Evolution, 58(6), 2004, pp. 1225–1241

SPECIATION ON THE COASTS OF THE NEW WORLD: PHYLOGEOGRAPHY AND THE EVOLUTION OF BINDIN IN THE SEA URCHIN GENUS *LYTECHINUS*

KIRK S. ZIGLER[1,2,3] AND H. A. LESSIOS[1,4]
[1]*Smithsonian Tropical Research Institute, Box 2072, Balboa, Panama*
[2]*Department of Biology, Duke University, Durham, North Carolina 27708*
[4]*E-mail: lessiosh@naos.si.edu*

Abstract.—Beginning with E. Mayr's study in 1954, tropical sea urchins have played an important role in studies of speciation in the sea, but what are the processes of cladogenesis and divergence that give rise to new species in this group? We attempt to answer this question in the genus *Lytechinus*. Unlike the majority of other tropical sea urchin genera, which have circumtropical distributions, *Lytechinus* is mostly confined to the tropics and subtropics of the New World. We sequenced a region of mitochondrial cytochrome oxidase I and the entire molecule of nuclear bindin (a sperm gamete recognition protein) of nearly all species in the genus, and we assayed isozymes of three partially sympatric closely related species and subspecies. We found that in both mitochondrial DNA (mtDNA) and in bindin the genus *Lytechinus* is paraphyletic, encompassing *Sphaerechinus granularis* as the sister species of *L. euerces*. The rest of the species are arranged in an Atlantic clade composed of *L. williamsi* and *L. variegatus*, and a Pacific clade containing *L. anamesus*, *L. pictus*, *L. semituberculatus*, and *L. panamensis*. Divergence between these clades suggests that they were separated no later than the closure of the Isthmus of Panama, and possibly before this time. Our data confirm that *L. anamesus* and *L. pictus* from California are a single species, and provide no evidence of differentiation between *L. variegatus variegatus* from the Caribbean and *L. variegatus atlanticus* from Bermuda. *Lytechinus variegatus variegatus* mtDNA is distinct from that of *L. variegatus carolinus* from the North American seaboard and the Gulf of Mexico, whereas their bindins are very similar. However, there is clear evidence of introgression of mtDNA between the two subspecies and they share alleles in all sampled isozyme loci. *Lytechinus williamsi* from the Caribbean shares mtDNA haplotypes with *L. variegatus variegatus*, and they also share isozymes in all assayed loci. Their bindin, however, is distinct and coalesces within each morphospecies. A private clade of mtDNA in *L. williamsi* may be indicative of former differentiation in the process of being swamped by introgression, or of recent speciation. Recent sudden expansions in effective population size may explain the predominance of a few mitochondrial haplotypes common to the two species. Despite the high divergence of bindin (relative to differentiation of mtDNA) between *L. variegatus* and *L. williamsi*, comparison of amino acid replacement to silent substitutions by various methods uncovered no evidence for positive selection on the bindin of any clade of *Lytechinus*. With the possible exception of *L. williamsi* and *L. variegatus*, our results are consistent with a history of allopatric speciation in *Lytechinus*. The molecular results from *Lytechinus*, along with those of other similar studies of sea urchins, suggest that the general speciation patterns deduced in the middle of last century by Mayr from morphology and geography have held up, but also have uncovered peculiarities in the evolution of each genus.

Key words.—Echinoid, gamete recognition, hybridization, selection, speciation.

Received July 21, 2003. Accepted February 11, 2004.

The multifaceted question of how speciation proceeds requires many kinds of data to be addressed. Mitochondrial DNA (mtDNA) sequences combined with geographical information can provide clues on the order of splitting between clades and on the possible extrinsic barriers that caused the observed patterns. DNA sequences of nuclear loci involved in reproductive isolation can be used to examine the role that divergence in such loci has played in perfecting reproductive isolation. Data on reproductive compatibility can provide information on the degree of completion of the speciation process between putative species. When reproductive isolation is incomplete, independent molecular datasets can identify introgression and prevent incorrect conclusions about species relationships. We carried out a study that combines these types of information in the sea urchin genus *Lytechinus*.

Unlike most genera of shallow water sea urchins that show very wide geographical distributions, *Lytechinus* is confined almost exclusively to the coasts of America, ranging from California to the Galapagos in the Pacific and from Bermuda to Brazil in the Atlantic (Mortensen 1943). Of the 11 species and subspecies in the genus, only *L. pallidus* is found outside the New World, at the Cape Verde Islands in the eastern Atlantic (Mortensen 1943; Serafy 1973). All the *Lytechinus* species in which developmental mode is known form planktonic feeding larvae from small (100–111 μm diameter) eggs, which can metamorphose into juveniles in as little as two weeks (Mortensen 1921, 1943; Harvey 1956; Mazur and Miller 1971; Cameron 1984, 1986; Emlet et al. 1987 ; Emlet 1995).The species of *Lytechinus* exhibit a variety of distribution patterns. The widespread and morphologically variable *L. variegatus* contains three subspecies: *L. variegatus atlanticus* at Bermuda; *L. variegatus carolinus*, ranging from North Carolina around the tip of Florida through the Gulf of Mexico to the Yucatan peninsula; and *L. variegatus variegatus* from southern Florida throughout the Caribbean all the way to southern Brazil (Serafy 1973). A morphologically different species, *L. williamsi*, is present in most localities in the Caribbean (Chesher 1968; Hendler et al. 1995, pp. 218–220). Other species are distributed over much smaller areas. *Lytechinus pallidus* has only been reported from the Cape Verde Islands. *Lytechinus euerces* and *L. callipeplus* are found in deep waters at the West Indies (Mortensen 1943; Lewis 1963). *Lytechinus pictus* and *L. anamesus*, two nominal species the distinctiveness of which has been questioned (Clark

[3] Present address: Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington 98250; E-mail: ziglerk@u.washington.edu.

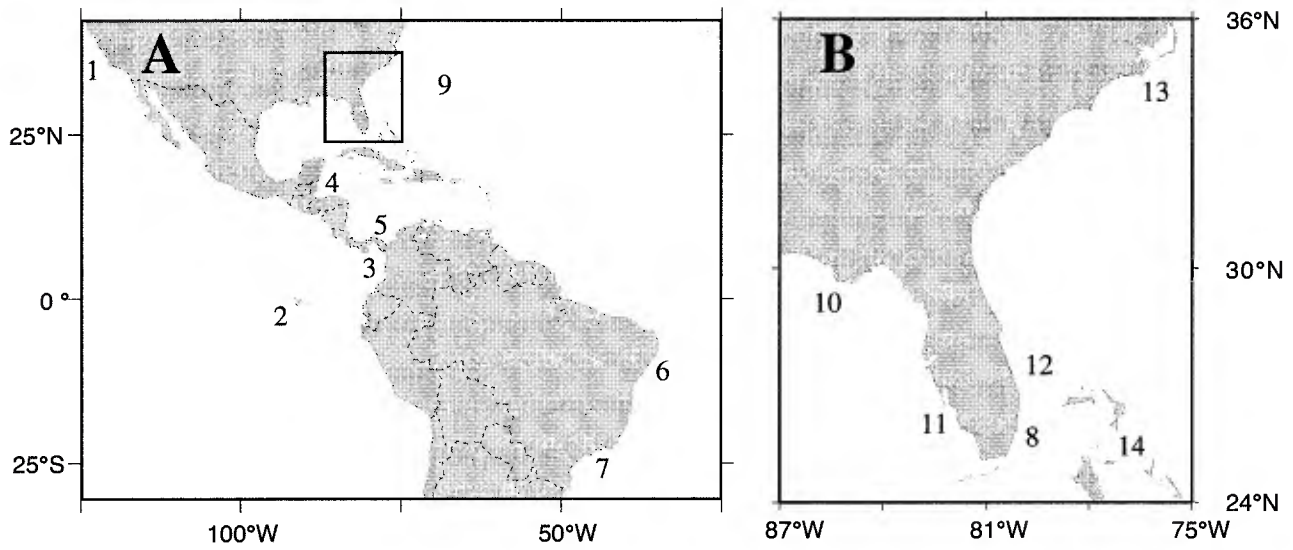

FIG. 1. Collection sites in (A) tropical America, with detailed inset (B) of southeastern North America. *Lytechinus anamesus* and *L. pictus* were collected at southern California (1); *L. semituberculatus* at the Galapagos (2); *L. panamensis* and *Toxopneustes roseus* at the Pacific coast of Panama (3); *L. williamsi* at Belize (4) and the Caribbean coast of Panama (5); *L. variegatus variegatus* at Belize (4), the Caribbean coast of Panama (5), Recife (6) and Rio de Janeiro (7), Brazil, and at Miami, Florida (8); *L. variegatus atlanticus* at Bermuda (9); *L. variegatus carolinus* at Tallahassee (10), Tampa (11) and Jupiter (12), Florida, and at Beaufort, North Carolina (13); *L. euerces* in the Bahamas (14). *Sphaerechinus granularis* was collected at the Canary Islands, Corsica, and the Aegean Sea (not shown).

1940; Cameron 1984), are found off the coast of California and in the Sea of Cortez. *Lytechinus panamensis* is known only from the Gulf of Panama (Mortensen 1921). *Lytechinus semituberculatus* is abundant at the Galapagos and is also known from the adjacent Ecuadorian coast. Thus, *Lytechinus* contains both sympatric and allopatric combinations of species.

Lytechinus pictus and *L. variegatus* have been used as model organisms for the study of fertilization and early development (e.g. Ettensohn 1985; Hardin and Cheng 1988; Ettensohn and McClay 1988; Sherwood and McClay 1999), but little is known about the levels of gametic compatibility between the various species of *Lytechinus*. Minor et al. (1991) found that gametes of the Atlantic *L. variegatus* and the Pacific *L. pictus* could cross-fertilize. Cameron (1984) found no evidence for gametic incompatibility between *L. pictus* and *L. anamesus* from the coast of California.

The sperm protein bindin plays a central role in sea urchin gamete interactions. It is the major insoluble component of the acrosomal vesicle and has been implicated in sperm-egg attachment (Vacquier and Moy 1977). A portion of the molecule functions as a membrane fusogen, suggesting that it may be involved in fusing sperm and egg membranes (Ulrich et al. 1998, 1999). This fusogenic activity is concentrated in an 18-residue portion of the 55-residue bindin "core" that is highly conserved among all bindins characterized to date (Ulrich et al. 1998; Zigler and Lessios 2003a). The pattern of bindin evolution has been examined in five genera of sea urchins. In three genera with sympatric species (*Echinometra*, *Strongylocentrotus*, and *Heliocidaris*) there are many sequence rearrangements, and indications of positive selection in "hotspot" regions on either side of the core (Metz and Palumbi 1996; Biermann 1998; Debenham et al. 2000a; Geyer and Palumbi 2003; Zigler et al. 2003). In *Arbacia* (Metz et al. 1998) and in *Tripneustes* (Zigler and Lessios 2003b), two genera in which all species are allopatric, there are fewer sequence rearrangements and no evidence for positive selection. One sequence of *Lytechinus* bindin has been published (Minor et al. 1991), but without information on the variation of the molecule, its mode of evolution within the genus remains unknown.

In this study we attempt to reconstruct the history of speciation in *Lytechinus*. We use mtDNA sequences to reconstruct the phylogeny of its species and subspecies and to determine whether there is geographic structure within the widespread Atlantic subspecies *L. variegatus carolinus* and *L. variegatus variegatus*. We also assess variation in bindin to see whether it has been evolving under positive selection, in particular in association with the evolution of reproductive isolation between sympatric species. Finally, we examine in more detail three closely related species and subspecies with overlapping ranges in Florida and the Caribbean through the use of isozymes.

MATERIALS AND METHODS

Samples

Individuals representing nine species and subspecies of *Lytechinus* were collected from various localities throughout the New World (Fig. 1). We were unable to obtain specimens of *L. callipeplus*, which is known from deep (125–300 m) waters from the West Indies (Mortensen 1943, p. 460) and of *L. pallidus* from the Cape Verde Islands. Mitochondrial sequences, intended for use as outgroups to root the mitochondrial phylogenetic tree, were obtained from *Sphaerechinus granularis* (from Gran Canaria Island, Corsica, and the Aegean Sea), and *Toxopneuestes roseus* (from the Gulf of Panama). We also used previously published sequences

from *Tripneustes* (Lessios et al. 2003) as additional outgroups. We rooted the bindin genealogy of *Lytechinus* with bindin sequences from *Tripneustes* (Zigler and Lessios 2003b). DNA was extracted from gonad samples stored in ethanol, NaCl-saturated 20% dimethyl-sulfoxide solution, or liquid nitrogen.

Mitochondrial DNA Phylogeny

A 640-bp fragment of the mitochondrial COI gene was amplified and sequenced using primers COIa (5′-AGTATAAGCGTCTGGGTAGTC-3′) and COIf (5′-CCTGCAGGAGGAGGAGAYCC-3′) as described in Lessios et al. (1999) from a total of 140 individuals of *Lytechinus*, six of *Sphaerechinus granularis*, and one of *Toxopneustes roseus*. The sequences have been deposited in GenBank (accession numbers AY183145–AY183291). We used MacClade version 4.0 (Maddison and Maddison 2000) to identify sequences that were identical. Among 147 individuals, we found 86 distinct haplotypes. We determined the simplest model of unique haplotype evolution that adequately described our data using Modeltest version 3.06 (Posada and Crandall 1998). Using this model (Hasegawa et al. [1985] with a gamma distribution of rates and invariant sites) and the parameters estimated by Modeltest we reconstructed the phylogeny by the neighbor-joining method and conducted a bootstrap analysis (1000 replicates) in PAUP* version 4.0b10 (Swofford 2001). We also conducted a Bayesian analysis using MrBayes (Huelsenbeck and Ronquist 2001) with six substitution types and with site-specific rates based on codon position. We calculated clade credibility values from 1500 trees by sampling every 100th tree of a total of 300,000, after discarding the first 1500 trees.

Population Structure and Demographic History

We calculated *F*-statistics in Arlequin version 2.0 (Schneider et al. 2000) to determine whether there was evidence of population structure within clades of the COI phylogeny. F_{ST} values were determined between populations within the following clades: one composed of *L. pictus* and *L. anamesus*; one composed of *L. variegatus carolinus*, and one composed of *L. variegatus variegatus*, *L. variegatus atlanticus*, and *L. williamsi*. The sample of *L. williamsi* from Belize was not included, because it consisted of only three individuals. We also used Arlequin to calculate Tajima's (1989) *D* and Fu's (1997) F_s measures of departure from molecular neutrality as indices of possible population expansion in *Lytechinus variegatus variegatus* and in *L. williamsi*, as well as mismatch distributions (Rogers and Harpending 1992; Rogers 1995), based on the COI data.

Characterization of Bindin

We designed primers flanking mature bindin of *Lytechinus* based on the sequence of *L. variegatus* published by Minor et al. (1991; SULBIND, GenBank accession no. M59489). *Lytechinus* bindin was amplified, cloned, sequenced, and edited as described for the bindin of *Tripneustes* (Zigler and Lessios 2003b), with the following modifications: mature bindin was amplified from genomic DNA with the forward primer Lv785 (5′-CCGCTACCGATTTCTTCAACTTC-3′), and the reverse primer Lv1597 (5′-CAAACGTCTTGAGACTGATCTGC-3′) for all species except *L. euerces* and *Sphaerechinus granularis*, for which the reverse primer was LER1 (5′-GCCCCACATGGCTTATGTAACG-3′). LER1 was designed based on *L. euerces* 3′ UTR sequences obtained by the 3′ rapid amplification of cDNA ends method (Frohman et al. 1988) from testis mRNA isolated from a specimen of *L. euerces* as described by Zigler and Lessios (2003b). Both strands of cloned bindin alleles were sequenced using an ABI 377 automated sequencer. Depending on the species, a combination of the primers Lv785, Lv1597, LER1, LYTIN-R (5′-GAAAACTAAAAGGTGCAGTTATG-3′), LYTIN-F (5′-AACTCACATAAGGTACCTTGACC-3′), LYTINF-R (5′-GGTCAAGGTACCTTATGTGAGTT-3′), LYTINR-R (5′-CATAACTGCACCTTTTAGTTTTC-3′), MB1136- (5′-ARGTCAATCTTSGTSGCACC-3′), and MB1130+ (5′-TGCTSGGTGCSACSAAGATTGA-3′) were used for sequencing. The sequences were edited in Sequencher version 4.1 (Gene Codes Corp., Ann Arbor, MI). They were deposited in GenBank under accession numbers AY183324–AY183355.

Bindin Gene Genealogy

We obtained 30 bindin sequences of *Lytechinus* and two of *Sphaerechinus granularis*. Bindin of all species of *Lytechinus* included in the COI phylogeny, except *L. panamensis*, was sequenced. These sequences, plus the single previously known *L. variegatus* sequence (Minor et al. 1991) and outgroup sequences from *Tripneustes* (Zigler and Lessios 2003b) were aligned in Se-Al (ver. 1.0, Rambaut 1996). This alignment of coding sequences included both partial preprobindin (29 amino acids) and the full length of mature bindin. Stretches of mature bindin in glycine-rich repeat regions (amino acids 37–85 and 210–220 in Fig. 2) were excluded from further phylogenetic analysis because they could not by unambiguously aligned. DnaSP (Rozas and Rozas 1999) was used to implement the four-gamete test for recombination (Hudson and Kaplan 1985) in the alignable portions of the bindin molecule. We reconstructed the *Lytechinus* bindin gene genealogy using the neighbor-joining method with maximum likelihood (ML) distances in PAUP* using a Tamura and Nei (1993) model with a gamma distribution of rates (as chosen by Modeltest). The tree was bootstrapped in 1000 iterations. We also conducted a Bayesian analysis using MrBayes with six substitution types and with site-specific rates based on codon position. We calculated clade credibility values from 1500 trees by sampling every 100th tree of 200,000 after discarding the first 500 trees.

Combined COI and Bindin Phylogeny for Lytechinus

To help resolve the phylogeny of *Lytechinus* we combined the COI and bindin data. For every individual for which we had bindin sequence, we also had COI sequence, with the exception of the previously known *Lytechinus variegatus* bindin sequence (Minor et al. 1991). When we had sequenced both bindin alleles from a single individual (three cases), we randomly chose one allele for this analysis. We reconstructed the phylogeny in PAUP* using a Tamura and Nei (1993) model of evolution with a gamma distribution of rates, as

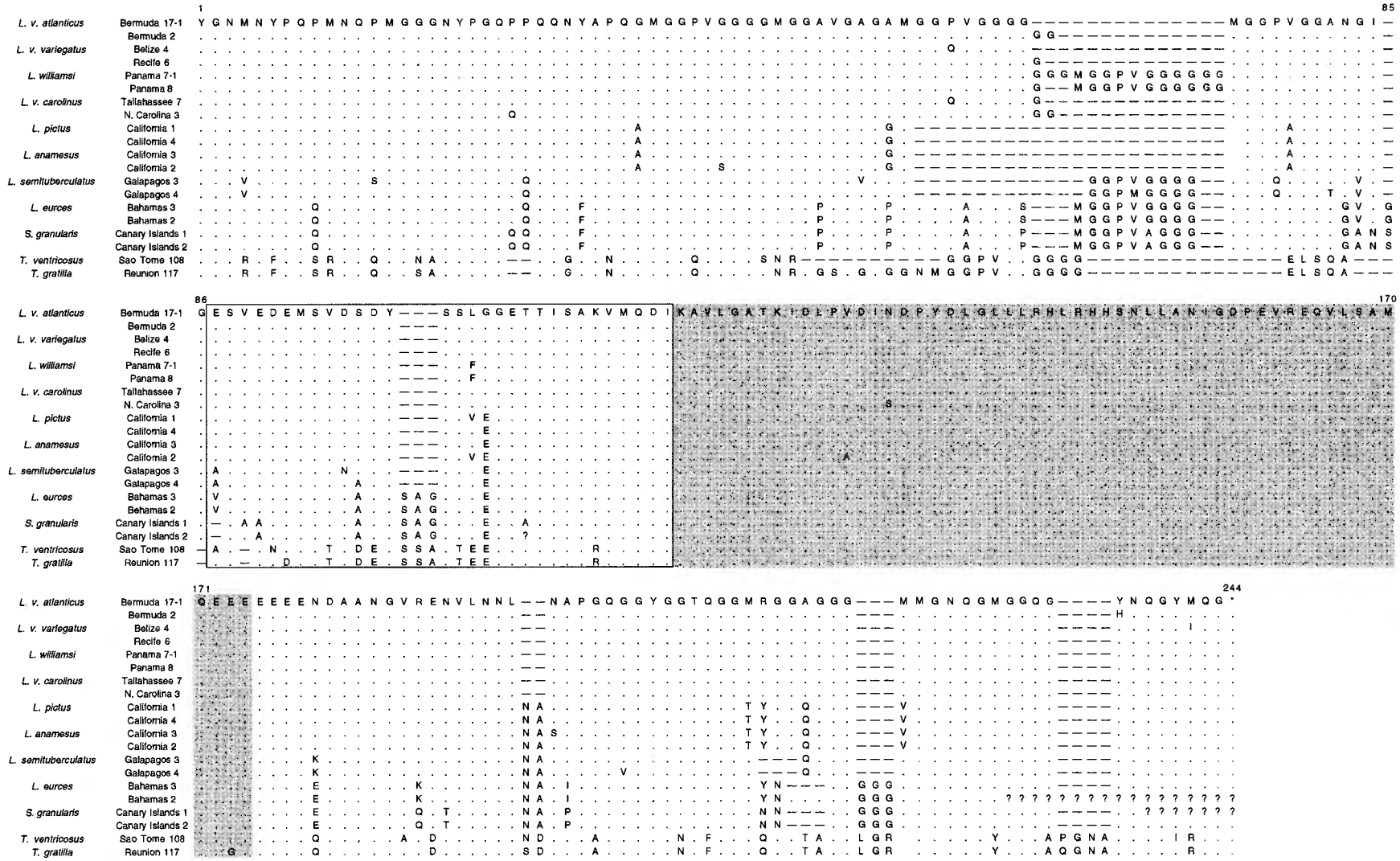

FIG. 2. *Lytechinus* mature bindin amino acid alignment. The sequences begin with the first amino acid after the presumed cleavage site at the end of the preprobindin. Identity to the first sequence is indicated by a period, gaps are marked with dashes, and missing data with question marks. When both bindin alleles for an individual were available, only one is included, identified with a number following the identification of the individual (e.g. Bermuda 17-1). The amino acids of the hotspot region (residues 86–118) are enclosed in a box. The amino acids of the core (residues 119–173) are shaded.

determined by log-likelihood ratios in Modeltest. Using this model and the parameter estimates from Modeltest, we reconstructed the phylogeny by the neighbor-joining method and conducted a bootstrap analysis (1000 replicates) in PAUP*. We also conducted a Bayesian analysis in MrBayes under a model of six substitution types and with site-specific rates based on codon position. We calculated clade credibility values from 1500 trees by sampling every 100th tree of a total of 200,000 after discarding the first 500 trees.

Tests of Selection on Bindin

We compared amino acid replacement and silent substitutions in bindin of *Lytechinus* (including *Sphaerechinus*) using MEGA version 2.1 (Kumar et al. 2001). We first divided the bindin sequences into three regions based on patterns of evolution of bindin observed in other genera (Metz and Palumbi 1996; Biermann 1998; Zigler and Lessios 2003b): (1) the 55-amino acid conserved core (amino acids 119–173 in Fig. 2); (2) a 33-amino acid "hotspot" 5′ of the core (amino acids 86–118 in Fig. 2); (3) the rest of the molecule (92 residues). We used MEGA to calculate the proportion of synonymous differences per synonymous site (d_S) and nonsynonymous differences per nonsynonymous site (d_N) by the Pamilo and Bianchi (1993) and Li (1993) method, and from these values calculated the d_N/d_S ratio (ω). An ω value significantly greater than one is considered to be evidence for positive selection (Zhang et al. 1997). We first calculated these values for all pairwise comparisons among *Lytechinus* plus *Sphaerechinus* bindin alleles. This method is useful for gaining an overall picture of the pattern of bindin evolution in this group, but ignores the shared history of sequences that belong to the same bindin clade. For this reason, we also calculated average ω values between sister species as identified in the COI and bindin phylogenies. We also conducted McDonald and Kreitman's (1991) tests comparing sister clades, using DnaSP version 3.51.

To test for the possibility that selection might be acting at sites scattered throughout the bindin molecule rather than on specific regions, we implemented a series of models in PAML version 3.0 (Yang 2000; Yang et al. 2000) based on the neighbor joining tree of 18 *Lytechinus* (two sequences from each species and subspecies, same alleles as those included in Fig. 2), and two *Sphaerechinus* mature bindin alleles. We calculated the likelihood of this tree under two neutral models (M1 and M7) that do not allow for positively selected sites, and under three alternate models (M2, M3, and M8) that permit selection (see Swanson et al. 2001). Then, we compared the log likelihoods between the neutral and selection models. We also used PAML to test for evidence of changing d_N/d_S ratios along different lineages of the neighbor-joining tree by first calculating the likelihood for a model that kept the d_N/d_S ratio constant across the tree (Model 0), and then calculating the likelihood for a model that allowed each branch to have a separate d_N/d_S ratio (Model b).

Isozymes

Because mtDNA and bindin gave conflicting results for the Caribbean species (see Results), we used isozymes as an independent nuclear marker. Using the methods of Lessios and Pearse (1996) we compared *L. variegatus carolinus* from Beaufort, North Carolina, *L. variegatus variegatus* from the San Blas Archipelago and from Isla Grande, Panama, and *L. williamsi* from the San Blas on the basis of 12 to 14 allozymic loci. The loci were: acid phosphatase (*Acph,* assayed only in *L. williamsi* and *L. variegatus variegatus* from the San Blas), α-glucosidase (αGlu), creatine kinase (*Ck*), glucose-6-phosphatase-dehydrogenase (*G6pdh,* not assayed in *L. variegatus variegatus* from the San Blas), aspartate aminotransferase (*Got*), isocitrate dehydrogenase (*Idh*), malate dehydrogenase (*Mdh*), octanol dehydrogenase (*Odh*), phosphoglucose isomerase (*Pgi,* not assayed in *L. variegatus variegatus* from the San Blas), phosphoglucose mutase (*Pgm-1* and *Pgm-2*), superoxide dismutase (*To*), triosephosphate isomerase (*Tpi,* assayed only in *L. williamsi* and *L. variegatus variegatus* from the San Blas), and xanthine dehydrogenase (*Xdh*). BIOSYS-1 (Swofford and Selander 1989) was used for statistical comparisons of allozyme frequencies (Workman and Niswander 1970) and for the calculation of Nei's (1978) unbiased genetic distance.

Results

COI Phylogeny and Population Structure

The COI phylogeny of *Lytechinus* is a polytomy with little phylogenetic structure (Fig. 3). Use of *Toxopneustes* and *Tripneustes* as outgroups revealed that *Sphaerechinus* is a sister group to *Lytechinus euerces*, with which it forms one of the four basal clades. Another basal clade is formed by the tropical Pacific species *L. semituberculatus* and *L. panamensis*, with no distinction between the two. Yet another clade is composed of the two Californian nominal species *L. pictus* and *L. anamesus*, which are also not distinguished phylogenetically. Finally, there is a well supported Atlantic clade that includes *L. williamsi* and the three subspecies of *L. variegatus*. Contrary to what would be expected from the current systematic placement of the taxa, the subspecies *L. variegatus carolinus* splits off first, whereas *L. williamsi* is nested within the subclade that also contains *L. variegatus variegatus* and *L. variegatus atlanticus*. The *L. variegatus variegatus* and *L. variegatus atlanticus* haplotypes are completely intermingled, with most (12 of 15) of the *L. williamsi* haplotypes. The three remaining *L. williamsi* haplotypes form a closely related sister group distinct from *L. variegatus variegates*, *L. variegatus atlanticus*, and the rest of the *L. williamsi* haplotypes.

There is evidence of mitochondrial DNA introgression between *L. variegatus carolinus* and *L. variegatus variegatus* in southern Florida where their ranges overlap. *L. variegatus variegatus* individuals have green or white spines, whereas *L. variegatus carolinus* have red tests and (almost always) red spines (Serafy 1973). Of the 21 white or green-spined animals collected near Miami, six had a mitochondrial haplotype that fell within the *L. variegatus carolinus* clade. We also found one individual with red spines and red test from Tampa with a haplotype characteristic of *L. variegatus variegatus*.

F-statistics give a picture of patterns of gene flow within and between the various nominal species and subspecies of *Lytechinus* (Table 1). A negative F_{ST} value in the comparison between *L. pictus* and *L. anamesus* indicates that there is more

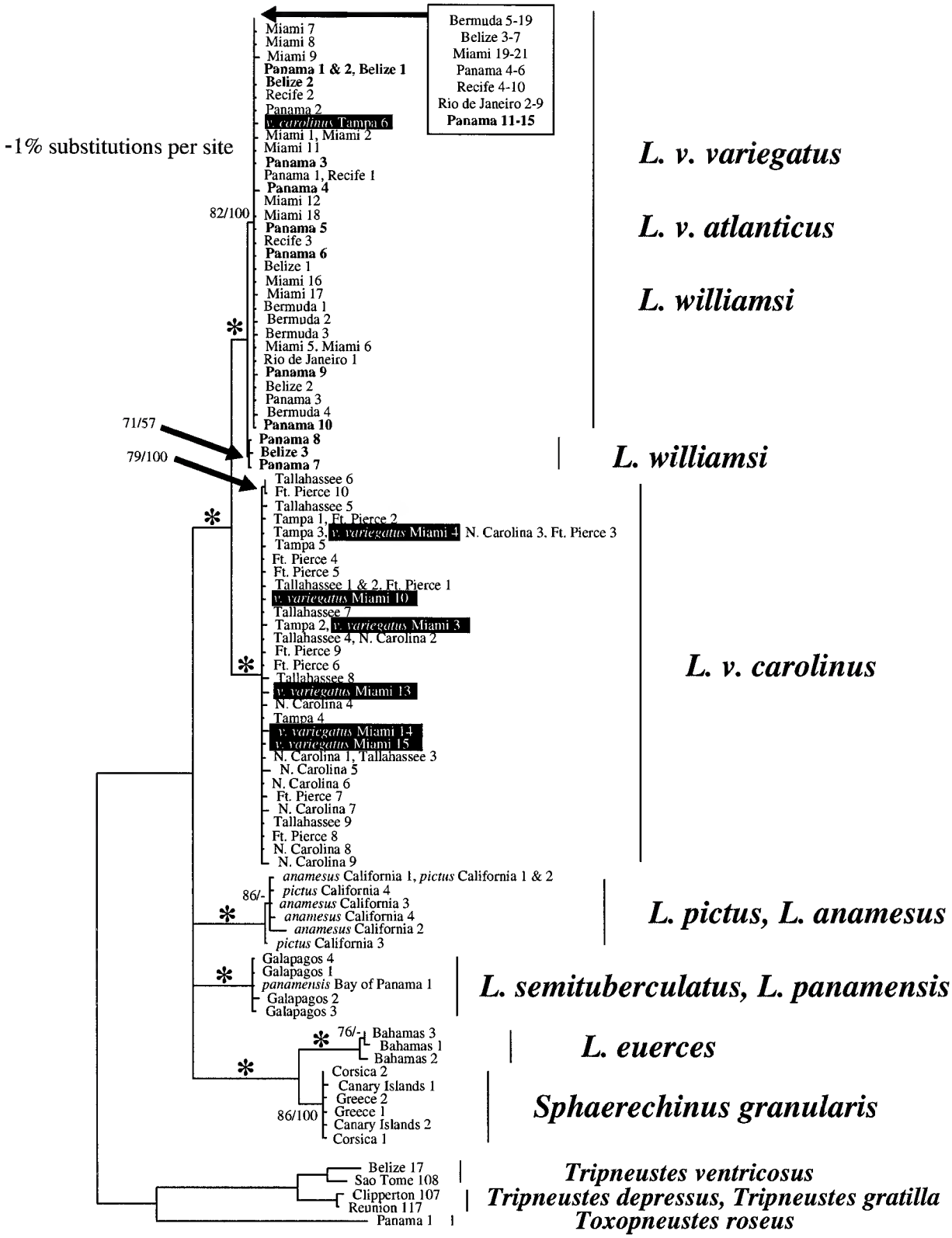

FIG. 3. *Lytechinus* cytochrome oxidase I gene genealogy. Neighbor-joining tree based on maximum-likelihood distances calculated under the Hasegawa et al. (1985) model of evolution, with a gamma distribution of rates and recognizing invariant sites. The tree has been bootstrapped in 1000 replicates. Clades with less than 70% bootstrap support have been collapsed. Nodes marked with an asterisk received support of at least 90% by both bootstrapping and Bayesian credibility values. Where this was not true, bootstrap values are indicated first, followed by Bayesian clade credibility values. Haplotypes are identified by the locality at which they were collected followed by a number, or a range of numbers when multiple identical haplotypes were obtained from the same locality. Individuals that

TABLE 1. *F*-statistics comparing COI sequences of populations within major mitochondrial clades. Individuals were classified based on morphology. F_{ST} values significant at $P < 0.05$ based on 3000 random reshufflings are indicated by an asterisk.

Lytechinus pictus and *L. ananiesus*		*n*	*L. pictus*
L. pictus	S. California	4	
L. anamesus	S. California	4	−0.07

L. variegatus carolinus		*n*	Tallahassee	Fort Pierce	North Carolina
L. v. carolinus	Tallahassee	9			
L. v. carolinus	Fort Pierce	10	−0.03		
L. v. carolinus	North Carolina	9	0.02	0.01	
L. v. carolinus	Tampa	6	−0.06	−0.06	0.02

L. v. atlanticus, *L. v. variegatus* and *L. williamsi*		*n*	Bermuda	Belize	Miami	Panama	Recife, Brazil	Rio de Janeiro
L. v. atlanticus	Bermuda	19						
L. v. variegatus	Belize	7	0.00					
L. v. variegatus	Miami	21	0.21*	0.12				
L. v. variegatus	Panama	6	0.05	0.01	0.10			
L. v. variegatus	Recife, Brazil	10	0.00	0.00	0.15	−0.01		
L. v. variegatus	Rio de Janeiro	9	−0.03	0.02	0.14	0.05	−0.01	
L. williamsi	Panama	15	0.05	−0.02	0.14	−0.04	0.00	0.00

mitochondrial DNA variability within each of these nominal species than there is between them. There is no geographic structure within *L. variegatus carolinus*, not even between populations from the west side of the Florida peninsula (Tallahassee, Tampa) and populations from the Atlantic seaboard. Nor is there any geographic structure within *L. variegatus variegatus*. Despite fairly large sample sizes, F_{ST} values comparing the two extremes of the subspecies range (Miami vs. Rio de Janeiro) are not significant. Indeed, the only large and significant value of F_{ST} among all the comparisons is between *L. variegatus atlanticus* from Bermuda and *L. variegatus variegatus* from Miami. However, this can hardly be considered as evidence of genetic differentiation between the subspecies, because the other four comparisons between *L. variegatus atlanticus* and *L. variegatus variegatus* show miniscule F_{ST} values. All comparisons between *L. variegatus variegatus* and *L. williamsi* are indicative of high rates of mitochondrial DNA exchange between the two species, despite the existence of a separate mtDNA clade within *L. williamsi*.

The presence of the same COI haplotype in 46 individuals of *Lytechinus variegatus variegatus* and *L. williamsi* morphology (Fig. 3) suggested a rapid population expansion in at least one of the two nominal species. To investigate this question further, we calculated Tajima's (1989) *D* and Fu's (1997) F_s tests and Rogers and Harpending's (1992) mismatch distributions, for each species separately and for both species together. Haplotypes of individuals with *L. variegatus carolinus* morphological characteristics were excluded from these calculations, as was the single individual with *L. variegatus variegatus* morphology but with *L. variegatus carolinus* mtDNA. Both Tajima's and Fu's tests produced values that were negative and significant (Table 2). Such values are indicative of either selection in a stable population, or of recent population expansion. As all the substitutions between the included haplotypes are silent, selection, if it affects this variation, could only do so through linkage with another mtDNA region. Mismatch distributions of the COI haplotypes are not significantly different from Rogers's (1995) sudden expansion model, whether they are calculated separately for each nominal species or for the two species together (Fig. 4). However, the presence of a separate clade of mtDNA in *L. williamsi* causes a second peak of 10–14 site differences in the mismatch distribution of this species and of the pooled data. *Lytechinus variegatus variegatus* COI, in contrast, shows a mismatch distribution that fits almost perfectly the parameters expected from sudden and very recent population expansion.

Bindin Genealogy and Evolution

The bindin gene genealogy (Fig. 5), like that of COI, places *Sphaerechinus granularis* as a sister species to *Lytechinus*

TABLE 2. Values of Tajima's (1989) *D* and Fu's (1997) F_s, calculated from COI for *Lytechinus variegatus variegatus* and *L. williamsi* separately, and for the two species together. Significance was determined by comparison to a distribution generated from Hudson's (1990) coalescent algorithm under the assumption of population equilibrium and selective neutrality, with 1000 iterations. * $P < 0.05$, *** $P < 0.0001$.

	Tajima's *D*	Fu's F_s
L. variegatus variegatus	−2.393***	−18.259***
L. williamsi	−1.528*	−3.453*
pooled	−2.454***	−26.285***

←

by morphology and locality of collection belong to *L. variegatus variegatus*, but possess COI haplotypes that fall in the *L. variegatus carolinus* clade are marked by "*v. variegatus*" preceding the locality code and are enclosed in a black box. The reverse notation is used for the single *L. variegatus carolinus* individual with a *L. variegatus variegatus* haplotype. Individuals of *L. williamsi* morphology are identified in bold text. Box encloses 46 individuals of *Lytechinus variegatus* and *L. williamsi* that had identical haplotypes.

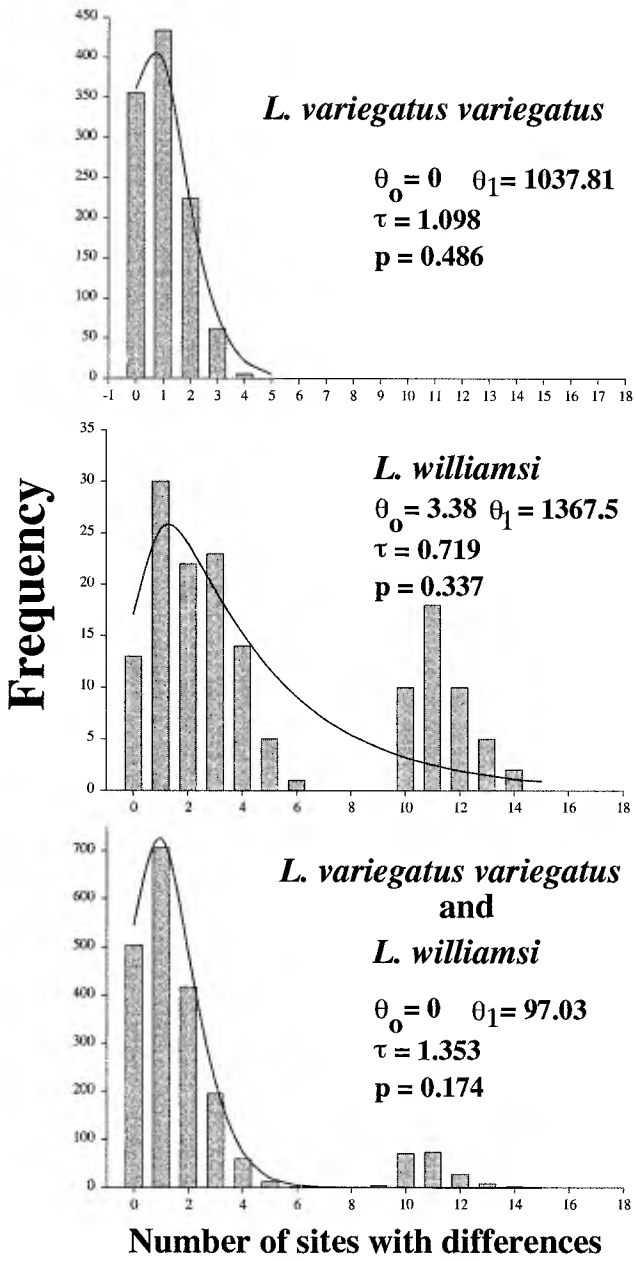

FIG. 4. Mismatch distributions (Rogers and Harpending 1992; Rogers 1995) of COI haplotypes of *Lytechinus variegatus variegatus* and *L. williamsi*, calculated for each species separately, and with the two species combined. The line depicts the mismatch distribution expected from a sudden expansion model with parameters shown in each figure. $\theta = 2N_e\mu$, where μ is the rate of mutation, and N_e is the effective population size. $\tau = 2\mu t$, where t is the number of generations between θ_o and θ_1. Probability values (P) for rejection of the sudden expansion model are based on a comparison of the sums of squares of expected and observed mismatch distributions, using parametric bootstrap with 1000 iterations (Schneider and Excoffier 1999).

euerces, but this time their clade is basal to the rest of *Lytechinus*. *Lytechinus variegatus carolinus*, which in the COI phylogeny was a well-supported sister group to the *L. variegatus variegatus*, *L. variegatus atlanticus* and *L. williamsi* clade, in bindin is intermixed with *L. variegatus variegatus* and *L. variegatus atlanticus*. As in the COI phylogeny, there is no differentiation in the bindin of *L. variegatus variegatus* and *L. variegatus atlanticus*. Finally, in the bindin tree the *L. williamsi* alleles form a distinct clade, whereas in the COI phylogeny most of the *L. williamsi* haplotypes were intermixed with the *L. variegatus variegatus* and *L. variegatus atlanticus* haplotypes. The bindin alleles of *L. williamsi* come from individuals Panama 3 and Panama 7, which belong to the COI clade that is private to *L. williamsi* (Fig. 3), but they also include alleles from five other individuals that in COI were indistinguishable from *L. variegatus variegatus*. All *L. williamsi* alleles are distinguished from all *L. variegatus* alleles by one amino acid change in the hotspot, and three synonymous substitutions in other regions of the molecule. In addition, *L. williamsi* has an extra copy of a 10-amino acid repeat in the 5′ glycine-rich repeat region (Fig. 2). As in COI, bindin alleles of *L. pictus* and *L. anamesus* do not form separate clades.

The glycine-rich repeat region (amino acids 32–82 in Fig. 2) on the 5′ side of the conserved core has undergone extensive evolution in *Lytechinus*. The repeats begin with MGG(A/P)(V/M/A) and are followed by four to seven glycine and alanine residues; occasionally other residues are included in repeats that range from nine to twelve residues. *Lytechinus pictus* and *L. anamesus* have three copies of the repeat, *L. williamsi* has five, and the rest of the species have four. Repeat number is constant within species of *Lytechinus*. *Tripneustes* contains two or three copies of this general motif in the same region (Zigler and Lessios 2003b). This interspecific variation in repeat number may have arisen by recombination between the glycine-rich repeats, as has apparently occurred intraspecifically in the bindin of *Echinometra* (Metz and Palumbi 1996; Geyer and Palumbi 2003). Outside the repeat region, the four-gamete test (Hudson and Kaplan 1985) identified one recombination event (which occurred somewhere between amino acids 30 and 182 in Fig. 2) within *L. variegatus*.

Evolution of different regions of bindin in *Lytechinus* follows the pattern typical of bindin in other genera of sea urchins (Metz and Palumbi 1996; Biermann 1998; Zigler and Lessios 2003b; Zigler et al. 2003). There is a conserved core of approximately 55 amino acids in which nonsynonymous changes accumulate at a very slow rate and a hotspot where changes accumulate rapidly, while the rest of the molecule evolves at an intermediate rate (Table 3). In the comparison between alleles of *L. williamsi* with those of *L. variegatus*, sequences are so similar that just a single substitution in a region can radically alter the ω value. In two cases the ω value in the hotspot exceeds 1, but, due to the small number of substitutions involved, Fisher's exact tests are not significant. McDonald and Kreitman (1991) tests on each of the three sister groups in Table 3 did not show a significantly higher ratio of amino acid replacement to silent substitutions between clades, relative to within clades. When substitution counts from all three comparisons were combined, the results (17 fixed silent, 30 polymorphic silent, 14 fixed replacement, and 27 polymorphic replacement sites) remained nonsignificant. One limitation of tests of selection through comparisons of synonymous and nonsynonymous changes is that they can only be carried out in regions that can be aligned. There

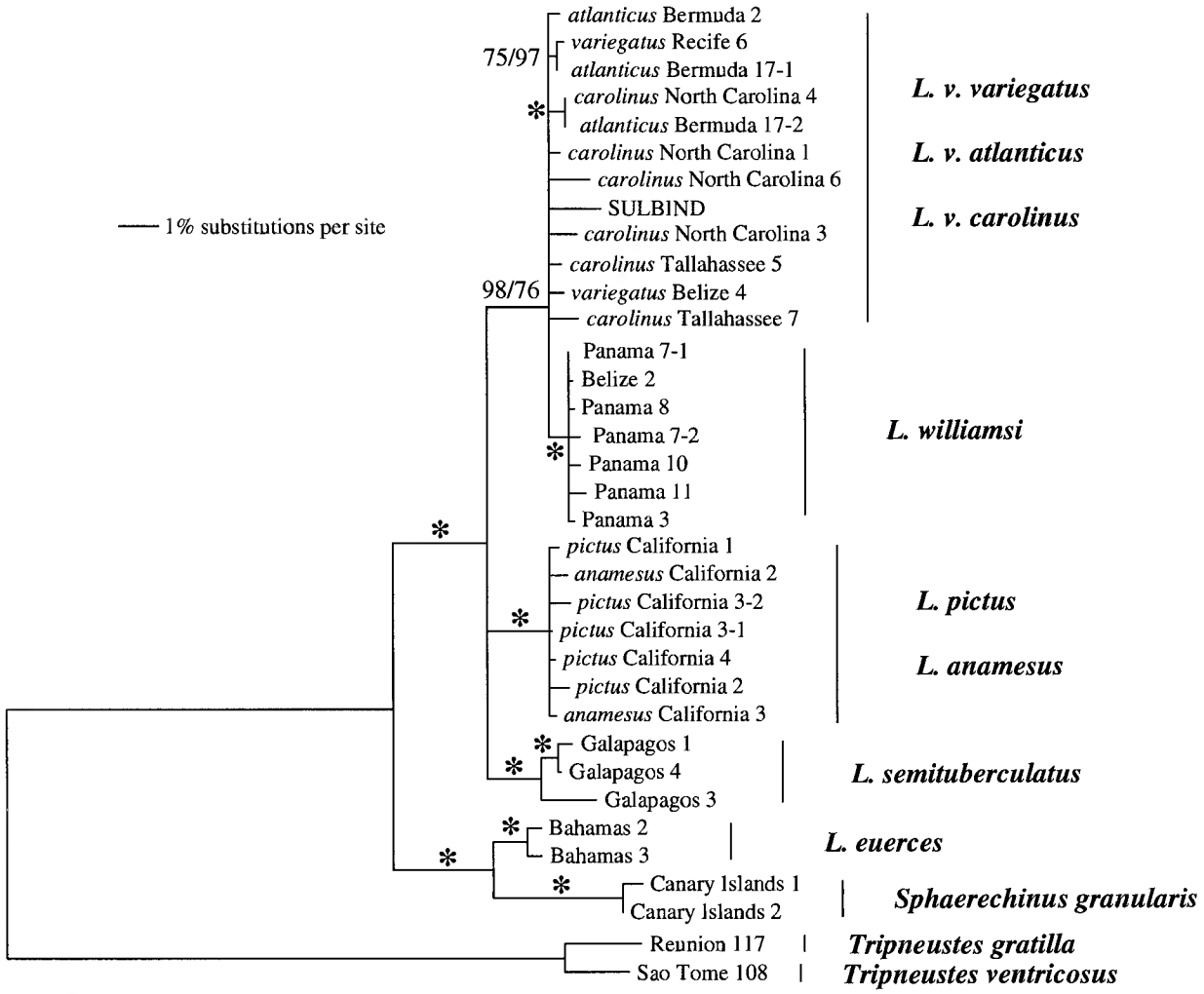

FIG. 5. *Lytechinus* bindin gene genealogy. Neighbor-joining bootstrap consensus tree using maximum-likelihood distances calculated under a Tamura and Nei (1993) model of evolution with a gamma distribution of rates (1000 bootstrap replicates). Clades with less than 70% bootstrap support have been collapsed. Also shown are Bayesian clade credibility values. The Bayesian analysis reproduced all the branches in the neighbor-joining tree. Branches marked with an asterisk are supported at or above 90% in both analyses; otherwise, bootstrap percentages are indicated before clade credibility values. When both bindin alleles were available for an individual, they are indicated with the same notation as in Figure 2. Identification of individuals follows Figure 3. SULBIND is the bindin sequence of *L. variegatus* obtained by Minor et al. (1991).

is no way to test whether extra repeats differing between species may be under selection, nor could we include the nonalignable glycine-rich regions in the tests.

Models implemented in PAML also failed to produce evidence for positively selected sites dispersed along the molecule. The likelihood of models that allowed for positively selected sites was not significantly higher than that of models that did not (Table 4). Nor did we find any evidence for significant variation in d_N/d_S ratios between lineages. Allowing a different d_N/d_S ratio for each branch in the phylogeny did not produce a significantly better model than a model with a single d_N/d_S ratio for the entire tree (Table 4). When a single ω value was estimated for the entire bindin tree (Model 0), it was much less than 1 (0.18); when each branch of the bindin tree was allowed to have a separate ω value (Model b), no branch with three or more changes occurring on it had an ω value greater than 0.68.

Combined COI and Bindin Phylogeny

The *Lytechinus* tree based on both COI and bindin (Fig. 6) is better resolved than trees based on each molecule alone. As in the COI and bindin trees, *L. euerces* and *Sphaerechinus granularis* form a basal clade as sister species. The better resolution of this tree shows that the rest of the species of *Lytechinus* are separated into Pacific and Atlantic clades. The Pacific lineage has split into well-supported northern (*L. pictus* and *L. anamesus*) and southern (*L. semituberculatus* and *L. panamensis*) clades. The Atlantic clade contains two species: *L. variegatus* and *L. williamsi*. The conflicting results

TABLE 3. Rates of mean nonsynonymous substition per nonsynonymous site (d_N) and synonymous substitution per synonymous site (d_S) and the d_N/d_S ratio (ω) for three regions of *Lytechinus* mature bindin. d_N and d_S calculated by the Pamilo and Bianchi (1993) and Li (1993) method.

Region	d_N	d_S	ω
All pairwise comparisons			
hotspot	0.033	0.104	0.32
core	0.006	0.089	0.07
rest of molecule	0.021	0.129	0.16
Total	0.018	0.107	0.17
L. semituberculatus vs. (*L. pictus* + *L. anamesus*)			
hotspot	0.037	0.104	0.36
core	0.005	0.130	0.04
rest of molecule	0.032	0.036	0.91
Total	0.025	0.073	0.34
L. williamsi vs. (*L. v. variegatus* + *L. v. atlanticus* + *L. v. carolinus*)			
hotspot	0.014	0.003	3.95
core	0.007	0.016	0.40
rest of molecule	0.005	0.035	0.18
Total	0.007	0.023	0.30
L. eurces vs. *Sphaerechinus granularis*			
hotspot	0.036	0.018	2.04
core	0	0.137	0.00
rest of molecule	0.025	0.108	0.23
Total	0.019	0.100	0.19

between the COI and bindin trees in the Atlantic most likely reflect the different histories of the mitochondrial and bindin markers (discussed below).

Isozymes

Results from protein electrophoresis indicate that *L. williamsi* and two subspecies of *L. variegatus* share alleles in all loci (Table 5) . Comparison of gene frequencies between *L. variegatus carolinus* from North Carolina, two populations of *L. variegatus variegatus* from the Caribbean, and *L. williamsi* by contingency chi-square analysis indicates that there are significant differences between gene frequencies in five loci: *G6pdh* ($P = 0.00001$), *Got* ($P = 0.0000$), *Mdh* ($P = 0.00016$), *Pgi* ($P = 0.0002$), and *Pgm-1* ($P = 0.00005$). A comparison between populations and subspecies of *L. variegatus* shows significant differences only in *Got* ($P = 0.047$) and *Pgm-1* ($P = 0.0075$). Nei's *D* values between populations of *L. variegatus* ranged from 0 to 0.001; *D* values between *L. williamsi* and each of the three *L. variegatus* populations, in contrast, ranged from 0.078 to 0.104. Thus, in allozymes, *L. williamsi*, though similar to *L. variegatus*, is more differentiated than the subspecies of *L. variegatus* are from each other.

DISCUSSION

Phylogeography and Systematics

There is a conflict between the COI and the bindin gene trees in the Atlantic clade, but for the rest of the cladogenetic events the better-resolved tree based on combined data is likely to contain the more reliable information on phylogeographic events in the history of *Lytechinus*. In this tree, the most basal split separates the clade composed of *L. euerces* and *Sphaerechinus granularis* from the rest of the genus. This predates the division of the rest of the genus into Atlantic and Pacific clades, which presumably occurred at (or before) the time of the completion of the Isthmus of Panama 3.1 million years ago (Coates and Obando 1996). The placement of *S. granularis* as sister to *L. euerces* makes *Lytechinus* polyphyletic. This can be corrected by redesignating *S. granularis* (which is presently in a monotypic genus) as *Lytechinus granularis*, or by moving *L. euerces* out of *Lytechinus*. Placing *Sphaerechinus* within *Lytechinus* would greatly extend the range over which this genus occurs, as *S. granularis* is widespread in the subtropical and temperate eastern Atlantic and Mediterranean, all the way north to the Channel Islands (Mortensen 1943, p. 525). No other tropical sea urchin genus ranges that far north, so it is probably preferable to remove *L. euerces* from *Lytechinus*. It remains to be seen where *L. pallidus* and *L. callipeplus* fit in this rearrangement.

The next split in the *Lytechinus* phylogeny might have been caused by the rise of the Isthmus of Panama. This split divides the species of *Lytechinus* into Atlantic and Pacific clades. Chesher (1972) suggested, on morphological grounds, that *L. williamsi* and *L. panamensis* represented a transisthmian pair, and indeed these two species in morphology resemble each other more than they do any other species of *Lytechinus*.

TABLE 4. Maximum-likelihood testing for variation in the ratio of replacement to silent substitutions among bindin sites and lineages. The testing follows Yang (2000) and Yang et al. (2000). Model M1 allows sites (amino acids) to be either conserved or neutral ($\omega = 0$ or $\omega = 1$), M2 adds a class of sites that can be under selection, and M3 recognizes three discrete site classes with proportions and ω values estimated from the data. M7 and M8 are based on the beta distribution, with M7 limiting ω to the interval (0, 1), and M8 adding a class of sites that can have ω values > 1. A significant difference in log likelihood of the compared models (and a class of sites with $\omega > 1$) would have indicated the presence of selection. Model 0 assumes a single value of ω for the entire tree, whereas Model b allows each branch to have a separate ω value. A significant difference in log likelihood of these models would have indicated variation in ω between lineages. L, log likelihood; $2(L_2 - L_1)$, the test statistic is twice the log-likelihood difference of the two models; *P*, probability from the chi-square distribution.

Models compared	L_1 (1st model)	L_2 (2nd model)	$2(L_2 - L_1)$	df	*P*
Variation among sites					
M1 vs. M2	−1313.52	−1311.91	3.22	2	0.20
M1 vs. M3	−1313.52	−1310.54	5.94	4	0.20
M7 vs. M8	−1312.54	−1310.64	3.80	2	0.15
Variation among lineages					
Model 0 vs. Model b	−1322.38	−1310.32	24.12	25	0.51

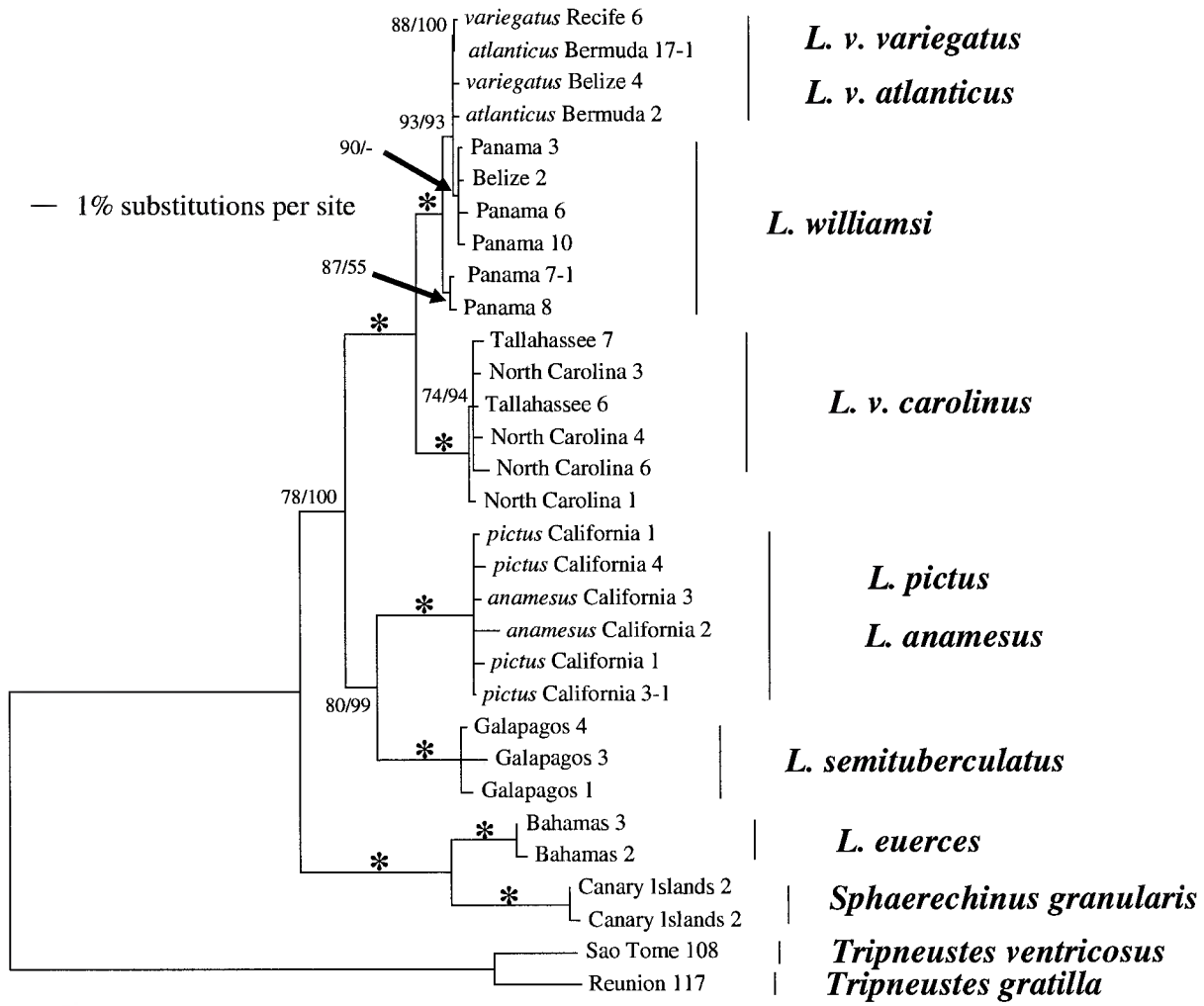

FIG. 6. *Lytechinus* phylogeny based on combined cytochrome oxidase I and bindin sequences. Neighbor-joining bootstrap consensus tree using maximum-likelihood (ML) distances calculated under a Tamura and Nei (1993) model of evolution with a gamma distribution of rates (1000 bootstrap replicates). Clades with less than 70% support have been collapsed. Also shown are Bayesian credibility values for each clade. Branches marked with an asterisk are supported at or above 95% in both analyses; otherwise, bootstrap percentages are indicated before clade credibility values. When both bindin alleles for an individual were available, the one used in this analysis is indicated (e.g. Bermuda 17-1).

The molecular phylogeny, however, indicates that these species are just part of their respective Atlantic (*L. williamsi* and *L. variegatus*) and Pacific (*L. panamensis*, *L. semituberculatus*, *L. pictus*, and *L. anamesus*) transisthmian clades. The genetic distance between these two clades in COI (13.44% Kimura [1980] two-parameter distance) is the greatest observed among six genera of echinoids with transisthmian phylogenetic relations (range 8.97–12.58% Kimura two-parameter distance; Lessios et al. 2001). Thus, it is possible that COI in *Lytechinus* evolves faster than in other tropical echinoids, or else that the split between Atlantic and Pacific *Lytechinus* predates the rise of the Isthmus of Panama.

The Pacific clade was divided into northern (*L. pictus* and *L. anamesus*) and southern (*L. semituberculatus* and *L. panamensis*) groups shortly after its separation from the Atlantic clade. *Lytechinus pictus* and *L. anamesus* are known from the Pacific coast of California and Baja California and from the Sea of Cortez (Mortensen 1943, p. 451). That they are separate species has been in question since Clark (1940) suggested that *L. anamesus* is a long-spined, deeper water form of *L. pictus*. Mortensen (1943, pp. 451–456) rejected this suggestion based on differences in spine length, spine color, and the shape of the spicules of the globiferous pedicellariae. Cameron (1984) noted that the two species are easily separated based on test color and spine length, but that spicule form was not a reliable character for distinguishing the two species. In support of Clark's (1940) view, Vacquier (quoted in Durham et al. 1980) reported that the two species are 100% cross-fertilizable, and that dissociated early blastomeres of the two species re-aggregate into mosaics of cells from both species. Cameron (1984) also found the two species to be readily cross-fertilizable (>90% fertilization success in heterospecific crosses), and raised the hybrid larvae through metamorphosis with >85% success. Our mitochondrial and nuclear data support the hypothesis that these two nominal species are merely ecotypes. Their COI haplotypes are not

TABLE 5. Number of sampled individuals and allele frequencies for 14 isozyme loci sampled from *Lytechinus v. variegatus* (from San Blas and Isla Grande, Panama), *L. v. carolinus* (Beaufort, NC), and *L. williamsi* (San Blas, Panama). *Ck*, *Idh*, *Odh*, *To*, and *Xdh* were monomorphic for 5–35 individuals in each species or subspecies. See the text for full names of locus abbreviations.

Locus	Allele	*L. v. variegatus* San Blas	*L. v. variegatus* Isla Grande	*L. v. carolinus* Beaufort	*L. williamsi* San Blas
Acph	*n*	35	—	—	35
	100	1.000	—	—	1.000
aGlu	*n*	35	5	20	35
	90	0.014	0.000	0.000	0.029
	100	0.986	1.000	0.975	0.957
	110	0.000	0.000	0.025	0.014
G6pdh	*n*	35	4	18	24
	100	1.000	1.000	0.917	0.458
	110	0.000	0.000	0.083	0.542
Got	*n*	35	4	7	35
	100	0.300	0.000	0.071	1.000
	110	0.700	1.000	0.929	0.000
Mdh	*n*	35	4	20	32
	90	0.029	0.000	0.000	0.234
	100	0.971	1.000	0.975	0.766
	110	0.000	0.000	0.025	0.000
Pgi	*n*	—	4	19	24
	90	—	0.125	0.000	0.000
	100	—	0.375	0.579	0.896
	110	—	0.500	0.421	0.104
Pgm-1	*n*	34	4	20	34
	90	0.059	0.000	0.250	0.074
	95	0.015	0.125	0.075	0.162
	97	0.000	0.000	0.050	0.000
	100	0.559	0.750	0.500	0.632
	105	0.044	0.125	0.050	0.059
	110	0.250	0.000	0.075	0.074
	115	0.074	0.000	0.000	0.000
Pgm-2	*n*	34	4	7	35
	90	0.074	0.000	0.071	0.000
	100	0.927	1.000	0.929	1.000
Tpi	*n*	35	—	—	35
	100	1.000	—	—	1.000

phylogenetically separated, and *F*-statistics indicate a high degree of gene flow. The high degree of cross-fertilizability between *L. anamesus* and *L. pictus* reported by Vacquier and by Cameron is in accordance with our observation that their bindin alleles are intermingled in the genealogy. There are no amino acid differences or indels that distinguish between their bindin alleles (Fig. 2).

No *Lytechinus* have been reported between the Sea of Cortez and the Gulf of Panama, so a geographic gap of about 3,500 km separates the northern Pacific group of *L. anamesus* and *L. pictus* from the southern group of *L. panamensis* and *L. semituberculatus*. *Lytechinus panamensis* is only known from the Gulf of Panama (Mortensen 1921, p. 41; 1943, p. 450), and *L. semituberculatus* is known from the Galapagos and the adjacent Ecuadorian coast (Mortensen 1943 p. 458), so there is yet another gap of about 1,000 km between these two species. *Lytechinus panamensis* is extremely rare; researchers at the Naos Marine Laboratory in the Gulf of Panama, despite persistent efforts, have collected only two specimens during the past ten years. That the COI sequence we obtained from one of these individuals falls within the clade of *L. semituberculatus* sequences casts doubt on the distinctiveness of these two species. However, the morphology, particularly that of the pedicellariae, which are extremely prominent in *L. panamensis*, is quite distinct. Mortensen (1943, p. 458) suggested that specimens found on the coast of Ecuador (but not in the Galapagos) do not belong to *L. semituberculatus*, but may be *L. panamensis*. In other genera of echinoids there are instances of both genetic continuity (in *Diadema*: Lessios et al. 2001; *Echinometra*: McCartney et al. 2000; and *Tripneustes*: Lessios et al. 2003), and discontinuity (in *Eucidaris*: Lessios et al. 1999) between mainland and Galapagos populations. More collections are needed to determine the status of these two nominal species.

The Atlantic clade contains two species: *L. variegatus* and *L. williamsi*. The bindin and COI trees suggest different relationships between these two species. The bindin tree suggests that *L. williamsi* and *L. variegatus* are sister species, whereas the COI tree suggests that *L. variegatus carolinus* split off first, and that there is no distinction between *L. williamsi*, *L. variegatus variegatus*, and *L. variegatus atlanticus*. It is possible that the close COI relationship of *L. williamsi* and *L. variegatus variegatus* is due to introgression of mtDNA between the two taxa. The hypothesis that *L. williamsi* is correctly placed outside all the *L. variegatus* subspecies (as it is in the bindin tree) is consistent with the observation of smaller isozyme divergence between *L. variegatus variegatus* and *L. variegatus carolinus* than between *L. variegatus variegatus* (or *L. variegatus carolinus*) and *L. williamsi*. Rosenberg and Wain (1982) also found very small

amounts of divergence in isozymes between the three subspecies of *L. variegatus*. Neither the bindin nor COI data indicate any distinction between *L. variegatus variegatus* and *L. variegatus atlanticus*.

Although the isozyme and bindin data provide no evidence for the distinctiveness of *L. variegatus carolinus* from *L. variegatus atlanticus*, differences in COI and morphology support their designation as distinct subspecies. This conclusion is supported by the observations of Pawson and Miller (1982), who raised larvae of *L. variegatus atlanticus* (from Bermuda) and *L. variegatus carolinus* (from Florida) through metamorphosis in a common garden experiment and found differences in the juveniles in color, spine length/test diameter ratio, and timing of genital pore formation, indicating underlying genetic differences.

COI data indicate high levels of gene flow in the Atlantic. *Lytechinus variegatus carolinus* populations appear to be genetically continuous from North Carolina on the Atlantic seaboard, to Tallahassee on the Gulf of Mexico. Populations of *L. variegatus atlanticus*, *L. variegatus variegatus*, and *L. williamsi* from Rio de Janeiro to Bermuda are not genetically distinct. The genetic continuity in *L. variegatus variegatus* between the Caribbean and Brazil matches that seen in *Eucidaris* (Lessios et al. 1999), and contrasts with the genetic break between the Caribbean and Brazil observed in *Echinometra* (McCartney et al. 2000), *Diadema* (Lessios et al. 2001), and *Tripneustes* (Lessios et al. 2003). The genetic uniformity of *Lytechinus* within the Caribbean resembles that of all other genera of sea urchins that have been similarly studied.

Speciation in the Caribbean

Besides *Lytechinus*, only one other shallow water genus of regular echinoids, *Echinometra*, has two species in the western Atlantic. This suggests that the present-day high levels of gene flow between Caribbean populations of sea urchins reflect an historical lack of barriers. As in *Lytechinus*, speciation between the two Caribbean species of *Echinometra* has been very recent, postdating separation from the Pacific by the Isthmus of Panama (McCartney et al. 2000); but unlike *Lytechinus*, the Caribbean species of *Echinometra* show gametic incompatibility, at least in one direction (Lessios and Cunningham 1990; McCartney and Lessios 2002). The various lines of evidence we present about *Lytechinus* appear to be in conflict with respect to the question of reproductive isolation and divergence between *L. variegatus* and *L. williamsi*. Mitochondrial haplotypes of *L. williamsi* are intermingled with those of *L. variegatus variegatus* and *L. variegatus atlanticus*, except for some that form a separate clade. Isozymes of the two species have significantly different gene frequencies, but no loci fixed for different alleles. We have uncovered no evidence of preference of eggs for sperm of their own species in competitive fertilization experiments (K. S. Zigler and H. A. Lessios, unpubl. data). And yet, bindin of *L. williamsi* forms a different clade than that of any subspecies of *L. variegatus*. How can these discrepancies be explained?

That there are differences in bindin supports Chesher's (1968) decision to designate *L. williamsi* as a species separate from *L. variegatus*, even though in our experience the morphological characters suggested as diagnostic by Chesher (color of pedicellariae and crenulation of the spines) are not consistently different between all individuals of the two species. There is a definite and large difference in adult size (*L. variegatus* grows to 85 mm horizontal diameter, whereas *L. williamsi* rarely exceeds 30 mm), but even juveniles of *L. variegatus* could not be confused in nature with *L. williamsi* because they inhabit different habitats. *Lytechinus variegatus* lives in sea grass beds, sandy bottoms, and reef flats, whereas *L. williamsi* inhabits live coral reefs (Chesher 1968; Lessios 1984, 1988; Hendler et al. 1995, pp. 216–220). Juvenile *L. variegatus variegatus* are common on coral reef flats, but adult members of either species are rarely found in the habitat of the other. This may limit the opportunities for the gametes of the two species to mix in nature, but apparently does not eliminate them, because the similarity of mitochondrial haplotypes and the lack of diagnostic isozyme loci suggest either a very recent time of splitting, or extensive hybridization. Certainly the two species could not be reproductively isolated temporally, because their annual (Lessios 1984) and lunar (Lessios 1991) reproductive cycles overlap. But if the two species have split so recently, or if they hybridize, why are their bindins distinct?

In the absence of selection, mitochondrial loci will, on average, coalesce more rapidly than nuclear loci (Moore 1995; Palumbi et al. 2001), yet between *Lytechinus variegatus* and *L. williamsi* the opposite is true; bindin distinguishes between the two species whereas mtDNA does not. This suggests that selection on bindin may have accelerated its coalescence after a recent speciation event, or else that it maintains its divergence in the face of ongoing hybridization. However, we found no evidence for selection on bindin at the amino acid level between *L. variegatus* and *L. williamsi*. The small number of changes between the bindins of the two species makes it difficult to detect selection by standard tests, and the indel differences between the two species cannot be analyzed for the signature of selection through comparisons of replacement and silent substitutions. It is, therefore, possible that selection on *Lytechinus* bindin exists, but was not detected. However, the lack of evidence of gametic incompatibility between the two species in sperm competition experiments suggests that the observed differences between bindin of the two species do not significantly affect gamete interactions. There may be subtle fertilization effects that we failed to detect, but it is also possible that the monophyly of bindin is simply a result of the stochasticity of coalescence processes (Hudson and Turelli 2003).

It is unclear whether *L. williamsi* and *L. variegatus variegatus* diverged in sympatry or allopatry. The distinct clade of *L. williamsi* COI may be a remnant of previous differentiation that occurred during a period of allopatry but is now in the process of being swamped by introgression of mitochondrial mtDNA from *L. variegatus*. The recent expansions of effective population size suggested by Tajima's and Fu's tests and by the mtDNA mismatch distributions could explain the prevalence of a single COI in both species. *Lytechinus variegatus*, in particular, has experienced documented extreme population fluctuations (Watts et al. 2001), sometimes suffering mass mortality (Goodbody 1961; Glynn 1968; Bed-

dingfield and McClintock 1994; Junqueira et al. 1997) and others tremendous population increases (Camp et al. 1973; Maciá and Lirman 1999; Rose et al. 1999). Such fluctuations could spread introgressed mtDNA through populations by means of the stochastic survival of particular haplotypes. Alternatively, the distinct clade may be a sign of increasing differentiation in COI occurring from restricted genetic exchange. VanDoorn et al. (2001) published a model, according to which the interaction of sexual selection and resource competition drives divergence of reproductive molecules, such as bindin, and ultimately results in the reproductive isolation of ecologically differentiated units in sympatry. It is conceivable that such a model could apply to *Lytechinus* because of the habitat separation between *L. variegatus* and *L. williamsi* and because of the complete sorting of their bindin alleles. Of course, whether these two species fulfill the other conditions of the model is uncertain, especially since we were unable to find any evidence of gametic isolation.

A less complicated incongruity between different sets of data exists between the two subspecies *L. variegatus variegatus* and *L. variegatus carolinus*. These subspecies are similar in bindin and isozymes and also show no tendency to fertilize their own eggs more efficiently (K. S. Zigler and H. A. Lessios, unpubl. data). But they differ in coloration and mtDNA, with occasional occurrences of the "wrong" haplotype in individuals of a particular morphology, particularly in the zone of contact in southern Florida. The history of *L. variegatus* that gave rise to this pattern can be most simply hypothesized as one of allopatric differentiation of northern *L. variegatus carolinus* and southern *L. variegatus variegatus* populations followed by a more recent period of secondary contact. During the initial period of isolation, mtDNA sequences sorted out while nuclear regions did not. Subsequent contact, and the lack of bindin divergence and reproductive isolation, has resulted in introgression of mtDNA.

Conclusion

Mayr (1954) pointed out that, except for the sympatry between *L. pictus* and *L. anamesus*, allopatric speciation has been the predominant mode of speciation in *Lytechinus*. Previous evidence (Cameron 1984), along with our bindin and COI sequences, make it clear that these two entities are ecotypes of the same species. Mayr (1954), however, could not take into account *L. williamsi*, which was described by Chesher in 1968. The occurrence of this species within the range of *L. variegatus* is the only instance of possible speciation in sympatry among the species of *Lytechinus*. The three eastern Pacific species are separated by large geographic gaps and, though the cause of their vicariance cannot be ascertained, fit an allopatric model of speciation. The subspecies of *L. variegatus* are a diagrammatic illustration of morphologically differentiated and geographically nonoverlapping populations envisioned as a stage in the process of speciation by distance. That the mtDNA of *L. variegatus variegatus* and *L. variegatus carolinus* is, in fact, differentiated adds evidence to what was previously suspected from morphology alone. The mtDNA introgression between *L. variegatus carolinus* and *L. variegatus variegatus* in the zone of contact off Florida is neither surprising, nor detracts from the picture of isolation by distance.

This predominantly allopatric pattern of speciation in *Lytechinus* without the development of prezygotic reproductive isolation is reflected in the evolution of bindin, where we find no evidence of positive selection. The lack of major differentiation in *Lytechinus* bindin is correlated with high levels of gametic compatibility between the taxa of *Lytechinus*. All the described species or subspecies of *Lytechinus* that have been tested for gametic compatibility appear able to fertilize each other. This is true not only for *L. pictus* and *L. anamesus* (Cameron 1984), but also for *L. variegatus carolinus* and *L. variegatus variegatus*, as well as *L. williamsi* and *L. variegatus variegatus* (K. S. Zigler and H. A. Lessios, unpubl. data). Even *L. pictus* and *L. variegatus*, which were separated more than three million years with the rise of the Isthmus of Panama, cross-fertilize each other at a high rate (Minor et al. 1991).

The study of molecular variation of mtDNA (Palumbi and Wilson 1990; Palumbi and Kessing 1991; McMillan et al. 1992; Bermingham and Lessios 1993; Palumbi 1996; Palumbi et al. 1997; Lessios et al. 1998, 1999, 2001, 2003; McCartney et al. 2000) and bindin (Metz and Palumbi 1996; Biermann 1998; Metz et al. 1998; Debenham et al. 2000a,b; Geyer and Palumbi 2003; Zigler and Lessios 2003a; Zigler et al. 2003) of shallow water sea urchins has provided insights on speciation. As often happens, the molecules revealed a number of cases of separate species that on morphological grounds had been lumped, and a few of species that on the basis of their morphology were thought to be separate, yet on the molecular level show no evidence of genetic divergence. Despite these new discoveries, the generalizations made by Mayr (1954) on the basis of morphology alone about how echinoids speciate have held up fairly well. As one would expect from allopatric speciation, closely related species tend to be distributed on either side of major barriers to marine larval dispersal. The phylogeny of each genus, however, also has its own interesting peculiarities, and that of *Lytechinus* is no exception. The challenge for future studies in this genus is to understand how the sympatric *L. williamsi* and *L. variegatus* have come to be, and how they maintain their separate genetic identities.

Acknowledgments

We thank P. Barnes, C. Damiano, A. Herrera, D. Levitan, M. McCartney, D. McClay, V. Vacquier, R. Ventura, S. Williams, and C. Young for collecting animals used in this study. A. and L. Calderón assisted in the laboratory. W. Lee, H. Reichardt, and M. E. Rice provided assistance at the Smithsonian Marine Station at Fort Pierce. R. Collin, C. Cunningham, D. McClay, a reviewer, and the associate editor provided helpful comments on the manuscript. This work was supported by National Science Foundation and Smithsonian predoctoral fellowships to KSZ, by the Duke University Department of Zoology, by the Smithsonian Molecular Evolution Program, and by the Smithsonian Marine Station at Fort Pierce.

Literature Cited

Beddingfield, S. D., and J. B. McClintock. 1994. Environmentally induced catastrophic mortality of the sea urchin *Lytechinus variegatus* in shallow seagrass habitats of Saint Josephs Bay, Florida. Bull. Mar. Sci. 55:235–240.

Bermingham, E. B., and H. A. Lessios. 1993. Rate variation of protein and mtDNA evolution as revealed by sea urchins separated by the Isthmus of Panama. Proc. Natl. Acad. Sci. USA 90:2734–2738.

Biermann, C. H. 1998. The molecular evolution of sperm bindin in six species of sea urchins (Echinoida: Strongylocentrotidae). Mol. Biol. Evol. 15:1761–1771.

Cameron, R. A. 1984. Two species of *Lytechinus* (Toxopneustidae: Echinoidea: Echinodermata) are completely cross-fertile. Bull. So. Calif. Acad. Sci. 83:154–157.

———. 1986. Reproduction, larval occurrence and recruitment in Caribbean sea urchins. Bull. Mar. Sci. 39:332–346.

Camp, D. K., S. P. Cobb, and J. F. van Breedveld. 1973. Overgrazing of seagrasses by a regular urchin, *Lytechinus variegatus*. Bioscience. 23:37–38.

Chesher, R. H. 1968. *Lytechinus williamsi*, a new sea urchin from Panama. Breviora 305:1–13.

———. 1972. The status of knowledge of Panamanian echinoids, 1971, with comments on other echinoderms. Bull. Biol. Soc. Wash. 2:139–158.

Clark, H. L. 1940. Eastern Pacific expeditions of the New York Zoological Society. XXI. Notes on echinoderms from the west coast of Central America. Zoologica 25:331–355.

Coates, A. G., and J. A. Obando. 1996. The geologic evolution of the Central American Isthmus. Pp. 21–56 *in* J. B. C. Jackson, A. G. Coates, and A. Budd, eds. Evolution and environment in tropical America. Univ. of Chicago Press, Chicago.

Debenham, P., M. A. Brzezinski, and K. R. Foltz. 2000a. Evaluation of sequence variation and selection in the bindin locus of the red sea urchin, *Strongylocentrotus franciscanus*. J. Mol. Evol. 51:481–490.

Debenham, P., M. Brzezinski, K. Foltz, and S. Gaines. 2000b. Genetic structure of populations of the red sea urchin, *Strongylocentrotus franciscanus*. J. Exp. Mar. Biol. Ecol. 253:49–62.

Durham, W. J., C. Wagner, and D. P. Abbott. 1980. Echinoidea: the sea urchins. Pp. 160–176 *in* R. Morris, D. P. Abbott, and E. C. Hadderlie, eds. Intertidal invertebrates of California. Stanford Univ. Press, Stanford, CA.

Emlet, R. B. 1995. Developmental mode and species geographic range in regular sea urchins (Echinodermata: Echinoidea). Evolution 49:476–489.

Emlet, R. B., L. R. McEdward, and R. R. Strathmann. 1987. Echinoderm larval ecology viewed from the egg. Pp. 55–136 *in* M. Jangoux and J. M. Lawrence, eds. Echinoderm studies. Vol. 2. Balkema Press, Rotterdam.

Ettensohn, C. A. 1985. Gastrulation in the sea urchin embryo is accompanied by the rearrangement of invaginating epithelial cells. Dev. Biol. 112:383–390.

Ettensohn, C. A., and D. R. McClay. 1988. Cell lineage conversion in the sea urchin embryo. Dev. Biol. 125:396–409.

Frohman, M. A., M. K. Dush, and G. R. Martin. 1988. Rapid production of full-length cDNAs from rare transcripts by amplification using a single gene specific oligonucleotide primer. Proc. Natl. Acad. Sci. USA 85:8998–9002.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

Geyer, L. B., and S. R. Palumbi. 2003. Reproductive character displacement and the genetics of gamete recognition in tropical sea urchins. Evolution 57:1049–1060.

Glynn, P. W. 1968. Mass mortalities of echinoids and other reef flat organisms coincident with midday, low water exposures in Puerto Rico. Mar. Biol. 1:226–243.

Goodbody, I. M. 1961. Mass mortality of a marine fauna following tropical rains. Ecology 42:150–155.

Hardin, J. D., and L. Y. Cheng. 1986. The mechanisms of archenteron elongation during sea urchin gastrulation. Dev. Biol. 115:490–501.

Harvey, E. B. 1956. The American *Arbacia* and other sea urchins. Princeton Univ. Press, Princeton, NJ.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22:160–174.

Hendler, G., J. E. Miller, D. L. Pawson, and P. M. Kier. 1995. Sea stars, sea urchins, and allies: echinoderms of Florida and the Caribbean. Smithsonian Institution Press, Washington, DC.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. Pp. 1–44 *in* D. Futuyma and J. D. Antonovics, eds. Oxford Surveys in Evolutionary Biology. Vol. 7. Oxford Univ. Press, New York.

Hudson, R. R., and N. L. Kaplan. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. Genetics 111:147–164.

Hudson, R. R., and M. Turelli. 2003. Stochasticity overrules the "three-times rule": Genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. Evolution 57: 182–190.

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Junqueira, A. D. R., C. R. R. Ventura, A. L. P. S. Decarvalho, and A. J. Schmidt. 1997. Population recovery of the sea urchin *Lytechinus variegatus* in a seagrass flat (Araruama Lagoon, Brazil): the role of recruitment in a disturbed environment. Invertebr. Reprod. Dev. 31:143–150.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120.

Kumar, S., K. Tamura, I. B. Jakobsen, and M. Nei. 2001. MEGA 2: molecular evolutionary genetics analysis software. Bioinformatics 17:1244–1245.

Lessios, H. A. 1984. Annual reproductive periodicity in eight echinoid species on the Caribbean coast of Panama. Pp. 303–310 *in* B. F. Keegan and B. D. S. O'Connor, eds. Proceedings of the Fifth International Echinoderm Conference. A.A. Balkema, Boston.

———. 1988. Population dynamics of *Diadema antillarum* (Echinodermata: Echinoidea) following mass mortality in Panama. Mar. Biol. 99:515–526.

———. 1991. Presence and absence of monthly reproductive rhythms among eight Caribbean echinoids off the coast of Panama. J. Exp. Mar. Biol. Ecol. 153:27–47.

Lessios, H. A., and C. W. Cunningham. 1990. Gametic incompatibility between species of the sea urchin *Echinometra* on the two sides of the Isthmus of Panama. Evolution 44:933–941.

Lessios, H. A., and J. S. Pearse. 1996. Hybridization and introgression between Indo-Pacific species of *Diadema*. Mar. Biol. 126:715–723.

Lessios, H. A., B. D. Kessing, and D. R. Robertson. 1998. Massive gene flow across the world's most potent marine biogeographic barrier. Proc. R. Soc. Lond. B. 265:583–588.

Lessios, H. A., B. D. Kessing, D. R. Robertson, and G. Paulay. 1999. Phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents. Evolution 53: 806–817.

Lessios, H. A., B. D. Kessing, and J. S. Pearse. 2001. Population structure and speciation in tropical seas: Global phylogeography of the sea urchin *Diadema*. Evolution 55:955–975.

Lessios, H. A., J. Kane, and D. R. Robertson. 2003. Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. Evolution 57: 2026–2036.

Lewis, J. B. 1963. The food of some deep-water echinoids from Barbados. Bull. Mar. Sci. Gulf Caribb. 13:360–363.

Li, W.-H. 1993. Unbiased estimation of the rates of synonymous and nonsynonymous substitution. J. Mol. Evol. 36:96–99.

Maciá, S., and D. Lirman. 1999. Destruction of Florida Bay sea-

grasses by a grazing front of sea urchins. Bull. Mar. Sci. 65: 593–601.
Mayr, E. 1954. Geographic speciation in echinoids. Evolution 8: 1–18.
Maddison, D. R., and W. P. Maddison. 2000. MacClade 4: analysis of phylogeny and character evolution. Ver. 4.0. Sinauer Associates, Sunderland, MA.
Mazur, J. E., and J. W. Miller. 1971. A description of the complete metamorphosis of the sea urchin *Lytechinus variegatus* cultured in synthetic sea water. Ohio J. Sci. 71:30–36.
McCartney, M. A., and H. A. Lessios. 2002. Quantitative analysis of gametic incompatibility between closely related species of neotropical sea urchins. Biol. Bull. 202:166–181.
McCartney, M. A., G. Keller, and H. A. Lessios. 2000. Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*. Mol. Ecol. 9: 1391–1400.
McDonald, J. H., and M. Kreitman. 1991. Adaptive protein evolution at the *Adh* locus in *Drosophila*. Nature 351:652–654.
McMillan, W. O., R. A. Raff, and S. R. Palumbi. 1992. Population genetic consequences of developmental evolution in sea urchins (genus *Heliocidaris*). Evolution 46:1299–1312.
Metz, E. C., and S. R. Palumbi. 1996. Positive selection and sequence rearrangements generate extensive polymorphism in the gamete recognition protein bindin. Mol. Biol. Evol. 13:397–406.
Metz, E. C., G. Gómez-Gutiérrez, and D. Vacquier. 1998. Mitochondrial DNA and bindin gene sequence evolution among allopatric species of the sea urchin genus *Arbacia*. Mol. Biol. Evol. 15:185–195.
Minor, J. E., D. R. Fromson, R. J. Britten, and E. H. Davidson. 1991. Comparison of the bindin proteins of *Strongylocentrotus franciscanus*, *S. purpuratus*, and *Lytechinus variegatus*: sequences involved in the species specificity of fertilization. Mol. Biol. Evol. 8:781–795.
Moore, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49:718–726.
Mortensen, T. 1921. Studies of the development and larval forms of echinoderms. G.E.C. Gad, Copenhagen.
———. 1943. A monograph of the Echinoidea III(2): Camarodonta. I. Orthopsidae, Glycocyphidae, Temnopleuridae and Toxopneustidae. C.A. Reitzel, Copenhagen.
Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.
Palumbi, S. R. 1996. What can molecular genetics contribute to marine biogeography? An urchin's tale. J. Exp. Mar. Biol. Ecol. 203:75–92.
Palumbi, S. R., and B. D. Kessing. 1991. Population biology of the transarctic exchange: mtDNA sequence similarity between Pacific and Atlantic sea urchins. Evolution 45:1790–1805.
Palumbi, S. R., and A. C. Wilson. 1990. Mitochondrial DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. Evolution 44:403–415.
Palumbi, S. R., G. Grabowsky, T. Duda, L. Geyer, and N. Tachino. 1997. Speciation and population genetic structure in tropical Pacific sea urchins. Evolution 51:1506–1517.
Palumbi, S. R., F. Cipriano, and M. P. Hare. 2001. Predicting nuclear gene coalescence from mitochondrial data: the three-times rule. Evolution 55:859–868.
Pamilo, P., and N. O. Bianchi. 1993. Evolution of the Zfx and Zfy genes: rates and interdependence between the genes. Mol. Biol. Evol. 10:271–281.
Pawson, D. L., and J. E. Miller. 1982. Studies of genetically controlled phenotypic characters in laboratory-reared *Lytechinus variegatus* (Lamarck) (Echinodermata:Echinoidea). Pp. 165–171 *in* J. M. Lawrence, ed. Proceedings of the International Echinoderm Conference, Tampa Bay. A.A. Balkema, Rotterdam.
Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.
Rambaut, A. 1996. Se-Al: sequence alignment editor. University of Oxford, Oxford, U.K. Available at http://evolve.zoo.ox.ac.uk/.
Rogers, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608–615.
Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9:552–569.
Rosenberg, V. A., and R. P. Wain. 1982. Isozyme variation and genetic differentiation in the decorator sea urchin, *Lytechinus variegatus* (Lamarck, 1816). Pp. 193–197 *in* J. M. Lawrence, ed. Proceedings of the International Echinoderm Conference, Tampa Bay. A.A. Balkema, Rotterdam.
Rose, C. D., W. C. Sharp, W. J. Kenworthy, J. H. Hunt, W. G. Lyons, E. J. Prager, J. F. Valentine, M. O. Hall, P. E. Whitfield, and J. W. Fourqurean. 1999. Overgrazing of a large seagrass bed by the sea urchin *Lytechinus variegatus* in Outer Florida Bay. Mar. Ecol. Progr. Ser. 190:211–222.
Rozas, J., and R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.
Schneider, S., and L. Excoffier. 1999. Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089.
Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin. Ver. 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
Serafy, D. K. 1973. Variation in the polytypic sea urchin *Lytechinus variegatus* (Lamarck, 1816) in the western Atlantic (Echinodermata: Echinoidea). Bull. Mar. Sci. 23:525–534.
Sherwood, D. R., and D. R. McClay. 1999. LvNotch signaling mediates secondary mesenchyme specification in the sea urchin embryo. Development 126:1703–1713.
Swanson, W. J., C. F. Aquadro, and V. D. Vacquier. 2001. Polymorphism in abalone fertilization proteins is consistent with the neutral evolution of the egg's receptor for lysin (VERL) and positive Darwinian selection of sperm lysin. Mol. Biol. Evol. 18:376–383.
Swofford, D. L. 2001. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Ver. 4. Sinauer Associates, Sunderland, MA.
Swofford, D. L., and R. B. Selander. 1989. BIOSYS-1. A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Illinois Natural History Survey, Champaign, IL.
Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.
Tamura, K., and M. Nei. 1993. Estimating the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512–526.
Ulrich, A. S., M. Otter, C. G. Glabe, and D. Hoekstra. 1998. Membrane fusion is induced by a distinct peptide sequence of the sea urchin fertilization protein bindin. J. Biol. Chem. 273: 16748–16755.
Ulrich, A. S., W. Tichelaar, G. Forster, O. Zschornig, S. Weinkauf, and H. W. Meyer. 1999. Ultrastructural characterization of peptide-induced membrane fusion and peptide self-assembly in the lipid bilayer. Biophys. J. 77:829–841.
Vacquier, V. D., and G. W. Moy. 1977. Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. Proc. Natl. Acad. Sci. USA 74:2456–2460.
VanDoorn, G. S., P. C. Luttikhuizen, and F. J. Weissing. 2001. Sexual selection at the protein level drives the extraordinary divergence of sex-related genes during sympatric speciation. Proc. R. Soc. Lond. B. 268:2155–2161.
Watts, S. A., J. B. McClintock, and J. M. Lawrence. 2001. The ecology of *Lytechinus variegatus*. Pp. 375–393 *in* J. M. Lawrence, ed. Edible sea urchins: biology and ecology. Elsevier, Amsterdam.
Workman, P. L., and J. D. Niswander. 1970. Population studies on

southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Hum. Genet. 22:24–29.

Yang, Z. 2000. Phylogenetic analysis by maximum likelihood (PAML). Ver. 3.0. University College London, London.

Yang, Z., R. Nielsen, N. Goldman, and A-M. K. Pedersen. 2000. Codon substitution models for heterogeneous selection pressure at amino acid sites. Genetics 155:431–449.

Zhang, J., S. Kumar, and M. Nei. 1997. Small-sample tests of episodic evolution: a case study of primate lysosymes. Mol. Biol. Evol. 14:1335–1338.

Zigler, K. S., and H. A. Lessios. 2003a. Evolution of the gamete recognition protein bindin across six orders of sea urchins. Biol. Bull. 205:8–15.

———. 2003b. Evolution of bindin in the pantropical sea urchin *Tripneustes*: comparisons to bindin of other genera. Mol. Biol. Evol. 20:220–231.

Zigler, K. S., E. C. Raff, E. Popodi, R. A. Raff, and H. A. Lessios. 2003. Adaptive evolution of bindin in the genus *Heliocidaris* is correlated with the shift to direct development. Evolution 57: 2293–2302.

Corresponding Editor: D. McHugh