

MINIMAL HYBRIDIZATION BETWEEN POPULATIONS OF THE
HARD CLAMS, *MERCENARIA MERCENARIA* AND
MERCENARIA CAMPECHIENSIS, CO-OCCURRING IN
SOUTH CAROLINA

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ABSTRACT

The Indian River of Florida is the only region where the natural co-occurrence of *Mercenaria mercenaria* and *M. campechiensis* has been documented prior to this report. In the Indian River, interspecific hybridization is extensive. Here I report that *M. campechiensis* is present at a frequency of about 0.1% in large beds of *M. mercenaria* inhabiting a tidal creek near Charleston, SC. Collections from a commercial belt-harvester yielded a total of 27 pure *M. campechiensis* and only 6 hybrids, as judged using shell morphology and isozyme frequencies at 4 diagnostic loci. Reproductive isolation is clearly more complete in South Carolina than previously observed in Florida. I suggest that the two species may spawn in response to differing environmental cues in South Carolina, and that such cues may weaken as seasonality diminishes further south.

Longstanding questions remain about the relationship between the two nominal taxa of commercially-valuable hard clams, *Mercenaria mercenaria* (L.) and *Mercenaria campechiensis* (Gme.). The distinguishing characters have traditionally been minor, although fairly consistent, differences in shell morphology (Abbott, 1974). The "northern hard clam," *M. mercenaria*, may be recognized by its more laterally-compressed shell, having thin concentric ridges that are easily eroded to leave smooth patches, and interior regions of purple nacre. In addition to its more rounded shell, the "southern hard clam," *M. campechiensis*, has thicker ridges and pure white nacre. There is considerable intrapopulation variation in these characters for both species, however.

A thorough review of *Mercenaria* distribution is provided by Menzel (1989). *Mercenaria campechiensis* is common in shallow water throughout the Gulf of Mexico and on the Atlantic coast of Florida. Dillon and Manzi (1989b) have demonstrated that Gulf of Mexico populations previously referred to as "*M. mercenaria texana*" are a subspecies of *M. campechiensis*. It occurs in deeper water offshore as far north as New Jersey. Specimens of *M. campechiensis* are occasionally retrieved off the coast of Charleston by divers.

Although the range of *M. mercenaria* broadly overlaps that of *M. campechiensis*, some authors have in the past questioned whether they are ever truly sympatric (Humphrey and Crenshaw, 1989). *Mercenaria mercenaria* inhabits shallow waters of the Atlantic and its estuaries from the Gulf of St. Lawrence to Florida. It is not reported offshore. There is some recent mitochondrial DNA evidence of hybridization between the two species in the vicinity of Oregon Inlet, North Carolina (Brown and Wolfinbarger, 1989). But the Indian River, a 150-km lagoon on the Atlantic coast of Florida, is the only region in which the actual co-occurrence of the two species has been critically examined, prior to this report (Dillon and Manzi, 1989a; Marelli and Arnold, 1990).

The two species readily hybridize in culture, and both F₁ and F₂ hybrids are fully viable and fertile (Menzel, 1977, 1989). Thus it was not surprising to find that a sample of 179 *Mercenaria* from the Indian River contained at least 10%

hybrids, judging by isozyme criteria, and possibly as many as 88% hybrids, if morphology is considered as well (Dillon and Manzi, 1989a). But disequilibrium between isozyme alleles and shell morphology in the Indian River is striking. Individuals with more compressed, smoother, more purple shells very significantly tend to carry *M. mercenaria* isozyme alleles, and a similar phenomenon is noted for *M. campechiensis*. Thus Dillon and Manzi characterized the Indian River as a "hybrid zone" between two species showing some, obviously limited, reproductive isolation.

Strictly speaking, hybrid zones are "narrow regions in which genetically distinct populations meet, mate, and produce hybrids" (Barton and Hewitt, 1985, 1989). Therefore, in the absence of data documenting more complete reproductive isolation between populations co-occurring elsewhere, our designation of the Indian River as a hybrid zone was premature. In late 1988, my attention was called to the rare occurrence of *M. campechiensis* in large beds of *M. mercenaria* inhabiting a tidal creek near Charleston, South Carolina. Here I report much greater reproductive isolation between these two populations than previously documented in Florida.

MATERIALS AND METHODS

The Isle of Palms, 13 km east of Charleston, SC, is bounded on the west by Hamlin Creek (32°47'N, 79°47'W). The clam populations examined in this study inhabited the creek about 1.7 km from its opening to the ocean at Breach Inlet. Absent any significant freshwater source, salinity at bottom almost certainly never drops below oceanic. On 14 December 1988, I obtained 10 individual clams, apparently pure *M. campechiensis*, from a commercial clammer operating a belt-harvester subtidally in Hamlin Creek. He estimated that *M. campechiensis* comprised at most 0.1% of the clams he had harvested. On 20 December, I accompanied him on a return trip to the same clam bed, during which time we harvested approximately 10,000 individual clams from a region of about 1 hectare. We watched the belt carefully, and set aside all clams with any *M. campechiensis* character. By the end of the day we had collected 20 clams which, in shell shape and ridging, appeared to be pure *M. campechiensis*. We also set aside the 30 individuals which appeared most likely to be hybrids. Most of this sample of 30 were purely *M. mercenaria* in ridging, although perhaps more laterally rounded. A few of the sample of potential hybrids did, however, show clear mixtures of traits.

I recorded data on shell nacre color and ridging for these 50 individuals, plus the 10 individuals previously collected, following the criteria of Dillon and Manzi (1989a). Shell ridging was scored as either thick, thin, or intermediate. (This has been found to be highly diagnostic for pure populations.) Nacre color was judged either white, purple, or intermediate (almost entirely white, but with a small amount of purple near the rim). These shells have been deposited as voucher specimens in the collection of the Academy of Natural Sciences of Philadelphia.

Samples of siphonal muscle were taken from all 60 individuals for horizontal starch gel electrophoresis. Details of the techniques employed are described elsewhere (Dillon, 1985; Dillon and Manzi, 1987). Although pure populations of the two hard clam species show at least statistical differences at almost all polymorphic loci examined to date, the differences are most striking at two loci encoding phosphoglucosyltransferase (*PgmF* and *PgmS*), and single loci encoding glucose phosphate isomerase (*Gpi*) and superoxide dismutase (*Sod*) (Pesch, 1974; Humphrey and Crenshaw, 1989). These loci contain "diagnostic" alleles, present in one species but absent from the other.

The genotypes and shell morphologies for the 60 Hamlin Creek individuals were compared to expectation from pure "reference" populations of *M. mercenaria* from a tributary of the Stono River 15 km south of Charleston and *M. campechiensis* from the Gulf coast of Florida (Dillon and Manzi, 1989a). Alleles diagnostic of *M. mercenaria* have been found to include *Gpi 100* and *PgmS 103*, while those diagnostic of *M. campechiensis* include *Gpi 50*, *PgmS 95*, *PgmF 97*, and *Sod 80*. On this basis, the 60 individuals were divided into three groups. One group was comprised of individuals that could not be distinguished from pure *M. campechiensis*. Individuals showing mixtures of alleles were placed in a second category, the apparent hybrids. The third group contained all the remainder of the individuals which, although perhaps initially appearing in shell shape as though they might have some *M. campechiensis* ancestry, nevertheless could not be distinguished from pure *M. mercenaria* by their isozymes.

Table 1. Shell phenotypes and genotypes at four enzyme loci in 60 *Mercenaria* sampled from Hamlin Creek, SC, compared to *M. campechiensis* and *M. mercenaria* reference populations. For the hybrids, "X" indicates that a trait or allele is shown, while "XX" denotes homozygotes

	Ref. <i>M. camp.</i>	N = 27		Hybrids							N = 27 Remaining sample	Ref. <i>M. merc.</i>
		Ref. <i>M. camp.</i>	Apparently pure <i>M. camp.</i>	C2	C12	C15	H4	H9	H31			
Ridges												
Thin	0.0	0.0					X	X			0.93	1.00
Inter.	0.01	0.0							X		0.07	0.0
Thick	0.99	1.00	X	X	X						0.0	0.0
Naacre												
Purple	0.02	0.0									0.55	0.80
Inter.	0.06	0.0							X		0.22	0.15
White	0.92	1.00	X	X	X	X	X	X			0.26	0.05
Gpi												
105	0.0	0.0	X	X	X						0.02	0.01
100	0.0	0.0	X	X	X						0.85	0.90
90	0.01	0.04									0.06	0.02
80	0.22	0.25									0.0	0.01
70	0.43	0.37	X		X						0.07	0.04
60	0.27	0.31							XX		0.0	0.0
50	0.03	0.04		X							0.0	0.0
other	0.03	0.0									0.0	0.02
PgmS												
103	0.0	0.0					X				0.04	0.01
100	0.16	0.16	X				X	XX			0.85	0.84
97	0.08	0.08							X		0.06	0.04
95	0.07	0.12	X								0.0	0.0
92	0.58	0.64							X		0.06	0.08
other	0.11	0.0		XX	XX						0.0	0.02
PgmF												
103	0.06	0.24		X							0.28	0.15
100	0.28	0.26	X	X	X			XX			0.72	0.85
97	0.64	0.44	X		X		XX				0.0	0.0
95	0.02	0.06									0.0	0.0
Sod												
100	0.02	0.19		XX	X		XX	X			0.73	0.77
90	0.70	0.42	X						XX		0.27	0.23
80	0.28	0.39	X		X			X			0.0	0.0

RESULTS

Table 1 shows that 6 of the total 60 *Mercenaria* collected from Hamlin Creek could be identified, with some certainty, as hybrids. Three of that number were among the 30 individuals originally believed to be pure *M. campechiensis*, judging by shell morphology. Although these 3 individuals (C2, C12, and C15) had thick ridges and white nacre, and although all carried at least some diagnostic *M. campechiensis* isozyme alleles, each was heterozygous for the diagnostic *M. mercenaria* allele *Gpi 100*. The enzyme genotypes shown in Table 1 for these three individuals are consistent with expectation for first generation hybrids, although they may certainly also be later-generation recipients of *Gpi 100* by introgression.

Table 1 also shows that the remaining 27 individuals collected as pure *M. campechiensis* had allele frequencies remarkably similar to those of reference *M. campechiensis* from the Gulf of Mexico, over 1,000 km distant. The only significant differences ($P < 0.001$ by chi-square) were the higher frequencies of *PgmF 103* and *Sod 100* in South Carolina. These two alleles are much more common in South Carolina *M. mercenaria*, and again, their higher frequency here may be due to introgression.

Only 3 of the 30 individuals chosen as most likely to be hybrids could be verified as such. Individual H4 was homozygous for *Gpi 100* and heterozygous for *PgmS 103*, both diagnostic of *M. mercenaria*. But H4 was also homozygous for *PgmF 97*, the most common allele at that locus in *M. campechiensis* but unknown in *M. mercenaria*. Individual H9 was similarly homozygous for *Gpi 100*, but carried a copy of the *M. campechiensis*-diagnostic *Sod 80*. Both H4 and H9 showed thin shell ridges but white nacre. Individual H31 did not carry any allele diagnostic for either species. The shell showed ridges of intermediate thickness, a trait very rare in either pure population. It was homozygous for *Gpi 70*, the most common allele in *M. campechiensis* but present at only 0.04 in reference *M. mercenaria*. But it was also homozygous for *Sod 100*, the most common *M. mercenaria* allele, present at 0.02 in reference *M. campechiensis*. Thus H31 is also included among the individuals almost certainly hybrids.

Unlike individuals C2, C12, and C15, the isozyme genotypes of individuals H4, H9 and H31 are not consistent with expectation for first generation hybrids. The genotype of individual H9 (homozygous for the *M. mercenaria* allele *Gpi 100* while heterozygous for the *M. campechiensis* allele *Sod 80*) could be interpreted most simply as resulting from a backcross between an F_1 hybrid and pure *M. mercenaria*. Individuals H9 and H31 are homozygous for *M. mercenaria* alleles at one locus and *M. campechiensis* alleles at a second. Short of some phenomenon such as self-fertilization or polyploidy, the only explanation for the genotypes of H9 and H31 would be that a hybrid has subsequently crossed to another hybrid.

The great majority of the remaining 27 individuals initially thought most likely to be hybrids were, almost certainly, pure *M. mercenaria* with unusually inflated shells. The sample did tend to show higher frequencies of white nacre, and two individuals among them did show intermediate ridging. But all combined, allele frequencies at the four loci examined did not differ significantly from expected in pure reference *M. mercenaria*.

Commercial clam harvesting gear will be expected to provide a sample strongly biased to larger individual sizes. Although the majority of the *M. campechiensis* and the hybrids had shell lengths greater than 10 cm, two pure *M. campechiensis* measured approximately 8 cm and two hybrids measured 7 cm. This was fairly representative of the size distribution of all clams being harvested. The evidence does not suggest a single, possibly artificial introduction of *M. campechiensis*, but rather a continuously reproducing population.

DISCUSSION

There have been very few reports of inshore *M. campechiensis* populations north of Florida. Their occurrence in Hamlin Creek is doubtless due at least in part to the creek's depth, the close proximity of Breach Inlet, and the absence of estuarine influences. I understand from fishermen that other small inshore populations of *M. campechiensis* may be found in similar environments scattered along the coast of South Carolina.

Clearly some hybridization is naturally ongoing between *M. mercenaria* and *M. campechiensis* populations in Hamlin Creek. But the presence of 27 apparently pure *M. campechiensis* among thousands of *M. mercenaria*, and the rarity of hybrids between the two species, obviously reflects very significant reproductive isolation. Although still incomplete, the reproductive isolation between these species is much stronger in South Carolina than previously observed in the Indian River of Florida. The previous designation of the Indian River as a hybrid zone would seem justified.

Given clear demonstrations of the viability and fecundity of hybrids between the two *Mercenaria* species, Dillon and Manzi (1989a) speculated that such reproductive isolation as is observed in the Indian River may be due to differing spawning time or habitat choice. The latter does not seem to be the case in Hamlin Creek, but there is some indirect evidence for the former. Differences in spawning time offer a plausible mechanism by which individuals with multiply hybrid parentage, such as H4, could be conceived from a bed of clams in which hybrids comprise so small a percentage.

Although certainly cued by the environment, substantial variance in the gametogenesis and spawning cycles of *Mercenaria* populations seems to be under genetic control (Knaub and Eversole, 1988). Natural populations of *M. mercenaria* in South Carolina show spring and fall spawning peaks, but seem to be capable of spawning through the summer as well (Eversole et al., 1980; Manzi et al., 1985). Nothing is known of the reproductive cycle of *M. campechiensis* from as far north as South Carolina. Dalton and Menzel (1983) monitored reproduction in a *M. campechiensis* population from the northern Gulf of Mexico, noting striking differences compared to a transplanted *M. mercenaria* population and F₁ hybrids. (The *M. campechiensis* did not spawn in the summer.) Hesselman and colleagues (1989) found the *Mercenaria* breeding season in the Indian River to be unusually prolonged, and could not distinguish between the reproductive cycles of *M. mercenaria* and *M. campechiensis*. But as seasonality increases further north, levels of hybridization between the two species might be expected to decrease.

Approximately 94% of the bivalve species known from the Miocene of Maryland are now extinct (Glenn, 1904). Included among the survivors are both *M. mercenaria* and *M. campechiensis*, apparently unchanged over 5 million years. Barton and Hewitt (1985, 1989) note that although there is rarely much evidence on the stability of hybrid zones, most available data suggest relative permanence. The extreme example offered by Barton and Hewitt involves a 3–4 million year divergence time between two species of hybridizing toads. With the confirmation of a hybrid zone in *Mercenaria*, the two hard clams should enter the list among the most ancient species known to be hybridizing worldwide.

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