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PHYLOGEOGRAPHY OF THE PANTROPICAL SEA URCHIN *TRIPNEUSTES*: CONTRASTING PATTERNS OF POPULATION STRUCTURE BETWEEN OCEANS

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Abstract.—To understand how allopatric speciation proceeds, we need information on barriers to gene flow, their antiquity, and their efficacy. For marine organisms with planktonic larvae, much of this information can only be obtained through the determination of divergence between populations. We evaluated the importance of ocean barriers by studying the mitochondrial DNA phylogeography of *Tripneustes*, a pantropical genus of shallow water sea urchin. A region of cytochrome oxidase I (COI) was sequenced in 187 individuals from locations around the globe. The COI phylogeny agreed with a previously published phylogeny of bindin that barriers important to the evolution of *Tripneustes* are: (1) the cold water upwelling close to the tip of South Africa, (2) the Isthmus of Panama, (3) the long stretch of deep water separating the eastern from the western Atlantic, and (4) the freshwater plume of the Orinoco and the Amazon rivers between the Caribbean and the coast of Brazil. These barriers have previously been shown to be important in at least a subset of the shallow water marine organisms in which phylogeography has been studied. In contrast, the Eastern Pacific Barrier, 5000 km of deep water between the central and the eastern Pacific that has caused the deepest splits in other genera of sea urchins, is remarkably unimportant as a cause of genetic subdivision in *Tripneustes*. There is also no discernible subdivision between the Pacific and Indian Ocean populations of this genus. The most common COI haplotype is found in the eastern, central, and western Pacific as well as the Indian Ocean. Morphology, COI, and bindin data agree that *T. depressus* from the eastern Pacific and *T. gratilla* from the western Pacific are, in fact, the same species. The distribution of haplotype differences in the Indo-Pacific exhibits characteristics expected from a sea urchin genus with ephemeral local populations, but with high fecundity, dispersal, and growth: there is little phylogenetic structure, and mismatch distributions conform to models of recent population expansion on a nearly global scale. Yet, comparisons between local populations produce large and significant F_{ST} values, indicating nonrandom haplotype distribution. This apparent local differentiation is only weakly reflected in regional divergence, and there is no evidence of isolation by distance in correlations between F_{ST} values and either geographical or current distance. Thus, *Tripneustes* in the Indo-Pacific (but not in the Atlantic) seems to be one large metapopulation spanning two oceans and containing chaotic, nonequilibrium local variation, produced by the haphazard arrival of larvae or by unpredictable local extinction.

Key words.—Benguela, cytochrome oxidase I, Eastern Pacific Barrier, gene flow, Isthmus of Panama, sea urchins, speciation.

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To understand how allopatric speciation proceeds, we must deduce the nature, efficacy, and timing of barriers to gene flow between populations. For marine organisms with planktonic larvae, identification of such barriers is difficult, because these propagules disperse to an unknown extent in unpredictable directions. In broad outline, obstacles to dispersal in the sea have been determined through the designation of biogeographic provinces, that is, areas that coincide with the ranges of many species with generally similar ecological requirements (Ekman 1953; Briggs 1974). However, the dividing lines of such provinces are often subjective and acknowledged not to apply to every species. They also depend on the assignment of regional populations to the correct species, which is sometimes difficult on the basis of morphology alone. Above all, species ranges by themselves cannot provide information on phylogenetic relations (e.g., Wallis and Trewick 2001), and without information on the order of splitting between species, little can be said about speciation.

Molecular comparisons between populations can overcome these problems and thus provide a basis on which concrete hypotheses about speciation within each taxon can be formulated. Each phylogeny, in addition to providing information about the particular taxon for which it was constructed, also permits an examination of the generality of each geographic barrier. Thus, it is useful to determine phylogenetic relations of populations spanning one or more putative barriers, but it is particularly beneficial to compare the phylogeography of several taxa to determine similarities and differences in the way geographic barriers have affected the history of populations (Avise 2000). Because of the wide dispersal of many marine species, phylogeographic analysis of taxa with planktonic larvae is most useful when done at the level of an entire ocean or, preferably, all the oceans in which the taxon is found. A number of molecular comparisons of populations on such a large scale have attempted to determine barriers that have caused speciation (e.g., McMillan and Palumbi 1995; Palumbi et al. 1997; Baldwin et al. 1998; Lessios et al. 1999, 2001a; McCartney et al. 2000; Waters et al. 2000; Bowen et al. 2001; Colborn et al. 2001; Muss et al. 2001; Coleman and Vacquier 2002; Ludwig et al. 2002; Waters and Roy 2002). Four of these studies involve sea urchins.

As Mayr (1954) was the first to realize, the relatively small number of extant sea urchin species and the adequate study of their alpha taxonomy make sea urchins a useful group for studies of speciation in the sea. Mayr plotted the species ranges of shallow water sea urchin genera as presented in

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Mortensen's (1928–1951) monograph, and reached the conclusion that they conformed to an allopatric mode of speciation. Mayr noted that the absence of phylogenetic information greatly hindered firm conclusions in cases of sympatric species. He did not stress another problem caused by reliance on traditional species designations in sea urchins to reach conclusions about speciation: early taxonomists tended to assign populations from different regions to different species even when no reliable morphological characters distinguished them, a practice that can artificially inflate the apparent number of allopatric speciation events. One such genus in which species designations are not firmly based on morphological divergence is the circumtropical shallow water sea urchin *Tripneustes*.

Tripneustes is common on sea grass beds, hard substrata, and reefs in the tropical Indo-Pacific and Atlantic Oceans, down to a depth of 75 m (Mortensen 1928–1951). Its fossil record extends back to the Miocene (Mortensen 1928–1951; Fell and Pawson 1966). Three species are generally recognized: *T. depressus* from the eastern Pacific, *T. gratilla* from the central Pacific to the African coast of the Indian Ocean, and *T. ventricosus* (= *T. esculentus*) from both sides of the Atlantic (Mortensen 1928–1951). The morphological differences of the three nominal species are so slight that Clark (1912, p. 285) suggested that ''there is probably but a single species'' of *Tripneustes*, and Mortensen (1928–1951, pp. 487–508) speculated that ''the time since they were separated into an Indo-Pacific, an Atlantic, and a West American group, has not been long enough to make them three very distinct species.'' Zigler and Lessios (2003) studied variation of the gamete recognition molecule, bindin, in *Tripneustes* from the Pacific, Atlantic, and Indian Oceans. They found that four amino acid changes and one insertion/deletion (indel) distinguish the bindin of the Atlantic *T. ventricosus* from those of the eastern Pacific *T. depressus* and the Indo-West Pacific *T. gratilla*, but that there are no fixed amino acid or indel differences between the bindins of *T. depressus* and *T. gratilla*, nor any geographic structure in the distribution of bindin alleles.

What is known about the ecology of *Tripneustes* indicates that its populations both in the Pacific and in the Atlantic are subject to high mortalities, offset by high fecundity and rapid individual and population growth (Lawrence and Agatsuma 2001). McPherson (1965) reported that *T. ventricosus* reaches sexual maturity at 40–44 mm and that the rate of growth of juveniles is 6–8 mm per month, which suggests that generation time is in the order of half a year. In the laboratory, the larvae of *T. ventricosus* reach metamorphosis in about a month (Mortensen 1921; Lewis 1958), those of *T. gratilla* in about 18 days (Mortensen 1937). Because of potential differences in culture conditions, these laboratory data can say little about dissimilarities in larval durations in nature, but they do indicate a range in the minimum time span during which single generation dispersal can occur.

We used sequence data from the cytochrome oxidase I (COI) region of mitochondrial DNA (mtDNA) of *Tripneustes* to address the following questions: (1) Given the morphological similarity of populations from different oceans, are all currently accepted species valid? (2) What biogeographic barriers are likely to have caused speciation between the species that are valid? (3) What degree of genetic structure exists within species and what can it tell us about the history of populations? (4) How do the observed phylogenetic patterns compare with those of other cosmopolitan sea urchins and other marine organisms?

MATERIALS AND METHODS

A total of 187 individuals of *Tripneustes* were collected from locations around the globe (Fig. 1); 48 of *T. ventricosus* (Atlantic), 35 of *T. depressus* (eastern Pacific), and 104 of *T. gratilla* (central and Indo-West Pacific). Samples were preserved in 95% ethanol, in high-salt DMSO buffer (Seutin et al. 1991), or in liquid nitrogen.

We carried out genomic DNA extractions, polymerase chain reaction (PCR) amplifications, PCR product purification, and DNA sequencing as described by Lessios et al. (1996, 1998), with one modification: primers used in the forward direction were either CO1f 5′CCTGCAGGAGGAGGAGAYCC or CO1p 5′GGTCACCCAGAAGTGTACAT, their 5′ end corresponding to positions 6448 and 6498 of *Strongylocentrotus purpuratus*, respectively (Jacobs et al. 1988). Reverse primers were either CO1TR1 5′GGCATTCCAGCTAGTCCTARAA (a *Tripneustes*-specific primer, designed for this study), CO1 k 5′ GTCSGARTAACGXCGSGGCAT, or CO1a 5′AGTATAAGCGTCTGGGTAGTC, corresponding to positions 7094, 7110, and 7128, respectively of the *S. purpuratus* mitochondrial genome. These primers amplified between 589 and 640 nucleotides from the COI region. We sequenced in both directions, at least once for each strand, starting at either position 6467 or 6518 and extending to either position 7072, 7089, or 7107, using a 377 automatic sequencer from Perkin-Elmer/Applied Biosystems (Foster City, CA). Sequences have been deposited in GenBank under accession numbers AY205373–AY205560.

Phylogenies were constructed using version 4.0b10 of PAUP* (Swofford 2002), with a sequence from *Lytechinus variegatus* as an outgroup. A neighbor-joining tree (Saitou and Nei 1987) was constructed using LogDet distances (Lockhart et al. 1994), and bootstrapped in 1000 iterations. We also employed Posada and Crandall's (1998) MODELTEST program version 3.06 to determine the simplest model of mtDNA evolution that produced the best fit of the tree to the data. Log-likelihood tests indicated that this condition was satisfied by Hasegawa et al.'s (1985) model of base substitution (HKY) with a gamma correction ($\alpha = 0.2025$). Because the LogDet and HKY models are not special cases of each other, it was not possible to statistically compare their likelihoods. We therefore carried out parallel analyses based on both models. To calculate confidence intervals for estimates of time since divergence, we used the mean path length method of Britton et al. (2002). This method incorporates rate variation between branches into the estimate of uncertainty.

Population structure was assessed by means of F_{ST} statistics, based on Kimura (1980) two-parameter distances. Only localities in which more than four individuals were sampled were included in this analysis. More detailed analyses were carried out with the sequences from the Indo-Pacific: to compare differentiation between regions over and above differ-

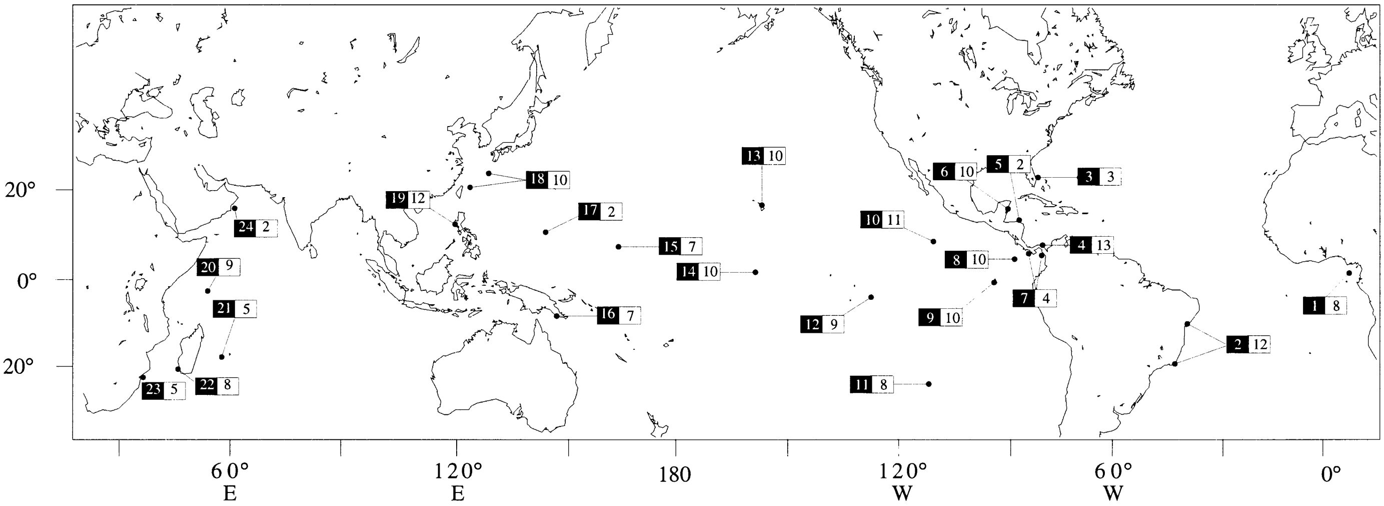

FIG. 1. Collection localities and sample sizes. Box with black background indicates locality code; box with white background the sample size. Locality codes: 1, São Tomé, Gulf of Guinea; 2, Salvador, Bahia (n = 11) and Arraial do Cabo, Rio de Janeiro, (n = 1), Brazil; 3, Fort Pierce, Florida; 4, San Blas Islands (n = 7) and Isla Margarita (n = 6), Panama; 5, Cochino Chico, Honduras; 6, Carrie Bow Cay, Belize; 7, Guayabo, Bay of Panama (n = 1), Uva Island, Gulf of Chiriquí (n = 2) and Jicarita Island, Gulf of Chiriquí (n = 1); 8, Isla del Coco; 9, Galapagos (three specimens each from Floreana and Genovesa, and two each from Isabella and Santiago); 10, Clipperton Atoll; 11, Easter Island; 12, Marquesas; 13, Hawaii (three specimens each from Hawaii and Oahu, and four from Maui); 14, Kiritimati, Kiribati; 15, Majuro, Marshall Islands; 16, Papua New Guinea; 17, Guam; 18, Sesoko (n = 9) and Ishigaki (n = 1), Japan; 19, Luzon Island, Philippines; 20: Mahé Island, Seychelles; 21, Reunión; 22, Tuléar, Madagascar; 23, Sodwana, South Africa; 24, Masirah Island, Oman.

entiation between local populations we used analysis of molecular variance (AMOVA; Excoffier et al. 1992). To determine the history of population size, we analyzed the distribution of pairwise differences between haplotypes with the models of Rogers and Harpending (1992) and Rogers (1995). History of effective population size was also assessed by means of Tajima's (1989), and Fu's (1997) tests. Version 2.0 of Arlequin (Schneider et al. 2000) was used for these analyses. To assess the possibility that variation was due to selection we used McDonald and Kreitman's (1991) comparisons of the ratio of replacement to silent substitutions within and between species. To examine the data for correlations between genetic differentiation and geographic distance we used Mantel (1967) tests carried out in version 2 of MANTEL by A. Liedloff, Queensland University of Technology (available at http://www.sci.qut.edu.au/NRS/mantel.htm) For these correlations, ''distance'' was defined either as the minimum number of kilometers of sea surface between two locations (i.e. straight-line distance, but avoiding land), or as ''current distance,'' that is, the number of kilometers a larva would have to traverse if it were traveling along the mean vectors of major currents (see Palumbi et al. 1997). Correlations were examined both between raw F_{ST} values and raw distance and between $\log_{10}(F_{ST} + 1)$ and $\log_{10}$(distance).

RESULTS

Phylogeny

The COI sequences from 187 individuals of *Tripneustes* contained 74 unique mtDNA haplotypes. Figure 2 presents a neighbor-joining tree of these haplotypes based on LogDet distances. The topology of a neighbor-joining tree based on HKY distances (not shown) was the same, except for details of the terminal branches. The reconstruction indicated that there was a deep split (8.83% LogDet distance) between mtDNA of *T. ventricosus* from the Atlantic and a clade composed of both *T. depressus* from the eastern Pacific and *T. gratilla* from central and Indo-West Pacific. There were 30 fixed nucleotide differences and one amino acid change between Atlantic and Indo-Pacific *Tripneustes*. No sites were fixed between *T. gratilla* and *T. depressus*, and thus these two nominal Pacific species did not sort out as separate clades.

The *T. ventricosus* clade was subdivided into two reciprocally monophyletic eastern and western Atlantic clades, the former containing all individuals from São Tomé and the latter encompassing Brazilian and Caribbean populations. LogDet sequence dissimilarity between eastern and western Atlantic populations was 1.66%. Six nucleotide sites were diagnostic between them. In the tree based on LogDet distances, the western Atlantic clade further subdivided into two subclades, one from Brazil, and the other from the Caribbean. The separation was shallow. The average genetic distance between these two subclades was 0.75%, and the nodes supporting the monophyly of each subclade received bootstrap support of <60% when LogDet distances were used, and <50% when HKY distances were used. A single nucleotide site was diagnostic between Brazilian and Caribbean populations of *T. ventricosus*.

The Indo-Pacific clade consisted of a polytomy composed of both *T. depressus* and *T. gratilla*. In fact, the two nominal species shared indistinguishable COI haplotypes. The most common mitochondrial haplotype was spread from the eastern Pacific to the western Indian Ocean. It was found in individuals of *T. depressus* from Galapagos and Clipperton (eastern Pacific); in individuals of *T. gratilla* from Kiritimati,

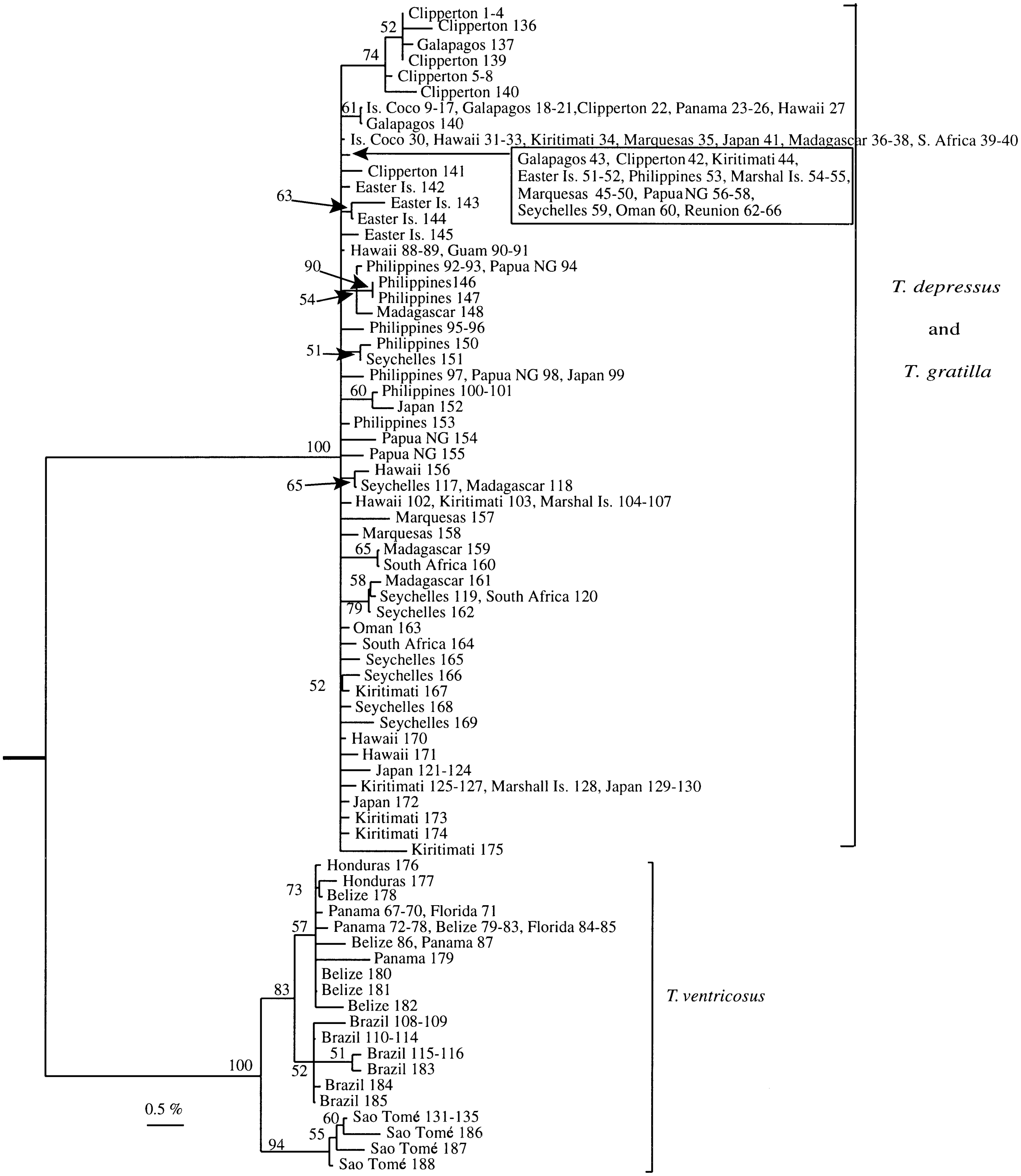

FIG. 2. Neighbor-joining tree (Saitou and Nei 1987) of unique COI haplotypes of *Tripneustes*, based on LogDet distances (Lockhart et al. 1994). Numbers after locality names indicate individuals that share each haplotype. Numbers next to nodes indicate bootstrap support from 1000 iterations. Nodes with <50% support have been collapsed. The tree is rooted on a COI sequence of *Lytechinus variegatus*.

the Marquesas, and Easter Island (central Pacific); in individuals from the Marshall Islands, Philippines, and Papua New Guinea (western Pacific); and in individuals from the Seychelles, Oman, and Reunión (Indian Ocean). Another haplotype was found both in *T. depressus* from Isla del Coco and in *T. gratilla* from the Marquesas and the Indian Ocean shores of South Africa.

A few haplotypes of *T. depressus* from Clipperton Atoll and one from the Galapagos formed their own fairly well-supported monophyletic entity on both the LogDet and HKY trees. Haplotypes from both of these localities were also present in the basal polytomy, so this clade was not necessarily indicative of geographic structuring in the eastern Pacific.

Gene Flow

That there has not been any gene flow for a long time between the eastern and western Atlantic sampled populations is apparent from their phylogenetic separation and presence of multiple diagnostic nucleotide sites. Within the western Atlantic, pairwise F_{ST} values agreed with the phylogeny in indicating that Brazil's *T. ventricosus* population was also genetically isolated from populations in the Caribbean (Table 1). Within the Caribbean, however, populations were genetically homogenous. The most common Caribbean haplotype was present in Panama, Belize, and Florida (Fig. 2).

Although haplotypes of *T. depressus* and *T. gratilla* from the Indo-Pacific formed a polytomy in the phylogeny, and though indistinguishable haplotypes were widely spread, large and significant values in pairwise F_{ST} statistics indicated that haplotype distribution was not random with respect to geographic location (Table 1). Little of this variation, however, was due to differences between major geographic regions. Analysis of molecular variance comparisons designating the eastern, central, and western Pacific and the Indian Ocean as separate groups indicated that 73.07% of the variation was within populations, 17.68% was between populations within groups, and only 9.25% was between groups. The Φ_{CT} value between groups was significant ($P = 0.005$), but small ($\Phi_{CT} = 0.093$).

When the eastern Pacific *T. depressus* was compared with *T. gratilla* from the rest of the Pacific and from the Indian Ocean, the Φ_{CT} value indicated a certain degree of divergence ($\Phi_{CT} = 0.15$, $P = 0.002$), but still less than the value of 0.33, which in mtDNA corresponds at genetic equilibrium to one propagule per generation. A migration rate of less than one propagule per generation is considered necessary for independent evolutionary development of populations (Avise 1994). Pairwise F_{ST} values between *T. depressus* populations from the eastern Pacific and *T. gratilla* populations from the rest of the Indo-Pacific were large and significant in every comparison, with the exception of comparisons between *T. depressus* from the Galapagos with *T. gratilla* from Reunión and from South Africa (Table 1). Analysis of molecular variance comparisons indicated that there was no barrier between the eastern and central Pacific, on the one hand, and the Indo-West Pacific, on the other ($\Phi_{CT} = 0.03$, $P = 0.14$). Nor was there any consistent differentiation between the Indian Ocean and the entire Pacific ($\Phi_{CT} = 0.03$, $P = 0.19$).

Within each of the major regions, populations generally exchanged genes at high rates, with some exceptions (Table 1). In the eastern Pacific, the distant and isolated Clipperton Atoll, 1000 km west of Mexico, was genetically isolated from Isla del Coco and Pacific Panama. *Tripneustes depressus* populations from Isla del Coco and Pacific Panama belonged to the same panmictic population. Although F_{ST} values between Galapagos and the other eastern Pacific populations were larger, they were not greater than those obtained by chance alone. In the central Pacific, F_{ST} values between Hawaii and every other island were always significant, although <0.33. In the western Pacific, F_{ST} values between the Marshall Islands and all other localities were large and always significant, suggesting that *Tripneustes* on this island are genetically distinct. In the Indian Ocean, Reunión was unusual in containing only one haplotype, the one that was also most common in the entire Indo-Pacific (Fig. 2).

As might be expected from the lack of strong regional divergence in the presence of large local differentiation, there was no correlation between geographic and genetic distance in the Indo-Pacific. Mantel correlations between F_{ST} and geographic distance showed them all to be nonsignificant $r = 0.029$, $P = 0.38$ for raw data; $r = 0.100$, $P = 0.16$ for log-transformed data). When current distance was substituted for geographical distance, the correlation was no better $r = -0.040$, $P = 0.37$ for raw data; $r = 0.091$, $P = 0.21$ for log-transformed data).

Demographic History

If, despite the occasionally large and significant F_{ST} values between populations, the entire Indo-Pacific is considered as a single metapopulation (see discussion for reasons of treating it in this fashion) and all 139 sequences from this ocean are analyzed together, they produce negative and significant values in both Tajima's (1989; $D = -2.199$, $P < 0.001$) and Fu's (1997; $F_S = -3.4 \times 10^{38}$, $P = 0.001$) tests. Such negative values can result from variation that is caused either by selection, or by recent population expansion. McDonald and Kreitman's (1991) test indicated that COI variation in *Tripneustes* cannot be distinguished from neutral expectations. When Atlantic and Pacific clades were compared, the ratios of replacement to silent substitutions within and between clades were not significantly different ($P = 0.559$, Fisher's exact test). In the absence of selection, the negative values of the Tajima and Fu tests suggest that *Tripneustes* populations in the Pacific may have recently expanded. Despite the geographic structuring indicated by the F_{ST} analysis, the differences between Indo-Pacific haplotypes fit almost perfectly the expectations of Roger and Harpending's (1992) model for the mismatch distribution expected from an expanding population (Fig. 3).

The Caribbean was the only area in the Atlantic for which sample size was large enough to be analyzed in the same manner as the Indo-Pacific. The Caribbean populations of *T. ventricosus* also conformed to a model of sudden expansion. Tajima's D was also negative and significant ($D = -1.73$, $P = 0.024$) and so was Fu's F_s ($F_s = -3.89$, $P = 0.001$). The mismatch distribution (Fig. 3) is also indicative of a sudden increase in N_e, but one much more recent and to a smaller population size than that of the Pacific.

DISCUSSION

Phylogeography

According to the mtDNA phylogeny presented here, barriers important to phylogenetic separation in *Tripneustes* are: (1) the cold water upwelling close to the tip of South Africa, (2) the Isthmus of Panama, (3) the 3500-km long stretch of deep water separating the eastern from the western Atlantic, and (4) the freshwater plume of the Orinoco and the Amazon

TABLE 1. Pairwise F_{ST} values between populations in the same mitochondrial DNA clade, in which $n > 4$. Asterisks mark F_{ST} values larger than 95% of those produced by 3024 random reshufflings of haplotypes between localities.

Western Atlantic

	Brazil	Panama
Panama	0.82*	
Belize	0.73*	−0.01

Indo-Pacific

	Eastern Pacific				Central Pacific				Western Pacific				Indian Ocean		
	Panama	Isla del Coco	Galpagos	Clipperton	Easter Island	Marquesas	Hawaii	Kiritimati	Marshall Islands	Papua New Guinea	Japan	Philippines	Reunión	Seychelles	Madagascar
Eastern Pacific															
Isla del Coco	−0.12														
Galpagos	0.17	0.25													
Clipperton	0.54*	0.60*	0.11												
Central Pacific															
Easter Island	0.48*	0.49*	0.19*	0.40*											
Marquesas	0.38*	0.41*	0.18*	0.40*	−0.02										
Hawaii	0.55*	0.56*	0.23*	0.38*	0.11*	0.16*									
Kiritimati	0.40*	0.44*	0.15*	0.33*	0.08	0.06	0.15*								
Western Pacific															
Marshall Islands	0.60*	0.64*	0.22*	0.33*	0.29*	0.27*	0.19*	0.06							
Papua New Guinea	0.44*	0.47*	0.22*	0.46*	0.13*	0.04	0.34*	0.13*	0.37*						
Japan	0.41*	0.46*	0.23*	0.44*	0.18*	0.11*	0.33*	0.10	0.30*	0.11*					
Philippines	0.24*	0.31*	0.21*	0.42*	0.12*	0.06	0.28*	0.15*	0.29*	0.00	0.07				
Indian Ocean															
Reunión	1.00*	0.74*	0.20	0.48*	0.14	−0.01	0.40*	0.11	0.46*	−0.06	0.10	−0.02			
Seychelles	0.38*	0.43*	0.18*	0.32*	0.04	0.07*	0.03	0.08	0.19*	0.20*	0.22*	0.19*	0.19*		
Madagascar	0.42*	0.47*	0.19*	0.35*	0.02	0.05	0.02	0.10	0.24*	0.18*	0.22*	0.16*	0.20*	−0.06	
South Africa	0.43*	0.51*	0.17	0.31*	0.02	0.06	0.03	0.10	0.23*	0.20*	0.23*	0.16*	0.25*	−0.08	−0.11

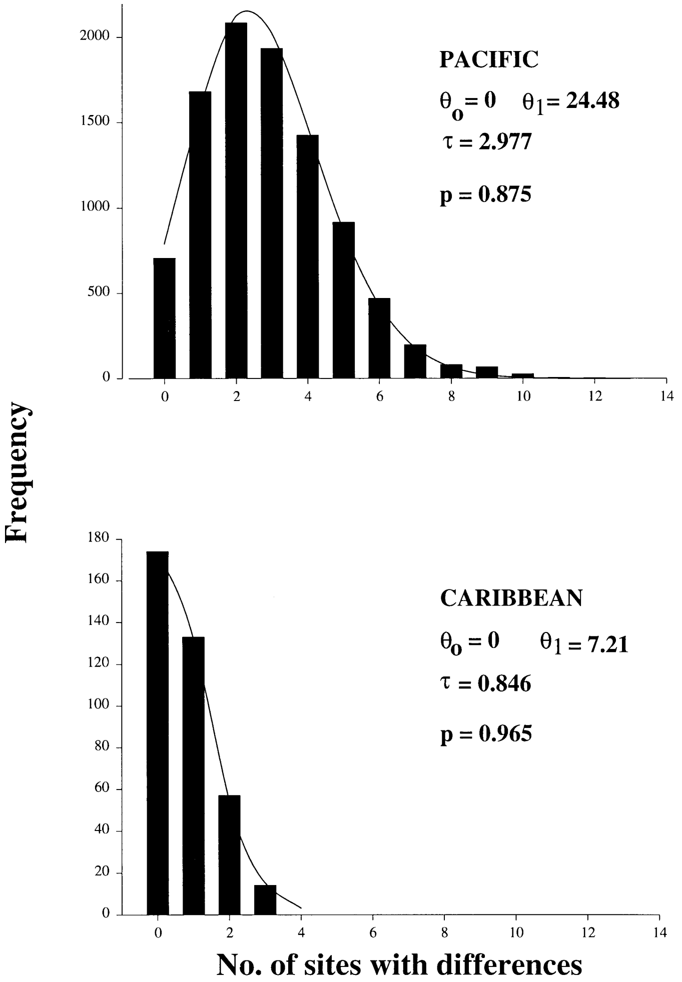

FIG. 3. Mismatch distributions (Rogers and Harpending 1992; Harpending 1994; Rogers 1995) of haplotypes of *Tripneustes* in the Indo-Pacific Ocean and in the Caribbean Sea. The line depicts the mismatch distribution expected from a sudden expansion model with parameters shown in each figure. $\theta = 2N_e\mu$, where μ is the rate of mutation. $\tau = 2\mu t$, where t is the number of generations between θ_0 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 10,000 iterations (Schneider and Excoffier 1999).

between the Caribbean and the coast of Brazil. Mayr (1954, p. 5) was led by morphological information presented in Mortensen (1928–1951) to speculate that there were recent connections between Indian Ocean *T. gratilla* and Atlantic *T. ventricosus* around the tip of South Africa and stated that ''the major break in this group would be between Hawaii and Galapagos.'' This did not turn out to be the case. The Eastern Pacific Barrier, 5000 km of deep water between the central and the eastern Pacific, is remarkably unimportant as a cause of genetic subdivision in *Tripneustes*.

The phylogeny of the male gamete recognition molecule, bindin, is compatible with the COI phylogeny (Zigler and Lessios 2003). The two trees agree in showing a separation between Atlantic and Pacific *Tripneustes* as the first split within the extant taxa of the genus and no geographic structure in alleles from the entire Indo-Pacific. The difference between the bootstrapped trees of the two molecules is that bindin (as expected from the slower evolution of a nuclear molecule [Moore 1995; Palumbi et al. 2001] that is not under positive selection [Zigler and Lessios 2003]) shows lower phylogenetic resolution. In the Atlantic, bindin sequences from Sâo Tomé form a monophyletic clade as they do in COI, but this clade is nested within the other Atlantic alleles, rather than being reciprocally monophyletic. Bindin alleles from the Caribbean and Brazil do not cluster separately.

That the Atlantic and the Pacific COI and bindin clades of *Tripneustes* are reciprocally monophyletic indicates the presence of blocks to gene flow both at the tip of South Africa and at the Central American isthmus. The cold water barrier that keeps biota of the Atlantic and the Indian Oceans separate is the Benguela upwelling off the southwest coast of Africa. The upwelling first appeared in the Miocene (Diester-Haass and Schrader 1979; Siesser 1980), intensified in the late Pliocene (Meyers et al. 1983), and assumed its present—weaker and fluctuating—levels at the Plio-Pleistocene boarder (Shannon 1985; Marlow et al. 2000). It has permitted relatively recent larval transport in the sea urchin *Diadema* (Lessios et al. 2001a) and in trumpetfishes (Bowen et al. 2001), but for other sea urchins, such as *Eudicaris* (Lessios et al. 1999) and *Echinometra* (McCartney et al. 2000), as well as for bonefishes (Colborn et al. 2001), it has been an effective barrier at least since the Pliocene. The Isthmus of Panama was completed in the Pliocene 3.1 million years ago (mya; Duque-Caro 1990) and split the ranges of a large number of previously continuous marine populations (Lessios 1998). It may, however, have been breached at times of high sea-level stands in the Pleistocene between 2.3 and 2.0 mya (Cronin and Dowsett 1996). The COI Kimura two-parameter distance between Atlantic and Pacific clades of *Tripneustes* (K_2 = 8.7%) is at the low end of the range of divergence values in six pairs of sea urchins presumed to have been split by the original Pliocene completion of the Isthmus (Lessios et al. 2001a). However, the discrepancy from the mean transisthmian divergence (K_2 = 10.97%) is small enough to suggest that it might be due to rate variation between taxa or error arising from the random survival of existing mitochondrial lineages (Edwards and Beerli 2000). Transisthmian COI divergence in *Tripneustes* is certainly not as low as in *Diadema* (K_2 = 4.57%) or *Meoma* (K_2 = 4.55%), the members of which may have established genetic connections during the Pleistocene breach of the isthmus. Thus, if it is assumed that *Tripneustes*, like other tropical marine biota, was split by the Isthmus of Panama, COI in this genus has diverged at a rate of 2.8% per million years (my), equivalent to a mean path length (Britton et al. 2002) of 8.98 substitutions per my.

By the calibration obtained from the Isthmus of Panama, the split in the Atlantic between sampled populations at the American and the African coasts occurred (by mean path length calculations) in the Pleistocene approximately 800,000 years ago, with a confidence interval of 400,000 years. A Pleistocene subdivision of Atlantic populations also characterizes *Diadema* (Lessios et al. 2001a), and the parrot fish *Sparisoma* (Bernardi et al. 2000), whereas eastern and western Atlantic populations of *Eucidaris* (Lessios et al. 1999) and *Echinometra* (McCartney et al. 2000) maintain genetic contacts to the present day. Presumably these separations of

Atlantic populations are related to current or temperature changes during Pleistocene glaciations, but it is not clear why haplotypes of some genera did not cross to the other side of the Atlantic after the barriers were removed.

The separation between Brazilian and Caribbean sampled populations of *Tripneustes* is even more recent, dating at approximately 455,000 years ago, with a confidence interval of 266,000 years. The barrier most likely consists of inhospitable habitat created by the freshwater plumes of the Amazon and the Orinoco (Froelich et al. 1978; Muller-Karger et al. 1988). This stretch of muddy habitat and low salinity water is proving to be a surprisingly strong barrier to gene flow in sea urchins, also manifesting itself in *Diadema* (Lessios et al. 2001a) and *Echinometra* (McCartney et al. 2000), though not in *Eucidaris* (Lessios et al. 1999). It is also a barrier to species of surgeonfish that prefer coral reef, although it permits gene flow in those that can occupy soft bottom habitats (Rocha et al. 2002). The Orinoco and the Amazon have been emptying at their present location since the late Miocene (Hoorn et al. 1995), but the small degree of divergence between Caribbean and Brazilian samples of *Tripneustes* suggests that their separation is much more recent. Thus, there must have been migration through the barrier after its present physical characteristics were established. One possibility, suggested by Joyeux et al. (2001) for fishes, is that the effectiveness of the barrier changed through time due to fluctuations of outflow water volume and sediment associated with Pleistocene glaciations.

Contrary to the geographic structuring of clades in the Atlantic, *Tripneustes* in the Indo-Pacific shows an extraordinary degree of haplotype similarity from the west coast of Central America all the way to the east coast of Africa. The most common haplotype is spread from Galapagos, through the central and western Pacific, all the way to Reunión off the African coast, a straight-line distance of more than 25,000 km. This is an unmatched degree of dispersal for a sea urchin, rarely seen even among strongly swimming pelagic vertebrates, such as sea turtles (Bowen et al. 1994; Dutton et al. 1999; but see Bowen et al. 1992), swordfish (Kotoulas et al. 1995; Rosel and Block 1996) or marlin (Graves and McDowell 1994; Buonaccorsi et al. 1999). The lack of any phylogenetic sorting between eastern Pacific and Indo-West Pacific *Tripneustes* in either mtDNA or bindin alleles (Zigler and Lessios 2003), along with the morphological similarity already noted by taxonomic authorities (Clark 1912; Mortensen 1928–1951), indicates that *T. gratilla* and *T. depressus* are a single species, the populations of which exchange alleles across the Eastern Pacific Barrier. This conclusion remains robust whether one subscribes to the biological (Mayr 1963), phylogenetic (Cracraft 1983), or cohesion (Templeton 1989) species concept.

The Eastern Pacific Barrier is considered second only to the continents as a biogeographic barrier for marine organisms (Ekman 1953; Briggs 1961; Grigg and Hey 1992). *Tripneustes*, in contrast to *Eucidaris*, *Echinometra*, and *Diadema*, shows no evidence of being affected by the Eastern Pacific Barrier, other than a difference in the distribution of haplotypes, evident in a significant but fairly small Φ_{CT} value in AMOVA comparisons between populations on either side of the barrier. Although haplotypes of the Indo-West Pacific *Echinometra oblonga* and *Diadema savignyi* are also occasionally found in the eastern Pacific (Lessios et al. 1996, 2001a; McCartney et al. 2000), the only other sea urchin whose larvae appear able to cross this long expanse of deep water en masse is *Echinothrix* (Lessios et al. 1998). *Echinothrix*, however, may have invaded the eastern Pacific very recently, possibly with the 1987 El Niño Southern Oscillation (ENSO) event (Lessios et al. 1996, 1998), and is only known from the outer islands of Isla del Coco and Clipperton. *Tripneustes*, on the other hand, can be found along the American mainland and is abundant in the Galapagos. The acceleration of the North Equatorial Countercurrent that occurs during ENSO events (Firing et al. 1983; Wyrtki 1985) probably facilitates invasion of the eastern Pacific from the central Pacific by making the crossing possible within the competent time period of planktonic larvae (Richmond 1990). In *Tripneustes*, the geographical extent and mixing of haplotypes suggests that transport of larvae is not the result of a few recent ENSO events, but must have been occurring over thousands of generations.

Why larvae of Pacific *Tripneustes* should be so much better at dispersing than those of other Pacific sea urchins is not clear, particularly in light of the genetic subdivision of this genus in the Atlantic. One possible explanation for the lack of divergence between eastern and western Pacific populations is that speciation across the Eastern Pacific Barrier—which occurred before the closure of the Isthmus of Panama in other sea urchin genera (Lessios et al. 1999, 2001a; McCartney et al. 2000)—was never completed in *Tripneustes*. Another possibility is that *Tripneustes* might have been extirpated from the eastern Pacific. When Kiritimati drifted into the path of the Northern Equatorial Countercurrent in the Pleistocene—thus facilitating west-to-east larval transport (Dana 1975)—genetic contact was reestablished, or the eastern Pacific was recolonized. The possibility, suggested by the evidence from Tajima's and Fu's tests and from the mismatch distribution, that *Tripneustes* populations have been recently expanding, would fit this hypothesis. Increase of effective population size due to range expansion would explain both the current geographical distribution of extant haplotypes of *Tripneustes* in the Pacific and their ''star phylogeny.'' How long ago did this event occur? Substituting the uncorrected rate of accumulation of mutations, as estimated from the Isthmus of Panama, (2.55×10^{-5} substitutions per site per millennium) into the equations of the mismatch sudden expansion model (Fig. 3) gives an estimate of approximately 106,000 years ago for the initiation of the Pacific expansion, with a 95% confidence interval ranging from 51,000 to 151,000 years ago. The apparent population expansion of *Tripneustes* in the Caribbean, dated from its mismatch distribution (Fig. 3) in the same manner, would have occurred in the last 30,000 years, with a 95% confidence interval ranging from the present to 75,000 years ago. This is a much more recent event than the presumed population increase of *Diadema antillarum* in the same area, which a similar genetic analysis has dated as having occurred more than 100,000 years ago. (Lessios et al. 2001b). These population expansions (that must have followed population crashes) are not entirely surprising in *Tripneustes*, a genus characterized by high mortality (including occasional mass mortality [Williams et al. 1996]), rapid growth, and high fecundity (Lawrence and Agatsuma 2001).

Tripneustes also forms an exception to the general pattern of genetic differentiation between marine populations from the western Pacific and the Indian Ocean. Such differentiation has been found among sea urchins in *Eucidaris* (Lessios et al. 1999), in *Diadema paucispinum* and *D. setosum* (but not in *D. savignyi*) (Lessios et al. 2001a), and also in starfish (Williams and Benzie 1997, 1998; Benzie 1999), fish (McMillan and Palumbi 1995; Miya and Nishida 1997; Colborn et al. 2001), and crustaceans (Lavery et al. 1996; Duda and Palumbi 1999; Barber et al. 2000). Past restrictions to water flow through the straits separating Australia from southeast Asia are presumably the historical barriers responsible for this differentiation. That *Tripneustes* populations from the African coast of the Indian Ocean are genetically undifferentiated from those of the Pacific and that they contain haplotypes also found in the central and eastern Pacific is also consistent with the hypothesis of population expansion of its populations starting from a recent arrival of propagules.

Thus, the phylogeography of *Tripneustes* in the Indo-Pacific exhibits characteristics expected from a sea urchin genus with ephemeral local populations but with high fecundity, dispersal, and growth. There is little geographic structure, the differences between haplotypes conform to models of recent population expansion on a nearly global scale, and the common haplotypes are spread more widely than in any sea urchin in which mtDNA has been sequenced to date. Yet, many comparisons between local populations produce large and significant F_{ST} values, indicating nonrandom haplotype distribution. This apparent local differentiation is only weakly reflected in regional divergence, and there is no evidence of isolation by distance. All of this is indicative of a metapopulation spanning two oceans and containing chaotic, nonequilibrium local variation. Lack of correlation between F_{ST} values and geographic or current distance suggests that historical factors, such as haphazard arrival of larvae or unpredictable local extinction, rather than contemporary gene flow, are responsible for patterns of haplotype geographic distribution. The serendipitous nature of this dispersal is also illustrated by the population in Reunión, which is composed of a single mitochondrial genotype. Such monomorphism is most likely the result of a recent colonization.

This picture of a very large metapopulation in the Pacific contrasts with regionally structured populations in the Atlantic. In contrast to *Echinometra* (McCartney et al. 2000) or *Eucidaris* (Lessios et al. 1999), eastern and western Atlantic populations of *Tripneustes* have been separated long enough for complete sorting of their mitochondrial haplotypes. Like other sea urchins (Lessios et al. 1999, 2001a; McCartney et al. 2000), *T. ventricosus* is homogeneous within the Caribbean, where the differences between its haplotypes are consistent with a model of very recent expansion. Beyond the Caribbean, differentiation is ordered in a separate clade on the coast of Brazil. Thus, despite the fact that the Atlantic species seems to be as subject to rapid population turnover as the Pacific one, it conforms to the usual biogeographic barriers. That Atlantic populations of *Tripneustes* are isolated on either side of this ocean, whereas the Indo-Pacific ones exchange genes across the Eastern Pacific Barrier and throughout the Pacific and Indian Oceans, could not have been predicted by any known characteristic of the biology of the genus. Because the times to metamorphosis in the two species appear to be roughly the same, the causes for this difference remain to be explained.

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