Marine Biology (2004) 145: 1167–1179
DOI 10.1007/s00227-004-1411-x

RESEARCH ARTICLE

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Population divergence in the sinistral whelks of North America, with special reference to the east Florida ecotone

Received: 8 December 2003 / Accepted: 18 May 2004 / Published online: 30 June 2004

Abstract This study evaluated models of species relationships among sinistral whelks in the genus *Busycon* in the Atlantic and Gulf of Mexico. Gene frequencies at eight polymorphic allozyme loci, shell morphology, anatomy, and partial DNA sequences for the cytochrome *c* oxidase 1 (COI) mitochondrial gene were examined in eight populations, ranging from New Jersey to the Yucatan peninsula, and from the dextrally coiled sister taxon *Busycon carica* (Gmelin, 1791). Whelks were collected in 1997 and 1998. The maximum COI sequence divergence recorded among 32 sinistral individuals was 1.96%, which together with the absence of any gross or qualitative morphological differences, suggested all eight populations should be considered conspecific. High levels of divergence between the allopatric western Atlantic and Gulf of Mexico populations, as revealed by fixed or nearly fixed differences at several allozyme-encoding loci were interpreted as evidence that the east Florida ecotone constitutes a significant barrier to gene flow. Size trimming also revealed several significant quantitative differences in shell and radular morphology between the three pooled Atlantic populations and five pooled Gulf populations. The Yucatan sample was the most distinctive conchologically, with heavy spines and tumid ridges, possibly related to stone crab predation. Based on the evidence all left-handed whelks of North America should be referred to the oldest available nomen, *Busycon perversum* (Linné, 1758), with three subspecies. *B. perversum perversum* along the Yucatan peninsula, *B. perversum sinistrum* (Hollister, 1958) in the northern and eastern Gulf of Mexico, and *B. perversum laeostomum* (Kent, 1982) in the Atlantic.

Communicated by J.P. Grassle, New Brunswick

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Introduction

The importance of the Florida peninsula as a biogeographic boundary has been well documented for many elements of the coastal biota of the eastern United States (Avise 1992, 2000). Four general patterns have been recognized. In the first case (e.g. eastern mud snail: Scheltema 1989; American shad: Brown et al. 1999) the combination of subtropical climate, carbonate sediments, mangrove-dominated ecosystems, and adverse currents encountered along the eastern Florida coast seems to have blocked migration between the Atlantic Ocean and the Gulf of Mexico entirely. More commonly, the east and west coasts of Florida are inhabited by cognate species, reproductively isolated although perhaps hybridizing in zones of overlap (e.g. stone crabs: Bert 1986; hard clams: Dillon and Manzi 1989a, 1989b; ribbed mussels: Sarver et al. 1992; salt marsh killifish: Duggins et al. 1995; coquina clams: Adamkewicz and Harasewych 1996; slipper shells: Collins 2000; toadfish: Freshwater et al. 2000; polychaetes: Schultze et al. 2000). In the third case, a genetic discontinuity has been detected corresponding to the Florida peninsula, but the distinction between Atlantic and Gulf populations has not been recognized taxonomically (e.g. oysters: Hare and Avise 1996; marsh and fiddler crabs: Felder and Staton 1994; croaker: Lankford et al. 1999, red drum: Seyoum et al. 2000; snapping shrimp: McClure and Greenbaum 2000; squid: Herke and Foltz 2002). The fourth situation, where a longitudinal survey of a coastal, shallow water, or intertidal population failed to uncover evidence that the east Florida ecotone consti-

tutes a significant barrier to gene flow (e.g. *Littorina irrorata*: Dayan and Dillon 1995), is quite unusual.

All previous studies of the east Florida ecotone as a potential barrier to gene flow in marine populations (including that of *L. irrorata*) have involved species able to disperse through the water column during at least some stage of their life history. Here, we survey genetic divergence in large carnivorous gastropods of the genus *Busycon*, a group that lays eggs in capsules anchored to the substratum, with young emerging as benthic juveniles. Busyconine whelks inhabit the continental shelf, ranging from Cape Cod to the Yucatan peninsula of Mexico. For such populations, with relatively limited dispersal, one might expect the barrier effect presented by the Florida peninsula to be profound.

Systematic treatments of the busyconine whelks have been in a state of flux for many years. There are approximately ten modern species and subspecies of dextrally coiled ("right-handed") busyconine whelks, most displaying "case 1" distributions. The best known are the knobbed whelk *Busycon carica* (Gmelin, 1791) and the channeled whelk *Busycotypus canaliculatus* (Linné, 1758), both ranging from Cape Cod, Massachusetts to Cape Canaveral, Florida. Three deep-water species of *Busycon* [*B. coarctatum* (Sowerby, 1825), *B. candelabrum* (Lamarck, 1816), and *B. lyonsi* (Petuch, 1987)] and the three subspecies of *Busycotypus plagosus* (Conrad, 1863) are restricted to the Gulf of Mexico. Only one species of dextral busyconine, *Busycotypus spiratus* (Lamarck, 1816), inhabits both the eastern coast of the United States and the Gulf of Mexico. One subspecies, *Busycotypus spiratus spiratus* (Lamarck, 1816) is restricted to the Yucatan peninsula, while the subspecies *Busycotypus spiratus pyruloides* (Say, 1822) ranges from North Carolina to Cedar Key, in northwestern Florida.

Currently, there are five recognized modern species of sinistral ("left-handed") whelks, defined entirely on the basis of shell characters, to which six names have been applied. The earliest of the sinistral whelks to be described was *Busycon perversum* (Linné, 1758), a form with long, broad spines and a pronounced tumid ridge midway along the siphonal canal, from the Bay of Campeche, in western Yucatan, Mexico. Conrad (1840) described a fossil form with "poorly developed" (short) spines from the Pliocene of North Carolina as *B. contrarium*. The use of *B. contrarium* for living sinistral whelks dates from Smith (1939), who noted: "The proper disposition of the recent slender sinistral Busycons is full of many difficulties. For the present, it seems best to regard them as a race of *Busycon contrarium* (Conrad)." Hollister (1958) suggested that the nomen *B. contrarium* be restricted to a small extinct species, and described three new species among the modern fauna: *B. sinistrum*, ranging from Cape Hatteras to the Yucatan peninsula, *B. aspinosum*, restricted to the area around Sarasota, Florida, and *B. pulleyi* ranging from Louisiana to northern Mexico. Pulley (1959) argued that all recent sinistral whelks were a single species with "considerable geographic variation," perhaps warranting some subspecific designation, although he did not elaborate. Abbott (1974) recognized only two extant left-handed species, *B. perversum* and *B. contrarium*. More recently, Kent (1983) described a new species of sinistral whelk, *B. laeostomum*, ranging from southern New Jersey to northern Virginia.

Surprisingly, no study has ever distinguished the left-handed whelks of the Atlantic coast (as a group) from those of the Gulf of Mexico. The present study had two goals. We examined traditional morphological characters of shell and soft part anatomy, as well as data from gene frequencies at allozyme loci and mitochondrial cytochrome *c* oxidase I (COI) gene sequences, to evaluate the several models of species relationships among the left-handed whelks of the western Atlantic. We then assessed the impact of the Florida peninsula as a biogeographical boundary among these populations.

Materials and methods

Sampling

Eight populations of sinistral whelks, spanning the geographic range of the group (Fig. 1), were sampled in 1997 and 1998 by collecting in the intertidal, trapping, or trawling (Table 1). All of the specimens were freshly collected, and either dissected alive, with tissue samples immediately frozen, or frozen whole while living, transported to the laboratory on dry ice, and maintained at −80°C until tissues were sampled for DNA extraction and allozyme analysis. The remaining tissues, radulae, and shells were then subjected to morphological examination. Collecting localities and their

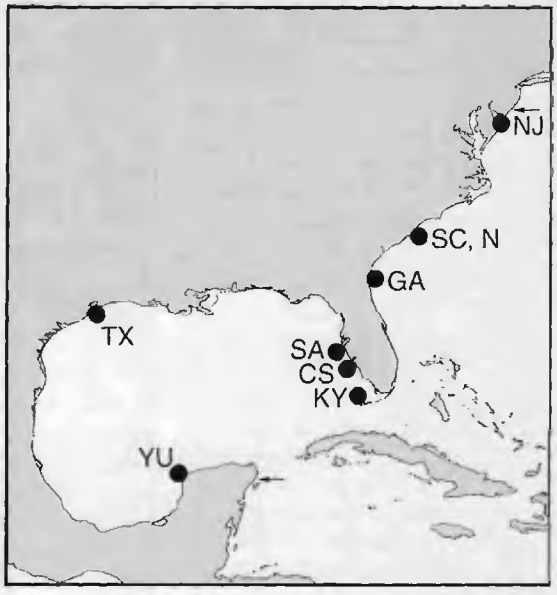

Fig. 1 *Busycon* spp. Sample sites for sinistral whelks. *B. carica* (N) was collected together with population SC. *Arrows* indicate limits of the range of sinistral whelks (site abbreviations, see Table 1)

Table 1 *Busycon* spp. Locality data, voucher material, sequenced specimens, and sequence accession numbers for taxa used in this study (*HMNS* Houston Museum of Natural Science; *USNM* National Museum of Natural History, Smithsonian Institution; N *B. Carica*)

Population abbreviation	*N*	Collection site and period	Voucher material	Specimens sequenced	GenBank accession number
NJ	26	Delaware Bay, Off Cape May, New Jersey (39°03.4′N; 74°45.9′W), trapped, 6–15 m, Aug 1998	USNM 1012273; HMNS 52423	3	AY194561–AY194563
SC	34	Between Georgetown and Charleston, primarily Debidue Island, South Carolina (33°22′N; 79°21.3′W), commercial trawlers, Feb 1998	USNM 1021631; HMNS 52424	5	AY194565–AY194569
GA	30	St. Simons Sound, near Brunswick, Georgia (31°07′N; 81°19′W), commercial trawler, Mar 1998	USNM 1021632; HMNS 52425	8	AY194570–AY194577
KY	40	Marvin Key (off Sugarloaf Key, Florida (24°38.9′N; 81°34.2′W), intertidal sand flats, Feb 1997	USNM 1021633; HMNS 52426	4	AY194578–AY194581
CS	33	Blind Pass, between Sanibel and Captiva Islands, Florida (26°31′N; 82°11′W), intertidal sand flats, Feb 1997	USNM 1021634; HMNS 52427	2	AY194582–AY194583
SA	30	Longboat Key, W of Sarasota, Florida (27°20′N; 82°33′W), intertidal sand flats, Feb 1997	USNM 1021635; HMNS 52428	2	AY194584–AY194585
TX	31	N side of Christmas Bay, Texas (29°05′N; 95°10′W), intertidal sand flats, Nov 1997	USNM 1021636; HMNS 52429	5	AY194586–AY194590
YU	21	Celestun, Yucatan, Mexico (20°40′N; 90°33′W), adults in 5–8 m, juveniles in 0–1 m, May 1997	USNM 854842; HMNS 52430	3	AY194591–AY194593
N	40	Between Georgetown and Charleston, primarily Debidue Island, South Carolina (33°22′N; 79°21.3′W), commercial trawlers, Feb 1998	USNM 1009402; HMNS 52431	1	AY578710
Busycon carica		Woods Hole, Massachusetts, USA	USNM 1021638	1	AY194560
Busycon carica[a]		Cape Henlopen, Delaware, USA	USNM 888705	1	U86306
Busycotypus canaliculatus[a]		Cape Henlopen, Delaware, USA	USNM 888706	1	U86307
Melongena melongena		Celestun, Yucatan, Mexico, 0–1 m	USNM 1021639	1	AY194558
Melongena corona		Sanibel, Florida, USA, 0–1 m	USNM 1021639	1	AY194559

[a]Data from Harasewych et al. 1997b

designations, sample sizes, voucher information, number of individuals sequenced for COI, and GenBank accession numbers for each of the sampled populations is provided (Table 1). The table also includes data on outgroup taxa used in DNA sequence–based phylogenetic analyses.

COI sequences

Snippets of red buccal muscle from 32 individual specimens of sinistral whelks (3 NJ, 5 SC, 8 GA, 4 KY, 2 CS, 2 SA, 5 TX, 3 YU), 2 specimens of *Busycon carica* (Gmelin, 1791) (1 from South Carolina and 1 from Massachusetts) and single specimens of *Melongena melongena* (Linné, 1758) and *M. corona* (Gmelin, 1791) were subjected to DNA extractions using a modified CTAB technique, following the protocol of Adamkewicz and Harasewych (1996), as modified by Harasewych et al. (1997a) (Table 1). Small shreds of muscle were ground in CTAB buffer at 60°C; the DNA was extracted twice with chloroform/isoamyl alcohol 24:1 and precipitated in a sodium acetate ethyl alcohol solution. The DNA pellet was washed in 70% ethyl alcohol, dried, and dissolved in TE buffer.

A fragment of the mitochondrial COI gene was amplified using the "universal" COI primers of Folmer et al. (1994). Typical amplification reactions of 50 µl contained 200–500 ng of genomic DNA (determined empirically), 1.25 U of Amplitaq Gold polymerase (Perkin-Elmer), 10 µl of dNTP, 0.25 µM of each primer, 1.5 mM $MgCl_2$, and 5 µl of 10× Perkin-Elmer PCR reaction buffer. Our thermal cycler parameters were 10 min at 95°C, followed by 30 cycles of 45 s at 94°C, 45 s at 50°C, and 90 s at 72°C. A 5 min final extension at 72°C followed the 30 cycles. Amplification of fragments of the expected size was verified by agarose gel electrophoresis. The PCR-amplified DNA samples were washed with isopropanol, concentrated with a microconcentrator, and sequenced on an Applied Biosystems 377A automated DNA sequencer using fluorescence dye terminator sequencing kits.

These newly produced sequences and previously determined sequences for *Busycon carica* and *Busycotypus canaliculatus* (Linné, 1758), both from Delaware Bay (Table 1), were aligned using CLUSTAL W (Thompson et al. 1994), with minimal manual modification. The aligned sequences were subjected to maximum-parsimony analyses (branch and bound) using PAUP version 4.0b10 (Swofford 1998), with characters equally weighted.

Bootstrap and jackknife analyses (1,000 replicates) were performed using the "fast" step-wise addition option.

Allozymes

For analysis of allozyme polymorphism, samples of foot muscle were homogenized in 0.05 M Tris tissue buffer (pH 7.5) and centrifuged, and the supernatant was subjected to horizontal starch gel electrophoresis (Dillon 1985, 1992). The 14% gels were a 1:1 mixture of Electrostarch (Otto Hillar, Madison, Wisconsin) and Sigma starch (Sigma Chemical, St. Louis, Missouri). Initially, we compared the allozyme phenotypes of small samples from 3 disparate populations [SC, SA, and *B. carica* (N)] resolved using 5 buffer systems and stained to provide 18 enzymes. The following combinations yielded clear polymorphisms interpretable as the products of codominant Mendelian alleles. The AP6 buffer of Clayton and Tretiak (1972) was used to resolve glucose-6-phosphate isomerase (*Gpi*), mannose-6-phosphate isomerase (*Mpi*), phosphoglucomutase (two loci, *PgmF* and *PgmS*), leucine aminopeptidase (*Lap*), hexanol dehydrogenase (*Hexdh*), and 6-phosphogluconate dehydrogenase (*6Pgd*). As *Busycon* spp. *6Pgd* apparently carries a positive charge at pH 6, adequate resolution required reversal of the normal gel polarity. The discontinuous buffer of Poulik (1957) was also used for *Hexdh*, as well as for octopine dehydrogenase (*Odh*). A tris-EDTA-borate buffer (pH 8, III of Shaw and Prasad 1970) was used to resolve *Mpi* and *Odh*.

The allele encoding the most common allozyme band at each of the eight putative loci analyzed for *B. carica* was named "100," and all other alleles were named by the mobility of their products relative to this standard, measured in millimeters. Where a polymorphism was apparent in one set of buffering conditions but obscured in another, alleles were designated F ("fast") and S ("slow"). Gene frequencies, fits to Hardy–Weinberg expectation (pooling rare alleles), values of Nei's (1978) unbiased genetic identity, and Cavalli-Sforza and Edwards' (1967) chord distances were calculated using Biosys-1 (release 1.7, Swofford and Selander 1981). Nei's distance assumes equal evolutionary rates of change among lineages, while chord distances do not (Weins and Servedio 1998). We used step "cluster" of Biosys-1 to analyze symmetric matrices of both these statistics using the unweighted pair group (UPGMA) method.

Morphology

Each snail was blotted dry, weighed, and its standard shell length measured from apex to tip of siphon. The number of spines on the final whorl was counted, and the length of the penultimate spine was measured with calipers. Shells were scored for presence or absence of apertural lirae and a tumid ridge. The color of the siphonal canal, parietal callus, and aperture were characterized as white, yellow, pale orange, orange, purple, brown, or multicolored.

Several individuals (male and female) from each population were dissected in toto, and structures of the alimentary and reproductive tracts were examined using a dissecting microscope. The busyconine whelks produce three teeth per radular row, a central rachidian tooth flanked by a pair of lateral teeth (Fig. 2A–C). The length and number of tooth rows on the radular ribbon were recorded, as was the number of tooth cusps in rows 10, 50, and 75 for 20 individuals per population. Our preliminary observations revealed substantial variation (both within single radular ribbons and among individuals) in cusp patterns on lateral teeth, but negligible variation within radular ribbons in cusp patterns on rachidian teeth. For each individual, the cusps counted on the three pairs of lateral teeth were added to produce a single variable, while the modal cusp count on a single rachidian tooth was recorded.

Morphological analysis was based on both qualitative anatomical observations and data on 13 quantitative characters per individual: live weight, 2 measurements and 1 count taken on the shell, 1 measurement and 3 counts taken on the radula, and 5 nominally scaled shell characters (2 binary and 3 with 8 states). Sample sizes were approximately 30 individuals per population for the shell characters, but loss or breakage reduced the radula data sets to 12–20 per population.

None of these variables were expected to be normally distributed. Even the continuously distributed variables were recorded from populations truncated by the sampling process, and hence were expected to be skewed. Nonparametric statistics were therefore employed throughout the analyses. Initial observations suggested that much variation in shell morphology might be correlated with overall individual size. As mean shell lengths and wet weights differed strikingly among the eight sinistral populations (partially as a consequence of the diverse sampling methods), it was necessary to examine each morphological variable for effects attributable to overall size using median tests. Individuals were pooled across populations, divided into two groups at their combined median shell length (142.5 mm), and each of the remaining 12 variables tested for a significant difference using chi-square statistics, with Yates correction in 2×2 cases.

The combined sample of three Atlantic populations had significantly greater shell length than the combined sample of five Gulf populations. In order to facilitate a comparison between these two combined groups for the 12 other variables, the two groups were plotted in 20-mm shell-length categories, overlain, and trimmed. Cases were excluded first on the basis of the completeness of their data record, then randomly. After trimming, Mann–Whitney *U*-tests were used to compare Atlantic and Gulf samples for the seven variables of ordinal or interval scale, and chi-square tests were used for the five variables of nominal scale.

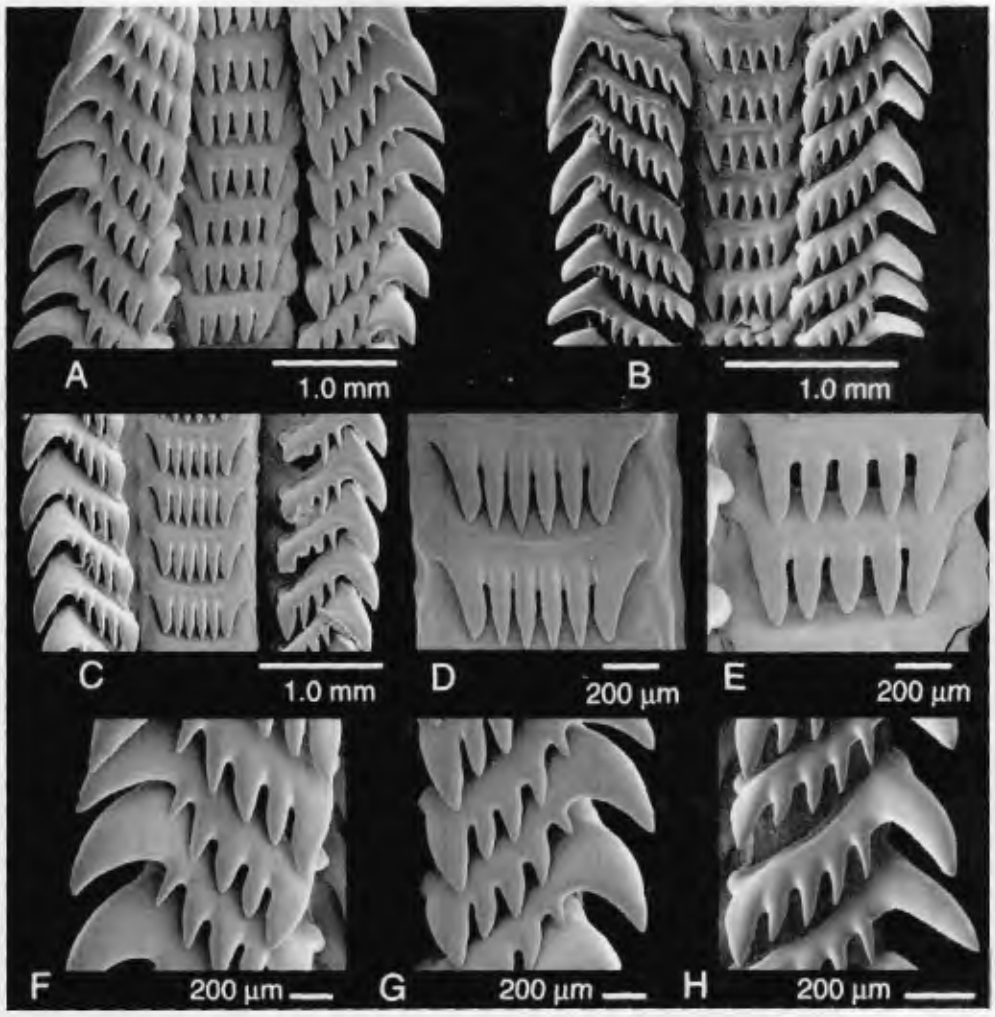

Fig. 2A–H *Busycon* sp. Scanning electron micrographs of radulae: **A–C** overall: **D, E** rachidian tooth detail: **F–H** lateral tooth detail. A, E, F and G—population CS; B—population TX; C, D, and H—population YU

Results

The sample of 32 sinistral whelks yielded 20 different COI sequences (presumed haplotypes) of 561 base pairs (bp). The maximum sequence divergence recorded between two individuals (KY23 and GA3) was 11 bp (1.96%). The minimum sequence divergence between an individual of *Busycon carica* [specimens from Delaware (DE) and South Carolina (SC) were equally divergent in tree 1] and the most similar sinistral whelk (TX17) was 21 bp (3.74%). Phylogenetic analysis yielded two most-parsimonious trees (differing only in the resolution among the three specimens of *B. carica*) of 208 steps (Fig. 3). The sinistral whelks grouped into a Yucatan clade, an Atlantic clade, and a clade from the Gulf coast of Florida, while the Texas snails lacked a unifying apomorphy to distinguish them as a clade, or to unite them with either the Atlantic clade or the Gulf Coast of Florida clade.

Gene frequencies at eight putative loci encoding allozyme polymorphisms are given in Table 2. Fits to Hardy–Weinberg expectation within populations were excellent in most cases. With pooling into three classes (homozygotes for the most common allele, common/rare heterozygotes, and other), a total of 53 goodness-of-fit chi-square tests were possible within populations. Only three of these tests returned values of chi-square nominally significant at the 0.05 level (*Lap* in populations KY and YU, *Gpi* in population NJ), a result we attribute to type I statistical error. The genetic divergence among the eight sinistral populations was highly significant. Even the most similar pair of populations, SC and GA, differed significantly at three loci (Fisher's exact tests for *Gpi* and *Mpi*, pooling rare alleles, a chi-square test for *6Pgd*, with 3 *df*). Most strikingly, there was a fixed difference between the three Atlantic populations and the five populations from the Gulf of Mexico at the *PgmF* locus, and differences were nearly fixed at the *Gpi* and *Mpi* loci (Table 2).

Our UPGMA cluster analyses of Nei's genetic similarity and of Cavalli-Sforza and Edwards' chord distance returned a pair of dendrograms identical in topology. The result of the analysis according to Nei is shown in Fig. 4, with a percent standard deviation of 10.7 and a cophenetic correlation of 0.975. The three south Florida populations in the Gulf of Mexico (sep-

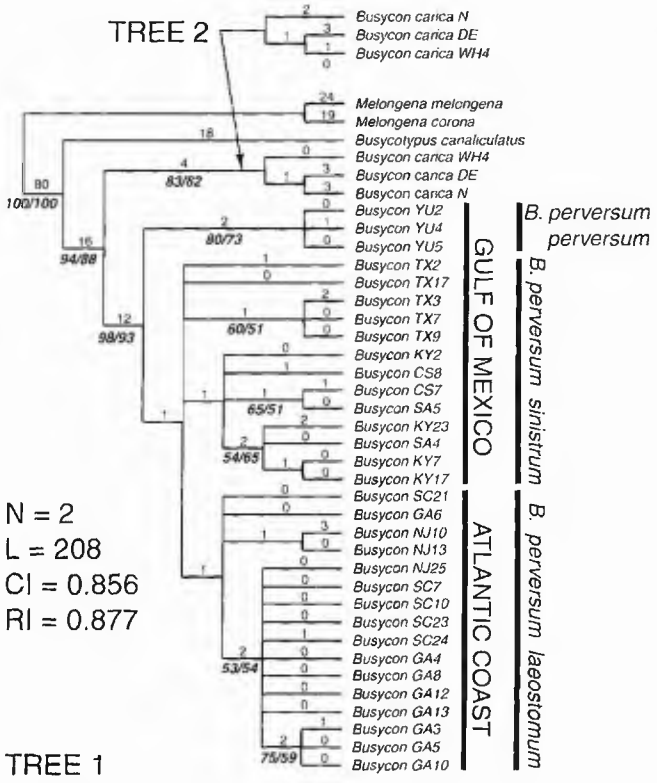

Fig. 3 Sinistral whelks. Two most-parsimonious trees resulting from maximum-parsimony analyses of partial cytochrome *c* oxidase I sequences of 32 sinistral whelks, and representatives of the outgroup genus (*Busycon carica*), genus, (*Busycotypus canaliculatus*), and subfamily (*Melongena* spp.). The two trees differed only in the resolution of the three specimens of *B. carica* (tree 2 *inset*)

arated by about 400 linear km) seem to constitute a fairly discrete group, as do the three Atlantic populations, separated by approximately 1,000 linear km. The Texas and Yucatan populations were more genetically distinctive, separated by 1,500 and 2,000 km from their Gulf of Mexico neighbors, respectively. Sinistral whelk populations from the Atlantic were more similar in their allozyme phenotype to the dextral *B. carica* population from the Atlantic than to sinistral whelk populations from the Gulf of Mexico. This phenomenon was noticeable at the *Gpi*, *Mpi*, *PgmF*, and *Lap* loci. Only a single fixed difference at the *Hexdh* locus was apparent between *B. carica* and Atlantic populations NJ, SC, and GA.

Anatomical observations revealed no gross differences in external morphology or in the alimentary and reproductive tracts within or between populations (Fig. 5). Summary statistics are given for the nine morphological variables of the shell and body in Tables 3 and 4, and four radular variables in Table 5. There was a striking diversity in the average size of the individuals among populations, from a median shell length of 98 mm (wet weight 58 g) in Texas to 178 mm (400 g) in New Jersey. The Texas whelks also seemed to show an unusually large number of spines per whorl and a high frequency of apertural lirae, while the New Jersey shells were distinguished by the absence of tumid ridges and apertural lirae. The Sarasota snails had exceptionally small spines (1.0 mm), while the Yucatan whelks were distinguished by a low count of strikingly long spines, and a higher frequency of individuals with a tumid ridge (Fig. 6).

Siphonal colors were primarily purple and brown, with pale orange and white in lesser frequencies, and others rare. The most common apertural colors were yellow and pale orange, with substantial fractions white, purple, and brown (Table 5). About half of the parietal callus observations were white, with multicolored the second most common and single colors (of any sort) more rare. Elongate ("lightning strike") stripes were present on all the shells of all five Gulf of Mexico populations. Stripes were somewhat less commonly observed on the shells of the Atlantic whelks —44% of the NJ, 89% of the GA, and 100% of the SC.

Laboratory rearing studies have suggested that *B. carica* develop first as males and that most individuals change sex after several years (Castagna and Kraeuter 1994). In addition, whelks from natural populations are expected to show strong sexual dimorphism in body size. In our study, the population with the smallest mean body size (TX) was 100% male, while the much larger (in size) Sarasota population was 79% female.

Median tests across all populations pooled showed strong relationships between shell length and 10 of the 12 other morphological variables. In addition to having greater body weight ($\chi^2 = 62.7$***), larger snails had fewer spines per whorl ($\chi^2 = 12.2$***), bore larger penultimate spines ($\chi^2 = 8.57$**) and more rarely displayed apertural lirae ($\chi^2 = 74.6$***). They tended to have longer radular ribbons ($\chi^2 = 56.5$***), with more rows of teeth ($\chi^2 = 7.73$**) and more cusps on the lateral teeth ($\chi^2 = 24.1$***). Larger snails tended to have more white and pale orange siphonal canals and apertures, while those of smaller individuals tended to be more purple or brown ($\chi^2 = 39.6$*** and $\chi^2 = 74.6$***, respectively). The parietal callus of larger whelks tended to be white, while that of smaller whelks was more likely to be colored, especially multicolored ($\chi^2 = 19.0$***).

Regardless of whether these trends are a function of sexual dimorphism or simply correlated with it, the effect of body size bias must be removed before a meaningful comparison of morphological variance can be made among populations. Figure 7 shows two combined groups from the Atlantic ($N = 87$, three populations) and from the Gulf ($N = 158$, five populations), plotted by shell length (SL). The Atlantic sample, collected by commercial gear, contained no snails with SL < 100 mm, while the mode of the hand-collected, intertidal Gulf group fell in this range. The central tendency of the SL of the Atlantic sample was significantly greater than that of the Gulf (Mann–Whitney $U = 4552$, $P < 0.001$). Trimming as shown (Fig. 7) removed 11 individuals from the Atlantic sample and 82 cases from the Gulf sample. Those Atlantic individuals remaining were approximately equally distributed among NJ, SC, and

Table 2 *Busycon* spp. Gene frequencies at eight enzyme-encoding loci in nine populations of whelks

Locus	Allele	*B. carica*			*Busycon* sp					
		N	NJ	SC	GA	KY	CS	SA	TX	YU
Gpi	(*N*)	39	24	34	30	38	33	30	31	21
	108	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.296
	106	0.112	0.365	0.103	0.333	0.075	0.045	0.233	0.000	0.167
	101	0.000	0.000	0.029	0.000	0.925	0.939	0.717	1.00	0.548
	100	0.850	0.635	0.868	0.667	0.000	0.000	0.000	0.000	0.000
	97	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	95	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
Mpi	(*N*)	40	26	34	30	40	33	30	31	20
	104	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.500	1.00	0.971	0.867	0.000	0.000	0.033	0.000	0.025
	96F	0.475	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	96S	0.000	0.000	0.029	0.083	0.688	0.606	0.383	0.177	0.425
	94	0.000	0.000	0.000	0.050	0.237	0.227	0.367	0.000	0.000
	92	0.000	0.000	0.000	0.000	0.025	0.061	0.083	0.048	0.000
	90	0.000	0.000	0.000	0.000	0.038	0.106	0.083	0.387	0.550
	89	0.000	0.000	0.000	0.000	0.013	0.000	0.050	0.145	0.000
	86	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.242	0.000
6Pgd	(*N*)	39	24	34	30	38	33	30	31	21
	106	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.339	0.143
	103	0.000	0.125	0.221	0.367	0.000	0.061	0.083	0.000	0.000
	100	0.923	0.563	0.382	0.517	0.842	0.864	0.900	0.661	0.786
	97	0.077	0.000	0.206	0.017	0.000	0.000	0.000	0.000	0.000
	95	0.000	0.313	0.191	0.100	0.158	0.076	0.017	0.000	0.071
Odh	(*N*)	39	26	34	30	40	33	28	31	21
	106	0.000	0.000	0.000	0.000	0.000	0.015	0.018	0.000	0.000
	103	0.487	1.00	1.00	1.00	1.00	0.939	0.982	1.00	1.00
	100	0.513	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000
PgmF	(*N*)	39	26	34	30	38	33	30	31	21
	108	0.000	0.000	0.000	0.000	0.184	0.015	0.000	0.000	0.000
	106	0.000	0.000	0.000	0.000	0.079	0.030	0.000	0.387	0.000
	103	0.500	1.00	0.971	0.950	0.000	0.000	0.000	0.000	0.000
	102	0.000	0.000	0.000	0.000	0.737	0.955	1.00	0.613	1.00
	100	0.500	0.000	0.029	0.050	0.000	0.000	0.000	0.000	0.000
PgmS	(*N*)	40	26	34	30	40	33	30	31	21
	103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000
	100	1.00	1.00	1.00	1.00	1.00	0.955	1.00	0.903	1.00
	97	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.065	0.000
Lap	(*N*)	40	26	34	30	39	33	29	31	21
	100	1.00	0.712	0.441	0.383	0.000	0.000	0.000	0.000	0.000
	98	0.000	0.212	0.559	0.550	0.154	0.106	0.138	0.000	0.000
	96	0.000	0.077	0.000	0.067	0.205	0.167	0.328	0.694	0.048
	93	0.000	0.000	0.000	0.000	0.641	0.682	0.448	0.306	0.571
	91	0.000	0.000	0.000	0.000	0.000	0.045	0.086	0.000	0.167
	90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.214
Hexdh	(*N*)	40	26	34	30	37	33	30	31	21
	103	0.175	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.825	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	98	0.000	0.019	0.015	0.050	0.081	0.015	0.033	0.000	0.000
	94	0.000	0.942	0.971	0.917	0.703	0.758	0.517	0.226	0.143
	91	0.000	0.038	0.015	0.033	0.176	0.197	0.450	0.435	0.857
	88	0.000	0.000	0.000	0.000	0.041	0.030	0.000	0.339	0.000

GA, but the TX population was somewhat under-represented among the trimmed Gulf sample.

The Atlantic and Gulf samples, after trimming by shell length, were compared on the basis of the eight variables of ordinal or interval scale. The lack of a significant difference in wet weight between the two samples offered some independent reassurance that the effects of overall body size had in fact been mitigated by shell length trimming. Yet very significant differences remained. Atlantic snails still had significantly larger shell spines and larger radulae, measured either as ribbon length or as number of tooth rows.

All the nominal shell variables differed significantly between the combined and trimmed Atlantic and Gulf samples. In the Atlantic, siphonal canal color was primarily (56%) purple, while in the Gulf only 10% of canals were purple, with brown (37%) the most common alternative ($\chi^2 = 38.0^{***}$). A multicolored parietal callus (as opposed to white or single-colored) was relatively more common in the Gulf (32%) than in the Atlantic

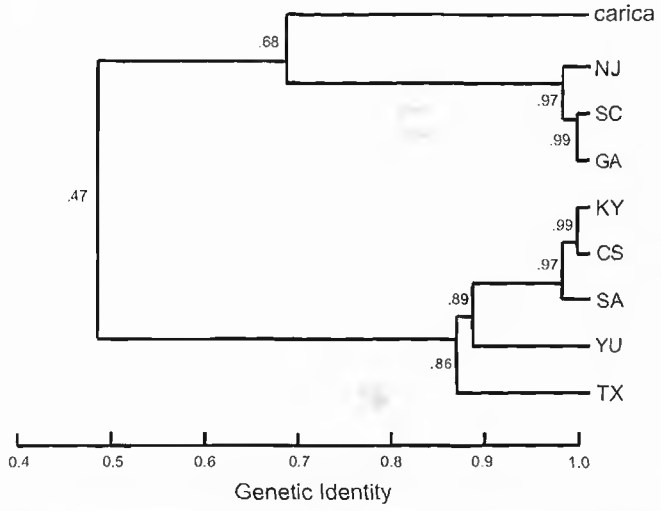

Fig. 4 *Busycon* spp. UPGMA cluster analysis according to Nei's (1978) unbiased genetic identity among eight populations of sinistral *Busycon* sp. and a *Busycon carica* outgroup. The clustering identity is given at each node

($\chi^2 = 8.3^{**}$), and apertures tended to be pale orange (31%) or brown (22%) in the Gulf, while purple apertures (32%) were more common in the Atlantic ($\chi^2 = 40.0^{***}$). Both apertural lirae (64%) and tumid ridges (31%) were significantly more common in the Gulf sample ($\chi^2 = 22.2^{***}$ and $\chi^2 = 5.4^{**}$, respectively).

Discussion

The level of COI sequence variation found in these left-handed whelks was surprisingly low. Atlantic populations were distinguished from Gulf of Mexico populations by a single unique nucleotide substitution. By comparison, sequence divergence within the Atlantic samples ranged up to nine nucleotides, and that within the northern Gulf ranged up to eight. The maximum divergence recorded between any pair of sinistral whelks was 11 nucleotides or < 2.0%.

Our COI data are consistent with the levels of sequence divergence recorded among conspecific populations of the marine gastropods *Notoacmaea fascicularis* (Simison and Lindberg 1999), *Crepidula plana* (Collin 2000), *Littorina* spp. (Wilding et al. 2000), and *Hydrobia* spp. (Wilke and Davis 2000), as well as the marine bivalves *Mytilus* spp. (Geller et al. 1993), *Mercenaria* spp. (O'Foighil et al. 1996), *Crassostrea* spp. (O'Foighil et al. 1998), and vesicomyid clams (Baco et al. 1999). Our data are inconsistent with models, suggesting that the living left-handed whelk populations of the western Atlantic represent multiple species. Levels of mitochondrial sequence divergence among even closely related species of marine mollusks are typically 3% or greater (O'Foighil et al.1995; Jozefowicz and O'Foighil 1998; Baco et al. 1999; Collin 2000), a figure confirmed by the minimum 3.7% sequence divergence we measured between any sinistral individual and *Busycon carica*.

Our allozyme data do reflect substantial regional differentiation, however. The level of interpopulation divergence at enzyme-encoding was strikingly greater than those obtained at similar loci for populations of *Crassostrea virginica* (Buroker 1983), *Mercenaria* spp. (Dillon and Manzi 1987, 1989a, 1992), or *Littorina irrorata* (Dayan and Dillon 1995), surveyed over similar geographic ranges. We do not interpret these data as evidence of reproductive isolation. Rather, we suggest that divergence of this magnitude is to be expected among gastropod populations ranging over 20° of latitude, given dispersal occurs via benthic crawling.

The fixed or nearly fixed differences we recorded at the *PgmF*, *Gpi*, and *Mpi* loci between the three Atlantic populations and the five Gulf of Mexico populations are attributable to the well-documented biogeographic boundary on the east Florida coast. Whelks of all species are rarely reported between Cape Canaveral and the Florida Keys. The adverse currents, altered substrate, changing climate, and salt marsh/mangrove ecotone apparently combine to present a 500-km barrier to dispersal through which sinistral whelks rarely penetrate.

The high levels of interpopulation divergence we have uncovered at allozyme loci, especially striking at the east Florida ecotone, seem incongruent with the low levels of COI sequence divergence. This discordance is somewhat reminiscent of the situation in oysters, where high levels of DNA sequence divergence are associated with low divergence at allozyme loci (Karl and Avise 1992). Such findings prompted Karl and Avise to hypothesize that allozyme frequencies in Atlantic and Gulf of Mexico oyster populations might be held constant by balancing selection. The situation in sinistral whelks could be interpreted to imply diversifying selection at allozyme loci by a reversal of this logic. But it is perhaps more likely that the well-studied region of the COI gene we chose to sequence is abnormally conservative in our sample of whelks and that other genes might reflect greater divergence.

We detected no gross differences in shell, radula, or soft-part anatomy, supporting the older model that North American populations of sinistral *Busycon* spp. comprise multiple species. We were, however, able to document extensive quantitative morphological diversity both within and among populations, as has been reported for *B. carica* (Edwards and Humphrey 1981; Edwards 1988; Anderson et al. 1989). The morphological difference between our size-trimmed pools of Atlantic and Gulf populations was subtle; snails of the former group have larger radulae and shells with longer spines, less ridging, and more purple coloration. Whelk shells from the west coast of Florida were nearly spineless and more diverse in coloration. Elongate stripes were less commonly observed on the shells of Atlantic whelks, although these stripes were not entirely absent, as reported by Kent (1983).

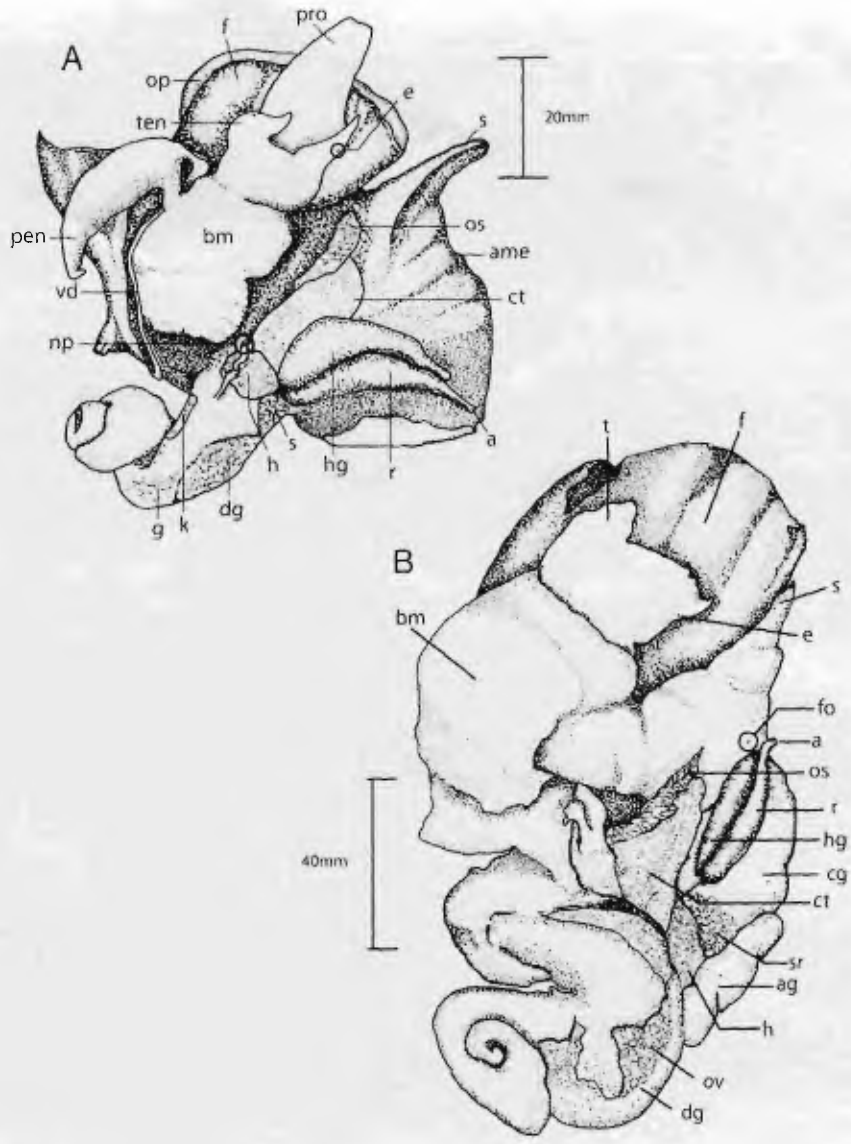

Fig. 5A, B *Busycon* sp. Diagrams of external anatomy of male and female sinistral whelks representative of the eight populations examined in this study: **A** male (*a* anus; *ame* anterior mantle edge; *bm* buccal mass; *ct* ctenidium; *dg* digestive gland; *e* eye; *f* foot; *g* gonad; *h* heart; *hg* hypobranchial gland; *k* kidney; *np* nephridio pore; *op* operculum; *os* osphradium; *pen* penis; *pro* proboscis; *r* rectum; *s* siphon; *ten* tentacle; *vd* vas deferens) and **B** female (*a* anus; *ag* albumen gland; *bm* buccal mass; *cg* capsule gland; *ct* ctenidium; *dg* digestive gland; *e* eye; *f* foot; *fo* female opening; *h* heart; *hg* hypobranchial gland; *os* osphradium; *ov* ovary; *pro* proboscis; *r* rectum; *s* siphon; *sr* seminal receptacle; *t* tentacle)

Table 3 *Busycon* sp. Shell and body measurements or counts for eight populations. Statistics reported are medians (lower quartile–upper quartile)

Pop.	*N*	Shell length (mm)	Wet weight (g)	Spine count	Spine length (mm)
NJ	23	178 (153–205)	400 (230–531)	12 (12–14)	6.2 (4.6–7.1)
SC	34	142 (126–186)	205 (149–496)	13 (12–15)	7.1 (6.0–9.3)
GA	30	168 (140–184)	435 (223–603)	13 (12–14)	9.4 (8.0–10.8)
KY	46	117 (92–147)	94 (45–197)	13 (11–14)	2.5 (1.5–5.0)
CS	33	140 (113–184)	156 (88–431)	13 (12–14)	2.9 (1.5–5.0)
SA	30	173 (143–247)	294 (180–992)	13 (12–15)	1.0 (0.5–1.5)
TX	31	98 (89–128)	58 (43–138)	16 (14–18)	2.5 (2.1–3.5)
YU	21	145 (123–175)	229 (110–371)	11 (9–13)	15.0 (8.5–19.6)

Shells of the Yucatan snails were the most distinctive among the eight populations. They had a very small number of exceptionally large spines and a high frequency of tumid ridges. Field observations suggested to us that this morphology might be a response to stone crab predation, as has been documented in previous studies of many other gastropods (Vermeij 1987). Heavy spines and tumid ridges were not noticeable in the smaller, younger individuals collected by wading in the shallow, perhaps somewhat brackish waters at the mouth of an estuary. Such traits were found only in the larger, older whelks collected offshore at depths of 5–8 m by divers ordinarily employed by the stone crab fishery.

The shells of the Yucatan whelks collected by diving were marked with repaired breaks from multiple attacks

Table 4 *Busycon* sp. Nominally scaled shell variables recorded for eight populations

Pop.	*N*	Aperture ridges (%, SD)	Tumid ridges (%, SD)	Modal siphonal canal color	Modal parietal callus color	Modal aperture color
NJ	23	0.0	0.0	Orange	Multicolor	Yellow
SC	34	45.5 (0.09)	21.2 (0.07)	Purple	White	Purple
GA	30	10.0 (0.06)	16.7 (0.07)	Purple	White	White
KY	46	86.7 (0.05)	13.3 (0.05)	Brown	White	Brown
CS	33	45.0 (0.09)	10.0 (0.05)	White	Multicolor	White
SA	30	43.3 (0.09)	13.3 (0.06)	Brown	White	Pale orange
TX	31	93.5 (0.04)	16.1 (0.07)	Purple	Multicolor	Yellow
YU	21	81.0 (0.09)	71.4 (0.10)	Pale orange	Pale orange	Pale orange

Table 5 *Busycon* sp. Radular variables for eight populations. Unless otherwise stated, statistics reported are medians (lower quartile–upper quartile)

Pop.	*N*	Radula length (mm)	Radula rows	Cusps on six laterals	Modal rachidian cusps (range)
NJ	19	69 (62–72)	120 (114–133)	36 (30–36)	5 (3–8)
SC	19	61 (52–74)	123 (115–131)	36 (30–36)	5 (3–6)
GA	20	74 (61–82)	130 (123–141)	37 (36–39)	5 (4–6)
KY	14	33 (30–43)	101 (84–120)	30 (28–30)	4 (4–5)
CS	20	53 (41–65)	109 (106–119)	36 (31–36)	5 (3–6)
SA	12	58 (47–77)	111 (105–123)	36 (34–40)	5 (2–6)
TX	20	24 (23–30)	103 (92–115)	33 (30–36)	5 (3–6)
YU	20	52 (37–65)	119 (110–129)	33 (30–36)	5 (4–7)

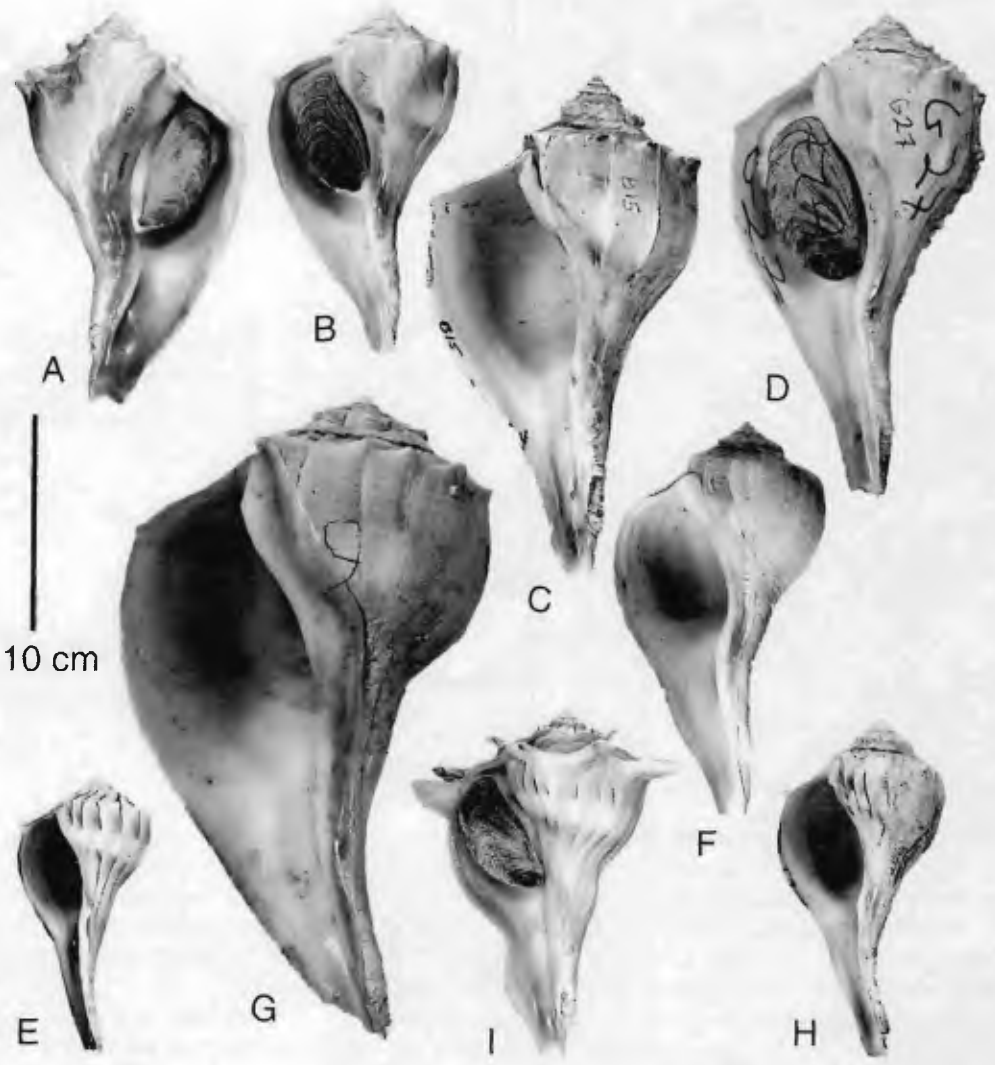

Fig. 6A–H *Busycon* spp. Representative shells from populations used in our study, with collection locations: **A** *B. carica* (N), **B** *B. perversum laeostomum* (NJ), **C** *B. perversum laeostomum* (SC), **D** *B. perversum laeostomum* (GA), **E** *B. perversum sinistrum* (KY), **F** *B. perversum sinistrum* (CS), **G** *B. perversum sinistrum* (SA), **H** *B. perversum sinistrum* (TX), and **I** *B. perversum perversum* (YU). All specimens to same scale

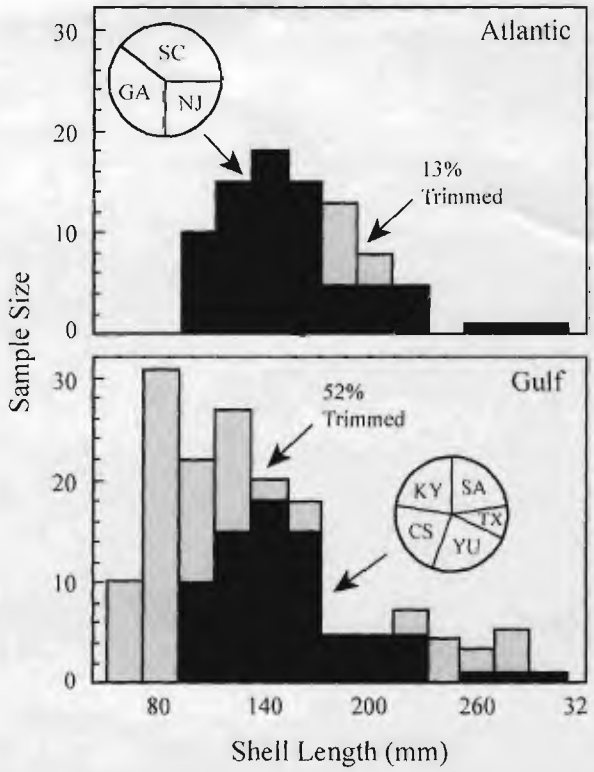

Fig. 7 *Busycon* spp. Shell lengths of sinistral whelks, pooled across populations, divided by the Florida peninsula. Length categories are minima. Fractions in the *filled regions* were analyzed for differences with respect to 12 other morphological variables; fractions in *open regions* were excluded

by crushing predators, almost certainly stone crabs, while those from the shallows were not. We subsequently examined a second sample of 58 individuals from varying depths off the Yucatan Peninsula (shell only) to test for a relationship between heavy spines and evidence of attack by crushing predators. Although 25 of the 42 shells bearing heavy spines and tumid ridges did in fact display observable shell damage, the trend was not significant (Fisher's exact test, $P = 0.36$).

The shells of the Texas whelks also appeared morphologically distinctive in several respects, most notably in the larger number of spines per whorl and shorter radular ribbon. We were concerned, however, that these differences might be a consequence of the smaller mean shell length of our Texas sample. Twenty larger Texas shells from the collection at the Houston Museum of Natural Science, with a median shell length of 174.5 mm, had a median spine count of just 12.0 mm. This is not different from similar values obtained in other populations (Table 3), suggesting that the Texas shell form is not distinctive.

In summary, our data suggest that all sinistral whelks of the west Atlantic are conspecific, *Busycon perversum* (Linné, 1758) being the oldest available nomen. The morphological and genetic diversity displayed by these populations is best modeled at the subspecies level: *B. perversum perversum* (L) inhabiting the coast of the Yucatan peninsula, *B. perversum sinistrum* (Hollister 1958) inhabiting the northern Gulf of Mexico, and *B. perversum laeostomum* (Kent 1983) on the Atlantic coast. Our allozyme data further suggest that Atlantic populations of *B. perversum laeostomum* are more similar to the (dextral) populations of *B. carica* with which they co-occur than to Gulf populations of *B. perversum sinistrum*.

The earliest sinistral whelks (assigned to the genus *Sinistrofulgur* Hollister, 1958 by Petuch 1994) appeared in the early Pliocene fossil record of the Carolinas, and, during the Pliocene, radiated north, south, and west across Florida and into the Gulf of Mexico (Leidy 1889; Grabau 1903; Puffer and Emerson 1954; Petuch 1994, 2003). There are at least 20 nominal Pliocene–Pleistocene fossil sinistral *Busycon* species from Florida and the Carolinas (Conrad 1845; Hollister 1958; Petuch 1994, 2003). The evolution of sinistrality in the busyconine whelks will be a challenging subject for future studies.

Acknowledgements For help with obtaining whelks we thank B. Anderson, D. Barges, G. Boone, W. Frank, J. Gault, R. Haggerty, D. Ingral, J. Leal, H. Lee, H. Murphy, J. Nace, H. Nash, Patricio of Celestun, H. and F. White, P. Williams, M. Yianopolous. Laboratory assistance was provided by H. Agger, R. Adams, M. Adams, B. McClintock, D. McMillan, and R. Rowe. Illustrations of sinistral whelks were drawn by S. Ryan. We thank M. Rice, S. Reed and the Smithsonian Station at Fort Pierce for hosting us in Florida. This is Smithsonian Marine Station at Fort Pierce contribution number 558. This paper is dedicated to Constance E. Boone for her many years of devotion to teaching others about mollusks. The research was supported by the Sterling Turner Research Grant, Houston Museum of Natural Science, Houston, Texas and the Visiting Scientist Award, Smithsonian Marine Station at Fort Pierce to J.W. and M.G.H.

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