# Morphometric Characterization of a *Mercenaria* spp. (Bivalvia) Hybrid Zone: Paleontological and Evolutionary Implications

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## (ABSTRACT)

Paleontological documentation of hybridization events has the potential to address a multitude of evolutionary and paleobiological issues unanswerable by purely biological means. However, previous studies of modern hybrids suggest that their morphology is often insufficient for their reliable discrimination. This study analyzes the morphology of an extant, genetically-identified *Mercenaria* spp. (Bivalvia: Veneridae) hybrid zone using Bookstein coordinates and multivariate methods to answer two questions: (1) can hybrid *Mercenaria* spp. individuals be identified based on morphology alone, and (2) would a *Mercenaria* spp. hybrid zone be recognizable in the fossil record?

Multivariate statistical procedures (principal components analysis, canonical variate analysis, etc.) using Bookstein coordinates demonstrate that, within the hybrid zone, hybrid individuals cannot be identified due to extreme overlap with the parental taxa. The hybrid zone as a whole, however, can be identified by comparison with pure-species populations sampled from outside the hybrid zone. Hybrid zones occupy parental species morphospace plus intermediate morphospace. The technique of using multiple pure-species populations to establish species morphospace is introduced to control for processes that may also result in morphological intermediates at ecological time scales (dimorphism, ecophenotypy, and geographic variation). Four alternative causal explanations of morphological intermediates through geological time (primary intergradation, uncoupled genetic and morphological divergence, time-averaged evolving populations, and developmentally instable populations) are evaluated. A literature survey strongly suggests that neither time-averaging nor developmental instability is occurring at the beginning of a lineage's evolutionary history, and that hybridization may be much more extensive than paleontological data suggest.

## Dedication

To Jenny,
my Catskill eagle

## Acknowledgments

I thank Michal Kowalewski for his constant instruction, support, and direction; without his guidance this study and my future as a paleontologist would be incomplete. I thank Andrew Bush for bringing the issue of hybrid zone morphology to my attention and for providing invaluable advice and patience during the completion of this study. Michal Kowalewski, Richard Bambach, and Bruce Turner contributed greatly to my understanding of paleobiology and evolution as well as providing editorial assistance during manuscript preparation. Theresa Bert and William Arnold graciously provided collections of *Mercenaria* spp. and genetic data.

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**Tables and Figures, continued…**

# Chapter 1: Introduction

Hybrid zones are geographic areas in which two genetically distinct species mate and produce viable offspring (Barton and Hewitt 1989). Hybrids, found in nearly all higher organisms (Barton and Hewitt 1985), result from incomplete reproductive isolation between two species, a condition that is believed to occur during and immediately following speciation events (Hodges et. al. 1995). Consequently, hybrid zones can provide insights into the process of speciation and the mechanisms of divergence (Barton and Hewitt 1985).

Paleontological documentation of hybrids and hybrid zones has the potential to provide information unobtainable by biological means. A sample of evolutionary and paleobiological issues that may be resolved by paleontological evidence include: (1) whether hybrid zones result from primary divergence or secondary contact between two species; (2) whether the attainment of reproductive isolation between fossil species was instantaneous or gradual; (3) whether the geographic position of hybrid zones migrates through time; (4) whether hybridization has a role in forming new species; (5) whether clade-specific differences in the frequency of hybridization (as a proxy for the relative strength of reproductive isolation) might relate to that clade's diversification; and, (6) whether the divergence of species was adaptive or not.

Given the frequency of extant hybridization (estimated at 9% for birds and probably higher for other groups [Jiggens et. al. 1996]), paleontological studies involving presumed hybrids are few, and largely focus on identifying hybrids rather than studying them. Geary (1992) reports intermediate morphs of the gastropod *Melanopsis* spp. following rapid divergence in the late Miocene. These intermediates co-occur with the diverged species for approximately one million years and then disappear. Geary rules out ecophenotypic effects but is ultimately unable to distinguish between dimorphism and hybridization. Ausich and Meyer (1994) identify possible early Mississippian hybrid crinoids (*Eretmocrinus* spp.) based on intermediate morphologies co-occurring with the parental species. Studies by Nichols (1959) and Shaw (1991) likewise document intermediate morphologies of echinoderms (*Micraster* spp.) and trilobites

(*Cryptolithoides* sp./*Cryptolithus* sp.), respectively. Goodfriend and Gould (1996) document two cases of apparent hybridization in the gastropod *Cerion* spp. First, they document unique morphological effects assumed to be due to ancient hybridization in a restricted geographic area of *C. columna*. Second, they document the transition from *C. excelsior* to *C. rubicundum* possibly by way of hybridization. In this case, however, it is worth noting that the gradual transition from one species to the other cannot be ruled out. Baker (1983) documents cladogenesis of the radiolarian *Theocorythium* spp. by way of apparent hybridization and subsequent introgression. Casey et. al. (1983) also reports suspected radiolarian hybrids.

The problem with all of these studies is that ultimately the identification of hybrids is inferential. Researchers must assume that the morphological criteria they used to identify hybridization events are valid proxies for genotypic intermediates, despite the problems inherent in using the morphological approach to identifying hybrids. Identifying hybrids based on morphology alone has been shown to be consistently uncertain (Baker 1947; Gottlieb 1972; Lamb and Avise 1987; Rieseberg and Ellstrand 1993; Bert et. al. 1996; Rieseberg and Linder 1999). For example, Lamb and Avise (1987) report over 40% misclassification of genetically identified tree frog (*Hyla* spp.) hybrids using the morphological approach, Rieseberg and Ellstrand (1993), in a review of 46 plant hybrid zones, report that F1 hybrids express intermediate morphologies only 45% of the time, and Bert et. al. (1996) report the inability to morphologically distinguish any hybrids from a stone crab (*Menippe* spp.) hybrid zone. These results are unfortunate for paleontology because morphology remains one of the most important raw materials upon which evolutionary paleobiological studies are conducted.

However, most morphometric studies of hybrid zones have been conducted using traditional measurements (*sensu* Rohlf and Marcus 1993a, such as distance measures, ratios, and angles). Few characters have been employed, further decreasing the discriminatory power of morphological data. Differences between incompletely reproductively isolated species are expected to be subtle, and therefore, the generally held view that hybrids cannot be identified morphologically should be considered tenuous pending rigorous morphometric treatment. This

is particularly true in light of the recent advances in morphometric methodology (Rohlf and Marcus 1993a). To date, no detailed morphometric study on a known hybrid zone has been conducted to determine if paleontologically-applicable characteristics for hybrid identification can be found.

This study utilizes an extant, genetically-identified hybrid zone of the bivalve *Mercenaria* spp. to accomplish two aims: first, to determine if identification of hybrids of the bivalve *Mercenaria* spp. is possible using morphological characters measured using sensitive landmark data and methods, and second, to determine if a *Mercenaria* spp. hybrid zone would be identifiable using morphological characters regardless of whether hybrids themselves could be identified on an individual basis. Because *Mercenaria* spp. is a widespread Cenozoic fossil and belongs to a diverse group of bivalve molluscs (Veneridae) with a rich fossil record, the specific objectives of this study should be of interest to both paleontologists and malacologists. However, the main objective of this study is much more general as it aims to provide morphological criteria for assessing the occurrence of hybridization in the fossil record, thereby opening the door to an important theme of evolutionary paleobiology that has been largely neglected in the past.

## Chapter 2: Materials and Methods

Study Organism.—*Mercenaria* spp. are commercially important venerid bivalves. Two species, *M. mercenaria* and *M. campechiensis*, occur in the United States. *M. mercenaria* inhabits shallow inshore embayments and estuaries of the U.S. Atlantic Coast, from Canada to central Florida, whereas *M. campechiensis* generally inhabits the coast of the Gulf of Mexico, although it is also found, uncommonly, in offshore open shelf environments along the Atlantic Coast from New Jersey southward (Dillon and Manzi 1989b). The two species are sympatric and hybridize in the northern part of the Indian River Lagoon, Florida (figure 1).

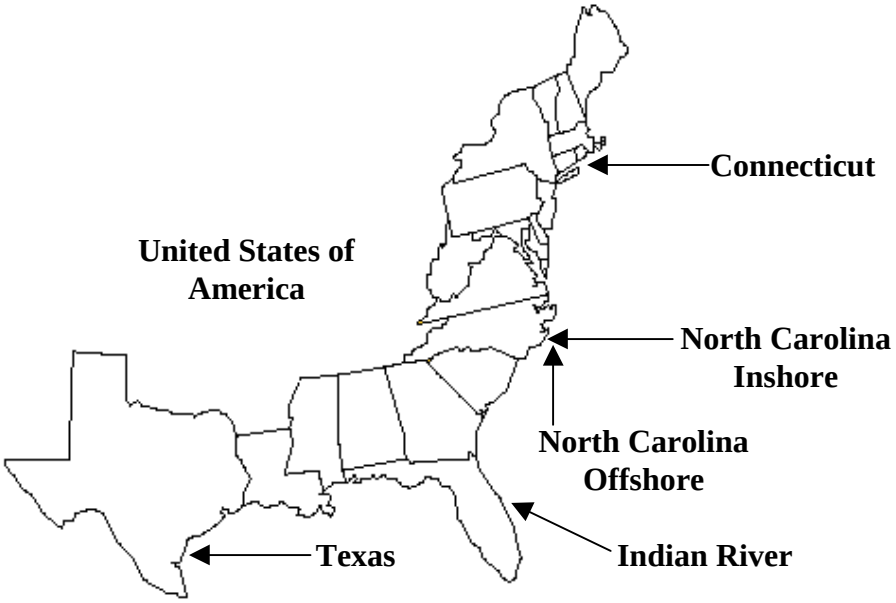



Figure 1. Locations of samples used in this study. Connecticut and North Carolina Inshore are pure *M. mercenaria*, Texas and North Carolina Offshore are pure *M. campechiensis*, and the Indian River Lagoon is an area of hybridization between the two species.

The Indian River hybrids are interfertile and can be backcrossed to parental lineages (Dillon and Manzi 1989b). Nevertheless, hybrids have reduced fitness due to a high frequency of gonadal neoplasia (Bert et. al. 1993). Both Bert et. al. (1993) and Dillon and Manzi (1989b) conclude that barriers to gene flow exist between *M. mercenaria* and *M. campechiensis*, and therefore, that

they are distinct species. Although hybridization occurs at low frequency wherever the species co-occur (Dillon 1992), hybridization is only extensive enough to constitute a hybrid zone (*sensu* Harrison 1990) in the Indian River Lagoon, Florida.

Study Area.—The Indian River, a 195 km-long, shallow, well-mixed, microtidal, polyhaline back-barrier lagoon, is located on the East coast of central Florida between the Eau Gallie River and Turkey Creek (Bader and Parkinson 1990; Bert and Arnold 1995). The Indian River Lagoon formed about 5,000 to 6,000 years ago when the Holocene transgression flooded a topographic depression (Bader and Parkinson 1990). Therefore, unless the hybrid zone has shifted in location, its maximum age cannot be more than 5,000 to 6,000 years. The well-defined symmetry of cuspate spits that lie along the seaward margin of the lagoon indicates that the lagoon has remained relatively stable over time (Bader and Parkinson 1990).

Samples.—The most extensive sampling was conducted in the hybrid zone in the Indian River Lagoon, Florida (n = 418). When mentioned in the text, the Indian River sample will be denoted parenthetically with "hz" to indicate its hybrid zone status.

This study not only utilizes a hybrid zone sample, but additionally pure-species samples. Although the study focuses on the hybrid zone sample, the additional pure-species samples, which do not contain hybrids, provide controlled information on the morphology of the pure species. Thus, in addition to the hybrid zone in Florida, specimens of live *Mercenaria* spp. (n = 187) were collected from four sites referred to here as Connecticut, North Carolina Inshore, North Carolina Offshore, and Texas. The samples were collected between July 1986 and June 1989 (figure 1) by William S. Arnold and colleagues as part of a series of studies concerning *Mercenaria* spp.

The North Carolina Inshore and Connecticut samples are composed of *M. mercenaria*. The North Carolina Inshore sample (n = 57) was collected from several locations in Bogue and Core Sounds around Morehead City. The substrate is typically a sand, mud, and shell mix with

seagrass beds of *Zostera marina* and *Halodule wrightii*. Water depth is generally 2 m or less. The Connecticut sample (n = 35) was collected from Long Island Sound in a water depth of approximately 5 m with a hard sand bottom covered with a thin layer of silt and shell.

The North Carolina Offshore and Texas samples are composed of *M. campechiensis*. The North Carolina Offshore sample (n = 43) was collected on the continental shelf off Morehead City in approximately 12 m water depth. The substrate was sandy with no vegetation. The Texas sample (n = 52) was collected from Ingleside Cove on the North side of Corpus Christi Bay, in less than 1 m water depth. The substrate was muddy with scattered patches of the seagrass *Halodule wrightii*.

When mentioned in the text, these four pure-species populations will be denoted by their sample name and, parenthetically, an abbreviated species designation: "*m*" for *M. mercenaria* populations, and "*c*" for *M. campechiensis* populations.

In a previous study by Bert et. al. (1993), gill and mantle tissue was used to genotype clams from each site by protein electrophoresis. *Mercenaria* spp. display pronounced (but not fixed) allelic differences at four enzyme loci (glucose phosphate isomerase [GPI], phenylalanine-specific dipeptidase-2 [DPEP2], aspartate aminotransferase [AAT], and phosphoglucomutase-2 [PGM2]). Clams were genotyped and classified as *M. mercenaria*, *M. campechiensis*, or a hybrid of the two by Theresa M. Bert, following the procedures described in Bert et. al. (1993).

Methods.—Morphological data were collected as 13 two-dimensional landmark points (figure 2). Landmarks are the Cartesian coordinates of discrete geometric locations that correspond between groups. Dryden and Mardia (1998) provide one useful categorization of landmark types: anatomical, mathematical, and pseudolandmark points (several other categorizations are also used [e.g. Bookstein 1991]). Anatomical landmarks, the most biologically informative category, are points that are biologically homologous between organisms; that is, a landmark on one organism biologically corresponds to the same landmark on another organism. Landmarks 1-9

(figure 2) used in this study are anatomical landmarks. Mathematical landmarks, generally less informative than anatomical landmarks, are defined by some mathematical or geometric property such as points of maximum curvature or extreme points. The mathematical landmarks used in this study (landmarks 10-13; figure 2) capture the general shell outline and, in a burrowing bivalve, a functionally important aspect of form. Pseudolandmarks, the least informative category, are points that are constructed according to some function, such as equally-spaced points along the outline of an object. No pseudolandmarks were used in this study. Anatomical landmarks are the most reliable to use because they are points of strict biological homology, generally the junction of tissues. Mathematical and pseudolandmark points are less reliable because, while they are definable between organisms, they are not necessarily biologically homologous (Bookstein 1991).

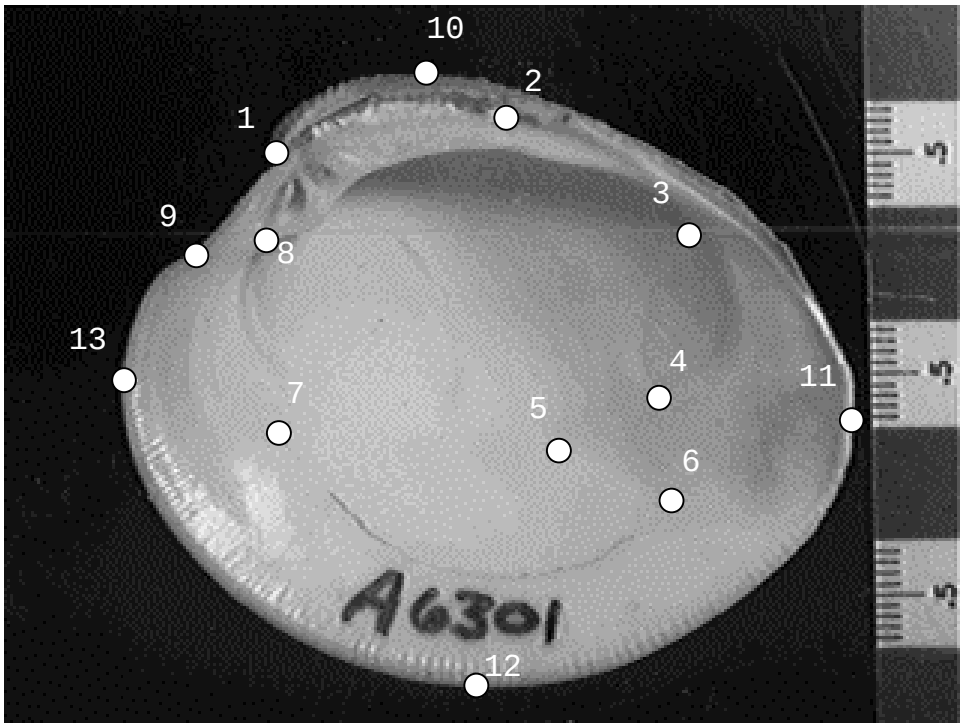



Figure 2. Landmarks used in this study. Anatomical landmarks (1-9): 1. umbo; 2. end of ligament; 3. junction of posterior retractor and posterior adductor; 4. junction of posterior adductor and pallial sinus; 5. inside of pallial sinus; 6. outside of pallial sinus; 7. junction of anterior adductor and pallial line; 8. junction of anterior retractor and anterior adductor; 9. end of lunule. Mathematical landmarks (10-13): 10. dorsal margin maxima; 11. posterior margin maxima; 12. ventral margin maxima; 13. anterior margin maxima.

Valves were oriented with a horizontal plane of commisure and then rotated the anterior and posterior retractor landmarks to a horizontal line. The interior of one valve per specimen was then digitally imaged. *Mercenaria* spp. valves are symmetrical, allowing the database to be maximized by using both right valves and digitally-mirrored left valves. Landmarks were collected as *x* and *y* Cartesian coordinates using Scion Image software (available as freeware from "www.scioncorp.com"). Repeated calibration and measurement of one specimen indicates that digitizing error accounts for a very small proportion of the total variation and does not affect the results.

Raw coordinates were transformed to Bookstein coordinates (also known as shape coordinates) by the following formulas (Dryden and Mardia 1998):

$$x^B = [(x_2 - x_1)(x_j - x_1) + (y_2 - y_1)(y_j - y_1)] / [(x_2 - x_1)^2 + (y_2 - y_1)^2]$$

$$y^B = [(x_2 - x_1)(y_j - y_1) - (y_2 - y_1)(x_j - x_1)] / [(x_2 - x_1)^2 + (y_2 - y_1)^2]$$

Where $(x^B,y^B)$ is the resulting Bookstein coordinate pair; $(x_1,y_1)$ and $(x_2,y_2)$ are the baseline coordinates; and, for $j = 3...k$ (for $k$ landmarks), $(x_j,y_j)$ are the coordinates to be transformed.

Bookstein coordinates result when a group of $k$ landmarks is subdivided into $k - 2$ triangles with the same baseline, and each triangle is translated, rotated, and rescaled so that the baseline coordinates become (0,0) and (1,0). The relationship between Bookstein coordinates that have different baselines is approximately linear provided the variability in the data is small (Dryden and Mardia 1998). The precise definition of "small" is debatable but a good rule of thumb is that the variability is small enough for the approximation to be reasonable if the standard deviation at the landmarks is less than 1/10 the mean length of the baseline (Dryden and Mardia 1998). (The approximation results because Bookstein coordinates do not exist in shape space, but in a linearized approximation of that space; details can be found in Dryden and Mardia 1998.) In addition, in graphical displays of Bookstein coordinates, variability will appear larger in landmarks further from the origin. For these reasons, it is best to choose baseline landmarks that are along the long axis of the organism and not too close together (Abe et. al. 1988; Dryden and

Mardia 1998). To fulfill the above considerations, the baseline was chosen to be landmarks three and eight, the posterior and anterior pedal retractor muscle scars, respectively.

Bookstein coordinates are one method in a suite of new and powerful geometric morphometric techniques, all of which use landmark data to explore shape differences (Rohlf and Bookstein 1990; Bookstein 1991; Foote, M. 1991; Rohlf and Marcus 1993a; Marcus et. al. 1996). Geometric morphometric techniques are more powerful than traditional morphometric techniques because they take into account the geometrical relationships among the measurements (Rohlf and Marcus 1993a). Bookstein coordinates were chosen because they are mathematically straightforward and easier to interpret than the various other geometric morphometric techniques (Corti 1993; Rohlf and Marcus 1993b). Analyses were also run using the generalized least-squares Procrustes procedure. For all analyses performed here, Procrustes analysis and Bookstein coordinates are fully consistent. Results presented here are limited to Bookstein coordinate data; no conclusions would change were the Procrustes procedure employed instead.

The size of each individual was reported as centroid size (in units of the original variables). Centroid size is the sum of squared interlandmark distances (also, the square root of the sum of squared Euclidean distances from each landmark to the centroid [Dryden and Mardia 1998]). Centroid size is the only size measure that is not correlated with shape when using landmark or any other morphometric data (Bookstein 1991); for this reason it is the size measure most used in geometric morphometrics (Dryden and Mardia 1998).

Statistical tests, except where explicitly stated, assume an alpha level of 0.05. All analyses were performed using SAS and SAS/IML software.

# Chapter 3: Results

The realization of the objectives of this study (morphological identification of individual hybrids and a hybrid zone) is contingent upon the ability to discriminate pure *M. mercenaria* from pure *M. campechiensis*; that is, if it is not possible to discriminate the pure-species from one another then it will not be possible to discriminate hybrids of those species. Therefore, an analysis of species-level differences, including an analysis of allometric effects on shape, precedes hybrid-level discrimination analyses.

Species Discrimination.—Previous studies based on traditional morphometric methods have shown that *M. mercenaria* and *M. campechiensis* can be discriminated morphologically on a number of characters, including thickness of ribbing, nacre color, and the height to width ratio of the lunule (Dillon and Hadley 1994; Dillon and Manzi 1989a). Populations of the same species were pooled (Texas [C] with North Carolina Offshore [C] and Connecticut [M] with North Carolina Inshore [M]) to form two samples: a representative *M. mercenaria* sample and a representative *M. campechiensis* sample. To determine if the two species form distinct morphogroups, a principal components analysis on the covariance matrix of the Bookstein coordinates was performed. Principal components analysis (PCA) is a commonly-used multivariate ordination procedure that creates new variables called principal components (PC). PCA captures the variability in the data using fewer dimensions than the original variables and is most often used to explore natural groupings within the data. A principal components ordination (PC 1 vs. PC 2) indicates that the species have overlapping but distinct morphospaces (figure 3). Together, the first two principal components account for 67% of the variation. The overlap of morphospace using the landmark data is not as severe as estimates using non-landmark data (e.g. Dillon & Manzi 1989a: fig. 2).

To confirm that the species morphogroups are statistically distinguishable and to determine characters that are most important in distinguishing the two species, a canonical variate analysis

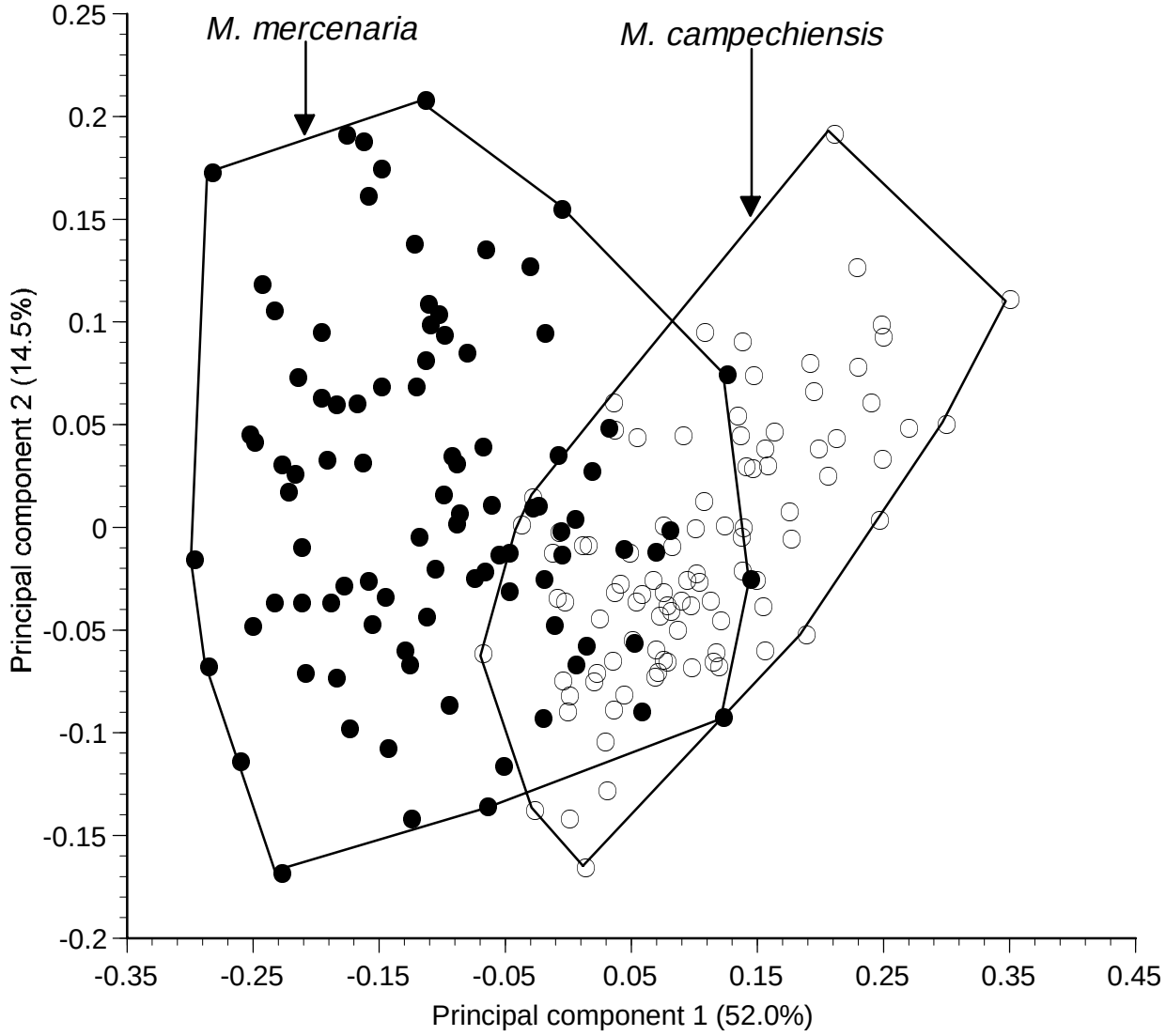



Figure 3. Principal component ordination (PC 1 vs. PC 2) of pooled non-hybrid zone (pure-species) samples (*M. mercenaria*, closed circles, n = 92; *M. campechiensis*, open circles, n = 95).

on the Bookstein coordinates of the pure-species was performed. Canonical variate analysis (CVA) is a multivariate ordination procedure that maximizes separation between *a priori* defined groups. A canonical variate ordination (can 1 vs. can 2) confirms that the two species are indeed non-overlapping (figure 4) (df = 22, F = 54.4, $p < 0.0001$; Wilks' Lambda MANOVA).

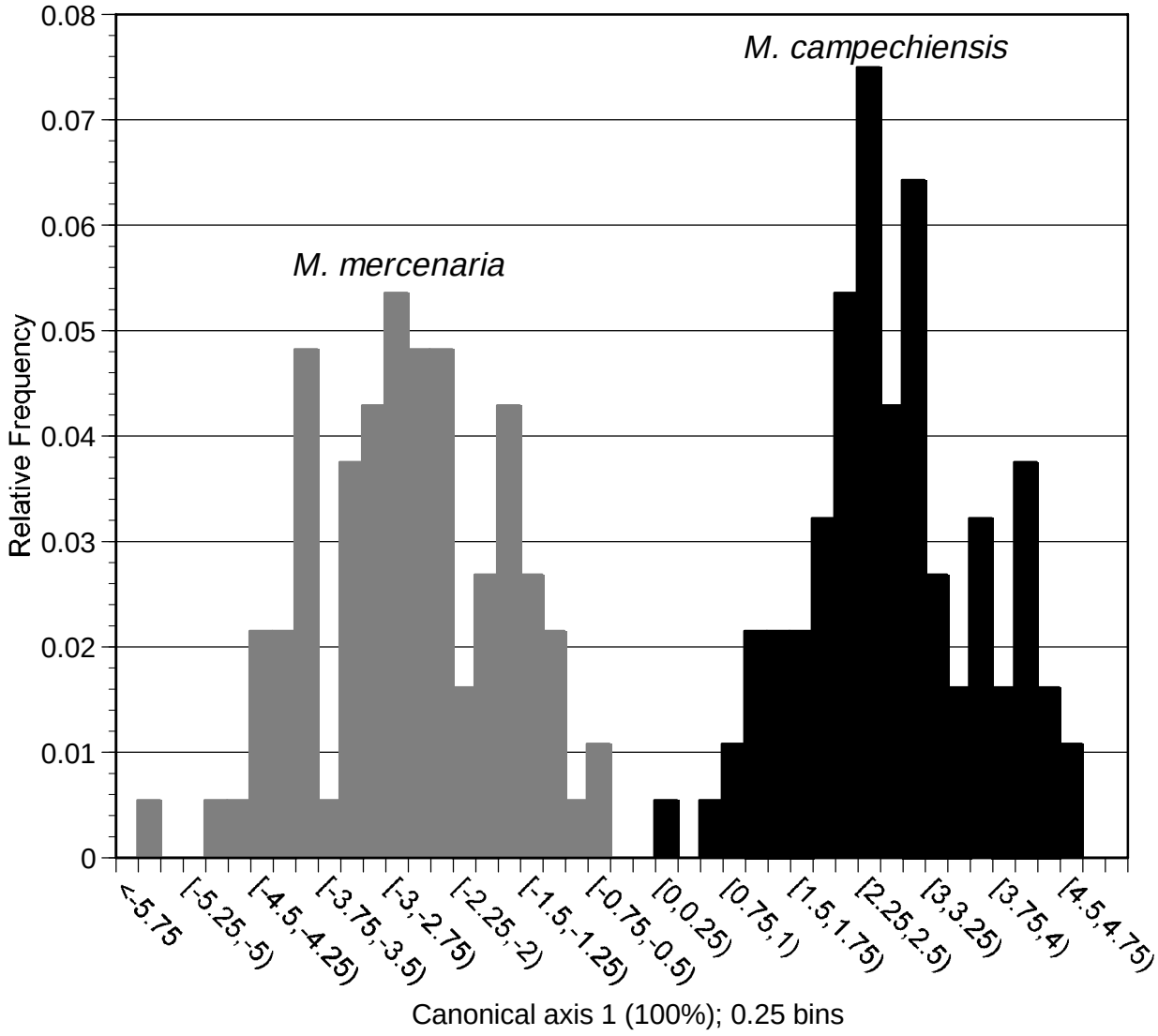



Figure 4. Histogram of canonical scores (can 1) of *M. mercenaria* (gray; n = 92) and *M. campechiensis* (black; n = 95). The axis accounts for 100% of the variability (for two groups, canonical variate analysis results in one axis).

Together, the two axes account for 91% of the total variance. Variables with the highest correlation with the first canonical axis indicating characters that were most important in discriminating the species were the lunule, ligament, ventral margin, and dorsal margin (figure 5).

The PCA and CVA show that species-level discrimination using the landmark data set is possible; in fact, landmarks provide more information to distinguish the species than traditional

measurements. However, the discrimination may be due to allometry (size-dependent shape effects) if the sampled populations happen to be at different ontogenetic stages. Bookstein (1991) suggests multiple regression of the Bookstein coordinates on centroid size as a simple test for allometry. Both *M. mercenaria* and *M. campechiensis* show statistically significant allometry (*M. mercenaria*: df = 91, F = 25.19, $p < 0.0001$; *M. campechiensis*: df = 94, F = 2.22, $p = 0.006$), indicating that size-dependent shape variation is present in the samples. When the effects of allometry are removed (by regression of each Bookstein coordinate on centroid size), the species cannot be discriminated. The worst-case scenario is that allometric effects are creating false shape differences between samples that have different sizes. A number of arguments, presented below, show that this is not the case.

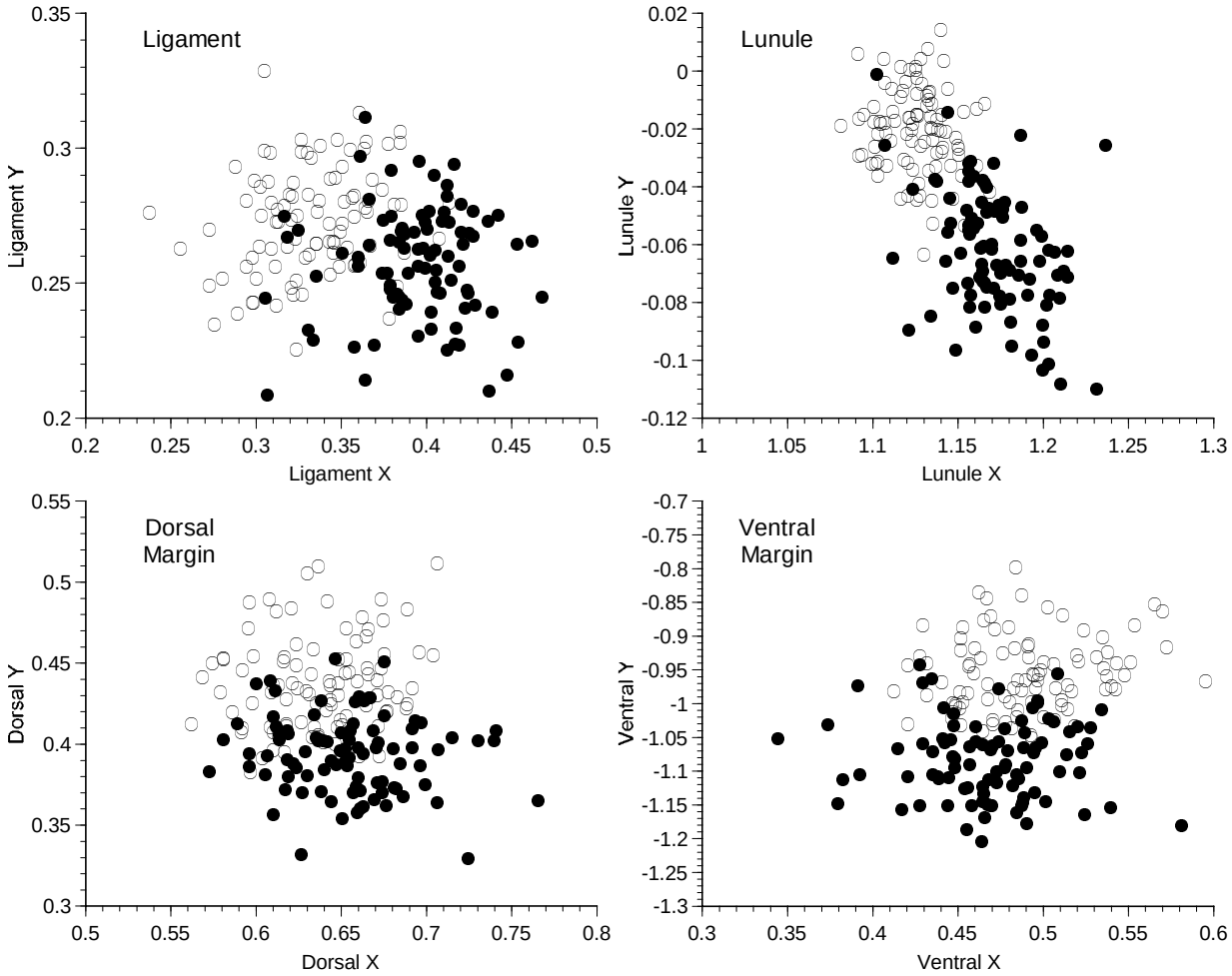



Figure 5. Bookstein coordinate plots of the four variables that are most important in discriminating *M. mercenaria* (closed circles; n = 92) from *M. campechiensis* (open circles; n = 95).

The samples used in this study do show size differences that correspond to the results of the discriminant analysis. Size distributions were indistinguishable from normal but have unequal variances; therefore, a Welch ANOVA for differences between the mean size was used (Zar 1999). The differences between the mean centroid sizes of the samples are statistically significantly different at the $\alpha = 0.05$ level (table 1). The Tukey-Kramer HSD (Honestly Significant Difference) test (Zar 1999) was used to determine which means were different (the Tukey-Kramer HSD test is conservative when sample sizes are different [Hayter, 1984]).

The above results show that *M. mercenaria* and *M. campechiensis* are morphologically distinct, but there are allometric effects in the data and the size distributions of the samples are different. To evaluate whether these factors acting together are introducing false shape differences between the species, the allometric trajectory of each species was examined.

Table 1: Descriptive Statistics. Means marked by the same letter are not statistically significantly different at the $\alpha = 0.05$ level using the Tukey-Kramer HSD test (Zar, 1999)

| Sample | Taxon | N | Centroid Size | | Shapiro-Wilk Normality Test | |
|---|---|---|---|---|---|---|
| | | | $\xi$ | $\sigma$ | W | *p* value |
| Connecticut | *M. mercenaria* | 35 | 106.3 [a] | 18.8 | 0.95 | 0.14 |
| North Carolina Inshore | *M. mercenaria* | 57 | 63.2 [b] | 20.4 | 0.97 | 0.13 |
| North Carolina Offshore | *M. campechiensis* | 43 | 135.2 [c] | 15.3 | 0.98 | 0.67 |
| Texas | *M. campechiensis* | 52 | 126.1 [c] | 12.0 | 0.98 | 0.51 |
| Indian River Hybrid Zone | *M. campechiensis* | 20 | 95.0 [d] | 16.9 | 0.92 | 0.091 |
| | Hybrids | 110 | 91.7 [d] | 22.9 | 0.99 | 0.92 |
| | *M. mercenaria* | 288 | 89.1 [d] | 21.6 | 0.98 | 0.0036 |
| | (Indian River Total) | (418) | (90.0 [d]) | (21.8) | (0.99) | (0.068) |
| Total | | 605 | | | | |

Allometric trajectories, the within-species pattern of growth during ontogeny (McKinney and McNamara 1991), are traditionally examined by using allometric coefficients to determine which

characters are allometric and then by visually inspecting the allometric growth patterns of the two species. Multivariate allometric coefficients are calculated by dividing the first principal component loading for each variable by the mean first principal component loading (Jolicoeur 1963). Unfortunately, allometric coefficients cannot be computed for Bookstein coordinates because the first principal component cannot be explained by size, as size is factored out when calculating Bookstein coordinates. Therefore, to determine if the species have different allometric trajectories, Pearson's correlation coefficient was calculated for each Bookstein coordinate against centroid size to measure the allometry for each coordinate. The higher the correlation coefficient the higher the association between the Bookstein coordinate and centroid size, and consequently, the stronger the allometry. The allometric pattern for each species is shown by the pattern of statistically significant correlations. It is appropriate to compare statistical significances because sample sizes for *M. mercenaria* (n = 92) and *M. campechiensis* (n = 95) are nearly identical ( values are also dependent on sample size). *M. mercenaria* is allometric for 17 of the 22 Bookstein coordinates, while *M. campechiensis* is allometric for only 3 of the 22 Bookstein coordinates (figure 6). Within-species populations show slight variations from the all-inclusive species allometric patterns, but generally are the same. The two species differ significantly in the proportion of statistically significant allometric characters ($p < 0.0001$; Fisher's Exact test).

Thus, the species have been shown not only to have size differences, but also to follow different allometric trajectories. This suggests that the morphological discrimination between *M. mercenaria* and *M. campechiensis* is not an accidental differentiation of populations that differ in size-frequency distribution; instead, it reflects real differences in patterns of growth during ontogeny. To confirm this, a canonical variate analysis was performed on a restricted data set consisting only of individuals within the size range in which the species co-occur. This tests whether the species can still be discriminated after size differences are eliminated.

All four *Mercenaria* spp. populations' size ranges overlap between 100 mm and 114 mm. Within this range, each population is represented and the two species have comparable sample

| *M. mercenaria* | | *M. campechiensis* | |
|---|---|---|---|
| BC 1 0.26 $p = 0.01$ | BC 12 0.29 $p = 0.01$ | BC 1 0.38 $p < 0.001$ | BC 12 0.03 $p = 0.77$ |
| BC 2 -0.39 $p < 0.001$ | BC 13 -0.56 $p < 0.001$ | BC 2 -0.11 $p = 0.29$ | BC 13 0.01 $p = 0.92$ |
| BC 3 -0.57 $p < 0.001$ | BC 14 0.16 $p = 0.12$ | BC 3 -0.13 $p = 0.21$ | BC 14 -0.13 $p = 0.21$ |
| BC 4 0.37 $p < 0.001$ | BC 15 0.16 $p = 0.12$ | BC 4 0.05 $p = 0.61$ | BC 15 0.21 $p = 0.04$ |
| BC 5 -0.16 $p = 0.14$ | BC 16 0.53 $p < 0.001$ | BC 5 0.12 $p = 0.23$ | BC 16 0.22 $p = 0.03$ |
| BC 6 0.06 $p = 0.58$ | BC 17 -0.26 $p = 0.01$ | BC 6 -0.11 $p = 0.29$ | BC 17 0.06 $p = 0.55$ |
| BC 7 -0.72 $p < 0.001$ | BC 18 0.25 $p = 0.02$ | BC 7 -0.16 $p = 0.12$ | BC 18 0.06 $p = 0.54$ |
| BC 8 0.42 $p < 0.001$ | BC 19 -0.21 $p = 0.04$ | BC 8 0.03 $p = 0.76$ | BC 19 -0.14 $p = 0.17$ |
| BC 9 -0.68 $p < 0.001$ | BC 20 0.55 $p < 0.001$ | BC 9 -0.07 $p = 0.48$ | BC 20 0.18 $p = 0.09$ |
| BC 10 0.53 $p < 0.001$ | BC 21 -0.33 $p = 0.001$ | BC 10 -0.02 $p = 0.88$ | BC 21 -0.10 $p = 0.34$ |
| BC 11 -0.62 $P < 0.001$ | BC 22 0.18 $p = 0.09$ | BC 11 -0.16 $p = 0.12$ | BC 22 0.06 $p = 0.53$ |

Figure 6. Allometric pattern of each *Mercenaria* species. Boxes contain the Bookstein coordinate and Pearson's correlation coefficient (for Bookstein coordinate vs. centroid size), which measures the degree of allometry for that Bookstein coordinate. Bookstein coordinates that are statistically significant at the $\alpha = 0.05$ level are shown in shaded boxes.

sizes (100 mm - 114 mm sample sizes: *M. mercenaria*, n = 19; *M. campechiensis*, n = 12). The within-species populations were pooled as before, and a histogram of the canonical scores indicates that the two species can be perfectly discriminated, despite the samples having the same size ranges (figure 7).

A highly conservative theoretical simulation agrees with the above results. This test is fashioned by artificially creating samples of clams that are all exactly the same. For each species, regression analysis was used to predict the value of each Bookstein coordinate for a 100 mm clam. Then, samples (each n = 30) were created from each Bookstein coordinate value by

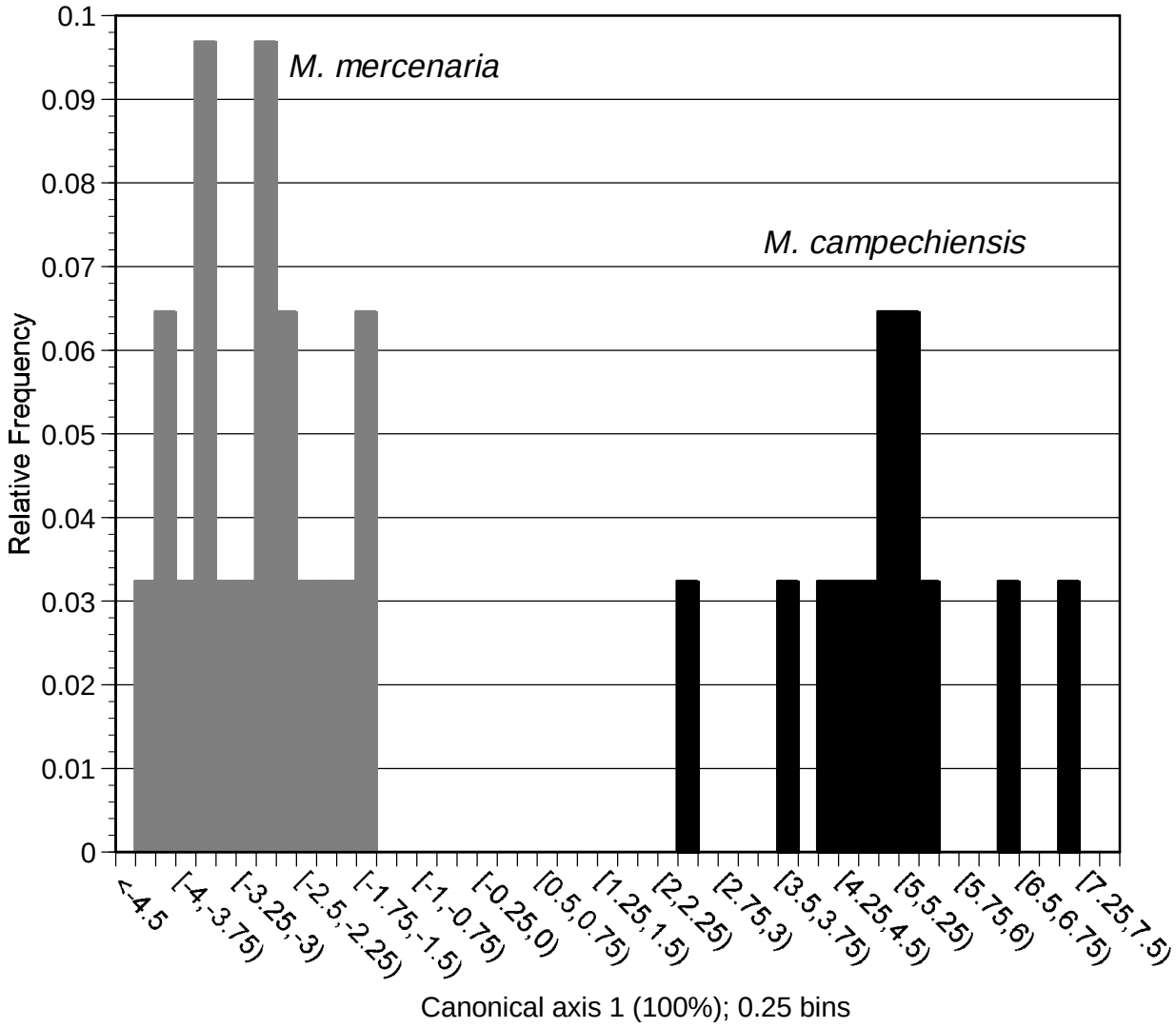
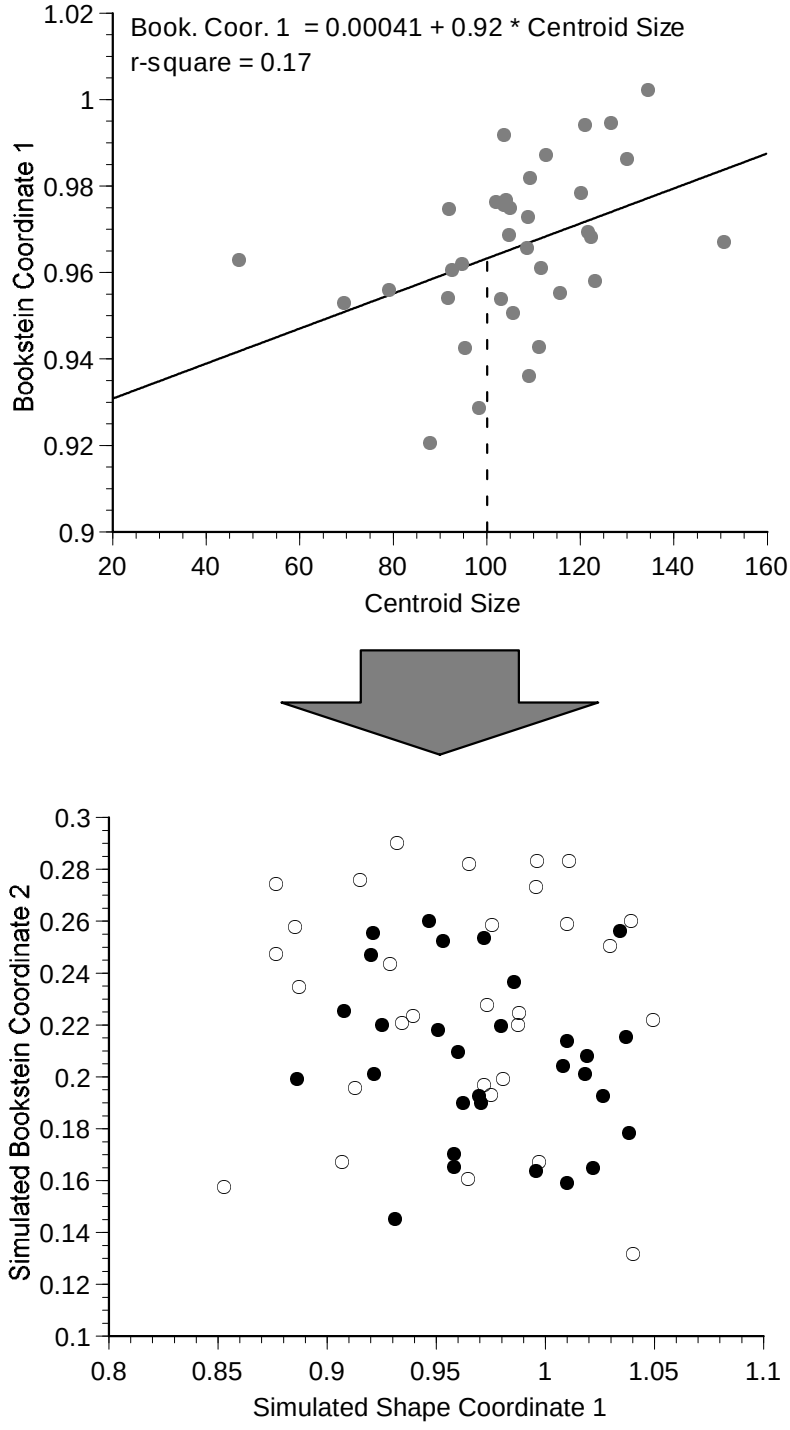



Figure 7. Histogram of canonical scores (can 1) for size-restricted (100 – 114 mm) *M. mercenaria* (gray; n = 19) and *M. campechiensis* (black; n = 12).

"jittering" the Bookstein coordinate according to a uniform distribution, constrained to stay within the true range of the Bookstein coordinate (figure 8). Using a uniform distribution makes the test conservative because each Bookstein coordinate has an equal probability of lying at the end of its range as at the midpoint; in a true case, most values would be clustered around the midpoint with a few extreme values at the edge. The uniform density of points is less likely to be discriminated because more points overlap one another. In addition, the entire range of the Bookstein coordinate was used as the boundary, not just the range observed at 100 mm. This,




Total range used in simulation

Figure 8. Explanation of the simulation of theoretical populations of 100 mm *Mercenaria* spp. Top: each Bookstein coordinate is predicted for a 100 mm specimen. Bottom: coordinate is randomized 30 times using a uniform distribution, constrained to the actual range of the Bookstein coordinate.

too, creates highly overlapping distributions of points, which are much less likely to be discriminated. Despite the highly conservative nature of the test, the simulated 100 mm samples could be discriminated with minimal overlap (figure 9).

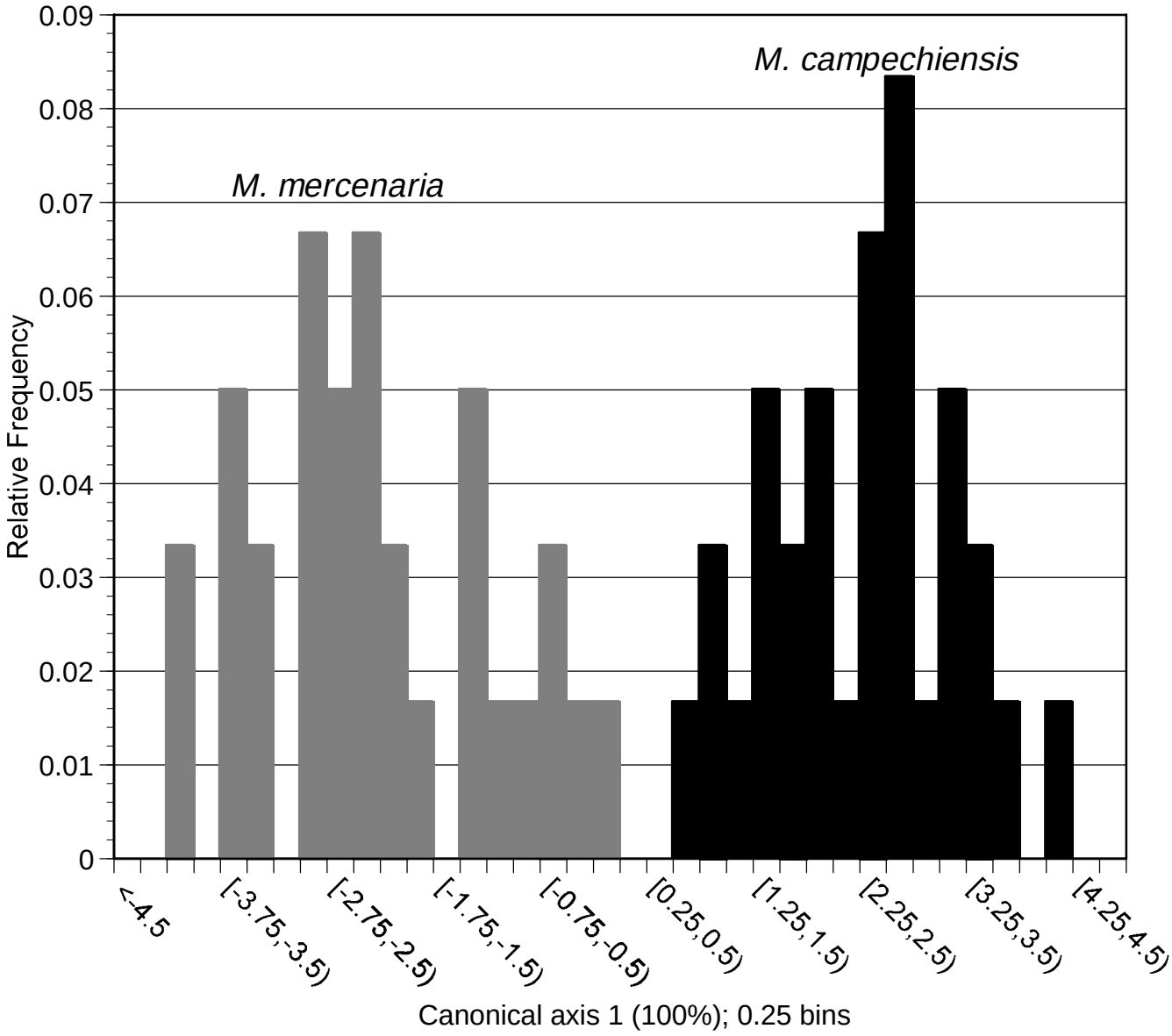



Figure 9. Histogram of canonical scores (can 1) for simulated 100 mm *M. mercenaria* (gray; n = 30) and *M. campechiensis* (black; n = 30).

The analyses above show that the sampled populations of the two species differ in size, but also differ in their allometric trajectories. Therefore, allometry was not factored out prior to analysis because the differences between the two taxa are produced by differences in allometric change in the two species. This choice raises many interesting points about the nature of evolutionary change between species.

Allometry is often dismissed by researchers without further explanation; the result shown here, however, indicates that the story is not so simple. While discarding size-dependent shape change is always the most conservative course of action, it is not necessarily the most biologically meaningful and can result in loss of explanatory power if the study's aim is to explore the nature of differences between groups. Allometry is the endpoint of heterochronic change (McKinney and McNamara 1991). In this case, it appears that *M. mercenaria* and *M. campechiensis* have diverged morphologically by differentially modifying their developmental pathways. However, with the available information, it cannot be determined what type of heterochronic change took place during the divergence of the species.

Hybrid Discrimination.—The first objective of this study is to determine if individuals within the *Mercenaria* spp. hybrid zone can be discriminated based on morphology. A principal components analysis was performed on the Indian River (hz) sample using Bookstein coordinates (figure 10). A principal component ordination (PC 1 vs. PC 2) demonstrates that all three taxa (*M. mercenaria*, *M. campechiensis*, and hybrids) are almost completely overlapping in morphospace. Exclusion of hybrids from the calculation of principal components does not affect the outcome—*M. mercenaria* and *M. campechiensis* from within the hybrid zone overlap completely. This result contrasts with the results obtained for non-hybrid zone samples, which formed distinct morphogroups. The lack of discrimination observed within the hybrid zone is probably due to the clinal nature of the hybrid zone. Despite genetic analysis of a few alleles forcing membership into one of three taxa, it is very likely that individuals from the hybrid zone make up a genetic gradient from 100% *M. mercenaria* to 100% *M. campechiensis* because *Mercenaria* spp. hybrids are fully fertile with one another and with the parental species. This

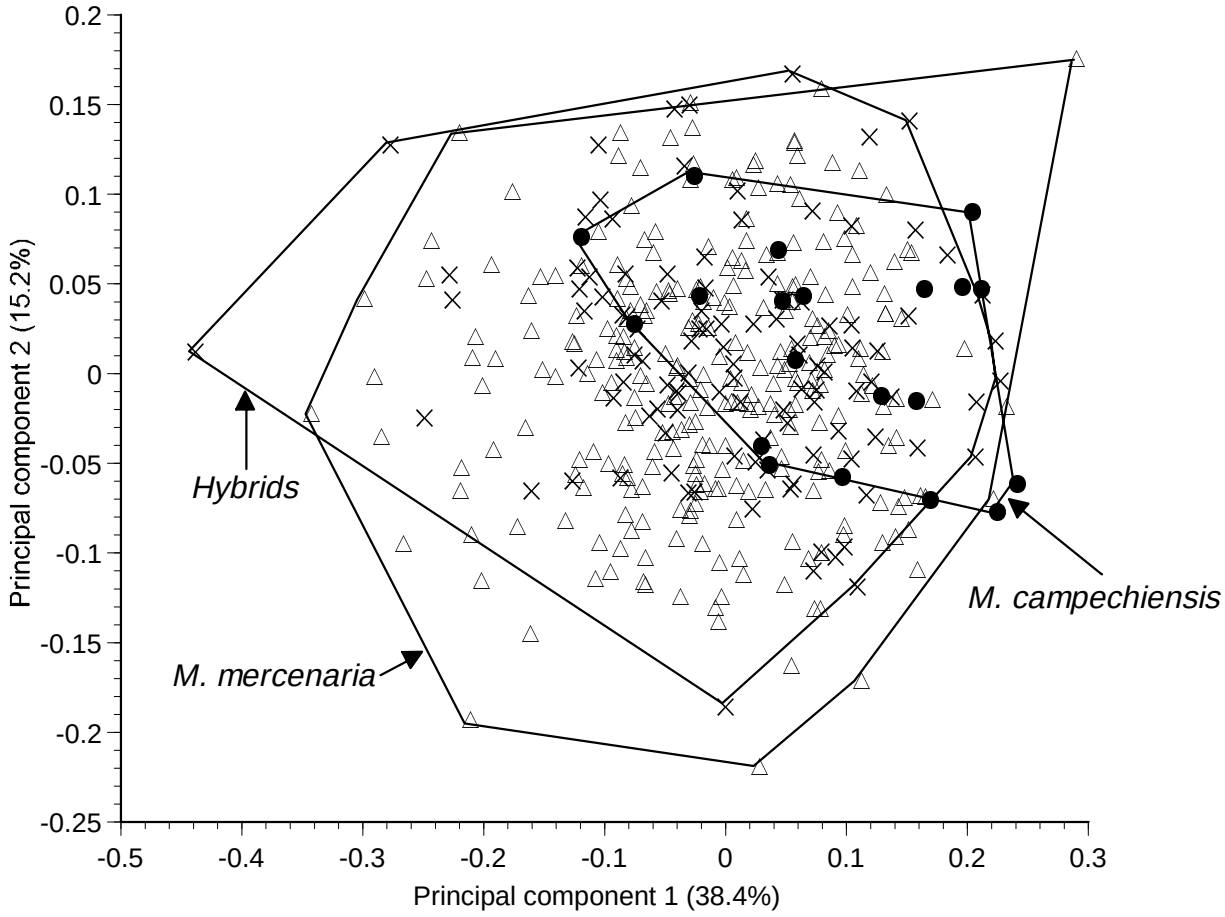



Figure 10. Principal component ordination (PC 1 vs. PC 2) of hybrid zone taxa: *M. mercenaria* (triangles; n = 288), hybrids (crosses; n = 110), and *M. campechiensis* (closed circles; n = 20).

point agrees with data from most hybrid zones, which are typically clines; that is, parallel gradients of one or many characters (Barton and Hewitt 1989).

Canonical variate analysis cannot be applied appropriately to identify group membership of individuals when no *a priori* groupings are known. Therefore, this procedure is not useful to determine if fossil individuals within the hybrid zone can be discriminated based on morphology. However, the analysis is of interest from a biological perspective because it can be used to evaluate morphological discrimination of genetically pre-defined groups and because it provides a "best case" scenario for morphological discrimination. A canonical variate ordination (can 1 vs. can 2) indicates that hybrids overlap with both of the parental species (figure 11); therefore,

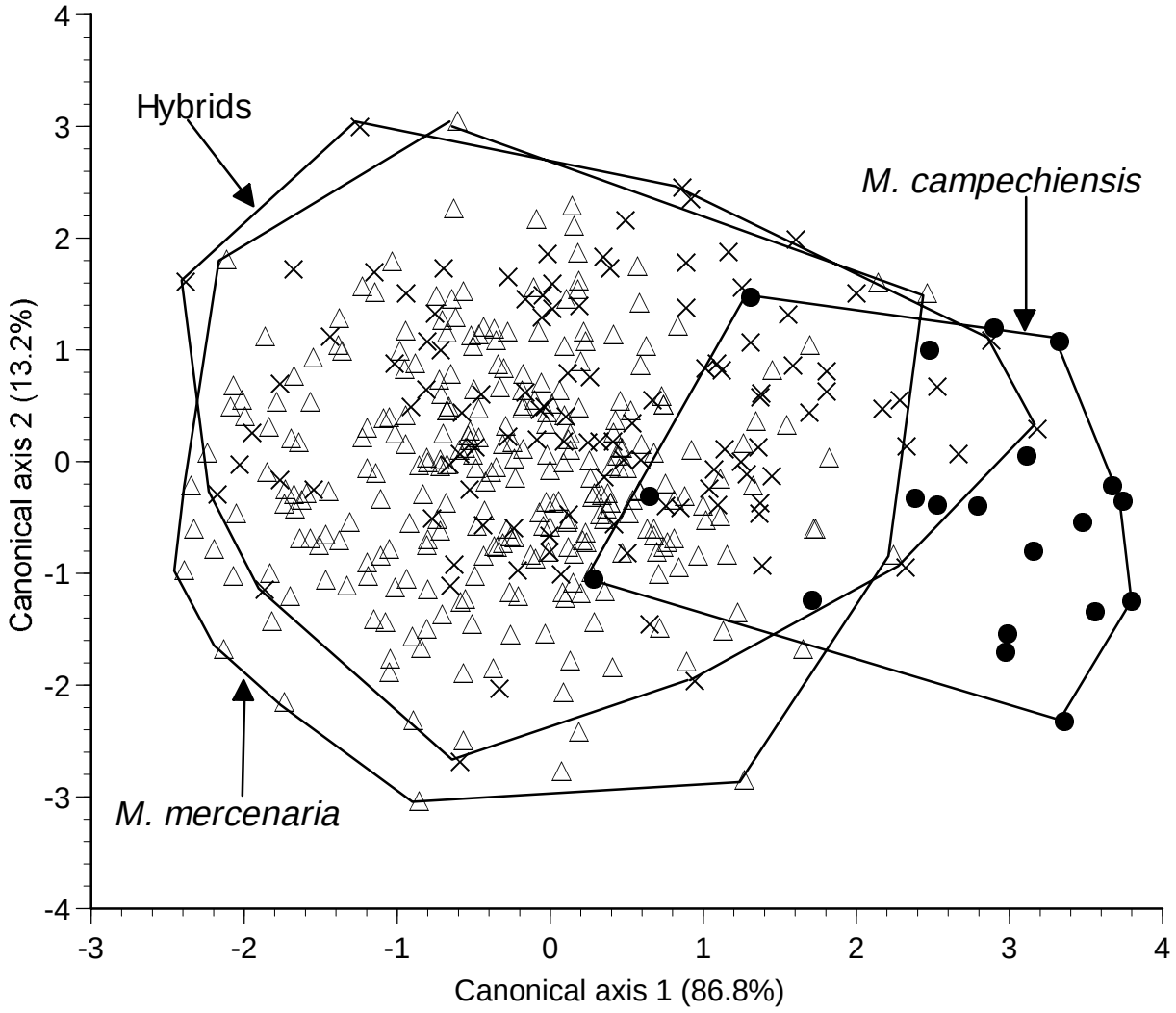



Figure 11. Canonical variate ordination (can 1 vs. can 2) of hybrid zone taxa: *M. mercenaria* (triangles; n = 288), hybrids (crosses; n = 110), and *M. campechiensis* (closed circles; n = 20).

even under optimum conditions, when genetic information and powerful multivariate methods are combined, there is still a high error rate in distinguishing the pure species from hybrids using this data set.

Detection of the Hybrid Zone.—Although the results above demonstrate that individuals from within the *Mercenaria* spp. hybrid zone cannot be identified morphologically, the possibility still exists that the hybrid zone as a whole can be identified by comparison with geographically separated populations. A canonical variate analysis of the Bookstein coordinates was performed

on the Texas (*c*) and Connecticut (*m*) samples (figure 12a). Individual scores on the canonical axis were plotted on a histogram rather than a bivariate plot, as above, because for two groups, canonical variate analysis results in one canonical axis (now called a discriminant axis; see Marcus 1990 for a discussion of the semantics involved). The Texas (*c*) and Connecticut (*m*) samples are disjunct on the discriminant axis. The Indian River hybrid zone was then classified *a posteriori* according to the discriminant function developed from the Texas (*c*) and Connecticut (*m*) samples. The Indian River hybrid zone occupies the canonical space of both pure *M. mercenaria* and pure *M. campechiensis*, as well as the intermediate canonical space, which is occupied by hybrids (figure 12b).

The observation that hybrid zones occupy both the pure-species morphospace as well as intermediate morphospace is consistent with biological views concerning the morphological and genetic makeup of hybrids as compared to pure species. Hybrids, because they are a mix of genotypes, are usually intermediate both phenetically and genetically (Barton and Hewitt 1985; Gardner 1997).

The distribution of morphologies within the hybrid zone is not perfectly centered between the pure-species morphospace, but skewed toward that of the most abundant parental species, *M. mercenaria*. This is probably not a product of asymmetrical introgression of alleles, but rather a reflection of the fact that the probability of backcrossing with a parental species is higher with the more abundant parental species. This is particularly true in bivalves because they do not have mate choice (bivalves reproduce by dispersing their gametes into the water). Though the right tail of the hybrid zone distribution is firmly within the *M. campechiensis* morphospace, indicating that many hybrids have *M. campechiensis*-like morphologies, the number of *M. campechiensis* is rather small (only 20 of 418 individuals from the hybrid zone).
The general pattern of morphospace occupation demonstrated above—that the *Mercenaria* spp. hybrid zone occupies the parental species morphospace plus intermediate morphospace—is not exclusive to hybrid zones. If three fossil samples (i.e. two putative pure-species samples and one

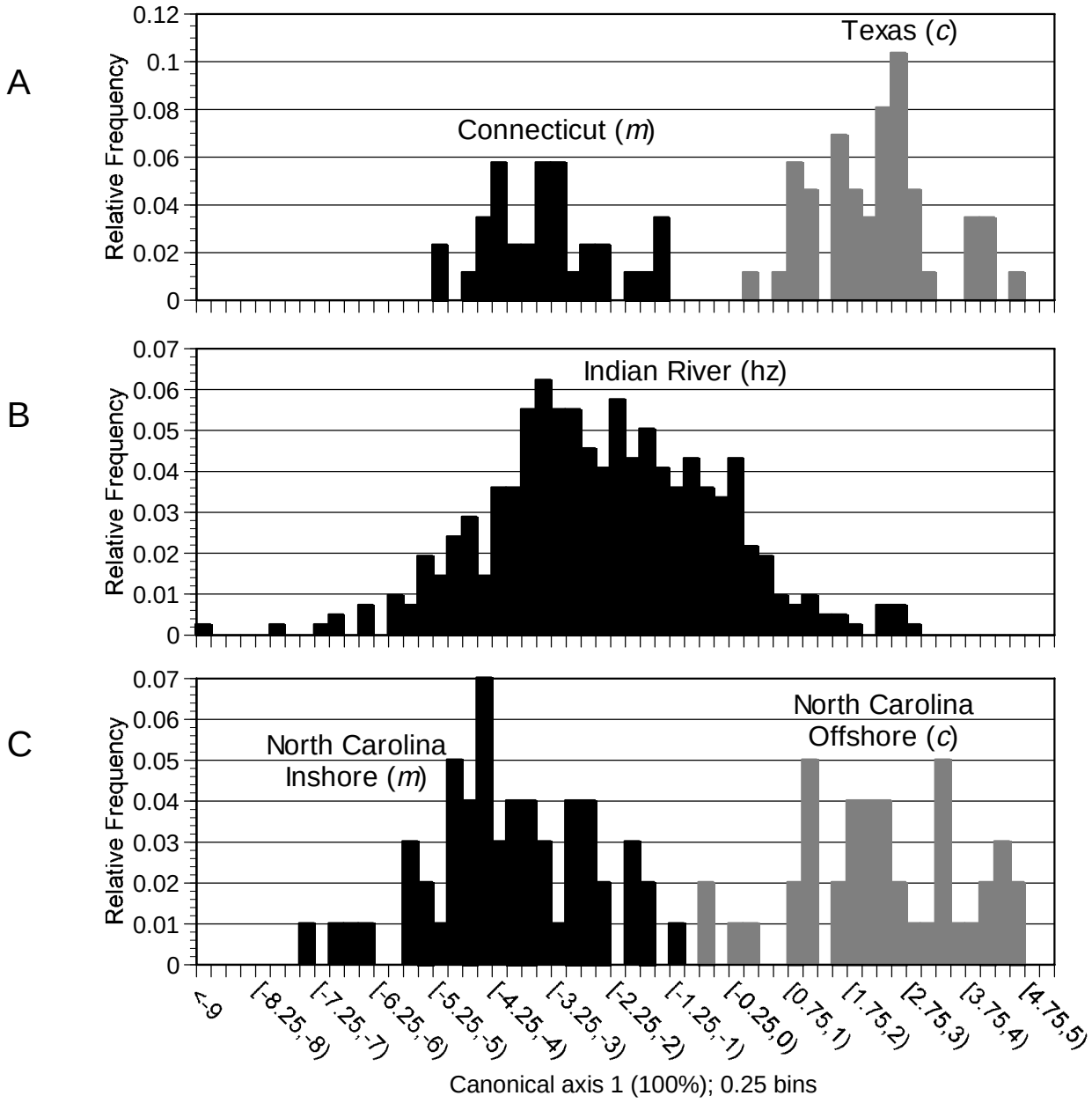



Figure 12. (A) Discriminant analysis of Connecticut (*m*) and Texas (*c*) samples. (B) *A posteriori* classification of the Indian River hybrid zone. (C) *A posteriori* classification of the North Carolina Inshore (*m*) and North Carolina Offshore (*c*) samples.

sample showing the above pattern of morphospace occupation) are taken from different geographic areas, without *a priori* knowledge that one of the samples is from a hybrid zone,

three alternative explanations of the pattern exist: polymorphism, population-level genetic differences, and ecophenotypy.

The first explanation, polymorphism, occurs when a population contains more than one recognizable form (Ridley 1996). Gender difference (resulting in dimorphism) is one common type of polymorphism. Strictly speaking, dimorphism will result in a bimodal morphological distribution, which is not observed in the *Mercenaria* spp. hybrid zone and thus would not present a problem for identifying a *Mercenaria* spp. hybrid zone. The issue is worth considering in a general sense, however, because, if hybrids are not abundant, a hybrid zone will be bimodal. Geary (1992) presents a bimodal distribution of fossil gastropod morphology and is ultimately unable to determine whether its cause is dimorphism or hybridization. Independent environmental evidence, such as used in Geary (1992), is not useful to distinguish betweeen dimorphism and hybridization because, though hybrid zones are typically situated at or near environmental transitions (Hewitt 1988), heterogenous environments can also give rise to polymorphisms (Ridley 1996). The question of dimorphism versus hybridization can be resolved by using samples from outside the putative hybrid zone. These samples will act as a control on whether that species is, in general, dimorphic. Though different morphs may appear in different environments (e.g. swallowtail butterflies [Ridley 1996]), it is unlikely that dimorphism will express itself in only one population of a species. Had the *Mercenaria* spp. hybrid zone had a bimodal morphological distribution, then comparison with the distributions of non-hybrid zone samples would have indicated that the species is not dimorphic.

The second alternative explanation is that the sample that shows parental plus intermediate morphospace occupation differs from the reference samples due to within-species (population-level) genetic differences. The magnitude of population-level differences within one species can range from no difference to the magnitudes traditionally seen between fully reproductively-isolated species (Mayr 1963), and therefore population-level differences may present a significant difficulty when attempting to distinguish hybrid zones in the fossil record. *Mercenaria* spp. populations do show population-level morphological differences. If the

samples used in this study were treated as fossil samples (i.e. the genetic designations of the samples were not known), then, based on morphology alone it is equally likely that the Texas (*c*) and Connecticut (*m*) samples are geographic variants of the same species, and that the Indian River (hz) is a highly-variable geographic variant of the same species.

The third alternative explanation is that the difference between all three samples is due to ecophenotypic effects on morphology, and that one of the three environments allows greater morphological variability (perhaps because it is itself more variable than the other two environments). Studies of the growth rate of *Mercenaria* spp. indicate that it is at least partially environmentally influenced (Jones et. al. 1990; Arnold et. al. 1996). The hypothesis of ecophenotypic population differences is analogous to the above case of population-level genetic differences, but instead the differences result from environmentally-controlled rather than genetically-controlled morphology. Environmental information collected independently of morphology can be used to infer ecophenotypy, but that requires knowledge of how environmental factors affect morphology, and assumes that the environmental factors that influence morphology are preserved in the fossil record.

Both population-level genetic differences and ecophenotypy can be ruled out by sampling additional populations from other localities and environments. If the additional populations fall within the same discriminant space as the reference populations, then it is unlikely that the sampled population is a geographic (i.e. genetic) or ecophenotypic variant. Thus, the addition of more populations tests the assumption that the morphospaces established by the first two reference populations are the true "species morphospaces", not "population morphospaces" specific to only those populations. To test this, the two additional samples of *Mercenaria* spp., North Carolina Offshore (*c*) and North Carolina Inshore (*m*), were classified according to the discriminant function developed from the Texas (*c*) and Connecticut (*m*) samples and plotted as a histogram of discriminant scores (figure 12c). Both of these samples are geographically distant from the other population of its species, and from different environments. The two groups are disjunct despite the fact that the discriminant function was optimized for the differences between

the Texas (*c*) and Connecticut (*m*) samples, providing a conservative estimate of morphological distance. In addition, the North Carolina Offshore (*c*) and the North Carolina Inshore (*m*) samples overlap the Texas (*c*) and Connecticut (*m*) samples, respectively, establishing that the occupied zone of canonical space is in fact species morphospace rather than population morphospace. The addition of more populations can, of course, continue indefinitely, with increasing power at each addition. Using multiple populations rules out both polymorphism and ecophenotypy in determining the cause of the pattern of parental species plus intermediate morphospace occupation.

# Chapter 4: Discussion

Discrimination of Hybrid Individuals.—This study substantiates the accumulating evidence that hybrid individuals cannot be identified based on morphology. Previous studies of hybrid morphology have relied on less powerful traditional morphometric methods, and yet the use of sophisticated geometric morphometric techniques based on landmark data results in the same conclusion.

*Mercenaria* spp. hybrids cannot be morphologically identified because of the morphological intergradation between hybrids and parental species. This intergradation is most likely the result of full hybrid fertility. Hybrid fertility causes the individuals within the Indian River hybrid zone to form a genetic gradient from 100% *M. mercenaria* to 100% *M. campechiensis*, despite genetic analysis forcing membership into one of three groups.

Various studies have demonstrated that there is no consistent correlation between morphology and genetics (Lessios 1981; Budd et. al. 1994; Jackson and Cheetham 1995; Kowalewski et. at. 1997; and references therein). Even if that were not so, hybrid individuals would not be morphologically identifiable because there are no discrete groupings (morphological or genetic) within a cline. Crespin et. al. (1999) point out the futility of attempting to distinguish putative hybrids from parental species for taxonomic purposes when there is a continuum of hybrids between parental species, a remark that is mirrored here. Even with molecular markers, it may not be possible to identify the genealogy of any individual taken from a hybrid zone (Rieseberg and Linder 1999).

The inability to distinguish *Mercenaria* spp. hybrids contrasts with the conclusions of Ausich and Meyer (1994), who report individual hybrids of the Mississippian crinoid *Eretmocrinus* spp. This contrast may point to taxon-specific differences in hybrid identification or may relate to the degree of divergence between the parental groups. However, Ausich and Meyer's conclusions are based on only three specimens that occur only in the largest samples, and these specimens

fall fully within the discriminant space occupied by one of the parental species. An equally likely explanation is that these specimens are extreme phenotypes, and therefore it is difficult to evaluate their result in the context of this study.

The most important implications of the inability to discriminate individual *Mercenaria* spp. hybrids are twofold. First, this result supports the long-held notion in biological literature that hybrids cannot be reliably morphologically identified, despite many studies that use morphometric indices of hybridity (Rieseberg and Linder 1999). The support of this study is particulary noteworthy because of the use of rigorous geometric morphometrics that are more likely to detect subtle differences between groups (Rohlf and Marcus 1993a). This study cautions that conducting paleontological studies that require identification of hybrid individuals will probably prove unprofitable.

Second, this result corroborates definitions of morphospecies by furthering the evidence that biological species do form discrete morphogroups. Pure-species samples of *M. mercenaria* and *M. campechiensis* form distinct morphogroups despite being closely related species that have not yet evolved reproductive isolation. Speculatively, the nature of species integrity in *Mercenaria* spp. appears to be a result of gene flow acting to homogenize the species (and therefore minimizing between-species differences). *M. mercenaria* and *M. campechiensis* are more distinct outside of the hybrid zone (in allopatry) than they are within the hybrid zone (in sympatry). The presence of gene flow within the hybrid zone is acting to homogenize the species, and where there is no gene flow, the species are more distinct. This contrasts with the predictions of character displacement (Schluter et. al. 1985; Coyne and Orr 1989; Schluter 1995) as the mechanism of the maintenance of species integrity. Character displacement between these species cannot take place outside of the hybrid zone because the species do not interact. In any case, pure *Mercenaria* species are the smallest units that are morphologically discrete. Hybrids do not represent a discrete morphogroup.

Discrimination of the Hybrid Zone.—This study also documents the pattern of morphology in the *Mercenaria* spp. hybrid zone as a whole. The revealed pattern strongly suggests that whereas *Mercenaria* spp. hybrids cannot be morphologically identified at the individual level, hybrid zones can be recognized. Hybrid zones are morphologically unique in that they violate the general assumption of species discontinuity (Dobzhansky 1951; Gingerich 1985). This is the most basic testable pattern for paleontologists interested in documenting hybrid zones. However, biologists have long recognized alternative explanations for morphological intermediacy that would discourage identifying hybridization events in the fossil record (Rieseberg and Linder 1999, and references therein). This paper advises the use of multiple pure-species populations as a more rigorous technique for establishing hybridization as the cause of morphological intermediacy. The use of multiple pure-species populations from different geographic areas and environments establishes species boundaries rather than population boundaries and thereby controls for polymorphism and ecophenotypy. This control reduces the error in identifying fossil hybrid zones and increases the utility of paleontological documentation of hybridization. However, the observation of morphological intermediates through time, as in the fossil record, requires further alternative explanations than those presented above. Alternative explanations of morphological intermediates through time include: primary intergradation, uncoupled genetic and morphological divergence, time-averaging, and the breakdown of developmental stability.

Primary intergradation occurs when a population differentiates due to selection along an environmental gradient. Primary intergradation occurs between populations in continuous contact; hybridization occurs between species (coadapted gene pools) that have been previously isolated (Mayr 1964). Barber and Jackson (1957) suggest the use of selection coefficients to rule out one or the other, a difficult if not impossible proposition to apply to fossil data. Distinguishing between primary intergradation and hybridization is difficult even when genetic data is available. However, Mayr (1964) notes that primary intergradation is generally considered the gradual merger of populations which are no more variable than neighboring populations, whereas hybrid zones are typically abrupt changes, and that the population in the area in which the abrupt change occurs is highly variable.

The imperfect correlation between genetics and morphology has been documented in several case studies (see references cited earlier). If genetic divergence occurs prior to morphological divergence (e.g. by non-morphological differences such as behavior), then the moment of speciation has not been preserved in the fossil record. Morphological divergence of fully reproductively isolated species then takes place by character displacement or drift. At the beginning stages of that divergence, morphological characters will overlap and form what appears to be a hybrid zone. Selection and/or gene flow will act to maintain species integrity (Ridley 1996) and therefore it is unlikely that morphological divergence would be localized as would a hybrid zone. Further evidence is available that again utilizes control samples. In general, populations in sympatry will show greater divergence than the populations in allopatry because of character displacement (Schluter et. al. 1985; Coyne and Orr 1989; Schluter 1995). However, in a hybrid zone, gene flow acts to homogenize the species and therefore the opposite prediction results—populations in allopatry will show greater divergence than populations in sympatry. Note that this is the case for the *Mercenaria* spp. hybrid zone, within which the species could not be discriminated, but outside of which the species could be discriminated. By comparing sympatric to allopatric populations, morphological divergence of fully reproductively isolated species can be ruled out as an alternative explanation of morphological intermediates.

Time-averaging (Kowalewski 1996) can amalgamate a sequence of rapidly-changing populations, inflating the variability at the point of speciation and making it appear as if intermediate morphologies are present alongside parental morphologies. Time-averaging of populations in stasis will not inflate the variability (Bush 1999), but during rapid speciation time-averaging will increase the variability of a sample.

Developmental stability is one component of developmental homeostasis, by which organisms reduce phenotypic variation due to developmental accidents (Alibert et. al. 1997). The allopatric model of speciation predicts the breakdown of developmental stability during speciation due to founder effects accompanied by a genetic revolution (Mayr 1963; Levin 1980; Williamson

1981), although these mechanisms remain largely theoretical (Ridley 1996). Both hybrid zones and developmentally unstable populations result in a localized increase in phenotypic variance at the beginning of a species' evolutionary history.

Both time-averaging and the breakdown of developmental stability predict the same morphological pattern as a hybrid zone—an increase in phenotypic variability. However, time-averaging and the breakdown of developmental stability should occur in both anagenesis and cladogenesis, whereas hybrid zones can only form during cladogenesis. Although there is no way to distinguish between these three causal mechanisms of increased phenotypic variability on a case-by-case basis, it is possible to assess the relative frequency of these events in the fossil record.

To do this, all articles that described morphological evolution through geological time published in the journal Paleobiology were examined (table 2). Out of 53 articles found, 22 papers provided 31 examples of a lineage's evolutionary history with sufficient information to make them useful for assessing the frequency of increases in phenotypic variation during speciation. These 31 cases included 22 examples of anagenesis and 9 examples of cladogenesis. Only 3 of the 22 (14%) examples of anagenesis showed a within-sample increase in variability prior to speciation, while 7 of the 9 (78%) examples of cladogenesis showed a within-sample increase in variability. This dramatic difference in proportion is statistically significantly different ($p < 0.001$; Fisher's Exact test).

Both time-averaging of rapidly changing populations and the breakdown of developmental stability predict an increase in phenotypic variance during both anagenesis and cladogenesis. The literature search strongly suggests that neither of these processes are occurring during speciation. Instead, what is suggested is that a process that results in an increase in phenotypic variance is operating solely during cladogenesis. Differences in objectives, methods, and study organisms preclude unambiguously identifying that causal process as hybridization. However, the results strongly suggest that neither time-averaging nor the breakdown of developmental

stability affect interpretations of hybridization, and that hybridization may have occurred at a much higher frequency than current paleontological studies document.

Table 2. Summary of literature search. "Variability Increase" column indicates whether morphological variability increased prior to or during speciation.

| Author | Year | Type of Evolution | Organism | Variability Increase |
|---|---|---|---|---|
| Kellogg and Hays | 1975 | Anagenesis | Radiolaria; *Calocycletta* sp. | No |
| Kellogg and Hays | 1975 | Anagenesis | Radiolaria; *Pterocanium* sp. | No |
| Kellogg and Hays | 1975 | Anagenesis | Radiolaria; *Pseudocubus* sp. | No |
| Kellogg and Hays | 1975 | Cladogenesis | Radiolaria; *Eucyrtidium* spp. | No |
| Malmgren and Kennett | 1981 | Anagenesis | Foraminifera; *Globorotalia* sp. | No |
| Reyment | 1982 | Cladogenesis | Ostracod; *Oertiella* spp. | Yes |
| Baker | 1983 | Cladogenesis | Radiolaria; *Theocorythium* spp. | Yes |
| Casey et. al. | 1983 | Cladogenesis | Radiolaria; *Lamprocytis* spp. | Yes |
| Malmgren et. al. | 1983 | Anagenesis | Foraminifera; *Globorotalia* spp. | No |
| Arnold | 1983 | Anagenesis | Foraminifera; *Globorotalia* spp. | No |
| Reyment | 1985 | Anagenesis | Ostracod; *Echinocythereis* spp. | Yes |
| Reyment | 1985 | Anagenesis | Ostracod; *Echinocythereis* spp. | No |
| Lazarus | 1986 | Cladogenesis | Radiolaria; *Pterocanium* sp. | Yes |
| Chaline and Laurin | 1986 | Anagenesis | Vole; *Mimomys* spp. | No |
| Stanley and Yang | 1987 | Anagenesis | Bivalve; *Dosinia* spp. | No |
| Geary | 1990 | Anagenesis | Gastropod; *Melanopsis* spp. | Yes |
| Geary | 1992 | Cladogenesis | Gastropod; *Melanopsis* spp. | Yes |
| Clyde and | 1994 | Anagenesis | Primate | No |

| | | | | |
|---|---|---|---|---|
| Gingerich | | | | |
| Lazarus et. al. | 1995 | Cladogenesis | Foraminifera; *Globorotalia* spp. | Yes |
| Chiba | 1996 | Anagenesis | Gastropod; *Mandarina* sp. | No |
| Renaud et. al. | 1996 | Anagenesis | Rodent; *Stephanomys* sp. | No |
| Bralower and Parrow | 1996 | Anagenesis | Coccolith; *Cruciplacolithus* sp. | No |
| Bralower and Parrow | 1996 | Anagenesis | Coccolith; *Chiasmolithus* sp. | No |
| Bralower and Parrow | 1996 | Anagenesis | Coccolith; *Sullivania* sp. | No |
| Norris et. al. | 1996 | Anagenesis | Foraminifera; *Globorotalia* spp. | No |
| Kucera and Malmgren | 1998 | Anagenesis | Foraminifera; *Contusotruncana* sp. | Yes |
| Chiba | 1998 | Anagenesis | Gastropod | No |
| Chiba | 1998 | Anagenesis | Gastropod | No |
| Chiba | 1998 | Anagenesis | Gastropod | No |
| Chiba | 1998 | Cladogenesis | Gastropod; *Mandarina* spp. | No |

# Chapter 5: Conclusions

This study demonstrates that it is not possible to morphologically discriminate individuals taken from a *Mercenaria* spp. hybrid zone. By using sensitive geometric morphometric data and methods, this study strengthens the consistently-demonstrated biological view that hybrid individuals cannot be identified. The lack of discriminatory power is most likely due to the full fertility of hybrid *Mercenaria* spp., which results in a genetic cline from 100% *M. mercenaria* to 100% *M. campechiensis*, despite genetic analysis forcing membership into groups. This genetic cline is reflected in the morphology.

Though hybrid individuals cannot be morphologically identified, it is possible to morphologically distinguish hybrid zones. This is accomplished by the use of pure-species samples, which establish the parental morphospace. Hybrid zones occupy both parental morphospace as well as intermediate morphospace. The strength of this study lies in its use of multiple pure-species populations from different geographic locations and environments to establish species boundaries, thus ruling out population-level genetic differences, ecophenotypy, and dimorphism as possible explanations of morphological intermediacy.

Although alternative causal explanations of morphological intermediates through time exist, they do not present severe problems in identifying hybridization events in the fossil record. Primary intergradation of populations is difficult to distinguish from hybridization even when employing genetic data. Distinguishing hybridization from morphological divergence following reproductive isolation can be accomplished by comparing sympatric to allopatric populations. Distinguishing morphological intermediates formed by time-averaged evolving populations or developmentally unstable populations cannot be accomplished on a case-by-case basis, but the frequency of their occurrence in the geologic past can be assessed. A literature survey suggests that neither time-averaging nor developmental instability are operating at the beginning of a lineage's evolutionary history, and that hybridization may have occurred at a much higher frequency than current paleontological evidence suggests.

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