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MOLECULAR GENETIC ANALYSIS OF A STEPPED MULTILOCUS CLINE IN THE AMERICAN OYSTER (*CRASSOSTREA VIRGINICA*)

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Abstract.—Gulf of Mexico versus Atlantic populations of several coastal species in the southeastern United States are known to differ sharply in genetic composition, but most transitional zones have not previously been examined in detail. Here we employ molecular markers from mitochondrial and nuclear loci to characterize cytonuclear genetic associations at meso- and microgeographic scales along an eastern Florida transitional zone between genetically distinct Atlantic and Gulf populations of the American oyster, *Crassostrea virginica*. The single- and multilocus cytonuclear patterns display: (1) a cline extending along 340 km of the east Florida coastline; (2) a pronounced step in the cline centered at Cape Canaveral (shifts in allelic frequencies by 50–75% over a 20 km distance); (3) a close agreement of observed genotypic frequencies with Hardy-Weinberg expectations within locales; and (4) mild or nonexistent nuclear and cytonuclear disequilibria in most local population samples. These results imply: (1) considerable restrictions to interpopulational gene flow along the eastern Florida coastline; (2) within locales, free interbreeding (as opposed to mere population admixture) between Gulf and Atlantic forms of oysters; and (3) localized population recruitment in the transition zone localities. These findings demonstrate that marine organisms with high dispersal potential via long-lived pelagic larvae can nonetheless display pronounced spatial population genetic structure, and more generally they exemplify the utility of pronounced genetic transition zones for the study of population level processes.

Key words.—Cline, cytonuclear genotype, gene flow, mtDNA, oyster, RFLP, RSP, scnDNA.

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The central and northeastern coast of peninsular Florida has long been recognized as transitional between temperate and subtropical maritime faunas (Briggs 1974). In the general vicinity of Cape Canaveral, roughly coincident species' distributional limits (e.g., in many fish and mollusk species) define a boundary between the Carolinian and Caribbean zoogeographic provinces. In some cases, particular species pairs with range limits that contribute to this regional zoogeographic pattern also have populations in the boundary region that display morphological or genetic intermediacy (Briggs 1958; Dillon and Manzi 1989; Bert and Arnold 1995; Duggins et al. 1995). In other cases, recognized species are distributed more or less continuously along the length of the Florida coast, yet their populations may display pronounced genetic differences between Atlantic versus Gulf of Mexico locales (tiger beetle, Vogler and DeSalle 1993; stone crab, Bert 1986; Bert and Harrison 1988; southern oyster drill, Liu et al. 1991), with the differences sometimes localizing to the east-Florida coastline (e.g., horseshoe crab, Saunders et al. 1986; ribbed mussel, Sarver et al. 1992). In several species, the genetic distances between Atlantic and Gulf populations are several-fold greater than those typically found within either the Atlantic or the Gulf regions. Collectively, these observations suggest that historical vicariant processes in addition to contemporary ecological factors have had a rather pervasive influence on the zoogeographic and population structures of many maritime taxa in the region (Avise 1992, 1996).

Some disjunct Carolinian taxa with relatively isolated conspecific populations in the Atlantic and Gulf of Mexico (e.g., black sea bass, Bowen and Avise 1990; seaside sparrow, Avise and Nelson 1989) show a pattern of Atlantic and Gulf genetic divergence roughly similar to that described above. In such species, contemporary environmental barriers to gene flow presumably help to maintain the deeper phylogeographic structures that appear to have been initiated by historical population separations. However, for eurythermal species continuously distributed around southern Florida, the roles of historical versus contemporary processes that shape population structure are less clear. Of particular interest in these continuously distributed species is how latitudinal ecotones in eastern Florida contribute to the maintenance or dissolution of any Atlantic/Gulf genetic differences. Despite the zoogeographical and environmental attributes that make Florida a "natural laboratory" for clinal variation, few studies have focused in meso- or microgeographic detail on the contemporary genetic, demographic, and ecological processes associated with transitional ecotone populations (Bert and Arnold 1995; Duggins et al. 1995)

The American oyster (*Crassostrea virginica*) provides an intriguing case study. Individuals of this species have a great capacity for dispersal via a several-week planktonic larval stage that precedes permanent settlement on a hard substrate (Korringa 1952). Perhaps related to this larval biology is the broad and nearly continuous distribution of this eurythermal species from New Brunswick, Canada to the Yucatan Peninsula, Mexico (Galtsoff 1964). Although physiological differences exist among oyster populations along this latitudinal gradient (Stauber 1950; Loosanoff and Nomejko 1951), no subspecific distinctions have been made based on these or on anatomical characters. Furthermore, a lack of significant structure among populations from Cape Cod to mid-Texas, as registered by polymorphic allozyme loci, reinforced the impression that this species' larval biology maintains gene flow at levels that retard population differentiation (Buroker 1983).

However, subsequent macrogeographic surveys based on mitochondrial (mt) DNA (Reeb and Avise 1990) and anon-

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ymous single copy nuclear (scn) DNA loci (Karl and Avise 1992) provided considerable evidence for genetic differentiation between Atlantic and Gulf populations of *C. virginica*. In addition, transitional populations along the mid-Atlantic coast of Florida displayed intermediate frequencies of these genetic characters in preliminary surveys. Thus, based on macrogeographic patterns, a working hypothesis has been that present-day marine currents, perhaps in conjunction with latitudinal selection pressures associated with water-mass differences, maintain a genetic distinction between Atlantic and Gulf oyster populations that probably was initiated by earlier vicariant population separations. However, the possibility also exists that genetic properties of the transitional populations evidence primary zones of selection rather than (or in addition to) secondary contact after allopatric differentiation (Endler 1977). An absence of pronounced differentiation at multiple allozyme loci (Buroker 1983) and at some other scnDNA gene regions (McDonald et al. 1996) raises the possibility that gene flow between the Gulf and Atlantic coastal regions has been (and may continue to be) reasonably high, such that natural selection would have to be invoked to explain the genetic differences at the geographically differentiated loci.

This study characterizes the geographic pattern of genetic transition between Atlantic and Gulf oyster populations in eastern Florida. To address the role of contemporary forces in the maintenance of the Atlantic/Gulf differences, we test two hypotheses (endpoints of a continuous spectrum of possibilities) about the reproductive and genetic interactions of individuals in this transitional area: (1) populations along eastern Florida might represent a noninterbreeding admixture of two cryptic species with distributions otherwise confined to the Atlantic and Gulf of Mexico, respectively; or (2) the transitional forms might show full reproductive compatibility. In the latter case, the geographic patterns of genetic variation under neutrality would be a function of gene flow rates and (assuming secondary contact) time since population joining. Other scenarios with genetic outcomes likely intermediate to those listed above include partial reproductive incompatibility and/or selection acting on individuals of mixed ancestry.

Materials and Methods

In eastern Florida, adult *C. virginica* occur only in the shallow, semiclosed estuarine lagoons that are connected to the Atlantic Ocean by passages between barrier islands (i.e., the conduit waters for the intracoastal waterway). Sampling sites (Fig. 1) included 18 locales spaced at roughly 40 km intervals that span the region previously suspected as genetically transitional for American oysters based on macrogeographic sampling (Karl and Avise 1992). Geographic sampling of eastern Florida adults was done in 1991, except for a 1995 collection of 38 oysters from Merritt Island. This study also includes analyses of 276 adult oysters collected at nine sites from Massachusetts to Louisiana in 1990 (Karl and Avise 1992). Sample sizes for all locales are available in Hare et al. (1996), and averaged 25 individuals per site (see also Table 1). To examine within-population aspects of variation in greater detail, at one locale (Sebastian, Florida) additional collections of 236 adults and 51 juvenile spat (3–10 mm shell diameter) were made in 1993 and 1994, respectively, near the original 1991 Sebastian site (i.e., 2 km distant, toward the mouth of Sebastian Creek).

To collect and preserve spat, clean oyster shells were deployed intertidally in February, retrieved in May, and kept on wet ice for $\leq$ 72 h until individual spat could each be transferred to a 500 μL centrifuge tube containing 10 μL of distilled, autoclaved water. Samples were then frozen in liquid nitrogen and stored at −70°C.

To develop a readily assayed mtDNA marker, we mapped some of the restriction sites described by Reeb and Avise (1990) from their total-mtDNA survey of restriction fragment length polymorphisms (RFLPs). This facilitated the cloning of a mtDNA fragment containing a *Bst*NI restriction site polymorphism (RSP). From the terminal sequences of this clone, two primers were designed (OY73a = 5′-GGAACCA-GAAAAATCTCGACC-3′ and OY73b =5′-AAATAGGT-TAGGGGGACTCAGC-3′) that amplified an 800 bp product from *C. virginica* total genomic DNA in polymerase chain reactions (PCRs). Using PCR to screen all of the Reeb and Avise (1990) samples with the OY73 primers and *Bst*NI, we confirmed that this RSP is diagnostic for the Atlantic and Gulf haplotypic clades described previously (data not shown).

A restriction site polymorphism also was assayed at each of two anonymous scnDNA loci as described in Karl and Avise (1992). Locus designations used here (CV-32.4 and CV-7.7) differ from those in Karl and Avise (1992, 1993) to indicate the current use of internal primers that amplify a smaller product containing the informative restriction site (see Hare et al. 1996). Observations were restricted to the CV-32.4 and CV-7.7 nuclear genes because, of the four loci described in Karl and Avise (1992), these have allele frequencies that best differentiate the Atlantic and Gulf oyster populations.

Adult oysters were returned live to the laboratory, where total genomic DNA was phenol extracted from gill and mantle tissue as reported in Karl and Avise (1992). DNA preparations from oyster spat involved thawing each sample, replacing the water with 10 μL of 10X PCR buffer (Promega), and incubating for 5 min at 95°C under mineral oil. After pelleting the tissue for 10 sec in a microcentrifuge, 1 μL of this crude DNA preparation was used for PCR amplification (adjusting buffer concentration to vendor recommendations). Nuclear DNA amplification procedures were as described in Hare et al. (1996). PCR amplification of mtDNA with the OY73a/b primers was similar except that $MgCl_2$ was at 2.5 mM concentration, and the 35 cycles each consisted of three 1-min steps (94°, 62°, and 72°C).

For each of the three loci (two nuclear, one cytoplasmic), a single RSP provided diallelic data. Contingency tests and 2 × 2 Fisher's exact tests were calculated using Statview (Abacus). Genotypic cytonuclear disequilibria were calculated and tested for significance with Fisher's exact test using a computer program written by C. Basten (Asmussen and Basten 1994). Composite gametic-phase nuclear disequilibria (Weir and Cockerham 1989) were calculated with the LD86 program of Weir (1991), and standardized to their maximum

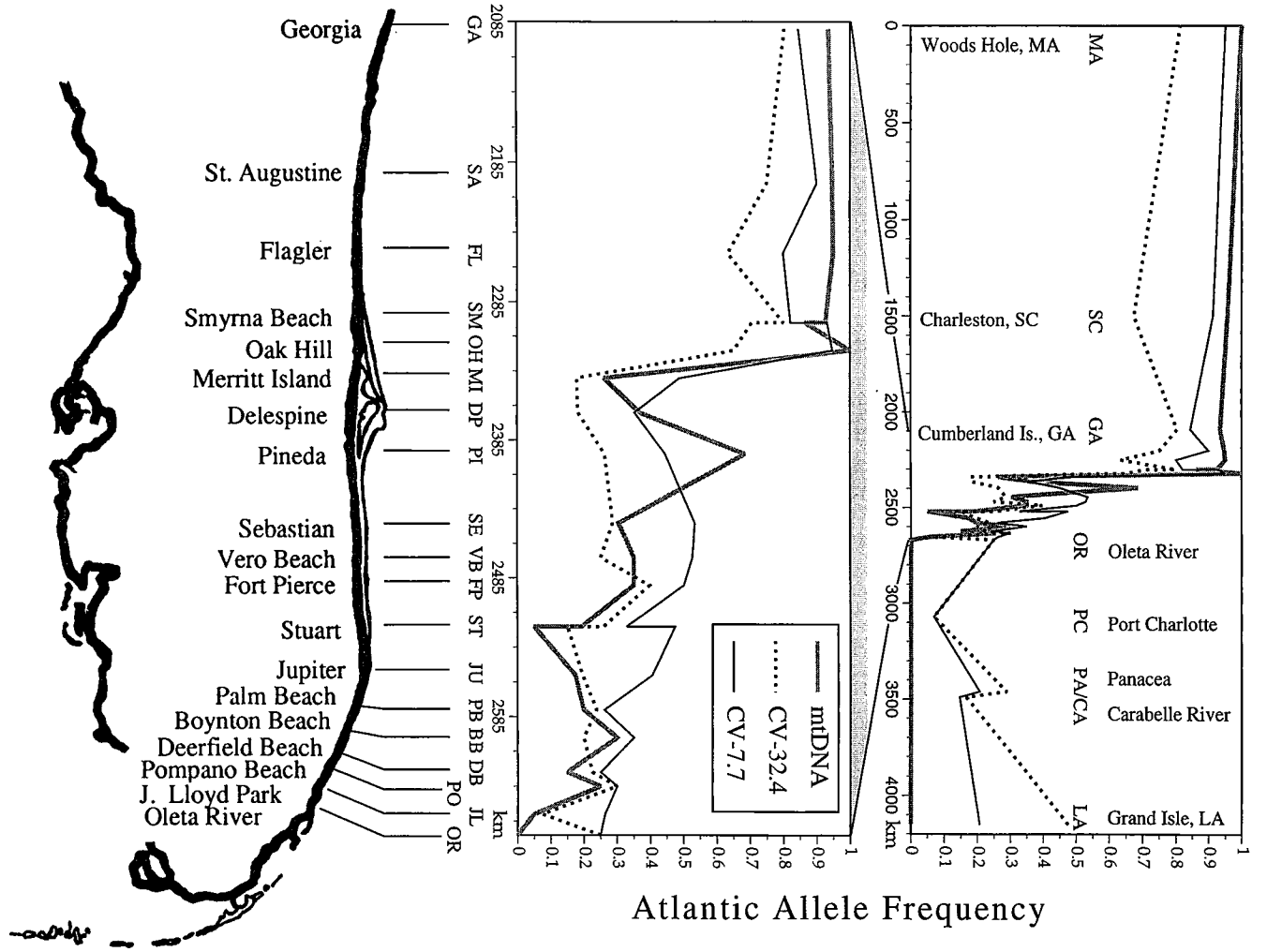

FIG. 1. Allelic frequency distributions for three RSPs from Massachusetts to Louisiana (top), an expanded view from Georgia to Miami (middle), and a map of Florida indicating collection localities along the intracoastal waterway.

or minimum possible values given the allele frequencies at the two loci (Lewontin 1964).

RESULTS

Static Observations

Geographic distributions of allele frequencies in adult oysters are shown in Figure 1 (genotype frequencies are available in Hare et al. 1996). From a broad geographic perspective (Fig. 1, top), the transition in allele frequencies is evident along the eastern Florida coast. The width of the oyster mtDNA cline was estimated at 340 km (95% support limits = 300–410 km) using the procedures of Szymura and Barton (1986; the genetic data were fit to a stepped cline using maximum likelihood in a computer program provided by N. Barton). At a mesogeographic scale (Fig. 1, middle), all three loci had coincident and major steps in allele frequency centered at Cape Canaveral between the Oak Hill (OH) and Merritt Island (MI) sites. Over this distance of 20 km, the frequency of the Atlantic allele at all three loci dropped precipitously (by 50–75%). Also apparent at the mesogeographic scale were spikes and reversals of gene frequency change. For example, after dropping in frequency from 1.0–0.26 between OH and MI, the frequency of the Atlantic mtDNA haplotype increased to 0.68 over the next 50 km to the south, and then reversed itself again over a similar distance further south (Fig. 1).

Due in part to these irregularities in the patterns of clinal change, the three loci had statistically distinguishable allele frequencies at 13 of the 27 locations sampled (three pairwise Fisher's exact tests at each locality, adjusting the significance level for multiple comparisons; Rice 1989). Nonetheless, the shifts in allele frequency in the transitional area were broadly consistent across the three loci. Outside of the area of pronounced clinal transition in eastern Florida, a degree of spatial concordance also was displayed by the two nuclear loci in the Gulf of Mexico, where roughly parallel increases in frequency of Atlantic alleles were exhibited in populations from Port Charlotte (PC) Florida to Louisiana (LA) (Fig. 1, top).

In agreement with Reeb and Avise (1990; but using independent samples), the two mtDNA haplotypes were alternately fixed in the Massachusetts and Gulf of Mexico collections (Fig. 1, top). However, in the current study based on

TABLE 1. Cytonuclear disequilibrium estimates and sample sizes for collections with nonzero marginal frequencies. Boldface values have $0.01 \leq P \leq 0.05$ without correcting for multiple tests. Locality names as in Figure 1, except numbers refer to year of collection and asterisks indicate collections from Karl and Avise (1992). Not all values within a locus are independent because $D_1 + D_2 + D_3 = 0$.

	CV-32.4				CV-7.7			
Locality	N	D_1	D_2	D_3	N	D_1	D_2	D_3
*SC	23	−0.03	0.03	0.00	23	0.02	−0.02	0.00
*GA	31	−0.03	0.03	0.00	30	−0.01	0.01	0.00
SA	19	−0.02	0.02	0.00	19	−0.01	0.01	0.00
FL	30	0.01	−0.01	0.00	29	0.02	−0.02	0.00
*SM-90	22	−0.02	0.02	0.00	22	0.05	−0.05	0.00
SM-91	27	0.02	−0.04	0.01	25	0.03	−0.03	0.00
MI	36	0.00	−0.02	0.02	38	−0.02	0.05	−0.03
DP	19	0.00	0.02	−0.02	17	−0.02	0.03	−0.01
PI	19	0.02	0.03	−0.04	18	−0.01	−0.07	0.08
SE-91	23	0.01	0.01	−0.02	23	0.02	0.01	−0.03
SE-93	235	0.20	0.09	−0.11	219	0.07	0.09	**−0.27**
SE SPAT	50	0.00	−0.07	0.06	49	0.03	**−0.08**	0.04
VB	20	−0.02	0.06	−0.04	19	−0.03	−0.06	0.09
FP	29	0.01	0.02	−0.03	29	0.07	−0.03	−0.04
*ST-90	20	0.00	−0.02	0.02	19	0.00	0.00	0.00
ST-91	27	−0.01	−0.02	0.02	27	−0.03	**0.08**	−0.05
JU	23	0.00	**0.11**	**−0.11**	21	−0.02	0.07	−0.05
PB	20	−0.02	0.04	−0.02	20	−0.02	−0.02	0.04
BB	20	0.00	−0.02	0.02	20	0.02	−0.02	−0.01
DB	20	0.00	−0.02	0.02	20	−0.02	−0.05	0.06
PO	20	−0.01	0.03	−0.01	20	0.04	−0.03	−0.01
JL	20	0.00	0.04	−0.04	19	0.00	−0.02	0.03

more and larger samples, mixtures of Atlantic and Gulf haplotypes were found in most collections from South Carolina to Miami, Florida. For example, a moderate proportion of Atlantic haplotypes was found in samples as far south as Miami. To the north of Cape Canaveral, Gulf haplotypes were never more than a minor proportion (0.14) of the samples. This asymmetry in the mtDNA cline was also observed with the CV-7.7 RSP (Fig. 1, middle). Both nuclear loci displayed wide tails on each end of the cline relative to mtDNA, and never reached alternate fixation.

Genotypic frequencies at the two nuclear loci did not differ significantly from Hardy-Weinberg expectations in any population sample (Hare et al. 1996). Fisher's method of combining probabilities (Sokal and Rohlf 1981) applied across localities also provided no evidence of an overall deviation. Although the power to detect Hardy-Weinberg departures in these tests was generally low due to the moderate sample sizes used (Ward and Sing 1970), genotypic frequencies in the large 1993 sample from Sebastian ($N = 235$ and $N = 220$ for CV-32.4 and CV-7.7, respectively) were nearly identical to Hardy-Weinberg expectations at both loci. The 1994 sample of oyster spat from Sebastian also conformed to Hardy-Weinberg genotype proportions ($N = 51$ and 49, $P = 1.0$ and 0.78 for CV-32.4 and CV-7.7, respectively).

For dilocus tests of gametic phase disequilibrium, reasonable statistical power to reject the null hypothesis ($D = 0$) when it is false was provided only by the 1993 Sebastian sample of adults. In this large sample ($N = 220$), genotypes at the two nuclear loci were not independent by contingency analysis ($\chi^2 = 15.3$, $P = 0.004$). In contrast, Sebastian spat had dilocus frequencies not significantly different from independence expectations ($N = 49$, $\chi^2 = 4.2$, $P = 0.38$). The same results were obtained using Weir and Cockerham's (1989) composite digenic disequilibrium estimates, shown (standardized) in Figure 2 for all samples across localities.

Although meaningful dilocus tests of gametic phase disequilibrium require larger sample sizes than were analyzed in this study from most Florida locales, the possibility of spatial patterning among samples in the disequilibrium sign estimates can be examined. For example, the tension zone model of hybrid zone dynamics predicts positive gametic phase disequilibrium (i.e., an excess of parental allelic combinations) in the center of the zone due to the dispersal and admixture of parental genotypes into this area and selection against hybrids (Barton and Hewitt 1985). From this perspective, the pattern of excess parental types (positive D) in the 1993 sample of Sebastian adults is not strongly registered across localities in the transitional zone in eastern Florida (Fig. 2). Instead, both excesses and deficits of parental genotypes (none significant) characterized these smaller collections.

Chi-square contingency tests of mtDNA versus nuclear genotype frequencies were also conducted, and failed to reject the null hypothesis of independence between the mtDNA haplotype and nuclear genotype at either locus in the large 1993 Sebastian sample (both $P \geq 0.09$). Fisher's exact tests of the null hypothesis $D_x = 0$ were then performed for three cytonuclear disequilibrium parameters, D_1, D_2, and D_3, corresponding to mtDNA haplotype associations with the Atlantic-type homozygote, heterozygote, and Gulf-type homozygote, respectively (Asmussen et al. 1987; Asmussen and Basten 1994). At the Sebastian locale, significant mtDNA associations were observed only with the CV-7.7 locus, and only for D_3 in the 1993 adult sample and for D_2 in 1994 spat ($P = 0.04$ in each case; Table 1). Overall, there are few D_x-values significantly different from zero, and these all become

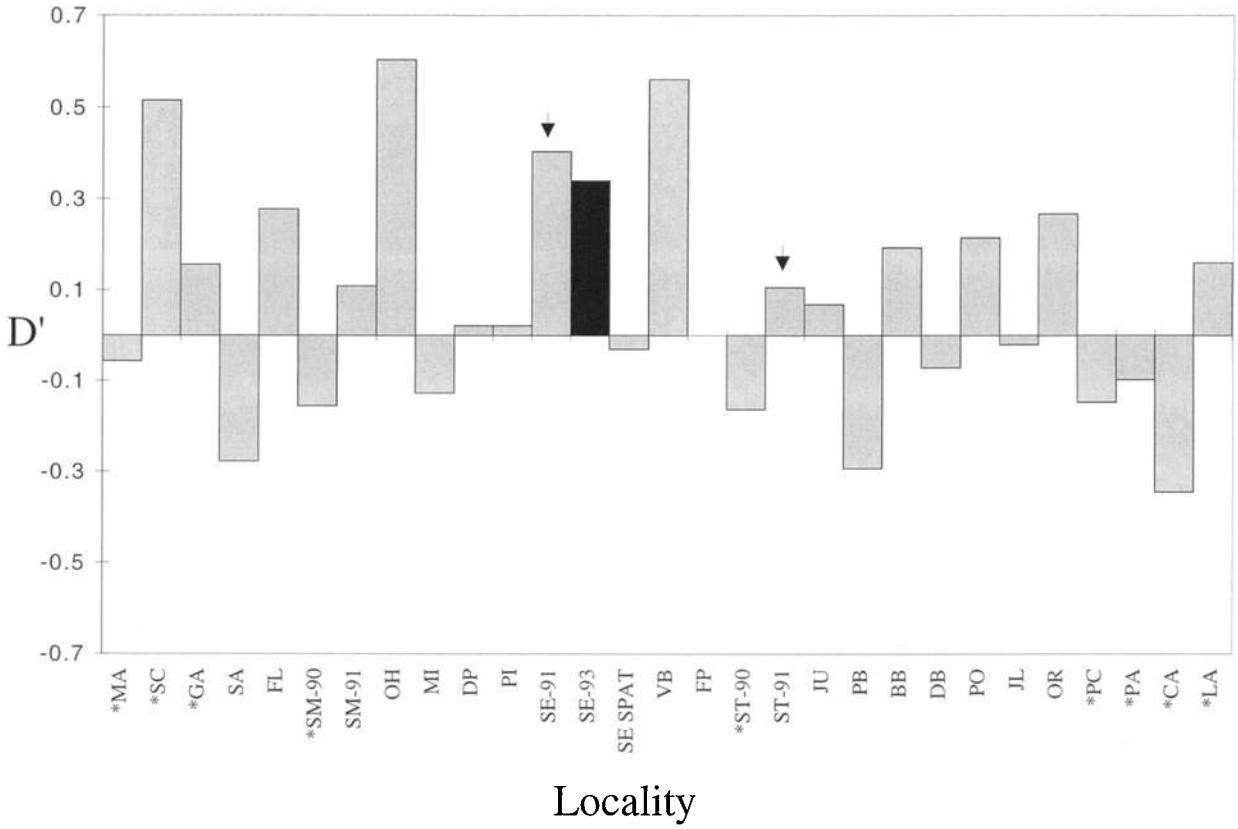

FIG. 2. Standardized digenic nuclear disequilibrium (D') values for all collections of the American oyster from Massachusetts to Louisiana. Significant ($P \leq 0.05$) digenic disequilibrium is shown in black, and arrows above columns indicate significant tri-and/or quadra-allelic disequilibria. All statistical tests are nonsignificant at the tablewide Bonferroni alpha level of 0.0017 (SE-93 $P = 0.0028$). Locality abbreviations as in Table 1.

nonsignificant with Bonferroni correction across localities. Furthermore, there appears to be no consistent pattern to the sign of cytonuclear disequilibria across localities (Table 1), as might otherwise be expected from an excess of parental types or asymmetrical introgression.

Temporal Observations

For a comparison of gene frequencies over time, the greatest ability to detect an effect of gene flow would be at the steepest part of the cline (OH–MI). Unfortunately, attempts to collect spat in this area were unsuccessful (oysters are rare in this area, see Discussion). Instead, we examined temporal change at the nearby Sebastian locale because it is geographically bounded by two adjacent collections that had very different 1991 adult mtDNA allelic frequencies (Fig. 1). At Sebastian, nearly identical allele frequencies were observed in 1993 adults and 1994 spat (Fig. 3). The adult sampling site in 1991 was not identical to that used for these later Sebastian collections (see Materials and Methods), thus partially confounding assessments of spatial and temporal heterogeneity. (Also, the mean shell size of oysters in the two collections of adults differed significantly; 1991 mean = 68.8 mm, $s = 14.1$; 1993 mean = 55.5 mm, $s = 14.1$; $t = 4.41$, $P \ll 0.001$.) Nonetheless, no significant differences were found in allele frequencies between any Sebastian collection for any of the three loci over this three year interval (Fig. 3; 2 x 3 contingency test and pairwise Fisher's exact tests within loci).

DISCUSSION

We have shown that a pronounced genetic transition between Atlantic and Gulf of Mexico oysters previously reported in macrogeographic surveys localizes upon meso- and microgeographic analysis to a dramatic step cline centered at Cape Canaveral on the east Florida coast. In accounting for this pattern, by hard criteria the current data cannot discriminate between scenarios of primary selective influence along an environmental gradient versus secondary contact between allopatrically differentiated populations. In principle, both situations can result in concordant spatial clines across independent loci and sharp genetic transitions (Endler 1977). Furthermore, the two scenarios need not be mutually exclusive, for environmental selection along an ecotonal gradient can reinforce clinal patterns established through secondary contact of differentiated populations (Bert and Arnold 1995; Arnold in press). Nonetheless, a case for proceeding here under a working hypothesis of secondary contact is provided by independent phylogeographic and geologic evidence for historical population separations involving multiple maritime taxa along this same coastline in the southeastern U.S. (Avise 1996). A provisional assumption that these transitional oyster populations along Florida's east coast result

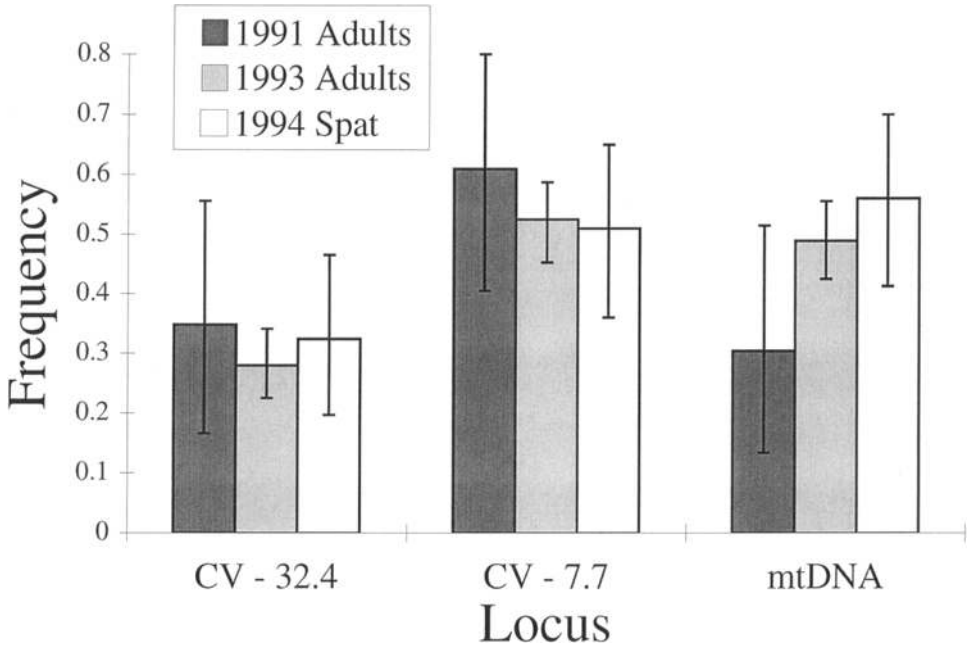

FIG. 3. Comparison of three collections at the Sebastian locale for allelic frequencies at three loci. Error bars show 95% confidence intervals. Only the 1993 and 1994 collections were made at exactly the same site.

from secondary contact also permits two heuristically useful scenarios to be critically evaluated.

Admixture versus Random Mating

Three observations of *C. virginica* made prior to this study gave plausibility to a hypothesis of population admixture (as contrasted to an alternative of full reproductive compatibility and random mating) in the genetic transition zone. First, numerous congeneric species pairs have parapatric or narrowly sympatric coastal juxtapositions along this coast (Briggs 1958; Frey 1965; Shipp and Yerger 1969; Duggins et al. 1986, 1989). Second, the spatial position of the changeover in the genetic composition of oysters was generally coincident with the northern and southern range limits for many subtropical and warm-temperate maritime species (Briggs 1974). Third, in preliminary sampling the oyster cline appeared steep relative to the capacity for dispersal inherent in this species' life history. Thus, a narrow zone of admixture might be due to competitive exclusion or differential selection between two overlapping species, or perhaps to long distance dispersal of larvae into demographically suboptimal ("sink") habitats (Pulliam 1988) from peripheral, reproductively incompatible "source" stocks in the Atlantic and Gulf of Mexico.

The genetic data presented in this report eliminate such admixture scenarios for the American oyster in eastern Florida, and instead are more consistent with the view that Atlantic and Gulf oysters are fully compatible reproductively. Support for this conclusion comes from: (1) the lack of significant departures from Hardy-Weinberg equilibrium in local population samples (heterozygote deficits would be expected to result from admixture due to a Wahlund effect); and (2) a lack of consistent pattern in dilocus disequilibrium for marker alleles and genotypes within collections from the transition zone. (It should be noted that with similar sample sizes, statistically significant disequilibria have been reported in hybrid zones of some other species, e.g., Barton 1982; Kocher and Sage 1986; Howard and Waring 1991; Scribner and Avise 1993.) Although significant nuclear and cytonuclear disequilibria were observed in some of our oyster population samples, the associations overall were modest and their directions not maintained across localities or years. Thus, deviations from random mating and/or selection against hybrids do not appear to be overriding phenomena in the transition zone.

In the large sample of Sebastian adults, predictions of the pure admixture versus random mating models with respect to the observed dilocus genotypic counts can be examined more critically (Fig. 4). Strictly speaking, both scenarios are statistically rejected overall ($\chi^2 = 14.9$, $P < 0.005$ for random mating; $\chi^2 = 212.1$; $P \ll 0.001$ for admixture), with empirical outcomes for particular genotypic classes often intermediate between expectations of the two models. For example, mild excesses were observed in the counts of "parental" genotypes (AA/BB and aa/bb) over random mating expectations, but strong deficits existed relative to strict admixture predictions. Among particular "nonparental" genotypic classes, deviations of genotypic counts above (e.g., Aa/Bb) and below (AA/Bb) both of the model's expectations raise the possibility of genotype-specific selection as well. Nonetheless, overall the empirical results far more closely approximate the expectations of random mating than they do those of simple population admixture.

Location and Steepness of the Step Cline

Under a "tension zone" model of secondary population contact and hybridization, the equilibrium shape of a cline is determined by a balance between the dispersal of parental types into the zone and selection against their hybrid progeny

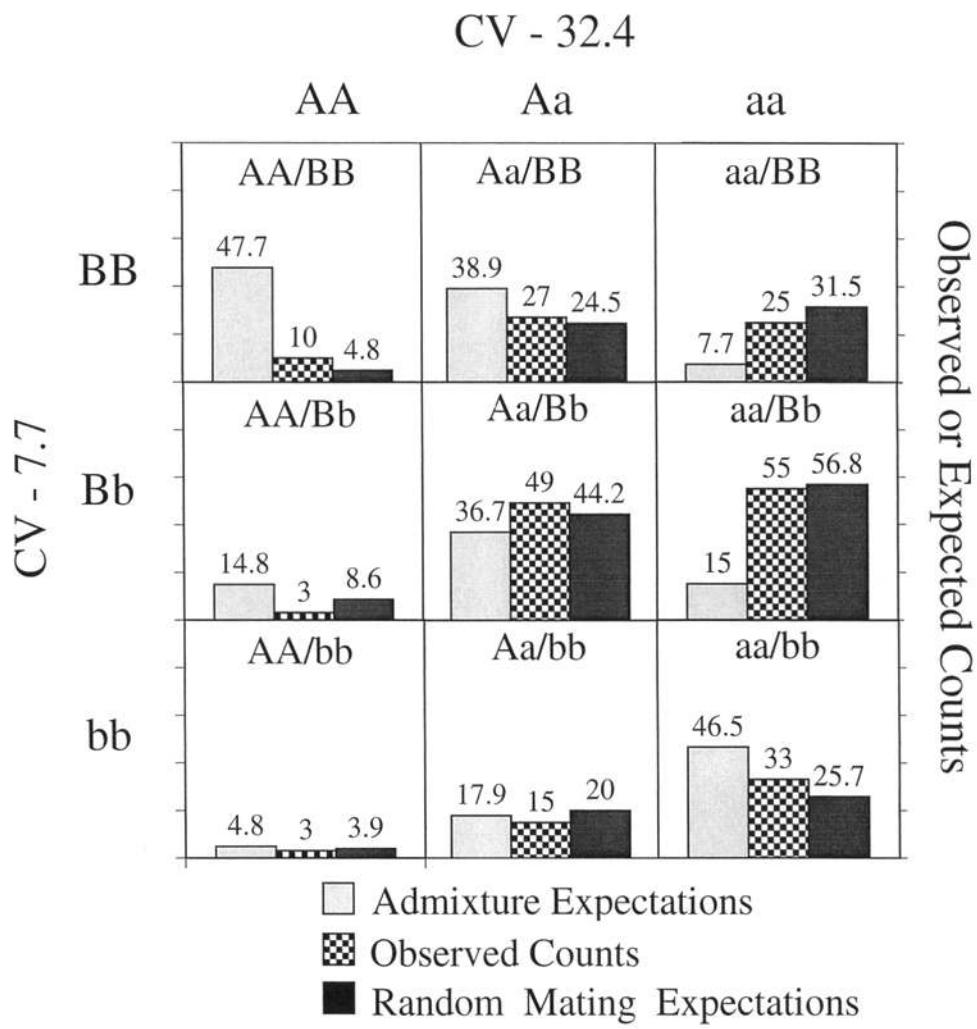

FIG. 4. Observed counts for the two-locus genotypic classes in the 1993 Sebastian collection of adult oysters, compared in a nine-cell contingency table format to expected counts based on random mating and admixture models. Random mating expectations were generated using Hardy-Weinberg expected genotypic counts from Sebastian in the marginals. Admixture expectations were constructed with a migration rate for Atlantic alleles into Sebastian defined from the mtDNA data: $m_A = (p_S - p_G) / (p_A - p_G)$, where $m_A + m_G = 1$, p_S = the mean of 1993 and 1994 Sebastian mtDNA allele frequencies, p_G = the mean Gulf (OR -LA) allele frequency, and p_A = the mean Atlantic (GA -OH) allele frequency. Normalizing to the total sample size ($N = 220$) available for digenic comparisons at Sebastian, observed counts from an Atlantic 3 × 3 contingency table were combined with those in a Gulf table using the migration rate determined from the equation above ($m_A = 0.56$). Chi-square values for the random mating and admixture models, when tested against observed counts, were 14.9 and 212.1, respectively (both $P < 0.005$).

(Barton and Hewitt 1985). The present data cannot exclude the possibility of weak or moderate selection against "hybrid" genotypes (breeding and transplant experiments would be required to further investigate this possibility). Nonetheless, the approximation to random associations of alleles within and between loci observed for local population samples suggests that selection against hybrids is not a formidable force molding within-population variation in this contact region. Given the steep latitudinal allele frequency change in oyster populations, this in turn implies, under tension zone theory, that secondary contact was recent and/or that impediments exist to dispersal and gene flow through the contact zone.

With regard to the second possibility, dispersal distances realized by planktonic veligers depend on larval behavior and physical hydrography. Along the eastern Florida coast, *C. virginica* spawns from approximately April to August (Butler 1953). Perhaps *C. virginica* larvae are often entrained within their natal estuary (Scheltema 1975; Mann 1988). American oyster populations in the Gulf of Mexico, for example, do show detectable genetic differences geographically (Groue and Lester 1982; Buroker 1983; Grady et al. 1989; King et al. 1994), raising the possibility of inhibitions to gene flow in this area. In the transitional eastern Florida region, the pronounced genetic differences on a mesogeographic scale in conjunction with the genetically inferred near-random mating within locales strongly implies highly localized oyster recruitment along this shoreline. The physical and ecological mechanisms of local entrainment in contemporary time must, however, be further assessed by direct field studies of larval movement to complement the perspectives provided by these genetic analyses.

In addition to estuarine entrainment, impediments to dispersal can result from local hydrographic barriers or patchy habitat conditions. The eastern Florida intracoastal waterway consists of a series of lagoons where currents are largely driven by wind and by tides entering and ebbing through 11 major oceanic inlets (Sternberger 1983; Smith 1993a). This nearly closed, linear coastal configuration may amplify the effects of dispersal barriers produced by habitat or hydrographic anomalies. The greatest distance between two oceanic inlets in eastern Florida, 150 km, spans the Mosquito, Banana, and Indian River lagoons behind Cape Canaveral (Fig. 5). Between these two inlets tidal ranges are minimal and currents resulting from wind shear are weak (Smith 1987, 1993b). In Mosquito Lagoon, lower *C. virginica* areal coverage to the south was strongly correlated with a diminishing tidal range (Grizzle 1990), and very low population densities characterize the entire Cape Canaveral lagoon system (Futch 1967; pers. obs. MPH). Thus, these lagoons may be poor habitat for adult oysters as well as poor contributors to larval "stepping-stone" transport. These observations suggest that local environmental factors likely play an important role both in the positioning of the step cline at Cape Canaveral, and in molding contemporary genetic features of the transitional populations. Of course, the broader evolutionary theater that set the stage for these local and contemporary population genetic forces no doubt includes historical events (such as vicariant separations and secondary contact) and macrogeographic influences (such as the Gulf Stream).

Timing of Secondary Contact

With regard to the possibility of recent contact of oyster populations in eastern Florida, the Cape Canaveral lagoons were not a continuous waterway until construction of the "Haulover Canal" between the Mosquito and Indian River Lagoons in the mid-1860s (Fig. 5). Prior to that time, the presence of a 2 km wide spit of land prevented any contact between oyster populations north versus south of this latitude, perhaps excepting transport by occasional hurricane storm tides or oceanic larval exchange via inlets. The fate of larvae exiting southern Florida inlets is speculative, but the major northbound current, the Gulf Stream, normally veers offshore near Cape Canaveral (Atkinson et al. 1983), making deposition of larvae at suitable habitats to the north unlikely. The near-shore hydrography in northeastern Florida is seasonal and variable, discouraging predictions about the fate of larvae exiting inlets north of the canal (Bumpus 1973; Stapor 1980; Weber and Blanton 1980).

In any event, both the historical Haulover-spit barrier and the contemporary low tidal flux are no doubt ephemeral features of the Cape Canaveral lagoons given the dynamic nature of many barrier island coastal landforms over geologic time. Multiple previous episodes of north-south contact in oysters probably have occurred at different locations along eastern Florida as hurricanes and sediment deposition changed the configuration of barrier islands along the coast. Indeed, this is one possible explanation for the deviation of the allelic cline in eastern Florida oysters from a smooth sigmoidal curve: Some departures may represent relict signatures of past contact events. Alternative possibilities include heterogeneous or sporadic effects of selection along the cline, and stochastic or idiosyncratic demographic events such as occasional long distance dispersal (Nichols and Hewitt 1994). Evaluation of these alternatives will require further temporal sampling of oysters.

Cline Shape and Introgression

In a zone of secondary contact, the cline shape of a diagnostic locus can be informative about introgression, and variation in that shape across genes can in theory be used to infer the relative intensities of selection on particular loci (Slatkin 1973; Barton 1983). Unfortunately for these purposes, neither of the two nuclear markers used in this study proved completely diagnostic for the Atlantic and Gulf populations of oysters. Thus, the fact that both nuclear loci have Atlantic alleles at moderate frequency in the Gulf could result from: (1) considerable introgression relative to mtDNA; (2) mutational convergence to an Atlantic RSP phenotype on a Gulf allelic background; (3) the parallel retention (and drift) of an ancestral polymorphism; or (4) similar selective gradients for the two loci.

The macrogeographic discordance between the diagnostic mtDNA and clinal nuclear allelic distributions could also, in principle, be due to selection against mtDNA on heterologous nuclear backgrounds. Under this hypothesis, cytonuclear incompatibilities would have developed such that the Atlantic and Gulf oysters cannot exchange cytotypes without incurring selective costs (regardless of environmental gradients). The mtDNA distributional asymmetry (Gulf mtDNA alleles are in low frequency when found north of the step cline, whereas Atlantic alleles are at moderate frequencies south of the step) suggests, under this hypothesis, that selective costs may be greater for the Gulf cytotype on Atlantic nuclear backgrounds. Asymmetrical cytonuclear incompatibilities have been reported from other hybridizing taxa (reviewed in Arnold 1993). However, if selection was an important evolutionary force shaping the mtDNA cline in *C. virginica*, a stronger signal of cytonuclear disequilibria might be expected than was observed in the transitional zone. Furthermore, all of the previously suspected examples of asymmetrical mtDNA-nuclear incompatibilities involve either asymmetries in mating patterns or cytoplasmic male sterility (Arnold 1993). Neither phenomenon would appear to be as likely in *C. virginica*, which is a sequential (protandrous) hermaphrodite (Galtsoff 1964).

Because of nearly fixed differences in haplotype frequency between Atlantic and Gulf populations, the mtDNA molecule becomes singularly informative about gene flow regimes under a strictly neutral model. Then, assuming Cape Canaveral is an original location of secondary contact, the geographic asymmetry in the mtDNA cline could result from different strengths of dispersal vectors moving larvae north versus south (Nichols and Hewitt 1994). In other words, northward water movements (e.g., the Gulf Stream) appear to have been less effective in moving oyster larvae past Cape Canaveral than have been opposing vectors in dispersing oysters southward of this region. However, other scenarios certainly are possible. For example, if a secondary contact originally occurred in south Florida, then the asymmetry would suggest

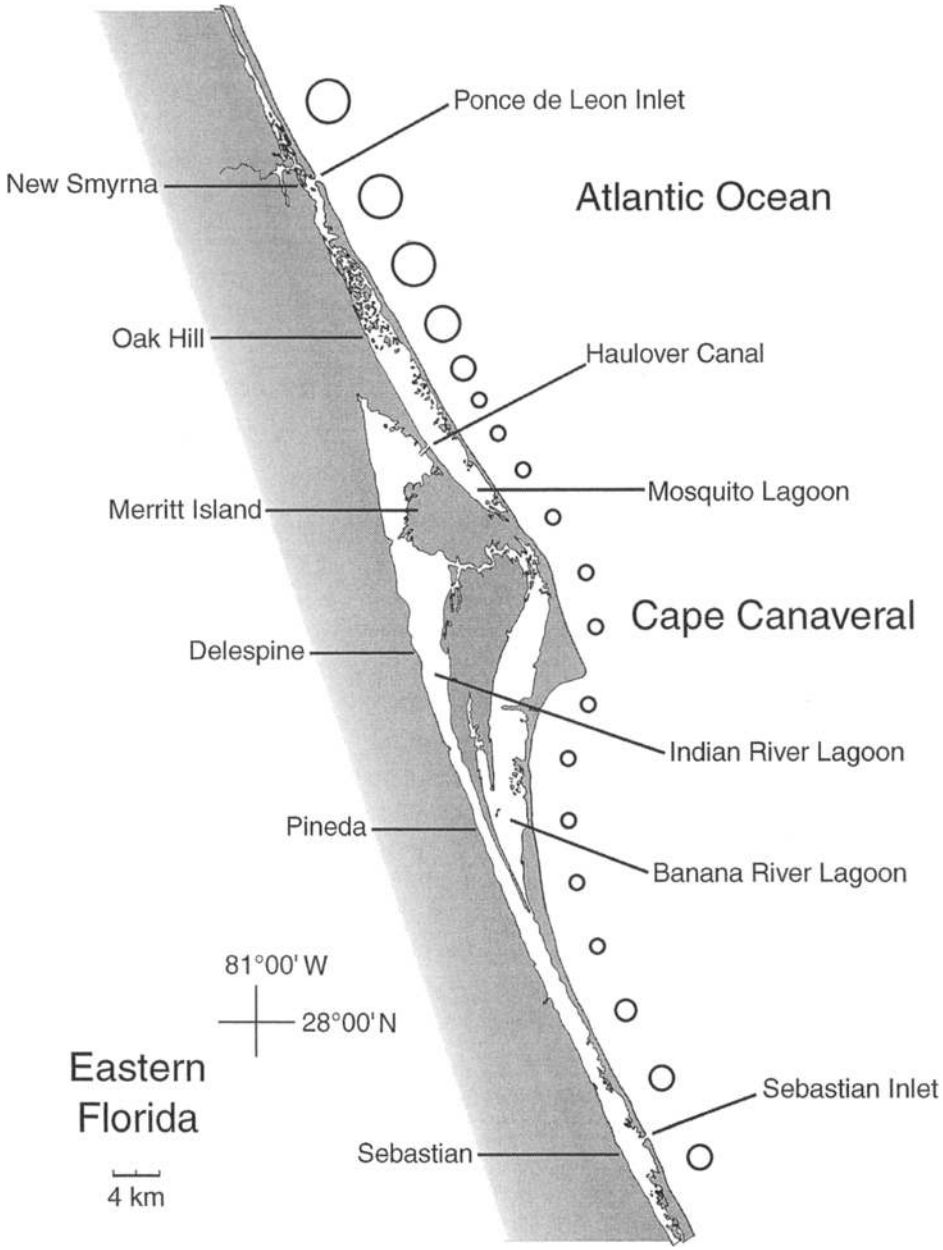

FIG. 5. Map of the Cape Canaveral coastline showing the oyster collection localities and the Haulover Canal. Circles (drawn in the ocean for ease of viewing) provide diagrammatic representations of relative oyster densities in the relevant adjoining lagoons of the intracoastal waterway.

that the contact zone (or "tension zone"; Barton and Hewitt 1985) has moved northward until halted (and perhaps steepened) by the conditions existing at Cape Canaveral. In this scenario, the Gulf Stream might have been one of the dispersal agents for oyster larvae, and thereby a contributor to the movement of the tension zone northward.

Genetic Transitions in Coastal Florida Ecotones

It is of interest to review two other cases in which genetically transitional populations have been examined in microgeographic detail along the eastern Florida coast. The hard clam *Mercenaria campechiensis* occurs in the Gulf of Mexico and in nearshore open-ocean waters along the Atlantic coast, whereas a related species *M. mercenaria* occupies inshore embayments and estuaries along the Atlantic coast. In a restricted area of the northern Indian River (between the Haulover Canal and Sebastian Inlet), hybrid populations between these species have been described on the basis of morphological and allozyme characters (Dillon and Manzi 1989; Bert and Arnold 1995). In this area near Cape Canaveral, strong evidence for selection against hybrids was documented, and found to be environment related for some hybrid genotypes and independent of environmental conditions for others (Bert et al. 1993; Bert and Arnold 1995).

The coastal killifish *Fundulus majalis* occurs along the Atlantic coast from New Hampshire to northeastern Florida

where it is replaced by *F. similis*, which occurs from there southward as well as along the coastline of the Gulf of Mexico. In the northeastern Florida contact area of these species a sharp step cline was reported for allozyme allele frequencies (Duggins et al. 1995). In the Matanzas River (part of the brackish intracoastal waterway), allozyme frequencies shifted dramatically over a 30 km distance at a location approximately 100 kms north of Cape Canaveral. The general absence of single- and multilocus disequilibrium in local samples of *Fundulus* supported the hypothesis that there are no intrinsic (i.e., genetic) barriers to hybridization. Instead, geographic coincidence of the step cline with an ecotonal transition between salt marsh and mangrove habitats led the authors to favor environmental selection as an explanation for the clinal shift.

Thus, exogenous environmental selection (*Fundulus*), endogenous genetic selective forces, or both (*Mercenaria*) have been identified as important factors limiting introgression in contact zones in northeastern Florida for other coastal taxa. In the transitional populations of *C. virginica* along the east Florida coast, we have uncovered little population genetic evidence for overt incompatibilities between Atlantic and Gulf genomes. Thus, hydrographic and physiographic barriers to gene flow must be instrumental in maintaining population genetic distinctions in this area. Although the precise role of the ecotonal variation in the transition zone in eastern Florida is not entirely clear from current data, the genetic patterns observed have illuminated aspects of the contemporary biology and recruitment dynamics of oysters that suggest that local environmental factors can shape microgeographic population structure to a degree that is remarkable given the species' life history.

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