Genetics and Shell Morphology of Hard Clams (Genus *Mercenaria*) from Laguna Madre, Texas*

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ABSTRACT

Texas *Mercenaria* were originally described as a subspecies of *M. campechiensis*, but are now generally regarded as a subspecies of *M. mercenaria*, primarily based on aspects of shell ridging. We used isozyme frequencies at seven loci, six morphometric variables, shell ridging, and nacre color to compare Texas populations to reference populations of *M. campechiensis* and *M. mercenaria*. Texas populations were indeed distinct, but much more similar to the former. Hard clams from Texas should be considered *Mercenaria campechiensis texana* (Dall, 1902).

Key words: Hard clams; Texas; electrophoresis; morphometrics.

INTRODUCTION

Venerid clams of the genus *Mercenaria* (variously known as quahogs, cherrystones, hard clams, etc.) are of such commercial importance that it is surprising their systematic relationships are not better understood. Most authors follow Abbott (1974) in recognizing two North American species, the northern *Mercenaria mercenaria* (Linné, 1758) and southern *Mercenaria campechiensis* (Gmelin, 1791). Three criteria have been used to distinguish the species. *Mercenaria campechiensis* is supposed to have thick concentric ribs, white nacre, and a lunule at least as wide as it is high, while *M. mercenaria* has thin, easily eroded ribs, purple nacre, and a narrower lunule (figure 1).

Abbott (1974:523) noted that *M. campechiensis* hybridizes with *M. mercenaria* in the wild, and "could well be considered a subspecies." It has recently been shown, however, that some reproductive isolation exists between the two species where they occur sympatrically in the Indian River, Florida (Dillon & Manzi, 1989). Thus we consider these species distinct.

Dillon and Manzi (1989) selected one population each from central portions of the ranges of *M. mercenaria* and *M. campechiensis* to serve as "references". These populations appeared to be typical both genetically and morphologically, with no evidence that either contained any hybrid genomes. We found that 100% of the *M. mercenaria* shells had thin, easily eroded concentric ribs, while over 99% of *M. campechiensis* had thick, resistant concentric ribs. Nacre color was also a useful discriminator—80% of the *M. mercenaria* had distinct purple color, while 92% of *M. campechiensis* were completely white. The ratio of lunule width to lunule height proved to be of limited utility. Over 86% of *M. campechiensis* in our sample had ratios less than 1.0, and thus would have been misclassified as *M. mercenaria* using this traditional criterion. We did find, however, that if measures of lunule height and width were combined with overall shell length, width, height, and weight, very accurate morphometric discrimination between the two species was possible (Dillon & Manzi, 1989).

Hard clam populations inhabiting the Texas coast of the Gulf of Mexico were originally described by Dall (1902) as *Venus* (now *Mercenaria*) *campechiensis texana*. Dall viewed the presence of thin, easily eroded concentric ribs in the Texas populations (figure 1) as justification for recognizing the subspecies. The subspecies was transferred to *M. mercenaria* by Abbott (1954), at least partly because the inshore fauna of the Gulf of Mexico is generally Carolinian, rather than Caribbean in affinity (personal communication to Joy Goodsell). Here we show that based on isozyme frequencies and all other shell characteristics besides ridging, *texana* is a subspecies of *M. campechiensis* as originally described, not *M. mercenaria*.

METHODS

We were able to obtain 29 *Mercenaria* of the subspecies *texana* from Laguna Madre, in the vicinity of Corpus Christi, Texas. Samples were taken of both siphon and foot tissue, and electrophoretic analysis performed as described elsewhere (Dillon, 1982, 1985; Dillon & Manzi,

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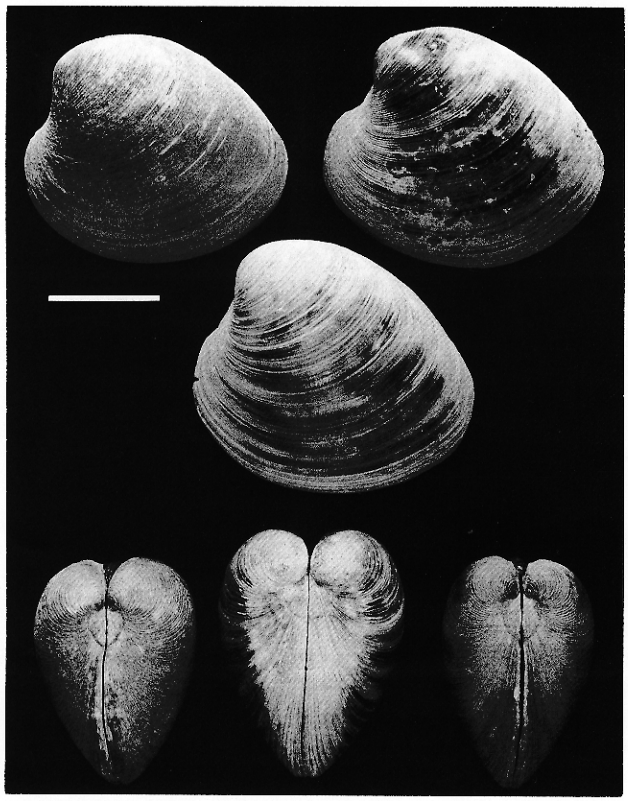

Figure 1. Left and anterior aspects of the three *Mercenaria* taxa. Left—*M. campechiensis* (Gmelin, 1791), Center—the subspecies *M. campechiensis texana* (Dall, 1902), Right—*M. mercenaria* (Linné, 1758). The scale bar is 50 mm.

1987). We estimated allele frequencies at the same seven enzyme loci that have been examined previously: glucose phosphate isomerase (GPI), leucine aminopeptidase (LAP), superoxide dismutase (SOD), 6-phosphogluconate dehydrogenase (6PGD), mannose phosphate isomerase (MPI), and phosphoglucomutase (two loci—PGMS and PGMF). Simple Mendelian inheritance of codominant alleles has been demonstrated at GPI, LAP, 6PGD, PGMS, and PGMF by Adamkewicz *et al.* (1984).

Gene frequencies at individual loci were compared using chi-square tests for two independent samples, corrected for continuity in 2 × 2 cases. Alleles with expected frequencies less than 5 were combined with other rare classes if possible, otherwise they were eliminated. The genetic distance over all 7 loci between each pair of populations was calculated using the method of Nei (1972).

Six measurements were made on the shells of most individuals. Maximum shell length, shell height (maximum dimension in the plane of symmetry perpendicular to shell length), shell width (maximum dimension perpendicular to the plane of symmetry), lunule height (along the line separating the valves), and lunule width (the maximum dimension perpendicular to lunule height) were measured with vernier calipers. The weight of both valves combined was recorded to the nearest gram. Nacre color and strength of concentric ridges were also noted. Five whole individuals and two single-valves were lost subsequent to tissue sampling. Thus sample sizes were N = 29 for isozyme frequencies, but only N = 24 for the morphological analyses.

We compared the Texas clams to the reference populations of 224 *M. mercenaria* and 194 *M. campechiensis* analyzed by Dillon and Manzi (1989). The *M. mercenaria* were sampled from a tributary of the Stono River, 15 km south of Charleston, South Carolina. The *M. campechiensis* were collected at Cedar Key, on the north-

Table 1. Allele frequencies at seven enzyme loci for clams of the subspecies *texana* compared to reference populations of *M. mercenaria* and *M. campechiensis* (data of Dillon & Manzi, 1989). Sample sizes were approximately 29 *texana*, 194 *M. campechiensis*, and 224 *M. mercenaria*.

Allele	*M. mercenaria*	*texana*	*M. campechiensis*
GPI 110	0.023	0.0	0.0
105	0.014	0.0	0.0
100	0.901	0.0	0.0
90	0.021	0.0	0.008
85	0.0	0.034	0.023
80	0.002	0.310	0.221
70	0.038	0.414	0.432
65	0.0	0.0	0.013
60	0.0	0.190	0.271
50	0.0	0.052	0.031
LAP 104	0.095	0.063	0.074
100	0.412	0.042	0.127
96	0.463	0.521	0.333
94	0.019	0.354	0.407
90	0.012	0.021	0.059
SOD 100	0.768	0.0	0.018
90	0.232	0.609	0.702
80	0.0	0.391	0.281
6PGD 110	0.030	0.077	0.084
100	0.622	0.481	0.517
95	0.0	0.0	0.011
90	0.348	0.442	0.388
MPI 110	0.0	0.0	0.032
108	0.059	0.370	0.484
105	0.389	0.304	0.267
100	0.300	0.304	0.174
95	0.253	0.022	0.043
PGMS 103	0.012	0.0	0.0
100	0.844	0.060	0.161
97	0.043	0.040	0.078
95	0.0	0.0	0.075
92	0.077	0.860	0.578
87	0.024	0.040	0.056
82	0.0	0.0	0.035
77	0.0	0.0	0.016
PGMF 103	0.148	0.021	0.061
100	0.852	0.417	0.282
97	0.0	0.562	0.636
95	0.0	0.0	0.021

Table 2. Results of the principal component analysis of shell morphometric data from *Mercenaria mercenaria, M. campechiensis,* and the subspecies *texana.*

Morphological character	Eigenvectors					
	PC1	PC2	PC3	PC4	PC5	PC6
Shell length	0.42	0.16	−0.43	−0.59	0.41	0.32
Shell height	0.44	−0.08	−0.25	−0.15	−0.27	−0.80
Shell width	0.42	−0.33	−0.10	0.10	−0.66	0.50
Lunule width	0.40	−0.05	0.85	−0.30	0.09	−0.02
Lunule height	0.34	0.84	0.05	0.40	−0.10	0.05
Shell weight	0.41	−0.39	−0.06	0.61	0.55	−0.01
Eigenvalue	4.96	0.56	0.25	0.11	0.08	0.03
Cumulative variance	0.83	0.92	0.96	0.98	0.99	1.00

central Gulf coast of Florida. All shells examined in this study have been deposited at the Academy of Natural Sciences of Philadelphia. Catalog numbers are as follows: Texas population 373466, *M. campechiensis* 373467, *M. mercenaria* 373468.

Following Dillon and Manzi (1989), we performed a discriminant analysis on principal component scores extracted from the six measurement variables. First a principal component analysis was performed on the correlation matrix calculated over all 442 individuals (the Princomp procedure, SAS, 1985). We disregarded variance on the first principal component (PC) as a method of factoring out size variance, and used factor scores on the remaining 5 PC's as new variables for nearest-neighbor discriminant analysis (the Neighbor procedure, SAS, 1985). This is a nonparametric discriminant analysis, not involving the calculation of discriminant functions. In our application there were 418 known clams, and only the 24 Texas clams were entered as unknowns. Each clam was classified as *M. mercenaria* if at least 19 of its 20 nearest Euclidean neighbors of known affinity were *M. mercenaria, M. campechiensis* if 19 of 20 were *M. campechiensis,* and intermediate if otherwise.

RESULTS

Table 1 compares allele frequencies at seven enzyme loci in the Texas clams to reference frequencies established for *M. mercenaria* and *M. campechiensis* by Dillon and Manzi (1989). The two reference populations are strikingly distinct at GPI, SOD, MPI, and PGMF, and in these four cases, the *texana* sample is not significantly different from *M. campechiensis* by chi-square. The two reference populations are also distinct at the LAP and PGMS loci, but although the *texana* sample is much more similar to *M. campechiensis,* significant differences exist. The frequency of LAP 100 is significantly lower in *texana* (chi-square = 8.01, 2 d.f.), and there seems to have been a significant loss of allelic diversity at the PGMS locus (chi-square = 13.5, 1 d.f.). The Texas population was not significantly different from either reference population at the 6PGD locus.

Calculated over all 7 polymorphic loci, Nei's genetic distance (D) between the two reference populations was 0.82. The Texas population showed $D = 0.041$ to *M. campechiensis* but $D = 0.83$ to *M. mercenaria.*

Results of the principal component analysis on shell morphometrics are given in table 2. Factor loadings were somewhat different from those obtained by Dillon and Manzi (1989), since 24 clams of the subspecies *texana* have replaced 170 individuals from the Florida hybrid zone. We discarded PC1, representing 83% of the variance, and used the remaining 17% for discriminant analysis.

Figure 2 shows that the two reference populations are quite distinct on PC2, even though this is not a discriminant function, with *M. campechiensis* scoring lower. Judging from the factor loadings on PC2 (table 2), *M. campechiensis* would seem to have a wider, heavier shell than *M. mercenaria.* In contrast to our previous findings, lunule height loads very strongly on PC2, while the contribution of lunule width is negligible. It would appear that *M. campechiensis* does not have an especially wide lunule for its size, but rather a distinctively short (or "lower") one. Nearest-neighbor discriminant analysis confirmed that the two reference populations are very distinct (table 3). One *M. campechiensis* was misclassified, to 95% confidence, as *M. mercenaria,* but no *M. mercenaria* were misclassified. The reference populations were both about 80% distinct.

Although more similar to *M. campechiensis* than *M. mercenaria,* Texas clams were quite diverse morphometrically (figure 2). The three lowest PC2 scores all belonged to *texana* specimens, suggesting that Texas pop-

Table 3. Classification (to 95% confidence) of clams by nearest-neighbor discriminant analysis on principal component scores, given the two reference populations as knowns and specimens of the subspecies *texana* as unknowns.

	M. mercenaria	Intermediate	*M. campechiensis*
M. mercenaria reference	180	44	0
texana	2	14	8
M. campechiensis reference	1	37	156

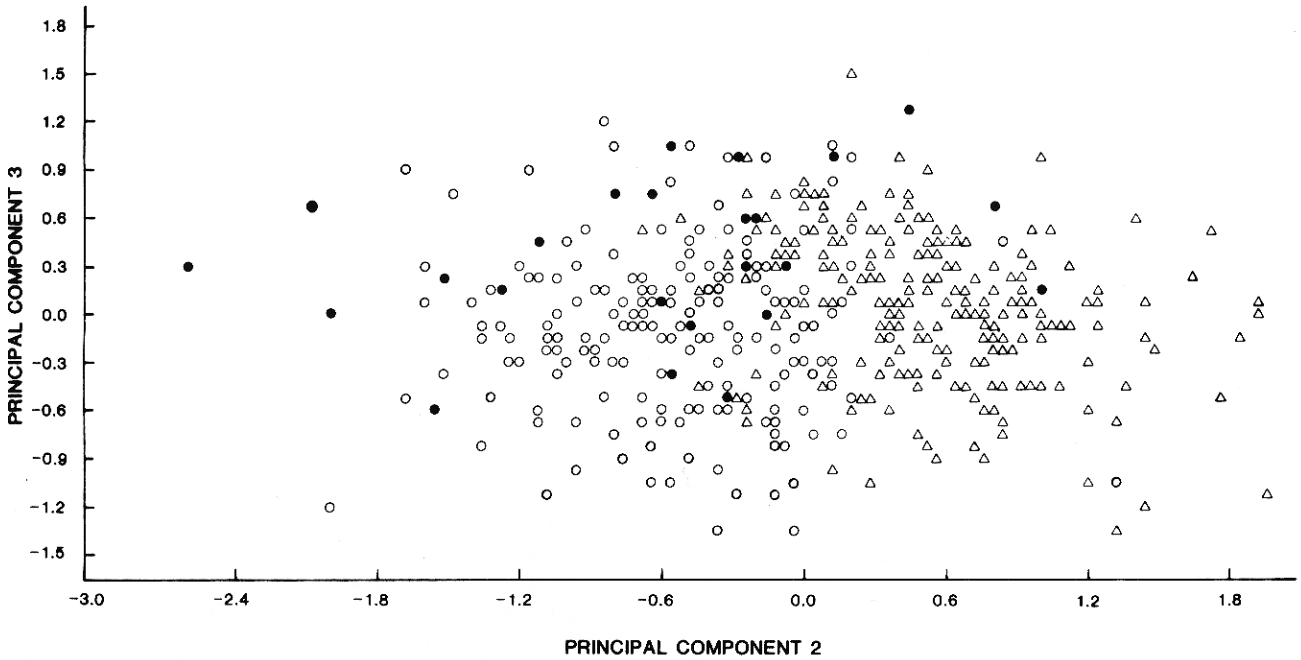

Figure 2. Factor scores on principal components 2 and 3. Triangles are *M. mercenaria* reference, open circles are *M. campechiensis* reference, and closed circles are *M. campechiensis texana*. A total of 51 reference individuals are obscured by overlap.

ulations may be distinguished by greater relative width and weight than reference *M. campechiensis*, and by even shorter lunules. Specimens of the subspecies *texana* also tended to be distinct on PC3, showing wider lunules and shorter shells overall. Table 3 shows that most shells from the Texas population could not be identified, to 95% confidence, as coming from either reference population. Among classifiable shells, however, those indistinguishable from *M. campechiensis* outnumbered those from *M. mercenaria* by a ratio of 4 to 1.

All individuals from the Texas population showed the typical *M. campechiensis* trait of purely white nacre. But the striking feature of the *texana* shells was the presence of thin, easily eroded concentric ridges or ribs. Ribs were eroded to leave bald patches on all 24 individuals examined, even though over 99% of the reference *M. campechiensis* population had strong, resistant ribs.

DISCUSSION

Isozyme frequencies clearly show that the Texas populations are much more similar to *M. campechiensis* than *M. mercenaria*. Considering overall genetic distance, it was in fact the reference *M. campechiensis* population that was intermediate, not the Texas population. It is difficult to compare our values of *D* to those collected from other taxa, since monomorphic loci were excluded from this study. But it appears that isozyme divergence between both species and subspecies of *Mercenaria* is unusually low (Avise, 1976).

It might be argued that the geographic distance between Texas and South Carolina populations makes a comparison of isozyme frequencies unfair. But extensive dispersal is apparently possible during *Mercenaria*'s veliger stage. Dillon and Manzi (1987) reported only a single significant difference at these seven loci in a comparison of Massachusetts and Virginia *M. mercenaria*. Only two significant differences were apparent between Virginia and South Carolina, and the approximately 20 clams from the Atlantic coast of Florida identified as pure *M. mercenaria* were not strikingly different from South Carolina populations (Dillon & Manzi, 1989). It seems unlikely that a difference of the magnitude reported here between the reference *M. mercenaria* and the Texas clams could be due to distance alone. The minor differences shown at two loci between the Texas population and the reference *M. campechiensis* population from northern Gulf Florida are of the magnitude we have observed from isolation by distance.

The reference populations were quite distinct in shell morphometrics, and again individuals of the *texana* subspecies tended to sort out with *M. campechiensis*. The nacre color of the Texas clams also clearly places them with *M. campechiensis*. But the presence of some peculiarities of shell shape, together with thin, easily eroded concentric ridges, makes Texas populations so distinct that they do warrant recognition as a subspecies, *Mercenaria campechiensis texana* (Dall, 1902).

Preliminary results from hybridization studies between standard *M. mercenaria* and *M. campechiensis* conducted in our facilities suggest that shell ridge thickness is primarily, perhaps entirely, under genetic control. The ridge thickness of F_1 hybrids (measured by mechanical filing) is intermediate between that of pure offspring from the two species spawned at the same time and reared in the same environment. The thinner, finer

ribs shared by *M. mercenaria* and *M. campechiensis texana* may be an adaptation for burrowing in the fine, terrigenous silt and mud found in the estuaries of the American Atlantic and northern Gulf coasts. The thicker, heavier ribs of typical *M. campechiensis* may be an adaptation for the coarser, carbonate sands offshore, in peninsular Florida, and the Caribbean Sea. Thin ridges are probably ancestral, with thicker ridges evolving after the divergence of *M. mercenaria* and *M. campechiensis*. Otherwise, one would need to postulate that thin ridges evolved separately in *M. mercenaria* and *M. campechiensis texana*.

Some attention has focused on *M. campechiensis texana* as a candidate for mariculture, especially in the Texas environment to which it is adapted (Craig *et al.*, 1988). Another possible source of commercial interest is in the hybridization of Texas populations to *M. mercenaria* as a method of increasing genetic variability (review by Dillon & Manzi, 1988). Recently Goodsell (1989) has made all reciprocal crosses between *M. mercenaria*, *M. campechiensis*, and *M. campechiensis texana*, demonstrating the feasibility of this approach. In any such future studies, the genetic relationships among these three taxa should be kept in mind.

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