

Morphological and Electrophoretic Evidence for Two Species of *Corbicula* (Bivalvia: Corbiculidae) in North America

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ABSTRACT: Two syntopic color forms of the Asiatic clam genus *Corbicula* which have become established in the lower Brazos River system in Texas were examined electrophoretically and morphologically. One form has a white nacre with purple highlights (the "white form") and the other form has a deep purple nacre (the "purple form"). The two forms have fixed allelic differences at six of 26 genetic loci and are remarkable in that both forms are homozygous at every locus. The two forms also differ in number of annuli and in shape of the shell (as determined by principal components analysis). Differences in ecological niche preference and in seasonal enzyme production are noted. In view of these data, a single-species concept proposed recently by Britton and Morton (1979) for North American populations of *Corbicula* cannot be justified. Two names (*C. fluminea* and *C. fluminalis*) that are applied to widely distributed Asian species may be applicable to the two North American species; however, conflicting morphological, reproductive and distributional data prohibit assigning either of these names (or any others) to the two introduced species until revisionary work is carried out on the genus *Corbicula* throughout its native range.

INTRODUCTION

The taxonomy of the Asiatic clam genus *Corbicula* has long been in a state of flux. *Corbicula* was first collected in North America from Vancouver Island, British Columbia, in 1924 (Counts, 1981); since that time *Corbicula* has become widespread and abundant in the United States (Britton and Morton, 1979). A number of different names have been applied to North American populations of *Corbicula* (including *C. fluminalis*, *C. fluminea*, *C. leana* and *C. manilensis*). Britton and Morton (1979) relegated all North American *Corbicula* to a single species, for which they chose the name *C. fluminea*. Their synonymy was based partly on electrophoretic data presented by Smith *et al.* (1979). Electrophoresis is a powerful tool for estimating relative amounts of protein variability within populations and levels of divergence between populations. Ayala (1975) reviewed data on the degree of protein relatedness of organisms at different levels and concluded that local populations have average genetic identities (\bar{I}) of $\bar{I} \pm 0.980$, subspecies have $\bar{I} \pm 0.850$ and semispecies have $\bar{I} \pm 0.800$. Considering that Smith *et al.* (1979) surveyed five populations of *Corbicula* in the United States from South Carolina to California and found $\bar{I} = 1.00$ for all populations, it is easy to understand the reasoning behind Britton and Morton's decision to recognize only a single species. Intriguingly, Smith *et al.* (1979) found the five U.S. populations not only were genetically identical, they also had no intrapopulation protein variability. Four Asian populations of *Corbicula* also examined by Smith *et al.* showed both interpopulation and intrapopulation protein variability. Due to the lack of variability found in the populations from the U.S., Smith *et al.* (1979) concluded that introduced *Corbicula* represent a single introduction of a very small population. The only report of any electrophoretic variability in North American *Corbicula* populations was by McLeod and Sailstad (1980)—they found very low heterozygosity ($H = 0.0049$) at three of seven loci in a population of *Corbicula* from North Carolina.

In a recent study of the distribution of *Corbicula* within the Brazos River system in Texas, Fontanier (1982) noted two color forms of *Corbicula* occurring syntopically within the lower portion of the Brazos River drainage. While the color of the nacre ap-

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peared to be diagnostic for the forms (one of these forms has a deep purple nacre whereas the other form has a white nacre with purple highlights), differences in exterior coloration were also noted. The only type of *Corbicula* found in the upper portion of the Brazos River drainage was the white form. The purpose of this study was to examine these two forms electrophoretically and morphologically to determine their degree of differences.

METHODS

One hundred *Corbicula*, 50 of each color form, were collected at the Hwy. 95 crossing of the San Gabriel River at Circleville, Williamson Co., Texas. Twenty-five of each form were collected on 10 January 1981, and another 25 of each were collected on 7 April 1981. The total collection area on both dates was less than 3 m². Smaller samples were also collected in Texas from the Guadalupe, Colorado, San Jacinto and Sabine rivers. Whole body extracts were prepared and subjected to horizontal starch gel electrophoresis, following procedures similar to those used by Selander *et al.* (1971) and Shaw and Prasad (1970). Genetic *I* was computed according to Nei (1972). Shells were dried and measured for length, depth, thickness (of the whole animal, not of one valve) and mass. The number of annuli (circuli) on each specimen was counted across the middle of one valve. The three linear measurements were included in a principal components analysis (program BMDP4M of the statistical package described by Dixon and Brown, 1979). See Schnell (1970) for a description of this technique.

RESULTS

Twenty-six presumptive loci were examined by electrophoresis: malate dehydrogenase (MDH-1,-2), malic enzyme (ME-1,-2), alpha-glycerophosphate dehydrogenase (GPD-1,-2), glucose-6-phosphate dehydrogenase (G6-PDH), adenylate kinase (ADK-1,-2), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), acid phosphatase (AP-1,-2), alkaline phosphatase (AKP), leucine aminopeptidase (LAP-1,-2,-3), peptidase (PEPT-1,-2,-3,-4) and esterase (EST-1,-2,-3,-4,-5). Both forms were homozygous at every locus that we examined. Six loci, ADK-2, AP-1 and -2, LAP-2, and PEPT-2 and -4, appear to be characterized by fixed differences between the two color forms (Table 1). The slow electromorph at ADK-2 did not stain for any of the purple form individuals collected on 10 January (ADK-1 was scorable for these individuals), but it stained for all of the purple form individuals collected on 7 April. Several of the purple form of *Corbicula* collected in January were run on the same gel as the April collection, but still nothing stained at ADK-2 within the purple form sample from the January collection. The electromorph at this locus may be seasonally produced in the purple form. Besides the electrophoretic differences, the two forms also differ morphologically in color, number of annuli (Fig. 1) and shape of the shell (Fig. 2 and Table 2).

All of the live *Corbicula* collected at the sites outside of the Brazos River drainage (Guadalupe, Colorado, San Jacinto and Sabine rivers, Texas) were morphologically and electrophoretically identifiable as the white form, although none of these sites were extensively sampled. None of these individuals showed any heterozygosity—all were fixed for the same alleles as the white form in the Brazos drainage. Both morphological types were found, however, among empty shells found at one site in the Colorado River drainage (Sandy Creek, Llano Co., Texas) and at one site in San Jacinto drainage (Huntsville State Park, Walker Co., Texas).

DISCUSSION

Morphological differentiation.— Besides color of the nacre, the two forms of *Corbicula* in the Brazos River drainage differ in number of shell annuli and in shape of the shell. The number of annuli is nonoverlapping between the two forms when plotted against mass of the shell (Fig. 1); the purple form has more annuli for a given shellmass than does the white form. This difference in number of annuli may reflect a difference in the

microhabitat preferences of the two forms. When the samples were collected, it was noticed that the purple form was more abundant than the white form in rocky areas of the riverbed (usually in the current), whereas the white form predominated closer to shore in areas with more silt. Downriver from the collection site, there are rocky areas where only the purple form is found, and muddy or sandy localities where only the white form was collected (C. E. Fontanier, pers. comm.).

The different shape of the shells of the two forms also may represent an adaptation to different microhabitats. The first factor (PC I) extracted in the principal components analysis was size-related and accounted for 97.9% of the total variance within the sample. PC I was positively correlated with all three of the linear measurements—length, depth and thickness of the shell. The second factor (PC II) accounted for only 1.9% of the variance, but completely separated the two species (Fig. 2). PC II was positively correlated with length and negatively correlated with depth and thickness (Table 3). Since individuals of the white form all had negative scores on PC II, whereas individuals of the purple form all had positive scores (Fig. 2), the white form is thicker and deeper in relation to length than is the purple form (*also see* Table 2). These differences in shape are readily apparent in the field and can be used to sort most individuals of the two forms without measurements.

Electrophoretic differentiation.—Since the two forms of *Corbicula* in the Brazos River drainage are fixed for different alleles at six of 26 loci ($\bar{I} = 0.769$), they are clearly not interbreeding. Even if both forms are simultaneous hermaphrodites, as has been suggested (for one form) by Kraemer (1979a, 1979b), Kraemer and Lott (1978) and

TABLE 1.—Allelic frequencies of white form and purple form *Corbicula* at 26 genetic loci. The fixed allele in the white form was designated the medium-speed electromorph for comparisons with Smith *et al.* (1979); purple form electromorphs were scored slow, medium or fast in relation to the white form allele. Abbreviations for buffer systems are as follows: TC 6.7 = Tris-citrate pH 6.7, TC 8.0 = Tris-citrate pH 8.0, TM-EDTA = Tris-maleic-EDTA, LiOH = Lithium hydroxide (Selander *et al.*, 1971). *See text* for enzyme abbreviations

Locus	Buffer type	White form		Purple form	
		M	S	M	F
MDH-1	TC 6.7	1.0		1.0	
MDH-2	TC 6.7	1.0		1.0	
ME-1	TC 6.7	1.0		1.0	
ME-2	TC 6.7	1.0		1.0	
GPD-1	TC 8.0	1.0		1.0	
GPD-2	TC 8.0	1.0		1.0	
G-6-PDH	TM-EDTA	1.0		1.0	
ADK-1	TC 6.7	1.0		1.0	
ADK-2	TC 6.7	1.0	1.0		
PGI	TC 6.7	1.0		1.0	
PGM	TC 8.0	1.0		1.0	
AP-1	TM-EDTA	1.0			1.0
AP-2	TM-EDTA	1.0			1.0
AKP	TC 8.0	1.0		1.0	
LAP-1	TC 8.0	1.0		1.0	
LAP-2	TC 8.0	1.0			1.0
LAP-3	TC 8.0	1.0		1.0	
PEP-1	LiOH	1.0		1.0	
PEP-2	LiOH	1.0			1.0
PEP-3	LiOH	1.0		1.0	
PEP-4	LiOH	1.0			1.0
EST-1	LiOH	1.0		1.0	
EST-2	LiOH	1.0		1.0	
EST-3	LiOH	1.0		1.0	
EST-4	LiOH	1.0		1.0	
EST-5	LiOH	1.0		1.0	

Sinclair and Isom (1963), some interindividual fertilization would be expected between syntopic clams (densities of *Corbicula* in the Brazos River drainage commonly range up to 3000 clams/m²). Further study is needed to determine whether this lack of hybridization is due to prezygotic isolation of the gametes (temporal isolation seems the most likely) or to hybrid inviability.

Smith *et al.* (1979) concluded that the form they examined in the United States (presumably the white form, J. C. Britton, pers. comm.) most likely is invariant for the 18 loci that they examined due to a small initial founding event. If *Corbicula* is capable of self-fertilization, this event could have involved a single individual. The presence of a second species of *Corbicula* in North America, also lacking observed heterozygosity, apparently is the result of a small introduction of a second species. This second species (the purple form) may also be a simultaneous hermaphrodite, judging from its lack of heterozygosity. In considering the spread of the white form across North America, Smith *et al.* (1979) stated that "the rapid expansion of a species with very little genetic variability is quite surprising and very instructive." The parallel invasion of a second species of *Corbicula*, also showing very little genetic variability (none observed), suggests that this pattern of invasion may not be unusual for species of *Corbicula*. Lack of detectable genetic variation also has been reported within introduced populations of another self-fertilizing mollusk, *Rumina decollata* (Selander and Kaufman, 1973).

In view of the fact that at least two introduced species of *Corbicula* occur in North America, it would be of interest to ascertain where each of these species occurs in Asia. Morton (1979) reported a widespread distribution in Asia of a largely freshwater species (*C. fluminea*) and a largely river-mouth species (*C. fluminalis*). Since these Asian

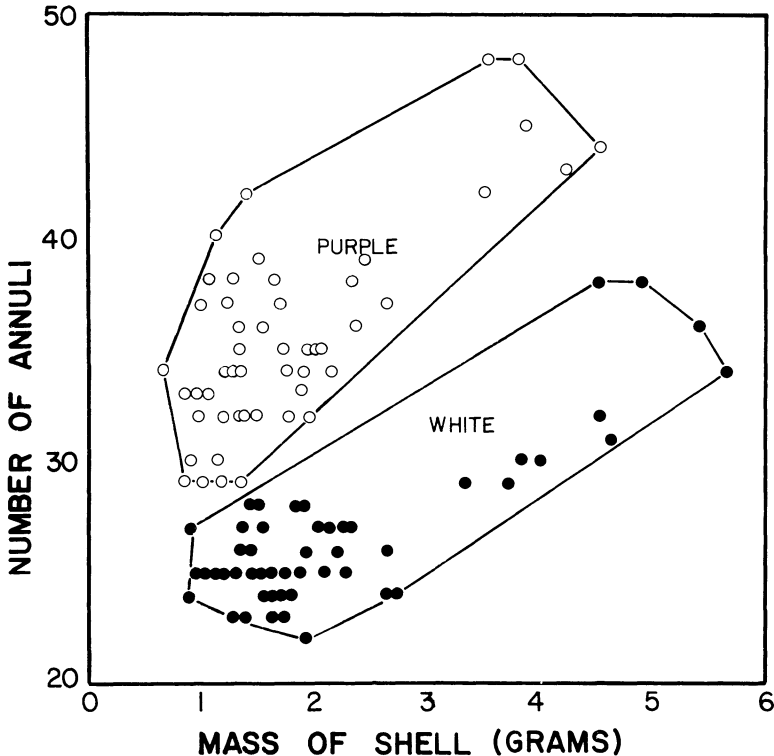


Fig. 1. — Number of annuli vs. mass of shell plotted for two forms of *Corbicula*. The open circles represent individuals of the purple form; the solid circles represent individuals of the white form

distributions seem analogous to the Brazos River distributions of the white and purple forms, it might be supposed that these names are applicable to the respective North American forms (that is, that the white form is *C. fluminea* and the purple form is *C. fluminalis*). However, there are two major problems with these applications. First, the morphological evidence suggests the opposite application: comparison of the type specimens of these two species described by Müller (1774) suggests that if these two names are appropriate, the purple form is *C. fluminea* and the white form is *C. fluminalis* (the types are figured in Britton and Morton, 1979; we have examined these photographs, not the actual specimens). This arrangement would also be preferable considering Morton's (1979) data on shell size and circuli configurations in Asian *C. fluminea* and *C. fluminalis*. Secondly, Asian *C. fluminea* are protandric consecutive hermaphrodites (Morton, 1977) and Asian *C. fluminalis* are apparently dioecious (Morton, 1979). Neither of these reproductive modes have been reported for North American *Corbicula* populations (of one or both species). North American *Corbicula* that have been studied are simultaneous hermaphrodites (Kraemer, 1979a, 1979b; Kraemer and Lott, 1978; Sinclair and Isom, 1963). These reported reproductive modes are supported by the electrophoretic data of Smith *et al.* (1979) and the present study. The *Corbicula* population that Smith *et al.* (1979) examined from Japan showed too much heterozygosity ($\bar{H} = 0.229$) to be a simultaneous hermaphrodite—or if it is, self-fertilization must be greatly reduced. Although the other Asian populations of *Corbicula* (one from Hong Kong and two from the Philippines) show heterozygosity, Smith *et al.* (1979) reported that heterozygosity in these populations "seemed reduced" (a value for \bar{H} was not specified). Since both species in North America show little or no heterozygosity (Smith *et al.*, 1979; McLeod and Sailstad, 1980; this study), both may be simultaneous hermaphrodites. Unless both *C. fluminea* and *C. fluminalis* are capable of alternate reproductive strategies (which has not been demonstrated), neither of these two Asian species' names may be appropriate for the two introduced North American species.

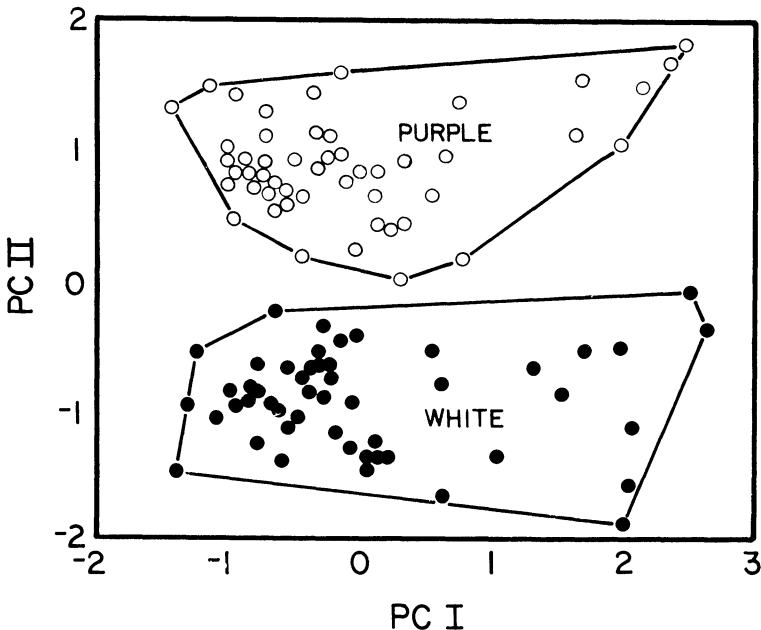


Fig. 2.—Scores on principal components I and II for two forms of *Corbicula*. See text and Table 3 for characters used in composing PC I and PC II

The genus *Corbicula* is obviously poorly understood in its native range. The taxonomic literature, including the most recent reviews, is generally contradictory. For instance, a recent symposium on *Corbicula* included these three disparate views: (1) *C. leana* and *C. japonica* of Japan are conspecific with *C. fluminea* and *C. fluminalis*, respectively (Britton and Morton, 1979); (2) the converse opinion expressed by the junior author of opinion #1 (Morton, 1979), and (3) the view that the Japanese "population" probably represents a species distinct from the Hong Kong and Philippine "populations" (Smith *et al.*, 1979—which includes the senior author of opinion #1 above).

Before names can be applied to the two introduced North American species, Asian *Corbicula* must be overviewed by distributional, morphological, biochemical and reproductive studies. Assigning names that are based upon short morphological descriptions of Asian *Corbicula* to the North American populations is unlikely to produce a meaningful nomenclatural arrangement. It is even less satisfactory to lump all North American *Corbicula* into a single convenient species in light of the fact that there are syntopic species that are morphologically, electrophoretically, ecologically and physiologically distinct.

CONCLUSIONS

Two introduced species of *Corbicula* occur syntopically in the lower Brazos River drainage in Texas. These two species are fixed for different alleles at six loci, and share a common electromorph over 20 other loci. We did not detect any heterozygosity in either species. The two species differ morphologically in color, number of shell annuli and in shape of the shell. They also differ ecologically in niche preference and may differ physiologically in seasonal enzyme production (ADK-2).

Since the genus *Corbicula* is poorly known in its native range, allocation of names to the introduced North American species is not possible at this time. Data that are available on the Asian species produce conflicting nomenclatural arrangements when applied to the North American species. Since two partly sympatric species that are morphologically, electrophoretically, ecologically and physiologically distinct are present in North America, the two must be differentiated in any biological study. Therefore, we propose that the two be referred to by the descriptive terms "purple form" and "white form" until sufficient study elucidates which of the more than 200 trivial names of *Corbicula* are appropriate.

Due in part to its high rate of reproduction, *Corbicula* has become a serious economic problem to industries using rivers (Sinclair, 1971). Although largely unstudied, *Corbicula* populations may also be competing with or displacing native clam species in North America. Considering the time, money and effort that will likely be expended in the near future in studying and controlling *Corbicula* populations, it is im-

TABLE 2. — Means, standard deviations and ranges of five characters for two forms of *Corbicula* collected syntopically

Character	White form	Purple form
	$\bar{x} \pm 1$ SD (range)	$\bar{x} \pm 1$ SD (range)
Length (mm)	18.8 ± 4.1 (12.8-29.1)	19.6 ± 3.5 (14.5-29.9)
Depth (mm)	17.7 ± 3.5 (12.5-26.7)	17.0 ± 3.2 (12.6-26.2)
Thickness (mm)	12.8 ± 2.1 (10.0-17.7)	11.9 ± 1.9 (9.0-16.6)
Number of annuli	26.8 ± 3.6 (22-38)	35.7 ± 4.6 (29-48)
Mass (g)	2.24 ± 1.2 (0.87-5.67)	1.76 ± 0.9 (0.66-4.57)

TABLE 3. — Factor loadings of PCI and PCII for the three morphometric measurements used in the principal components analysis in Figure 2

Character	PC I	PC II
Length	0.983	0.182
Depth	0.997	-0.033
Thickness	0.988	-0.147

perative that investigators dealing with these problems realize that they may be studying more than one species. Distributional studies defining the current ranges of these two species in North America and recording the earliest records for the purple form (to narrow down a point of introduction of this species) would greatly assist in evaluating the value of past studies of *Corbicula* biology in North America.

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