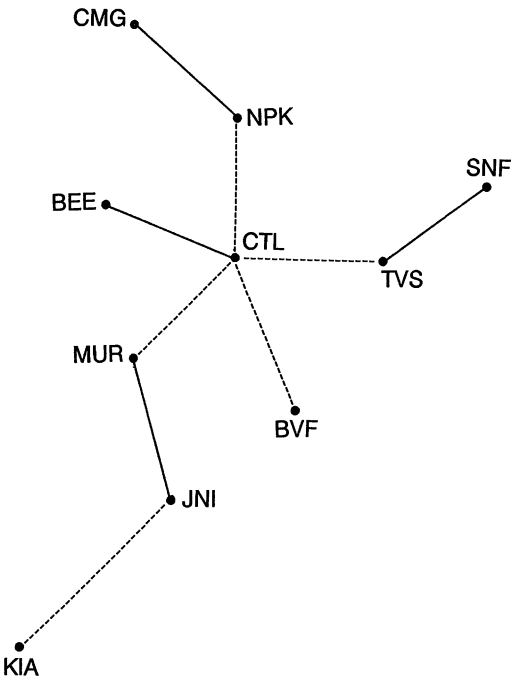

FIGURE 2. A minimally connected graph of the 10 *Physa* collection sites, calculated over the matrix of geographic distances using the method of Prim (1957). Solid segments link sites that share a land mass; dashed segments indicate that an estuary intervenes.

poor in many cases; seven populations showed striking deficits of heterozygotes at at least one locus. Both NPK and CMG populations showed deficiencies significant at the 0.05 level (uncorrected for the likelihood of Type I error) in three of nine polymorphic loci, and JNI showed similar deficiencies at two of five loci.

Seven alleles might be categorized as rare or local. Two are "private," i.e., held by single populations (*EST-3*^{104} and *LAP*^{105}), and the distribution of one allele (*ACON*^{102}) is scattered. The remaining four alleles seem to be associated with geography: the southern *ISDH*^{97}, the western *LAP*^{98}, the northwestern *6PGDH*^{95}, and the eastern *GPI*^{96}. Island boundaries seem to be of little consequence; among rare alleles only *GPI*^{96} appears to be associated with a particular land mass. Of the common alleles, *EST-2*^{96}, *EST-3*^{103}, and *LAP*^{103} are each absent from one site. *ISDH*^{94} is absent from three primarily southern populations, again regardless of land mass.

Gene diversity analysis identified large deviations from Hardy–Weinberg expectation both within and among populations. $F_{IS} = 0.198$ suggests high levels of inbreeding within populations, and $F_{ST} = 0.306$ reflects a high level of divergence among populations. After subdividing F_{ST}, $F_{LT} = -0.038$, surely not different from 0.0. Thus, none of the gene diversity among populations can be attributed to island structure, and $F_{SL} = 0.320 \approx F_{ST}$.

The matrix of Cavalli-Sforza and Edwards chord distances calculated from the data of Table 1 is shown in Table 2, along with the corresponding overland distances between all pairs of populations. Genetic divergence and geographic distance seem to be significantly correlated. The Spearman statistic comparing the upper and lower halves of Table 2 was 26,098 ($P = 0.035$), and the Mantel statistic was 2,931 ($P = 0.045$). Although the Kendall statistic was not quite significant ($P = 0.103$), it seems safe to conclude that genetic distances as estimated here are not entirely attributable to nondeterministic phenomena such as drift and sampling error.

If estuaries constitute significant biogeographic barriers to *Physa*, then the genetic distance between pairs of nearest neighbors separated by estuarine barriers should exceed the genetic distance between neighbors not so separated. The geographic distances between these two sets were comparable. The mean (of five) linear distance between pairs in the former category was 9.4 km, and the mean (of four) distance in the latter category was 8.1 km. However, the mean genetic distance between neighboring pairs separated by an estuary was 0.304, and that between pairs sharing the same land mass was 0.332, entirely counter to expectation. A Mann–Whitney *U*-test showed no significant difference in genetic distance between the two groups.

DISCUSSION

Although not the primary thrust of this investigation, there were significant het-

TABLE 1. Allele frequencies at 10 enzyme loci in 10 populations of *Physa heterostropha* from the vicinity of Charleston, South Carolina. Population names refer to Figure 1 and the Appendix. Alleles are named by the relative mobilities of their isozyme bands in standard gel conditions, setting the most common allele in population CTL to *100*.

		Study populations									
Locus[a]	Allele	CTL	NPK	SNF	TVS	BVF	JNI	MUR	BEE	CMG	KIA
EST-2	*100*	0.917	0.033	0.468	0.539	0.105	0.645	0.000	0.550	0.129	0.525
	103	0.071	0.098	0.032	0.442	0.465	0.081	0.000	0.067	0.855	0.450
	106	0.012	0.326	0.000	0.019	0.430	0.274	1.000	0.383	0.016	0.025
	94	0.000	0.543	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EST-3	*100*	0.846	0.732	0.777	0.742	1.000	0.662	0.875	0.607	0.375	0.518
	96	0.154	0.268	0.223	0.258	0.000	0.177	0.125	0.383	0.609	0.482
	104	0.000	0.000	0.000	0.000	0.000	0.161	0.000	0.000	0.000	0.000
	94	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000
EST-6	*100*	0.648	0.300	0.500	0.565	0.585	0.000	0.500	0.035	0.078	0.167
	102	0.041	0.457	0.205	0.177	0.000	0.000	0.000	0.000	0.000	0.000
	104	0.311	0.243	0.295	0.258	0.415	1.000	0.500	0.965	0.922	0.833
LAP	*100*	0.500	0.628	0.312	0.433	0.208	0.790	0.860	0.651	0.984	1.000
	103	0.500	0.372	0.391	0.567	0.792	0.210	0.109	0.262	0.016	0.000
	105	0.000	0.000	0.297	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	98	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.087	0.000	0.000
6PGDH	*100*	1.000	0.690	1.000	1.000	1.000	1.000	1.000	0.991	0.969	0.948
	95	0.000	0.310	0.000	0.000	0.000	0.000	0.000	0.009	0.031	0.052
ACON	*100*	0.867	0.782	0.828	0.967	0.386	1.000	0.562	0.966	0.703	0.333
	98	0.133	0.064	0.155	0.000	0.273	0.000	0.000	0.000	0.016	0.100
	96	0.000	0.128	0.017	0.000	0.341	0.000	0.000	0.000	0.281	0.567
	102	0.000	0.026	0.000	0.033	0.000	0.000	0.438	0.034	0.000	0.000
PGM-1	*100*	0.757	0.190	0.823	0.481	0.833	0.937	0.154	0.956	0.577	0.607
	104	0.243	0.810	0.177	0.519	0.167	0.063	0.846	0.044	0.423	0.393
PGM-2	*100*	1.000	0.983	1.000	0.963	0.992	1.000	1.000	0.951	0.641	0.783
	103	0.000	0.017	0.000	0.037	0.008	0.000	0.000	0.049	0.359	0.217
ISDH	*100*	1.000	0.321	0.625	0.712	0.600	0.532	0.937	0.033	0.393	0.429
	94	0.000	0.679	0.375	0.288	0.400	0.000	0.063	0.967	0.607	0.000
	97	0.000	0.000	0.000	0.000	0.000	0.468	0.000	0.000	0.000	0.571
GPI	*100*	1.000	1.000	0.953	0.952	1.000	1.000	1.000	1.000	1.000	1.000
	96	0.000	0.000	0.047	0.048	0.000	0.000	0.000	0.000	0.000	0.000

[a] *EST* = esterase; *LAP* = leucine aminopeptidase; *6PGDH* = 6-phosphogluconate dehydrogenase; *ACON* = aconitase; *PGM* = phosphoglucomutase; *ISDH* = isocitrate dehydrogenase; *GPI* = glucose phosphate isomerase.

TABLE 2. Cavalli-Sforza and Edwards (1967) chord distances based on the data of Table 1 (below the diagonal) and linear geographic distance (kilometers) (above the diagonal) between all pairs of *Physa* populations (abbreviations refer to Fig. 1 and the Appendix).

	CTL	NPK	SNF	TVS	BVF	JNI	MUR	BEE	CMG	KIA
CTL	—	8.1	15.3	8.5	9.6	14.7	8.3	8.2	14.8	26.0
NPK	0.373	—	15.0	11.8	17.5	22.7	15.2	9.2	8.1	37.8
SNF	0.246	0.290	—	7.6	17.3	26.0	22.9	22.3	22.6	42.6
TVS	0.195	0.296	0.245	—	10.2	18.7	15.6	16.5	19.9	35.2
BVF	0.292	0.338	0.319	0.282	—	8.8	9.9	16.4	24.5	24.1
JNI	0.291	0.433	0.372	0.336	0.378	—	8.6	17.7	27.9	12.4
MUR	0.358	0.340	0.411	0.340	0.359	0.384	—	9.1	19.4	18.3
BEE	0.353	0.360	0.344	0.308	0.344	0.297	0.382	—	10.6	26.4
CMG	0.377	0.348	0.377	0.300	0.348	0.369	0.410	0.298	—	41.4
KIA	0.346	0.414	0.403	0.358	0.381	0.302	0.427	0.390	0.257	—

erozygote deficiencies within local *Physa* populations. Because Mendelian inheritance of isozyme phenotype has been previously demonstrated at most of the loci we examined, the phenomenon is likely evidence of nonrandom mating. In some cases, the study ponds were fairly large and heterogeneous, and hence heterozygote deficiencies may be attributable to unintentional lumping across spatial subdivisions (a Wahlund effect). Occasional cases of outcross sterility among laboratory-reared *P. heterostropha* known to be self-fertile have been described (Wethington and Dillon, 1993). There also is some evidence (Dillon and Wethington, unpubl.) of sporadic self-fertilization even by individuals known to have outcrossed, a phenomenon that has been reported occasionally for other pulmonate species (Rollinson et al., 1989; Jarne et al., 1993). Regardless of whether such self-fertilization represents a mixed mating strategy or simply some inefficiency in the internal fertilization process, this process will clearly contribute to heterozygote deficiency in a natural population.

Genic diversity was high between and within populations. Divergence seems significant among most populations at the more polymorphic loci (*EST-2, EST-6, LAP, ACON, PGM-1, ISDH*), given sample sizes often as high as 60 (Table 1). Such high levels of interpopulation divergence in allele frequencies at isozyme loci are a common feature of the population genetics of freshwater snails (Chambers, 1980; Stoddart, 1983; Brown and Richardson, 1988; Jarne and Delay, 1991).

However, none of the gene diversity between populations, F_{ST}, could be attributed to the island structure of the study area. The four pairs of populations sharing land masses were no more similar to each other than were populations generally. Substitution of $F_{ST} = 0.306$ into the equation of Slatkin and Barton (1989) produces an estimate of the effective number of migrants, $Nm = 0.57$. Under the assumptions of most neutral models, Nm values <1 indicate that gene flow is not sufficient to prevent substantial differentiation due to genetic drift.

Both genetic drift and sampling error have surely contributed to the levels of gene diversity reflected in Table 1. However, the significant correlation between measures of genetic divergence and geographic distance confirms that the genetic data contain more than noise and is consistent with a minor role for estuarine boundaries. Three scenarios (not mutually exclusive) could account for this correlation: selection, vicariance, and dispersal.

First, a positive correlation between genetic divergence and geographic distance may not result from varying gene flow but may instead arise from natural selection in environmental clines. Taken to an extreme, environmental variation over 40 km might generate selection differentials sufficient to account for the entirety of the genetic variance reflected in Table 1 with no barriers or restrictions to gene flow whatever. The role of natural selection in maintaining isozyme polymorphism has been the subject of intense interest for over 25 years, and the issue is far from resolved (Lewontin, 1991; Karl and Avise, 1992). Although a correlation between geographic distance and isozyme divergence was also detectable among 25 populations of the freshwater snail *Goniobasis proxima*, Dillon (1984) found no significant correlation between divergence and environmental difference measured directly from physical, chemical, and biotic variables and thus attributed the divergence/distance correlation to variance in interpopulation gene flow alone, independent of effects from natural selection.

A vicariance hypothesis to explain the correlation between geographic distance and genetic divergence would emphasize gene flow prior to the isolation of these land masses, perhaps during the last glacial period. Although the current distribution of *Physa* seems fragmented into discrete ditches, drains, and ponds, prehistorically the Charleston area was likely of rather uniform relief, characterized by extensive swamps and marshes. Especially aided by sheet flooding during times of heavy rain, the movement of freshwater snails may have been relatively unrestrict-

ed except by salinity. The rise in sea level over the last 10,000 years may have created sea islands where none had existed previously, and the range of *Physa* may have become artificially fragmented by farming and settlement.

A dispersal hypothesis to account for these data would emphasize the efficacy of gene flow over estuarine barriers, especially by such agents as birds. Malone (1965) experimentally documented the ease by which freshwater snails may become attached to the feet of waterfowl. Boag (1986) showed that especially very young snails may be drawn to plumage by surface tension and that a substantial fraction of attached snails may survive several minutes of flight. These results and the several documented field observations of *Physa* on the feet and bodies of waterfowl (Roscoe, 1955; Rees, 1965) suggest that aerial dispersal over estuarine barriers is a factor in the geographic distance/genetic divergence correlation.

The island structure of the Charleston area does not seem to have affected the genetics of *Physa* populations. Genetic distance is significantly correlated with linear geographic distance, and $F_{LT} \approx 0$. Neighboring populations separated by putative estuarine boundaries are not significantly more divergent than populations sharing the same land mass, which comprises a third line of evidence arguing against the biogeographic integrity of sea islands. The relative contributions of selection, vicariance, and dispersal to establishing gene frequencies in this complex situation cannot at present be determined, but if estuarine boundaries cannot be shown to influence freshwater snails, any such effects on the genetics or distribution of other elements of South Carolina's sea island biota also would seem unlikely.

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APPENDIX

Locality data and sample sizes for *Physa* populations; all sites are located in Charleston County, South Carolina.

BEE: Pond by Bees Ferry Road, 1.4 km from its intersection with Ashley River Road, 2 km NW of Pierpont. $n = 57$.

BVF: Pond at Bay View Farms development on James Island, 6 km W of Fort Johnson. $n = 32$.

CMG: Pond at Carolina Memorial Gardens, 3.5 km N of airport. $n = 32$.

CTL: Pond at visitor's center, Charles Towne Landing State Park, "west Ashley" section of Charleston. See Dillon and Dutra-Clarke (1992) for site description. $n = 70$.

JNI: Ditch in cultivated field near Jenkins Farm Road, 3.5 km NE of Legareville, Johns Island. $n = 31$.

KIA: Pond by Kiawah Beach Drive near golf course, across from "Straw Market," Kiawah Island. $n = 28$.

MUR: Pond by Murraywood Road, 0.5 km from its intersection with River Road, 4 km SE of Limehouse Station, Johns Island. $n = 32$.

NPK: Pond at Park Circle in the city of North Charleston. $n = 56$.

SNF: Pond at entrance to Snee Farm development, 3.2 km E of Mount Pleasant. $n = 47$.

TVS: Pond 0.85 km W of the Cooper River bridge, Mount Pleasant. $n = 31$.