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POPULATION STRUCTURE AND SPECIATION IN TROPICAL SEAS: GLOBAL PHYLOGEOGRAPHY OF THE SEA URCHIN *DIADEMA*

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Abstract.—The causes of speciation in the sea are rarely obvious, because geographical barriers are not conspicuous and dispersal abilities or marine organisms, particularly those of species with planktonic larvae, are hard to determine. The phylogenetic relations of species in cosmopolitan genera can provide information on the likely mode of their formation. We reconstructed the phylogeny of the pantropical and subtropical sea urchin genus *Diadema,* using sequences of mitochondrial DNA from 482 individuals collected around the world, to determine the efficacy of barriers to gene flow and to ascertain the history of possible dispersal and vicariance events that led to speciation. We also compared 22 isozyme loci between all described species except *D. palmeri.* The mitochondrial DNA data show that the two deepest lineages are found in the Indian and West Pacific Oceans. (Indo-Pacific) *Diadema setosum* diverged first from all other extant *Diadema,* probably during the initiation of wide fluctuations in global sea levels in the Miocene. The *D. setosum* clade then split 3–5 million years ago into two clades, one found around the Arabian Peninsula and the other in the Indo-West Pacific. On the lineage leading to the other species of *Diadema,* the deepest branch is composed of *D. palmeri,* apparently separated when the climate of New Zealand became colder and other tropical echinoids at these islands went extinct. The next lineage to separate is composed of a currently unrecognized species of *Diadema* that is found at Japan and the Marshall Islands. *Diadema mexicanum* in the eastern Pacific separated next, whereas *D. paucispinum, D. savignyi,* and *D. antillarum* from the western and central Atlantic, and (as a separate clade) *D. antillarum* from the eastern Atlantic form a shallow polytomy. Apparently, Indo-Pacific populations of *Diadema* maintained genetic contact with Atlantic ones around the southern tip of Africa for some time after the Isthmus of Panama was complete. *Diadema paucispinum* contains two lineages: *D. paucispinum* sensu stricto is not limited to Hawaii as previously thought, but extends to Easter Island, Pitcairn, and Okinawa; A second mitochondrial clade of *D. paucispinum* extends from East Africa and Arabia to the Philippines and New Guinea. A more recent separation between West Indian Ocean and West Pacific populations was detected in *D. setosum.* Presumably, these genetic discontinuities are the result of water flow restrictions in the straits between northern Australia and Southeast Asia during Pleistocene episodes of low sea level. *Diadema savignyi* is characterized by high rates of gene flow from Kiribati in the central Pacific all the way to the East African Coast. In the Atlantic, there is a biogeographic barrier between the Caribbean and Brazil, possibly caused by fresh water outflow from the Amazon and the Orinoco Rivers. *Diadema antillarum* populations of the central Atlantic islands of Ascension and St. Helena are genetically isolated and phylogenetically derived from Brazil. Except for its genetic separation by the mid-Atlantic barrier, *Diadema* seems to have maintained connections through potential barriers to dispersal (including the Isthmus of Panama) more recently than did *Eucidaris* or *Echinometra,* two other genera of sea urchins in which phylogeography has been studied. Nevertheless, the mtDNA phylogeography of *Diadema* includes all stages expected from models of allopatric differentiation. There are anciently separated clades that now overlap in their geographic distribution, clades isolated in the periphery of the genus range that have remained in the periphery, clades that may have been isolated in the periphery but have since spread towards the center, closely related clades on either side of an existing barrier, and closely related monophyletic entities on either side of an historical barrier that have crossed the former barrier line, but have not attained genetic equilibrium. Except for *D. paucispinum* and *D. savignyi,* in which known hybridization may have lodged mtDNA from one species into the genome of the other, closely related clades are always allopatric, and only distantly related ones overlap geographically. Thus, the phylogenetic history and distribution of extant species of *Diadema* is by and large consistent with allopatric speciation.

Key words.—ATPase, biogeography, cytochrome oxidase I, gene flow, mitochondrial DNA, ocean barriers, phylogeny, sea urchins, speciation.

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It is often pointed out that marine invertebrates with long-lived planktonic larvae should rarely speciate because of their large effective population sizes and enormous capacities for dispersal (reviews in Palumbi 1992, 1994). The world's oceans are, after all, connected to each other, and planktonic larvae have the capacity to delay metamorphosis for long periods of time. That there are few recognized cosmopolitan species among echinoderms, molluscs, crustaceans, or annelids must therefore mean either that the sea does indeed contain present and past barriers to gene flow, or that sympatric speciation is common in marine invertebrates. The first alternative is supported by congruent distributions of many unrelated species, which permit the delineation of marine biogeographic provinces (Ekman 1953; Briggs 1974). Sympatric speciation can happen anywhere, and it is not expected to generate common geographic patterns. The second alternative has received recent attention because of the discovery that a few loci can code for molecules involved in marine invertebrate sperm-egg interactions (reviews in Foltz 1995; Vacquier et al. 1995; Palumbi 1998; Vacquier 1998). The relative ease with which mutations in such molecules can

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confer reproductive isolation, combined with the intangibility of marine barriers, has led to suggestions that new species may arise in the sea without geographic isolation (e.g., Wu and Johnson 1996; Hellberg 1998; Knowlton and Weigt 1998; Darling et al. 2000).

Ernst Mayr was an early and forceful proponent of the view that marine organisms in general and sea urchins in particular speciate allopatrically. His 1954 paper was an eloquent treatise in support of this view. By plotting the ranges of tropical echinoid species, Mayr (1954) was able to show that species distributions in most shallow water, tropical sea urchin genera conformed to a model of allopatric speciation, because they were arranged on either side of a land or open sea barrier. Mayr, however, needed an ad hoc hypothesis to fit the species distributions in one genus, *Diadema,* into a pattern of allopatric speciation. *Diadema* contains two morphologically similar species in the Indo-Pacific with ranges thought to be identical. Mayr speculated that this was the result of a ''double invasion,'' and called for a study to determine which of the two species was the original occupant of the Indo-West Pacific. He also predicted that a phylogenetic study would reveal whether the invader was the descendant of an eastern Pacific species having spread westward, or of an Atlantic species having dispersed eastward.

Questions such as this, with obvious relevance to speciation, can only be answered by reconstructing the phylogeny of the genus. If the phylogeny is built on the basis of molecules, it can also provide information on whether each morphospecies is a distinct genetic entity and on the time that species diverged from each other. We conducted a phylogeographic study of the genus *Diadema,* using partial sequences of mitochondrial DNA (mtDNA) and isozymes.

Diadema is one of the most abundant, widespread, and ecologically important shallow water genera of tropical sea urchins (reviews in Lawrence and Sammarco 1982; Lessios 1988; Birkeland 1989; Carpenter 1997). It is found in all tropical oceans, where it inhabits depths down to 70 m. (Mortensen 1940; see Pawson and Miller [1983] for a correction of the depth range reported by Mortensen for *D. antillarum*). The geographical distributions of its species, as recognized by morphology, are as follows: *D. mexicanum* occupies the tropical eastern Pacific from the Sea of Cortez to Ecuador, including the islands of Revillagigedos, Clipperton, Isla del Coco, and Galapagos. *Diadema antillarum* is found on both coasts of the tropical Atlantic, from Florida and Bermuda to Brazil and from Madeira (but, contrary to Mortensen [1940], not in the Azores; Wirtz and Martins 1993) to the Gulf of Guinea. *Diadema palmeri* is only known from the north coast of New Zealand (Baker 1967) and from the southeast coast of Australia (Rowe and Gates 1995). *Diadema paucispinum* is thought to be primarily a Hawaiian species. *Diadema setosum* and *D. savignyi* were considered until recently as having coincident ranges, extending from mid-Pacific to the East African coast. The morphological differences between species are so slight that specimens can usually not be identified without knowing where they were collected (H. L. Clark 1925, p. 42), and considerable confusion exists as to the true distributions of *D. savignyi* and *D. setosum* (Pearse 1998). These two species also form natural hybrids, but three isozyme loci fixed for alternate alleles indicate that introgression is low (Lessios and Pearse 1996). A single mtDNA haplotype of *D. savignyi* was found in the Clipperton Atoll in the eastern Pacific (Lessios et al. 1996). An additional species, *D. ascensionis,* was recognized by Mortensen (1909, 1940:279–280) from the central Atlantic islands of Ascension and St. Helena, and also from Fernando de Noronha off Brazil. Pawson (1978), however, demoted *D. ascensionis* to a subspecies of *D. antillarum* and doubted that the Fernando de Noronha populations were different from those on the coast of Brazil. Larval life span in the laboratory ranges from 34 to 90 days in *D. antillarum* (Carpenter 1997; Eckert 1998) and exceeds 42 days in *D. setosum* (Onoda 1936; Mortensen 1937). The fossil record of *Diadema* is extremely poor, consisting of only spines that may or may not belong to the genus, some of which go back to the Miocene, 5.3–23.7 million years ago (mya) (Smith and Wright 1990).

We sequenced mtDNA from all species of *Diadema* from around the world to answer the following questions: (1) To what extent do morphospecies coincide with mtDNA clades? (2) What is the true distribution of each species? (3) In what order did species diverge from each other, and what can this order reveal about the causes of speciation events? (4) Are there genetic subdivisions within species? (5) To what degree is the splitting of the clades consistent with that of other tropical echinoids, and what does this imply about speciation and the role of vicariant events in the sea?

Materials and Methods

Collections

Mitochondrial DNA was sampled in a total of 482 individuals of *Diadema.* The help of many people who collected samples around the world (see Acknowledgments) permitted very good geographic coverage. Samples were preserved in 95% ethanol, in high-salt DMSO buffer (Seutin et al. 1991), or in liquid nitrogen (N_2). Analyses of isozymes are based on previously published data (see below). They come from 21individuals of *D. paucispinum* from Honolulu, Hawaii; 23 individuals of *D. savignyi* and 28 of *D. setosum* from Okinawa, Ryukyu Islands; 10 individuals of *D. setosum* from Fantome Island, Australia; 16–44 (depending on the assayed locus) individuals of *D. mexicanum* from Guaymas, Mexico and 18–49 from the Bay of Panama; and 20–48 individuals of *D. antillarum* from the Atlantic Coast of Panama and 15–40 from Puerto Rico. All samples for protein electrophoresis were frozen in liquid N_2.

Mitochondrial DNA

Genomic DNA extractions, polymerase chain reaction (PCR) amplification, PCR product purification, and DNA sequencing were carried out as described by Lessios et al. (1998). In all 482 individuals, we amplified 642 nucleotides from the Lysine-tRNA, ATPase-6 and 8 region, starting at either position 8344 within the Cytochrome Oxidase II coding region, or position 8424 in the Lysine-tRNA region, and extending to position 9010 of the ATPase-6 region of sea urchin mtDNA as defined by Jacobs et al. (1988) for *Strongylocentrotus purpuratus.* Primers were: either CO2b 5′ GAATCTGTTCCTTTCTCTAC or LYSa 5′ AAGCTTTAAACTCTTA-

ATTTAAAAG and ATP6b 5′ GCCAGGTAGAACCCGAGAAT. After cycle-sequencing reactions using the same primers as in the amplification, 497 to 614 nucleotides of this fragment were sequenced on either a model 373A or a model 377 automatic sequencer from Perkin-Elmer/Applied Biosystems (Foster City, CA). Sequences were determined in both directions, at least once for each strand. We will refer to these 482 sequences as the ''0.6 Kb'' dataset. We also amplified 660 additional nucleotides from the Cytochrome Oxidase I (COI) region from two individuals from each major clade as defined from the 0.6 Kb data. Primers CO1f 5′ CCTGCAGGAGGAGGAGAYCC, corresponding to position 6448 of *S. purpuratus*, and CO1a AGTATAAGCGTCTGGGTAGTC corresponding to position 7128 were used. From 597 to 639 nucleotides from 20 individuals were sequenced in both directions. We will refer to the combined sequences of both the ATPase and the COI regions from these 20 individuals as the ''1.2 Kb'' dataset. Sequences have been deposited in GenBank under accession nos. AY012728–AY013241.

Phylogenies from mtDNA data were constructed with version 4.0b4a of PAUP* (Swofford 2000). As outgroups for rooting the phylogenetic trees, we used homologous sequences of the diadematids *Echinothrix diadema* from Isla del Coco, *E. calamaris* from Guam and from Okinawa, *Astropyga radiata* from Papua New Guinea (2 individuals), and *A. pulvinata* from the Bay of Panama. To determine the simplest model of DNA evolution that best fit our data, we used the procedure outlined by Swofford et al. (1996) and by Posada and Crandall (1998). We first determined a neighbor-joining tree (Saitou and Nei 1987) based on Log-determinant (LogDet) distances (Lockhart et al. 1994). Using this tree, we started with the simplest model of DNA evolution, and added parameters for the estimation of base frequencies and substitution rates. At each step, log likelihood scores were calculated and compared with those of the previous model. If the likelihood ratio indicated that the addition of the parameter significantly increased the fit of the data to the tree, the parameter was retained. Using Posada and Crandall's (1998) MODELTEST 2.1 program, the process was repeated until the addition of a new parameter did not produce a significant log-likelihood difference. MODELTEST indicated that the best DNA substitution model for both the 0.6 Kb tree and the 1.2 Kb tree was that of Tamura and Nei (1993) with a gamma correction. We added an additional step to our search, and found that maximum-likelihood (ML) estimation of base frequencies and substitution rates, applied to four site categories (one for each codon position, plus a separate one for the region coding for Lysine -tRNA) gave the best fit of the data. Using this model to estimate DNA distances, we constructed a neighbor-joining tree from the 0.6 Kb data after adding the outgroup taxa. For the 1.2 Kb data, we were able to conduct a full search for the best ML tree, because only 20 sequences were involved. Each set of data was bootstrapped in 1000 iterations.

To determine intraspecific genetic subdivisions among populations from different geographic regions we calculated F_{ST} statistics (Wright 1951, 1965), using version 2.0 of ARLEQUIN (Schneider et al. 2000). The program uses an analysis of molecular variance approach to arrive at an estimate of F_{ST} identical to Weir and Cockerham's (1984) $\hat{\theta}_w$. We used Tamura and Nei's (1993) distances to quantify the DNA differences upon which the F_{ST} statistics were based. The probability of obtaining these F_{ST} values by chance was estimated from 1023 random reshufflings of haplotypes between populations.

Isozymes

To examine the degree to which phylogenetic relationships suggested by mtDNA agreed with those obtained from nuclear markers, we used the allozyme frequencies obtained by Bermingham and Lessios (1993) for *Diadema antillarum* and *D. mexicanum* and by Lessios and Pearse (1996) for *D. setosum, D. savignyi,* and *D. paucispinum.* Only 22 loci listed in Lessios and Pearse (1996) (out of 34 assayed by Bermingham and Lessios [1993]) were included. Gels of the Indo-Pacific species were standardized by running individuals of *D. antillarum* in each. Individuals suspected of being hybrids (from their morphology or isozyme patterns) were excluded in the calculation of gene frequencies of each species. Calculation and comparison of jackknifed average Nei's (1978) genetic distances were performed according to Muellner and Ayala (1982), employing the program NEIC (Lessios 1990). Nei's distances were then used to construct a neighbor-joining tree. An alternate phylogenetic analysis was based on gene frequencies and followed Swofford and Berlocher (1987), using Swofford's FREQPARS program. In the latter analysis, the tree was jackknifed by successive removal and replacement of each locus.

RESULTS

Phylogenetic Lineages and Their Geographic Distribution

The 482 individuals in which the 0.6 Kb region was sequenced contained 209 unique mtDNA haplotypes. All 20 individuals in which the longer, 1.2 Kb section was sequenced had unique haplotypes. Figures 1A and 1B present reconstructions of the phylogeny of *Diadema* haplotypes as inferred from 0.6 Kb data, and Figure 2 the reconstruction from the 1.2 Kb data. The topologies of the two trees are consistent. Both reconstructions indicate that there is a deep split between the *D. setosum* clade and all other *Diadema.* The *D. setosum* clade is divided into two quite divergent groups. Whereas *D. setosum*-a is found in most of the Indo-West Pacific, subclade *D. setosum*-b is restricted to the areas around the Arabian Peninsula (Fig. 3). Thus, the two subclades are not only reciprocally monophyletic but also spatially nonoverlapping. The sequence dissimilarity between *D. setosum*-a and *D. setosum*-b is 8.18% when calculated from the 1.2 Kb data and 7.83% when calculated from the 0.6 Kb data (Table 1). There are 24 sites diagnostic between the two subclades in the 0.6 Kb sequence and another 74 such sites in the COI sequence.

In the sister clade of *D. setosum,* containing all other extant species of *Diadema,* the first group to diverge is *D. palmeri,* the only nontropical species of *Diadema.* Subsequent to this there is a mitochondrial DNA lineage (*Diadema*-sp) composed of 10 individuals from Japan and eight from the Marshall Islands. The morphological characters of most of these

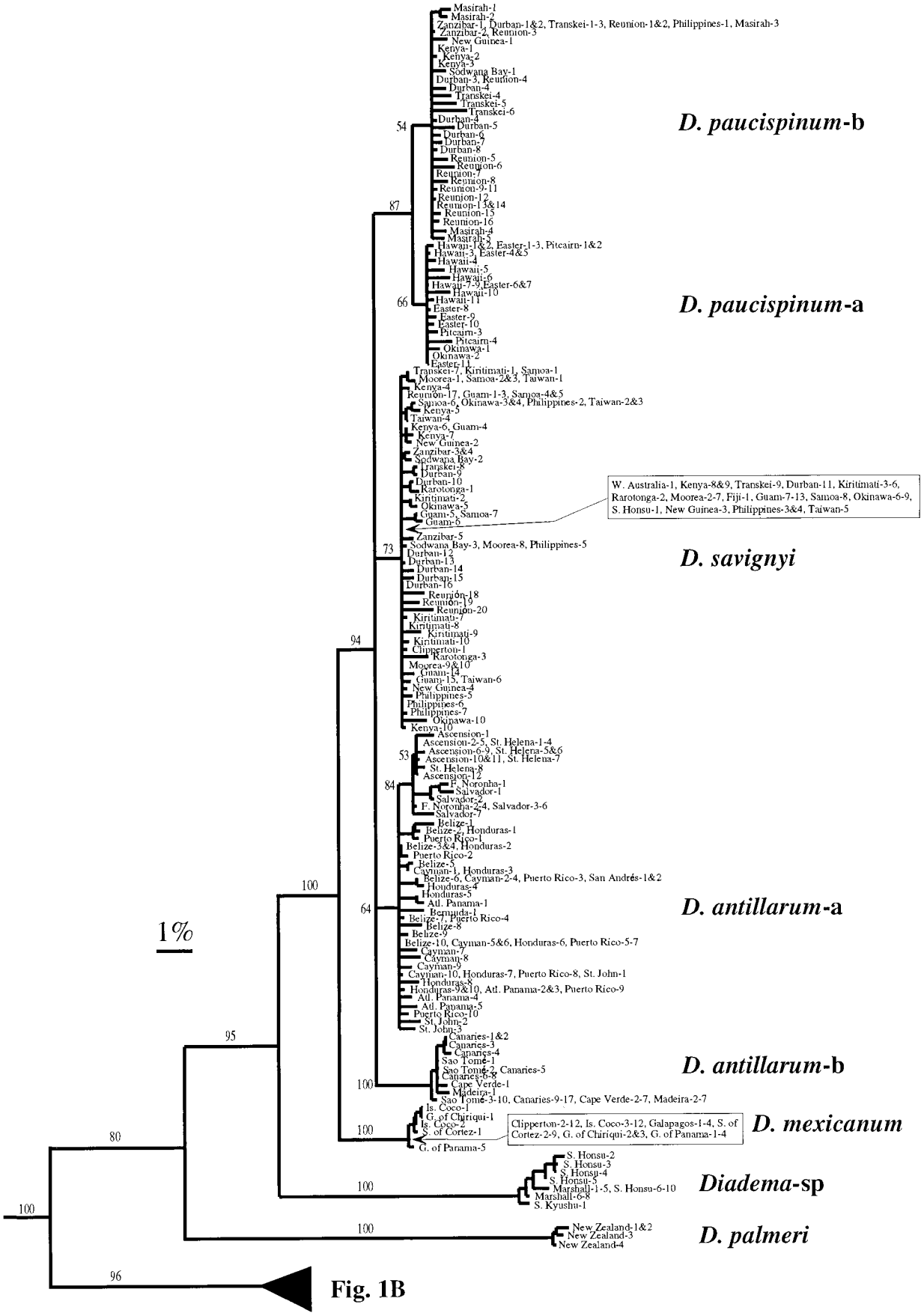
Masirah-1
Masirah-2
Zanzibar-1, Durban-1&2, Transkei-1-3, Reunion-1&2, Philippines-1, Masirah-3
Zanzibar-2, Reunion-3
New Guinea-1
Kenya-1
Kenya-2
Kenya-3
Sodwana Bay-1
Durban-3, Reunion-4
Durban-4
Transkei-4
Transkei-5
Transkei-6
Durban-4
Durban-5
Durban-6
Durban-7
Durban-8
Reunion-5
Reunion-6
Reunion-7
Reunion-8
Reunion-9-11
Reunion-12
Reunion-13&14
Reunion-15
Reunion-16
Masirah-4
Masirah-5
Hawaii-1&2, Easter-1-3, Pitcairn-1&2
Hawaii-3, Easter-4&5
Hawaii-4
Hawaii-5
Hawaii-6
Hawaii-7-9, Easter-6&7
Hawaii-10
Hawaii-11
Easter-8
Easter-9
Easter-10
Pitcairn-3
Pitcairn-4
Okinawa-1
Okinawa-2
Easter-11
Transkei-7, Kiritimati-1, Samoa-1
Moorea-1, Samoa-2&3, Taiwan-1
Kenya-4
Reunion-17, Guam-1-3, Samoa-4&5
Samoa-6, Okinawa-3&4, Philippines-2, Taiwan-2&3
Kenya-5
Taiwan-4
Kenya-6, Guam-4
Kenya-7
New Guinea-2
Zanzibar-3&4
Sodwana Bay-2
Transkei-8
Durban-9
Durban-10
Rarotonga-1
Kiritimati-2
Okinawa-5
Guam-5, Samoa-7
Guam-6
W. Australia-1, Kenya-8&9, Transkei-9, Durban-11, Kiritimati-3-6, Rarotonga-2, Moorea-2-7, Fiji-1, Guam-7-13, Samoa-8, Okinawa-6-9, S. Honsu-1, New Guinea-3, Philippines-3&4, Taiwan-5
Zanzibar-5
Sodwana Bay-3, Moorea-8, Philippines-5
Durban-12
Durban-13
Durban-14
Durban-15
Durban-16
Reunion-18
Reunion-19
Reunion-20
Kiritimati-7
Kiritimati-8
Kiritimati-9
Kiritimati-10
Clipperton-1
Rarotonga-3
Moorea-9&10
Guam-14
Guam-15, Taiwan-6
New Guinea-4
Philippines-5
Philippines-6
Philippines-7
Okinawa-10
Kenya-10
Ascension-1
Ascension-2-5, St. Helena-1-4
Ascension-6-9, St. Helena-5&6
Ascension-10&11, St. Helena-7
St. Helena-8
Ascension-12
F. Noronha-1
Salvador-1
Salvador-2
F. Noronha-2-4, Salvador-3-6
Salvador-7
Belize-1
Belize-2, Honduras-1
Puerto Rico-1
Belize-3&4, Honduras-2
Puerto Rico-2
Belize-5
Cayman-1, Honduras-3
Belize-6, Cayman-2-4, Puerto Rico-3, San Andrés-1&2
Honduras-4
Honduras-5
Atl. Panama-1
Bermuda-1
Belize-7, Puerto Rico-4
Belize-8
Belize-9
Belize-10, Cayman-5&6, Honduras-6, Puerto Rico-5-7
Cayman-7
Cayman-8
Cayman-9
Cayman-10, Honduras-7, Puerto Rico-8, St. John-1
Honduras-8
Honduras-9&10, Atl. Panama-2&3, Puerto Rico-9
Atl. Panama-4
Atl. Panama-5
Puerto Rico-10
St. John-2
St. John-3
Canaries-1&2
Canaries-3
Canaries-4
Sao Tome-1
Sao Tome-2, Canaries-5
Canaries-6-8
Cape Verde-1
Madeira-1
Sao Tome-3-10, Canaries-9-17, Cape Verde-2-7, Madeira-2-7
Is. Coco-1
G. of Chiriqui-1
Is. Coco-2
S. of Cortez-1
G. of Panama-5
Clipperton-2-12, Is. Coco-3-12, Galapagos-1-4, S. of Cortez-2-9, G. of Chiriqui-2&3, G. of Panama-1-4
S. Honsu-2
S. Honsu-3
S. Honsu-4
S. Honsu-5
Marshall-1-5, S. Honsu-6-10
Marshall-6-8
S. Kyushu-1
New Zealand-1&2
New Zealand-3
New Zealand-4
D. paucispinum-b
D. paucispinum-a
D. savignyi
D. antillarum-a
D. antillarum-b
D. mexicanum
Diadema-sp
D. palmeri
54
87
66
73
94
53
84
100
64
95
100
100
80
100
100
96
1%

Fig. 1B

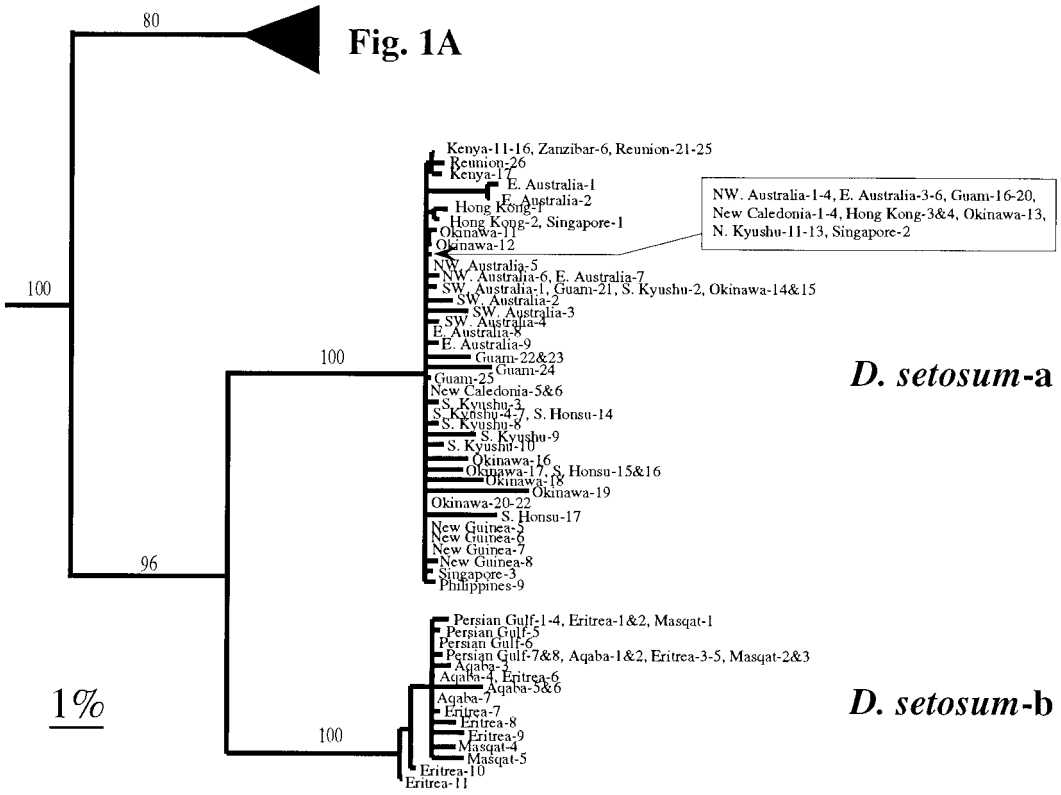

FIG. 1B. Second part of the neighbor-joining tree shown in Figure 1A.

individuals were consistent with those of *D. savignyi* (one was originally identified as *D. setosum*), but their mtDNA was quite divergent from either recognized species, with estimated distances exceeding 11.16% (Table 1).

The sister clade to *Diadema*-sp is composed of four species. The eastern Pacific *Diadema, D. mexicanum,* is an outgroup to the rest of the species in this clade, with a short but well-supported branch. In the tree based on the 0.6 Kb data, the other three species, *D. antillarum, D. paucispinum,* and *D. savignyi,* form a polytomy. *Diadema antillarum* is split into two clades, one from the western and central Atlantic (*D. antillarum*-a) and the other from the eastern Atlantic (*D. antillarum*-b). The average difference between these two clades is 2.99% in the 0.6 Kb data and 2.70% in the 1.2 Kb data, only slightly less than that between the recognized species *D. paucispinum* and *D. savignyi,* or between *D. savignyi* and *D. antillarum* (Table 1). In the ATPase region, there are nine diagnostically different sites between the two *D. antillarum* clades, and in the COI region there are another 24. In the only difference seen between the 0.6 Kb and the 1.2 Kb trees, the latter shows the western Atlantic *D. antillarum*-a as an outgroup to the other three taxa, and the eastern Atlantic *D. antillarum*-b as sister to the Indo-West Pacific *D. savignyi.* The relevant nodes, however, have bootstrap support of only 60%. The 0.6 Kb data also show that *D. antillarum* from the central Atlantic islands of Ascension and St. Helena is a separate clade, nested within the Brazilian clade, which itself is nested within the western Atlantic clade. There are three diagnostic sites that distinguish between Caribbean populations of *D. antillarum*-a, on the one hand, and Brazilian or central Atlantic ones, on the other; an additional site is unique to *D. antillarum ascensionis.*

Diadema paucispinum was until recently considered as endemic to Hawaii (Mortensen 1940, p. 279), or at the most to Hawaii and Kiribati (Clark 1954). Lessios and Pearse (1996), however, found isozyme alleles characteristic of this species in Okinawa. The 0.6 Kb data show that *D. paucispinum* mtDNA lineages extend not just south to Easter Island, but west all the way to the African Coast and north to the Arabian Peninsula (Fig. 3). The *D. paucispinum* clade is divided in two reciprocally monophyletic clades. One is mostly (but not exclusively) found in the central Pacific, the other mostly

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FIG. 1A. Part of the neighbor-joining phylogenetic tree (Saitou and Nei 1987) based on maximum-likelihood distances calculated from the 0.6 Kb data, that is, 497 to 614 nucleotides from the Lysine t-RNA-ATPase-6 and ATPase-8 region of *Diadema* mtDNA. The rest of the tree is shown in Figure 1B. Haplotypes of each species are coded by the locality of their collection and a number unique to the individual sea urchin. Series of identical haplotypes from the same locality are indicated by a range of numbers. Numbers next to branches indicate support from bootstrapping the tree in 1000 iterations. Branches with less than 50% support have been collapsed. Bootstrap values for nodes close to terminal clades have been omitted to maintain figure clarity. The tree was rooted on homologous sequences of *Echinothrix diadema, E. calamaris, Astropyga radiata,* and *A. pulvinata* as outgroups.

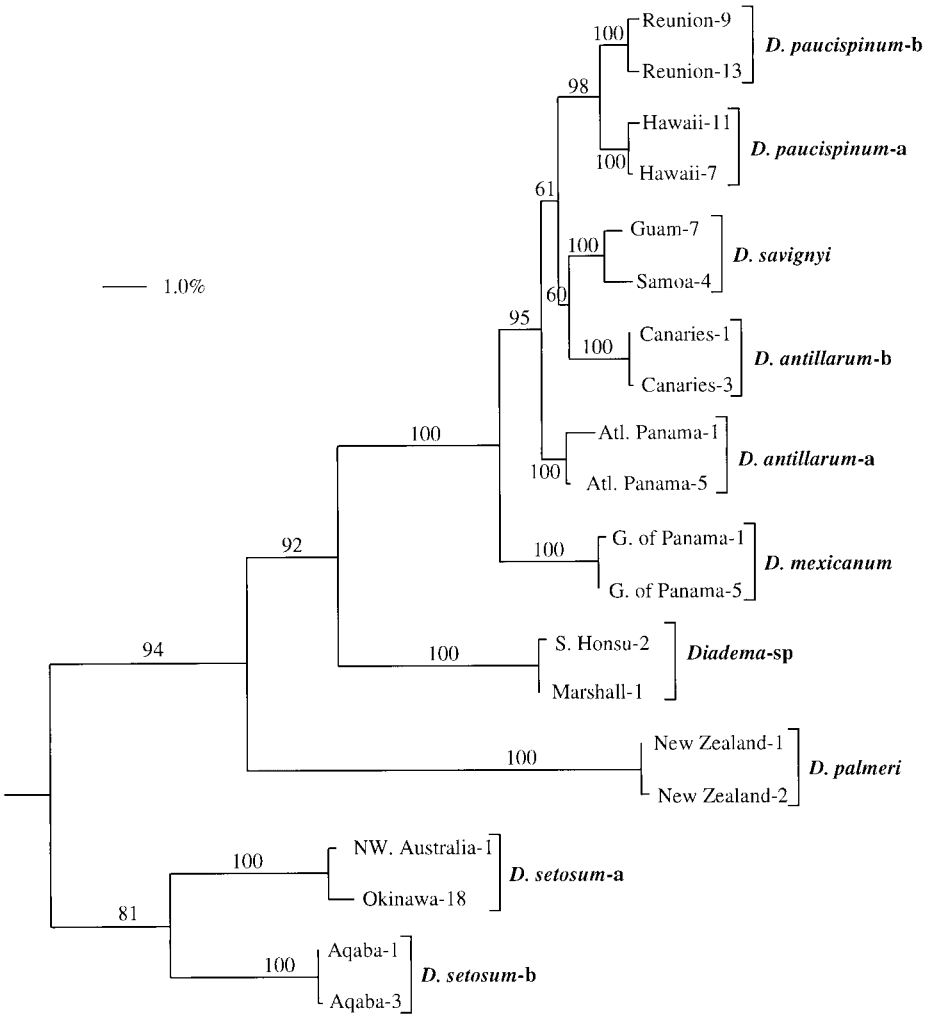

FIG. 2. Phylogenetic tree based on a maximum-likelihood analysis of the 1.2 Kb data, that is, sequences of 497 to 614 nucleotides from the Lysine t-RNA-ATPase-6 and ATPase-8 region, combined with 597 to 639 nucleotides of the COI region. Coding of haplotypes is the same as in Figure 1, with the same number indicating the same sea urchin. Numbers next to branches indicate support from bootstrapping the data in 1000 iterations. The tree was rooted on homologous sequences of *Echinothrix diadema, E. calamaris, Astropyga radiata,* and *A. pulvinata* as outgroups.

found in the Indian Ocean (Fig. 3). The estimated genetic distance between the two *D. paucispinum* clades is only 1.38% in the 0.6 Kb sequence and 1.76% in the 1.2 Kb sequence (Table 1); however, there are two diagnostic sites in the ATPase region and 15 in the COI region that distinguish the two clades.

In contrast to the extensive geographical coverage of mtDNA sampling, the samples available for the study of isozymes were limited by the need to obtain and transport frozen tissues. Phylogenetic resolution in the isozyme data was also low. As Lessios and Pearse (1996) have indicated, three loci in *D. setosum* carry completely different alleles than they do in *D. savignyi* and *D. paucispinum,* and as Bermingham and Lessios (1993) have found, none of the 22 loci included in this analysis is fixed for different alleles between *D. mexicanum* and *D. antillarum.* The combined data from the two studies indicated that there are also no diagnostic loci that can distinguish between *D. savignyi, D. paucispinum, D. antillarum,* and *D. mexicanum.* Thus, Nei's distances between all these closely related species are small (Table 2), and the phylogenetic trees generated by both the parsimony algorithm of Swofford and Berlocher (1987) and the neighbor-joining of Nei's D lack resolution. They group *D. setosum* consistently as different from all other species, but for the rest they produce extremely short branch lengths and different topologies, depending on the algorithm used and the loci that are included in the jackknifing (trees not shown). Thus, the value of the isozyme data lies in confirming the deep split of *D. setosum* from all other species of *Diadema,* and in indicating that the short branch lengths seen in the mtDNA gene tree of *D. mexicanum, D. paucispinum, D. savignyi,* and *D. antillarum* are likely to be a reflection of the species tree.

Genetic Discontinuities between Conspecific Populations

Complete absence of gene flow between East and West Atlantic populations of *Diadema antillarum* and between Arabian and non-Arabian populations of *D. setosum* is indicated

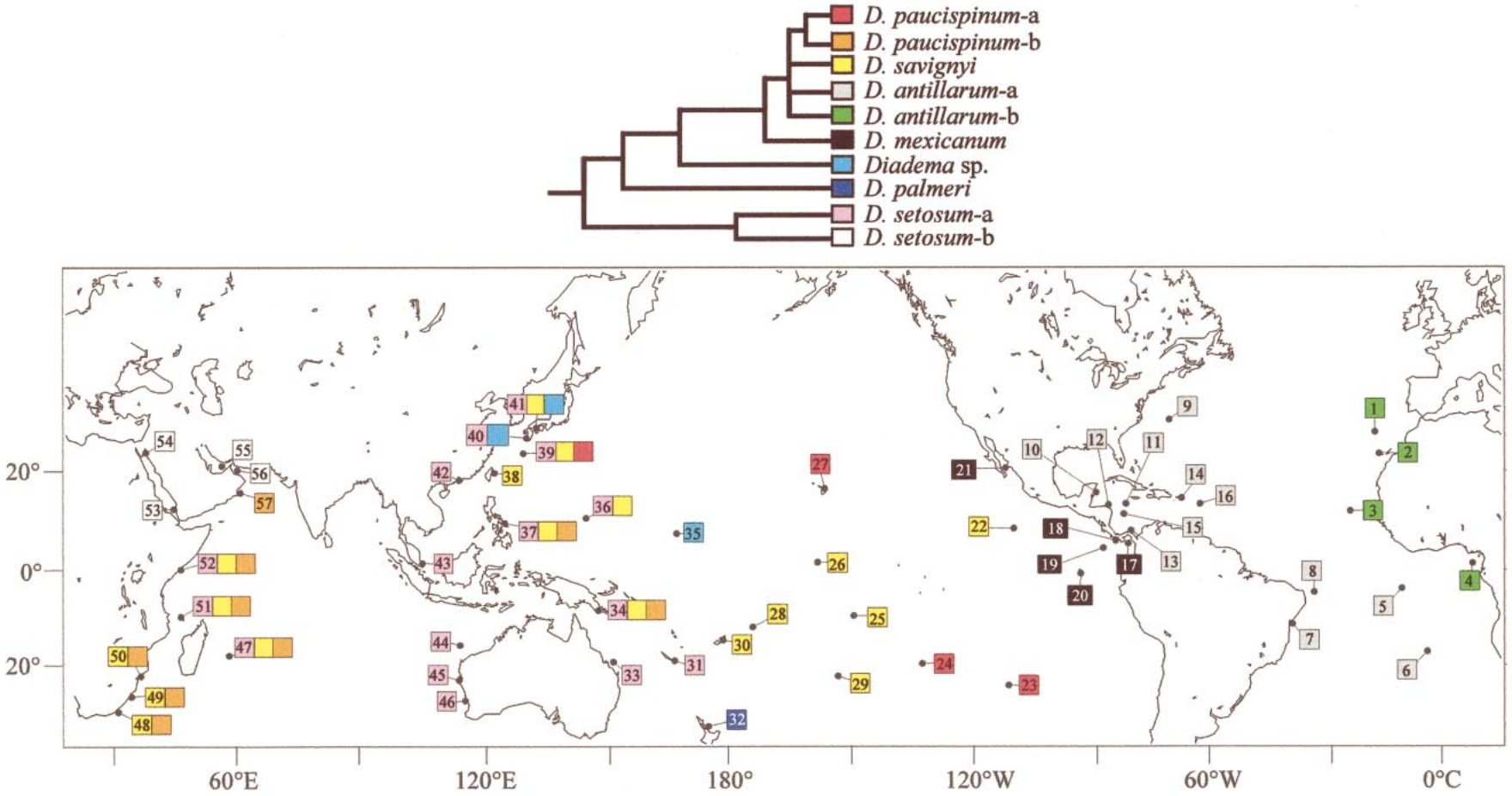

FIG. 3. Distribution of major *Diadema* mitochondrial clades in the world's oceans, and number of individuals sequenced from each locality. Locality codes: **1**: Madeira (*n* = 7); **2**: Canary Islands (*n* = 17 [Gran Canaria (*n* = 13), La Palma (*n* = 4)]); **3**: Boa Vista, Cape Verde (*n* = 7); **4**: Sâo Tomé (*n* = 10); **5**: Ascension (*n* = 12); **6**: St. Helena (*n* = 8); **7**: Salvador, Bahia (*n* = 7); **8**: Fernando de Noronha (*n* = 4); **9**: Bermuda (*n* = 1); **10**: Carrie Bow Key, Belize (*n* = 10); **11**: Cayman Brac, Cayman Islands (*n* = 10); **12**: Cayos Cochinos, Honduras (*n* = 10); **13**: Isla Margarita, Panama (*n* = 5); **14**: San Cristobál, Puerto Rico (*n* = 10); **15**: San Andrés (*n* = 2); **16**: St. John, Virgin Islands (*n* = 3); **17**: Bay of Panama (*n* = 5 [Isla Taboguilla (*n* = 3), Isla Bona (*n* = 2)]); **18**: Gulf of Chiriqui (*n* = 3 [Isla Montuosa (*n* = 2), Isla Ladrones (*n* = 1)]); **19**: Isla del Coco (*n* = 12); **20**: Galapagos (*n* = 4 [Isla Genovesa (*n* = 2), Isla Santiago (*n* = 2)]); **21**: Sea of Cortéz (*n* = 9 [Bahía San Carlos (*n* = 1), El Crestón (*n* = 4), Guaymas (*n* = 2), Isla Peruano (*n* = 1), Isla San Pedro (*n* = 1)]); **22**: Clipperton Atoll (*n* = 12); **23**: Easter Island (*n* = 11); **24**: Pitcairn (*n* = 4); **25**: Moorea (*n* = 10); **26**: Kiritimati, Kiribati (*n* = 10); **27**: Hawaii (*n* = 11 [Hawaii Island (*n* = 3), Oahu (*n* = 8)]); **28**: Upolu Island, Samoa (*n* = 8); **29**: Rarotonga, Cook Islands (*n* = 3); **30**: Suva Lagoon, Fiji (*n* = 1); **31**: Noumea, New Caledonia (*n* = 6); **32**: Bay of Plenty, New Zealand (*n* = 4); **33**: Fantome Island, Australia (*n* = 9); **34**: Motopure, Papua New Guinea (*n* = 8); **35**: Majuro, Marshall Islands (*n* = 8); **36**: Guam (*n* = 25); **37**: Philippines (*n* = 9); **38**: Kenting Reef, Taiwan (*n* = 6); **39**: Sesoko Island, and Motobu Harbor, Okinawa, Japan (*n* = 22); **40**: South side of Kyushu, Japan (*n* = 10); **41**: Seto, S. Honsu, Japan (*n* = 17); **42**: Lamma Island, Hong Kong (*n* = 4); **43**: Pulau Island, Singapore (*n* = 3); **44**: Lamarck Island and White Island, NW. Australia (*n* = 6); **45**: Ningaloo, W. Australia (*n* = 1); **46**: Geraldton, SW. Australia (*n* = 4); **47**: Ebang Salé, Reunión (*n* = 26); **48**: Transkei, East Cape Province, South Africa (*n* = 9); **49**: Isipingo and Durban, South Africa (*n* = 17); **50**: Sodwana Bay, South Africa (*n* = 3); **51**: Zanzibar (*n* = 6); **52**: Kanamai, Kenya (*n* = 17); **53**: Eritrea (*n* = 11 [Museri Island (*n* = 3), Norkra Island (*n* = 4), Tualot Island (*n* = 4)]); **54**: Eilat, Gulf of Aqaba (*n* = 7); **55**: Tarut Bay Reef, Persian Gulf, Saudi Arabia (*n* = 8); **56**: Masqat, Oman (*n* = 5); **57**: Masirah Island, Oman (*n* = 5).

TABLE 1. Mean percent substitutions between major clades of *Diadema,* calculated from maximum-likelihood estimations of base frequencies and rates of substitution as explained in the text. Values above the diagonal are based on the 0.6 Kb data, values below the diagonal on the 1.2 Kb data (see text). On the diagonal (in bold) is mean intraclade divergence based on the 06 Kb data.

	D. antillarum-a	*D. antillarum*-b	*D. mexicanum*	*D. palmeri*	*D. paucispinum*-a	*D. paucispinum*-b	*D. savignyi*	*Diadema*-sp	*D. setosum*-a	*D. setosum*-b
D. antillarum-a	**0.84**	2.99	4.24	17.47	2.51	2.95	2.56	10.79	16.81	17.92
D. antillarum-b	2.70	**0.07**	5.76	19.24	3.39	3.79	2.66	13.06	17.64	17.47
D. mexicanum	4.47	5.36	**0.03**	17.49	4.54	4.94	4.34	10.78	17.34	17.02
D. palmeri	19.37	20.30	19.45	**0.41**	17.65	18.79	17.30	18.62	21.92	23.52
D. paucispinum-a	2.91	3.60	5.34	19.82	**0.33**	1.38	2.61	12.01	18.35	17.97
D. paucispinum-b	3.23	3.48	5.61	19.97	1.76	**0.49**	2.63	11.96	18.38	18.09
D. savignyi	2.96	2.81	4.77	19.22	3.30	3.24	**0.39**	11.16	17.07	16.80
Diadema-sp	11.74	12.74	11.76	17.83	12.29	12.43	11.91	**0.28**	21.76	20.10
D. setosum-a	19.27	20.13	19.26	23.70	19.57	19.11	19.57	20.74	**0.37**	7.83
D. setosum-b	19.83	19.40	19.72	24.22	19.29	19.13	18.96	18.43	8.18	**0.31**

by reciprocally monophyletic lineages (Figs. 1, 2), and fixed diagnostic mtDNA sites. The phylogeny also points to a similar lack of genetic connections between *D. paucispinum*-a from the central Pacific and Okinawa, on the one hand, and *D. paucispinum*-b from New Guinea, Philippines, and the Indian Ocean, on the other. Phylogenetic reconstruction, however, can only detect cessation of gene flow that happened long enough in the past for lineages to have sorted. We used F_{ST} statistics, applied to the 0.6 Kb data, to estimate relative degrees of gene flow between geographic populations in the same mtDNA clade (Table 3). Populations from which less than four individuals were sampled were excluded from this analysis.

Within the eastern Atlantic, the overall F_{ST} value between populations of *D. antillarum*-b from Madeira, Canaries, Cape Verde, and Sâo Tomé is no larger than those obtained from random permutations of haplotypes between populations. Pairwise F_{ST} values are also very small. The probability of their being obtained by chance is high, except in one case, the comparison between Madeira and Cape Verde, in which most of the random permutations produced negative F_{ST} values. The dominant haplotype is found in six individuals from Madeira, nine from the Canaries, six from Cape Verde, and eight from Sâo Tomé. Thus, there is no genetic structuring over this stretch of nearly 5500 km along the African Coast.

The *D. antillarum*-a lineage shows very small pairwise values of F_{ST} between five widely spaced localities in the Caribbean, with the exception of the comparison between Panama and the Cayman Islands. The F_{ST} value for this comparison, though larger than the rest and unlikely to be due to chance, would still be equivalent to an exchange of 1.36 females per generation at genetic equilibrium, which is sufficient to place the two localities in the same genetic neighborhood. Moreover, each of these two localities show high levels of genetic exchange with each of the other localities. The overall F_{ST} between the Caribbean populations is 0.02, with a probability of 0.27 of being generated by chance. Thus, it is unlikely that *Diadema* either in Panama or the Caymans experiences any degree of genetic isolation from the rest of the Caribbean. This is in contrast to the consistent differences between populations from the Caribbean, on the one hand, and Brazil or the central Atlantic Islands, on the other. F_{ST} values between Fernando de Noronha or Salvador in Brazil and each of the Caribbean populations exceed 0.46. Those between Caribbean and Ascension or St. Helena exceed 0.62. Brazil and the central Atlantic Islands are also distinguished by large and significant F_{ST} values. Thus, F_{ST} statistics agree with the presence of diagnostic DNA sites that the three areas are genetically isolated from each other. In contrast, *Diadema* populations from St. Helena and Ascension show high rates of genetic exchange with each other and share indistinguishable haplotypes (Fig. 1A).

High levels of gene flow are also present among populations of *D. mexicanum* in the eastern Pacific. Sea urchins from Galapagos and Isla del Coco, despite their distance from the mainland and from each other, belong to the same genetic population as sea urchins from Panama and Mexico. The most common haplotype is found in every locality we sampled (Fig. 1A). Similarly the central Pacific populations of *D. paucispinum*-a from Hawaii, Easter Island, and Pitcairn are genetically connected, with no indications of isolation by distance, despite a straight-line distance of 7100 km between Hawaii and Easter Island. *Diadema paucispinum*-b in the Indian Ocean, however, shows a fairly high degree of isolation between the South African Coast and the island of Reunión, a distance of 2500 km. This is despite our pooling of samples from three South African collecting localities, which could have increased apparent within-population variation and thus decreased values of F_{ST}.

The most widespread species of *Diadema, D. savignyi,* shows only weak genetic structuring, as indicated by a significant but small overall F_{ST} value. The Indian Ocean populations at South Africa, Kenya, and Reunión appear to experience restrictions of gene flow towards a few of the western Pacific populations, such as Samoa and Okinawa. Samoa and Guam are genetically isolated from some of the western and central Pacific populations. However, there is no pattern

TABLE 2. Nei's (1978) unbiased D between populations of *Diadema,* based on 22 loci. Two populations per species have been sampled in *D. antillarum* and *D. mexicanum* (see Bermingham and Lessios 1993), but for the purposes of the present study, their frequencies have been pooled.

	D. antillarum	*D. mexicanum*	*D. paucispinum*	*D. savignyi*	*D. setosum* Okinawa
D. mexicanum	0.014				
D. paucispinum	0.120	0.095			
D. savignyi	0.076	0.066	0.047		
D. setosum (Okinawa)	0.246	0.284	0.277	0.234	
D. setosum (Australia)	0.237	0.273	0.263	0.236	0.006

in the F_{ST} values that would suggest isolation between the Indian Ocean, the West Pacific, and the central Pacific, nor any indication that isolation by distance has occurred in this species. This pattern of virtual panmixia over two oceans in *D. savignyi* contrasts strongly with the pattern seen in *D. setosum*-a. In *D. setosum*-a, there is a definite genetic break between Reunión and Kenya, on the one hand, and the rest of the populations (including West Australia), on the other. This break is obvious in the F_{ST} statistics, but is also supported by one diagnostic site found in the 0.6 Kb data between the western Indian Ocean *D. setosum* populations and those from everywhere else. Within these two groups, F_{ST} statistics show a great deal of genetic homogeneity. Finally, *D. setosum*-b is genetically continuous around the Arabian Peninsula, from the Gulf of Aqaba, to Eritrea, and to the Persian Gulf. Though the F_{ST} value between the Gulf of Aqaba and the Gulf of Oman is large, a larger value was obtained by chance 22% of the time.

Discussion

Correspondence between mtDNA Clades and Previously Described Morphospecies

The picture generated by the mtDNA data confirms some aspects of *Diadema* species distributions and relationships that were previously suggested by morphological or isozyme data, but also reveals some unexpected patterns. As most students of morphology have suggested (Clark 1925, p. 42; Mortensen 1940, p. 254; but see Clark 1966), *D. mexicanum, D. savignyi, D. paucispinum,* and *D. antillarum* are closely related to each other and quite divergent from *D. setosum. Diadema palmeri* is nested within the basal clades of *Diadema,* indicating that Baker's (1967) decision to include it in the genus despite its temperate distribution was correct. The separation of two clades of *D. setosum,* one around the Arabian Peninsula, the other in the Indo-West Pacific, was never suggested by any echinoderm systematist, despite the attention paid to Red Sea populations of *Diadema* (e.g., Tortonese 1936a,b, 1953; Clark 1966; James and Pearse 1969; Pearse 1970). Though *D. setosum* and *D. savignyi* are the easiest species to distinguish, because of the differences of their pedicellariae (see Lessios and Pearse 1996), there has been great confusion regarding their specific status and geographical distributions (Mortensen 1940, p. 254; Clark 1966; Pearse 1998). Both the mtDNA and the isozyme data confirm the conclusion of Mortensen (1940, p. 269) that these are different species, despite the occasional hybrids they produce (Lessios and Pearse 1996). The 0.6 Kb data also confirm Pearse's (1998) conclusions from morphological study of live animals that *D. setosum* is tied to continental margins and is probably entirely absent east of Tonga (Fig. 3). Thus, contrary to the original belief (Mortensen 1940; Mayr 1954) that the two species have identical ranges, they overlap only at the western margins of the Pacific and at the Australian and African shores of the Indian Ocean. H. L. Clark (1925, p. 44) noticed that some specimens of *Diadema* from Japan were distinctive, and Ikeda (1939) described them as a new species, *D. clarki,* but Mortensen (1940, p. 264) synonymized *D. clarki* with *D. setosum.* Ikeda's specimens came from N. Kyushu and S. Shikoku, therefore the geographic distribution of his species partly coincides with that of the *Diadema*-sp mtDNA clade. Thus, there may be morphological characters by which to recognize individuals that belong to *Diadema*-sp. However, this mtDNA clade is more similar to the lineage leading to *D. savignyi* than to *D. setosum,* and the presence of any morphotypes resembling Japanese *Diadema* in the Marshall Islands was not suggested by any morphological study. Thus, it may be premature to resurrect *D. clarki* on the basis of the present evidence.

As previously suggested by Lessios and Pearse's (1996) geographically limited isozyme survey, and contrary to what was believed from morphology (Mortensen 1940, p. 279; Clark 1954), *D. paucispinum* is not confined to Hawaii and Kiribati, but is abundant in the central Pacific and ranges widely all over the Indo-West Pacific (Fig. 3). Our results from Easter Island illustrate the unreliability of morphological characters for distinguishing between species of *Diadema.* Easter Island was said by Mortensen (1940, p. 277) to contain *D. mexicanum* and *D. savignyi.* F. J. Fell (1974) in a study of Easter Island echinoids, concluded that all *Diadema* at this remote island belonged to *D. savignyi.* Our sampling cannot exclude the possibility that either of the other two species is present, but the data show unequivocally that all 11 specimens we sequenced have *D. paucispinum* mtDNA.

Whether East Atlantic *Diadema* belong to *D. antillarum* or to a separate species is a question raised by Koehler (1914), Clark (1925, p. 42), and Mortensen (1940, p. 274). They all decided that the morphological differences were too slight to justify designating them as a separate species. Mitochondrial DNA of the two Atlantic lineages, however, is as different as it is between three accepted morphospecies, which suggests that they have been isolated for a long time. The derived position of the *Diadema* populations of Ascension and St. Helena as a monophyletic entity nested within *D. antillarum*-a fully supports Pawson's (1978) decision to demote them to a subspecies of *D. antillarum,* rather than accept them as a separate species, as thought by Mortensen (1909, 1940). Pawson was also correct in concluding that *Diadema* populations in Fernado de Noronha were identical to those on the Brazilian Coast and different from those in the Central Atlantic.

Timing of Cladogenic Events

There are no comprehensive previous hypotheses regarding the phylogeny of *Diadema,* but the two existing phylogenetic conjectures are in sharp conflict regarding the proposed timing of cladogenic events. Mortensen (1940, p. 254), impressed by the morphological similarity of *D. savignyi, D. mexicanum, D. antillarum,* and *D. paucispinum,* suggested that they were probably separated by the breakup of the Tethys, now known to have been completed between the Burdigalian, (16.6–21.8 mya; Adams 1981) and the Serravallian (11.2–15.1 mya ; Rögl and Steininger 1984; Vrielynck et al. 1997). Matsuoka (1989), on the other hand, compared isozymes of 15 loci between *D. setosum* and *D. savignyi* and obtained a value of Nei's (1972) D equal to 0.246, remarkably similar to the Nei's (1978) D value obtained by us from a different set of 22 loci (Table 2). Applying a molecular clock calibration obtained from *Drosophila,* of one unit of D for

TABLE 3. F_{ST} statistics comparing populations within major mitochondrial clades. Only populations in which four or more individuals were sampled are included. Comparisons in which less than 5% of 1023 random reshufflings produced larger values are indicated with asterisks.

D. antillarum-b
Overall F_{ST}: −0.10

	Sao Tome	Cape Verde	Gran Canaria
Cape Verde	−0.01		
Gran Canaria	−0.03	0.01	
Madeira	0.00	0.00*	0.01

D. antillarum-a
Overall F_{ST}: 0.61*

	Caribbean					West Atlantic		Central Atlantic
	Puerto Rico	Cayman Is.	Belize	Honduras	Panama	F. de Noronha	Salvador	Ascension
Caribbean								
Cayman Is.	0.02							
Belize	−0.06	0.02						
Honduras	−0.07	0.00	−0.04					
Panama	0.07	0.27*	0.09	0.68*				
West Atlantic								
F. de Noronha	0.55*	0.57*	0.46*	0.52*	0.67*			
Salvador, Bahia	0.56*	0.58*	0.50*	0.55*	0.65*	−0.16		
Central Atlantic								
Ascension	0.69*	0.71*	0.64*	0.68*	0.77*	0.50*	0.42*	
St. Helena	0.68*	0.71*	0.62*	0.67*	0.79*	0.55*	0.42*	−0.07

D. mexicanum
Overall F_{ST}: −0.02

	Clipperton	Is. del Coco	Galapagos	Mexico
Is. del Coco	−0.01			
Galapagos	0.00	−0.13		
Mexico	0.00	−0.02	0.00	
Panama	0.04	−0.01	−0.11	0.02

D. paucispinum-a
Overall F_{ST}: 0.04

	Hawaii	Easter Is.
Easter Is.	0.02	
Pitcairn	0.06	0.04

D. paucispinum-b
Overall F_{ST}: 0.10*

	Reunion	Gulf of Oman
Gulf of Oman	0.17*	
South Africa	0.19*	0.01

D. savignyi
Overall F_{ST}: 0.06*

	Indian Ocean			West Pacific					Central Pacific
	South Africa	Kenya	Reunion	Philippines	Guam	Samoa	Okinawa	Taiwan	Morea
Indian Ocean									
Kenya	0.01								
Reunion	0.01	−0.01							
West Pacific									
Philippines	−0.01	0.03	0.03						
Guam	0.01	0.00	0.03	0.05					
Samoa	0.19*	0.14	0.10	0.29*	0.19*				
Okinawa	0.03	0.05	0.11*	0.00	0.09*	0.31*			
Taiwan	0.06	0.00	0.04	0.04	0.09	0.11	0.02		
Central Pacific									
Morea	−0.03	0.03	0.09	0.01	−0.01	0.24*	0.07*	0.08	
Kiribati	−0.01	−0.01	0.02	−0.01	0.00	0.20*	0.02	0.05	−0.05

TABLE 3. Continued.

D. setosum-a
Overall F_{ST}: 0.42*

	Indian Ocean			West Pacific					
	Kenya	Reunion	West Australia	East Australia	New Guinea	Hong Kong	Okinawa	Kyushu	Guam
Indian Ocean									
Reunion	0.00								
West Australia	0.59*	0.58*							
West Pacific									
East Australia	0.56*	0.54*	0.03						
New Guinea	047*	0.43*	0.10	−0.03					
Hong Kong	0.68*	0.65*	0.17	0.15	0.07				
Okinawa	0.49*	0.45*	0.07	0.04	−0.01	0.06			
Kyushu	0.36*	0.34*	0.04	0.06	0.08	0.07	0.04		
Guam	0.59*	0.58*	0.00	0.03	0.09	0.17*	0.07	0.04	
New Caledoni	073*	0.71*	0.09	0.10	0.07	0.26	0.07	0.02	0.09

D. setosum-b
Overall F_{ST}: 0.02

	Gulf of Oman	Persian Gulf	Gulf of Aqaba
Persian Gulf	0.05		
Gulf of Aqaba	0.13	0.06	
Eritrea	−0.01	−0.01	−0.02

every five million years of separation, he inferred that *D. setosum* and *D. savignyi* diverged 1.2 mya. Thus, by one estimate the most recent cladogenic events in the genus occurred as early as 20 mya, while by the other the most ancient event occurred as recently as 1.2 mya. What estimates do our mtDNA sequence comparisons provide?

Obviously, the estimated times since divergence will be accurate only when rates of divergence are constant and when a calibration specific to the sequenced DNA region and the study organism is applied. We have data from many individuals for a shorter sequence and from fewer individuals for a longer sequence. To determine whether rate of change of either the 0.6 Kb or the 1.2 Kb sequences was linearly related with time, we compared the log likelihood values of a tree (without the outgroups) on which the assumption of constant rates is imposed to a tree in which rates were free to vary (Felsenstein 1988). In all cases, the topology of the fully resolved ML tree resulting from the 1.2 Kb data (Fig. 2) was used. By this test, there are no significant deviations from a constant rate in either set of data (Likelihood ratio test statistic δ: For the 0.6 Kb data: $\delta = 90.52$, df = 207, $P > 0.999$. For 1.2 Kb data: $\delta = 12.82$, df = 18, $P > 0.75$), so either could be used to estimate dates. To determine which set of data produced the least error, we compared divergences between each member of a clade and the outgroup of the clade. Under the assumption of a molecular clock, the differentiation between an outgroup and all ''ingroups'' should be equal. This ''relative rates test'' was applied to successively more inclusive clades, up to the comparison of *D. setosum* with all other clades. In the 1.2 Kb data, the maximum difference between values that should have been equal if the expectations of a molecular clock held was 29.14% of the mean divergence. In the 0.6 Kb data, the equivalent value was 30.10% of the mean. We, therefore, used the 1.2 Kb data for time calculations, and assumed that all time estimates derived from our data were likely to have a maximum error of 14.57% on either side of the value calculated from average sequence divergence between groups.

The second requirement for calculating times from molecular divergences is an estimate of how many base substitutions accumulate per unit time in the sequenced region. In data that include species from both sides of Central America, such a calibration is often obtained by assuming that eastern Pacific and Atlantic species were separated by the uplift of the Isthmus of Panama. The time of final isthmus closure is placed by geological evidence at about 3 mya (Coates and Obando 1996), with the more exact value of 3.1 mya considered as probable (Duque-Caro 1990). An isthmus-related vicariant event is a reasonable assumption in cases, such as those of the sea urchin genera *Eucidaris* (Lessios et al. 1999) and *Echinometra* (McCartney et al. 2000), in which clades that appeared more recently than the separation of the tropical eastern Pacific and the Atlantic are confined to either ocean. In *Diadema*, however, the phylogeny presents a problem of biogeographic as well as chronological importance: Given that the two clades of the Atlantic species are members of a polytomy that includes the western Pacific *D. savignyi* and *D. paucispinum* and that the eastern Pacific *D. mexicanum* is an outgroup to this clade, it is not immediately obvious which lineages were separated by the Isthmus of Panama. If we place our trust in the phylogeny generated by the 1.2 Kb data (Fig. 2), we will accept the traditional (Mortensen 1940; Chesher 1972; Lessios 1979, 1981, 1998; Bermingham and Lessios 1993; Gonzalez and Lessios 1999) assumption that the isthmus separated *D. mexicanum* from the western Atlantic populations of *D. antillarum.* According to this tree, gene flow around the tip of S. Africa continued between the eastern Atlantic clade of *D. antillarum* and *D. savignyi* after the rise of the Panama Isthmus. However, the node uniting *D. antillarum*-b and *D. savignyi* is supported only by 60% of

TABLE 4. Transisthmian mean divergence in four genera of sea urchins with lineages on either side of the Isthmus of Panama for approximately 640 bp of the COI region of mtDNA, RFLP estimates for the entire mtDNA molecule, and isozymes. K_2: Percent differences in all sites, corrected with Kimura's (1980) two-parameter model. K_s: Substitutions per silent site, estimated according to Pamilo and Bianchi (1993) and Li (1993). RFLP: Percent difference from Restriction Fragment Length Polymorphisms, estimated according to Nei and Miller (1990). A: *E. tribuloides* versus (*E. thouarsi* + *E. galapagensis*). B: *Echinometra vanbrunti* versus (*E. lucunter* + *E. viridis*). C: *Arbacia incisa* versus *A. punctulata.* D: *Diadema antillarum*-a and *D. antillarum*-b are included, except in the isozyme data. a: Data from Lessios et al. 1999. b: Data from Bermingham and Lessios (1993); values do not include *E. galapagensis* or *D. antillarum*-b. c: Data from McCartney et al. 2000. d: Data calculated from GenBank sequences of Metz et al. (1998); this is not the same region of COI as the one sequenced in other genera. —: No data.

				Diadema		
Character	*Eucidaris*[A]	*Echinometra*[B]	*Arbacia*[C]	*D. mexicanum* vs *D. antillarum*[D]	*D. savignyi* vs *D. antillarum*[D]	*D. paucispinum* vs *D. antillarum*[D]
COI (K_2) (%)	10.08[a]	11.37[c]	12.58[d]	4.57	3.11	3.33
COI (K_s) (%)	34.74[a]	39.68[c]	43.76[d]	14.35	9.38	10.63
mtDNA (RFLP) (%)	6.58[b]	7.03[b]	—	5.28[b]	—	—
Isozymes (Nei's D)	0.39[b]	0.48[b]	—	0.05[b]	0.08	0.12

the bootstrap replicates in the 1.2 Kb tree and receives <50% support in the 0.6 Kb tree. We, therefore, have to admit another possibility: The *D. savignyi*-*D. paucispinum* ancestor may have been present in the eastern Pacific during the Pliocene, speciated from *D. mexicanum* for unknown reasons, then became separated from *D. antillarum* by the Isthmus of Panama. The only empirical support for this hypothesis comes from the single individual of *Diadema* from the Clipperton Atoll that was found to carry a *D. savignyi* ATPase haplotype. The other 11 individuals from Clipperton and 33 individuals from the rest of the eastern Pacific belonged to *D. mexicanum.* Although the close nucleotide similarity of the Clipperton *D. savignyi* individual to those of the central and western Pacific identifies it as the descendant of a recent migrant (Lessios et al. 1996), it opens the possibility that *Diadema,* like another diadematoid sea urchin, *Echinothrix* (Lessios et al. 1998), has been able to occasionally cross the 5400 km of open water between Kiribati and the outer islands of the eastern Pacific (the Eastern Pacific Barrier). However, the hypothesis that the Isthmus of Panama separated Atlantic *Diadema* from the ancestor of a species that is generally absent from the eastern Pacific is less parsimonious than the alternative. It requires the postulation of two extinction events, one that would extirpate the ancestor of *D. savignyi* from the eastern Pacific, the other that would extirpate the geminate of *D. mexicanum* (which according to this hypothesis was already present as a separate species) from the Atlantic. Because of this, we regard the traditional assumption that *D. mexicanum* and *D. antillarum* are geminate species as most likely.

An additional problem in calibrating the *Diadema* mtDNA clock by the timing of the completion of the rise of the Isthmus of Panama comes from a comparison of transisthmian divergence in this genus to that in other genera of sea urchins. There are three other echinoid genera for which published data on divergence across the Isthmus in COI exist. Kimura (1980) two-parameter corrected sequence dissimilarity values (K_2) between Atlantic and eastern Pacific members of these genera are close to each other and average to 11.34% (Table 4). Comparisons between Pacific and Atlantic species of *Diadema,* on the other hand, have only half as much sequence dissimilarity. Three out of four additional sea urchin species pairs (*Astropyga, Tripneustes, Lytechinus*) have transisthmian divergences ranging from 8.97% to 13.02% (mean 10.61%), whereas the fourth (*Meoma*) shows a divergence of 4.55% (K. Zigler, J. Kane, B. D. Kessing, and H. A. Lessios, unpubl. ms.). The difference does not appear to be due to a selectively driven slow-down in *Diadema* or *Meoma* COI sequence evolution, because accumulation of synonymous mutations, unlikely to be affected by selection, is also lower in these genera (14.35% and 13.81%, respectively). Nor can it be due to longer generation time, because *Diadema* reaches sexual maturity earlier than *Eucidaris* or *Echinometra* (Lessios 1979). *Diadema* also shows one order of magnitude less isozyme divergence across the Isthmus. Only the value of RFLP transisthmian comparisons of mtDNA in *Diadema* approaches that in *Eucidaris* and *Echinometra,* but is still the lowest among the genera thus compared (Table 4). Therefore, it appears likely that separation of *Diadema* populations by the Isthmus of Panama occurred more recently than it did in other sea urchins. The question is whether the Isthmus completion, 3.1 mya, separated the geminate species of *Diadema* and *Meoma,* or of the other six sea urchin genera.

Faced with a similar situation of unequal transisthmian divergence in COI and isozymes in geminate species pairs of shrimp, Knowlton and Weigt (1998) assumed that the smallest transisthmian divergence value—among pairs of mangrove-inhabiting species—represents the differentiation expected to occur in 3 my. Accordingly, they concluded that another 14 species pairs, including a cluster of eight pairs with similar divergence values, must have been divided by restrictions to water circulation before the Isthmus was fully formed. This is one possibility. Cronin and Dowsett (1996), however, presented evidence from planktonic foraminifera that the Isthmus may have been breached twice during high sea level stands, the most recent event occurring 2 mya. Earlier work by Crouch and Poag (1979), Gartner et al. (1987), and Keller et al. (1989) had also suggested the existence of sea water connections between the Caribbean and the eastern Pacific that lasted until 1.8 mya. Could it be that *Diadema* larvae crossed from one ocean to the other during a relatively brief episode of interoceanic reconnection? We consider this possibility as likely as the alternative. The six sea urchin genera with similar values of transisthmian divergence form

a relatively tight cluster of transisthmian K_2 values in COI, with a mean of 10.97%. The eight pairs of alpheid shrimp in the Knowlton and Weigt (1998) data have COI divergences ranging approximately between 7.5% and 11%; eastern Pacific and Atlantic shrimp in the genus *Penaeus* have COI divergence values of about 13% (Baldwin et al. 1998). Although there is no reason to expect shrimp and sea urchins to have similar rates of molecular divergence, the coincidence of divergences within each group suggests a common vicariant event as having isolated their populations. If the split of *D. mexicanum* from *D. antillarum* were to be placed at 3.1 mya, under the assumption of a COI molecular clock the splits in the other sea urchin genera would be dated as having occurred between 6.1 and 8.5 mya. Stratigraphic and fossil biogeographic evidence indicates that shoaling of the Isthmus and changes in current patterns were occurring between 3.5 and 6 mya (Keigwin 1982; Duque-Caro 1990; Jackson et al. 1993; Farrell et al. 1995; Collins 1996; Collins et al. 1996a,b; Haug and Tiedemann 1998). At 8 mya, the depth in what is now the Panama Canal Basin may have been reduced to as little as 25 m, but the Atrato strait next to Colombia remained at least 150 m deep (Duque-Caro 1990). The depth of the Panama Canal Basin increased once again, so that at 6 mya it was at least 200 m (Collins et al. 1996b). Thus, sea water connections that would provide habitat for adults of shallow-water sea urchin species and would permit the passage of their long-lived larvae appear to have existed until 3.5–3.1 mya, when uninterrupted land severed all connections between the Caribbean and the eastern Pacific (Keigwin 1982; Duque-Caro 1990; Coates and Obando 1996). Different species are likely to have responded to these changes in a different manner, and thus become separated at different times. However, there is no known geological barrier in the time range of 6–8 mya that would universally restrict gene flow of shallow water organisms so as to produce concordant divergences in many species pairs. Conversely, a short-lived breach of the isthmus would be expected to affect a small number of species, which (because of their ecological attributes, or due to pure chance) either reestablished genetic connections with their geminates, or replaced the resident population. For *Diadema,* a replacement of the mitochondrial DNA lineage in either the eastern Pacific or the Caribbean may be a more likely hypothesis, because there is no evidence in either body of water of a second mtDNA variant that would have presumably become differentiated in the 1 my of isolation between the Isthmus completion and the breach. Thus, on the basis of the sea urchin divergence values, it seems possible that the cluster of similar values dates the isthmus completion (or near completion), whereas *Diadema* and *Meoma* crossed over at a time of subsequent breaching.

The comparisons between genera in Table 4 were made for COI alone, because these are the only data available for the other sea urchins. The dating obtained in *Diadema* from combined ATPase and COI sequences under the two calibrations is shown in Figure 4. For the reasons outlined above, we consider the dates produced by the calibration of 2.6% nucleotide sequence divergence per my as slightly more credible, but, of course, we cannot dismiss the possibility that it may be as low as 1.6% per my. Either calibration is adequate to reject both previously existing hypotheses regarding the time that new species in *Diadema* arose. The separation of *D. mexicanum, D. antillarum, D. paucispinum,* and *D. savignyi* occurred in the late Pliocene or early Pleistocene, far too recently to be of Tethyan origin as suggested by Mortensen (1940). The split between *D. setosum* and *D. savignyi* happened in the Miocene, far too early to be compatible with the 1.2 mya date suggested by Matsuoka (1989).

Possible Causes of Isolation

The phylogeny of the extant species of *Diadema* goes back to 9 my by the fast time scale and to 14 my by the slow one (Fig. 4). During this time, the world's oceans have undergone important changes (Adams 1981). What events can account for the distribution and divergence of mtDNA haplotypes we see today?

The first splitting event in the history of extant *Diadema* separated the *D. setosum* clade from all others, including two other species with present-day Indo-Pacific distributions. In this regard, the phylogeny of *Diadema* differs from the phylogeny of *Eucidaris* (Lessios et al. 1999) or *Echinometra* (McCartney et al. 2000), which show a deep split between species from the western Pacific, on the one hand, and species from the eastern Pacific and the Atlantic, on the other. The separation of the two deepest *Diadema* lineages is dated at 10.4–13.9 mya by the slow calibration, and 6.7–9.0 mya by the fast one (Fig. 4). Mayr (1954) had speculated that either *D. setosum* or *D. savignyi* invaded the Indo-West Pacific from the Atlantic or the eastern Pacific, but the phylogeny suggests no invasions from these oceans. The lineage terminating in *D. setosum* has not left behind any surviving species in another ocean basin; and the basal connection of the New Zealand *D. palmeri* to the lineage that culminates in *D. savignyi* implies that the ancestor of *D. savignyi* was present in the western Pacific shortly after it became separated from the ancestor of *D. setosum.* This, however, does not necessarily require that the overlapping geographic distributions of *D. setosum* and *D. savignyi* were the product of sympatric speciation. The 6.5–14.0 my age estimate of this split includes a period of drastic sea level fluctuations, starting with an extreme sea-level drop of some 80 m at approximately 10 mya (Haq et al. 1987). Such a low sea-level stand could cause isolation between marine populations in many areas of the Indo-West Pacific.

Diadema palmeri diverged from the lineage leading to *D. savignyi* (Figs. 1, 2), but its absolute time of splitting can only be estimated as overlapping with that of the separation of the two major lineages (Fig. 4). However, rather than being the product of a vicariant event, this species—the only one with such a restricted geographical distribution—could be the result of dispersal. Such dispersal could have occurred on the warm currents flowing southward throughout the Cenozoic along the coast of Australia, and being deflected eastward towards New Zealand since the Oligocene (Kennett and von der Borch 1986). Starting in the early Miocene, many tropical Indo-Pacific species appeared at the North Island of New Zealand (Fleming 1975; Knox 1980). Separation between *D. palmeri* and its sister lineage coincides, by our fast time scale, with the most intense Neogene extinction of New Zealand molluscs (Beu 1990). Both time scales are consistent

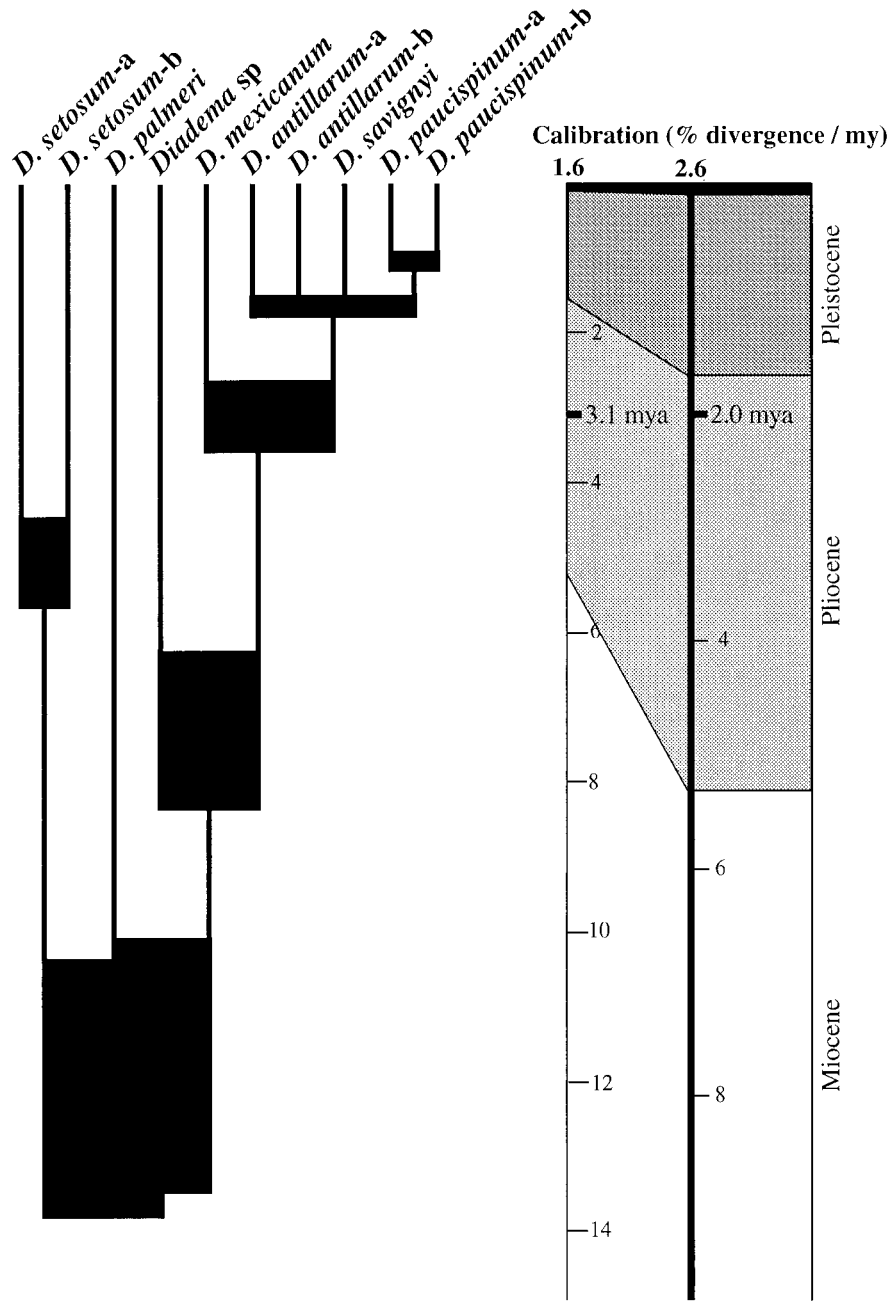

FIG. 4. Time scale for cladogenic events in *Diadema,* under the assumption that *D. antillarum* was split from *D. mexicanum* either at 2 or 3.1 mya. Rates of divergence of the combined COI and ATPase sequences (1.2 kb data) are shown above each scale. The point used for calibration in each scale is shown with a thick tick mark. Thickness of horizontal lines in the tree represents the maximum range of dating uncertainty as calculated from the maximum difference between divergence values that, under the assumption of a molecular clock, should have been equal (see text).

with the more gradual extinctions of other species of tropical echinoids and corals in New Zealand (Fell 1953; Fleming 1975; Knox 1980), presumably caused by severe cooling (Mercer 1983). Apparently, rather than going extinct, *Diadema* became adapted to the colder temperatures, but in the process was cut off from its tropical ancestral lineage.

It is pointless to speculate about a possible barrier that separated *Diadema*-sp from the rest of the lineage leading to *D. savignyi* 4.0–8.5 mya, because more extensive sampling is required to establish its true distribution. *Diadema*-sp haplotypes are abundant on the south coast of Honsu, but their presence at the Marshall Islands, 5000 km down-current from Japan, indicates a wider distribution in at least one direction.

The two mitochondrial lineages of *D. setosum* were separated from each other 2.7–5.7 mya (Fig. 4). Although it is possible that there is an area of overlap between Eritrea and Kenya, a region for which we have no samples, the two lineages appear to be allopatric. *Diadema setosum*-b may have had its origins in the Red Sea and then spread around the Arabian Peninsula into the Persian Gulf. The Red Sea first

opened to the Indian Ocean in the late Miocene (Girdler and Styles 1974; Adams 1981) or early Pliocene (Braithwaite 1987; Girdler and Southern 1987). Along with more widely distributed species, it contains an endemic fauna (Ekman 1953; Gohar 1954; Briggs 1974, 1995), including at least 19 endemic species of echinoderms (Campbell 1987). Klausewitz (1968), Por (1978), and Briggs (1995) have attributed this endemicity to possible isolation of the Red Sea during major glaciations. North of the strait of Bab-el-Mandab there is a sill only 125 m deep, which is likely to have become emergent during low sea-level stands and to have separated the Red Sea from the Indian Ocean. McMillan and Palumbi (1995) estimated from mtDNA sequence comparisons that a Red Sea endemic species of butterfly fish was separated from its Indian Ocean sister species no earlier than 850,000 years ago. Our dating of the separation of the two *D. setosum* lineages indicates that it occurred at pre-Pleistocene times. The fast time scale indicates the cladogenic event as roughly contemporaneous with early glaciations that started 3.2 mya and intensified 2.5 mya (Shackleton and Opdyke 1977; Shackleton et al. 1984). The sea-level drop about 3.5 mya was as drastic as those of the Pleistocene (Haq et al. 1987). Though it is not known whether the sill at the mouth of the Red Sea was present at that time, it is possible that the two *D. setosum* clades were separated by a reduction of sea level somewhere in the Red Sea, then spread around the Arabian Peninsula as the waters rose. That the Persian Gulf contains mtDNA haplotypes indistinguishable from those of the Red Sea is not surprising, considering that until 14,000 years ago the strait of Hormuz was closed, and the Gulf was lacustrine (Lambeck 1996). *Diadema* must have entered the Gulf with the marine intrusion that started 12,500 years ago. There is no obvious present-day barrier that would prevent *D. setosum*-a and *D. setosum*-b from invading the ranges of each other. Monsoon-related reversing currents (Wyrtki 1973) could easily bring larvae of each into the territory of the other, so geographic isolation is possibly due to ecological factors that prevent members of each clade from becoming established or surviving in the territory of the other.

The next cladogenic event is the separation of *D. mexicanum* shortly before the polyfurcation of all remaining major clades. As discussed in connection to the calibration of the *Diadema* molecular clock, the mtDNA phylogeny and the present-day distributions of the species are compatible with two hypotheses. The first hypothesis, that *D. mexicanum* speciated before the rise of the Isthmus, requires the postulation of two extinction events, one of the pan-Pacific ancestor in the eastern Pacific, the other of the *D. mexicanum* descendants in the Atlantic. There is also no obvious barrier that would account for speciation within the eastern Pacific shortly before the rise of the Isthmus. To be sure, an elaborate scheme can be constructed according to which *D. mexicanum* became isolated and speciated in the more isolated eastern Pacific islands, in a manner analogous to what occurred at a later time in *Eucidaris* (Lessios et al. 1999). But this scheme would require that, unlike *Eucidaris*, *D. mexicanum* would then invade the mainland coast after extinction of the ancestor of *D. savignyi*, *D. paucispinum*, and *D. antillarum*. The second hypothesis, that *D. mexicanum* and *D. antillarum* are indeed geminate species is consistent with present-day distributions, and thus does not need the postulation of any extinctions or of speciation events due to unknown barriers. It only requires that the isolation of the eastern Pacific from the central Pacific by the Eastern Pacific Barrier occurred before the rise of the Isthmus of Panama, which is consistent with geological (Grigg and Hey 1992) and biogeographic (Ekman 1953; Briggs 1974; Vermeij 1978, 1987) evidence. It is also consistent with what has been found in molecular phylogenies of *Eucidaris* (Lessios et al. 1999) and *Echinometra* (Palumbi 1996a; McCartney et al. 2000). Indeed, there would be no reason to doubt that the second hypothesis is the true one, if it were not for the indication that *D. antillarum* maintained connections with *D. savignyi* and *D. paucispinum* after the completion of the Isthmus of Panama, a pattern absent in other sea urchins. The only route by which this could have happened is gene flow around the southern tip of Africa. It is, therefore, necessary to discuss the importance of South Africa as a biogeographic barrier for tropical organisms.

The coast of Africa extends to 35°S, but its southeastern side is washed by the warm waters of the Agulhas Current (Peterson and Stramma 1991). This allows tropical organisms to spread southwest of Durban (Ekman 1953; Briggs 1974). Marshall et al. (1991) report that dense populations of *D. setosum* and *D. savignyi* are found at 32°S. The Agulhas Current divides, and part of it carries warm water into the southeastern Atlantic (Harris et al. 1978; Gordon 1985; Shannon et al. 1990). Intertidal communities (Brown and Jarman 1978) and inshore fishes (Penrith 1976) on the two sides of Cape Agulhas are similar, and populations of marine invertebrates are genetically continuous (Grant and Lang 1991; Grant et al. 1992). The biogeographic break between the Atlantic and Indian Oceans is caused by the Benguela cold current and upwelling system, which comes close to the southwestern coast of Africa at about 34°S (Shannon 1985). Organisms originating from the east coast of Africa cross from temperatures of 25°C at Durban (Heydorn 1978) to 9°C at Benguela (Andrews and Cram 1969). The Benguela upwelling first appeared in the Miocene (Diester-Haass and Schrader 1979; Siesser, 1980; Meyers et al. 1983), but it intensified in the late Pliocene (Meyers et al. 1983; Shannon 1985). At the Plio-Pleistocene boundary, 1.6 mya, the upwelling weakened, and its intensity has been experiencing cyclical fluctuations ever since (Meyers et al. 1983; Shannon 1985). The intensity of the Agulhas Current has also been fluctuating (Bé and Duplessy 1976; Hutson 1980), with substantial intrusions of warm water into the Atlantic seen to the present day (Shannon et al. 1990). Thus, the time at which gene flow between populations from the Indian Ocean and the eastern Atlantic ceased can vary according to thermal tolerance and chance. In *Eucidaris* and *Echinometra*, gene flow around the tip of Southern Africa had stopped before the establishment of the Isthmus of Panama. *Diadema* seems to have maintained connections by this route until a more recent time than the other echinoid genera. If its presence today at Madeira, the Canaries, and New Zealand, where other tropical sea urchin genera do not occur, is an indication of its thermal tolerance, then it might be understandable why its larvae could cross the Benguela barrier until 1–2 mya. Though modes of migration are very different, the contrast between *Eucidaris* and *Echinometra*, on one hand, and *Diadema*, on the other, is

reminiscent of the contrast between the tropical green turtle, *Chelonia mydas* (Bowen et al. 1992) and the more temperate loggerhead turtle, *Caretta caretta* (Bowen et al. 1994). The latter occasionally migrates between the Indian and the Atlantic Oceans, while the former does not.

The polyfurcation of *D. antillarum*-a, *D. antillarum*-b, *D. savignyi*, and *D. paucispinum* 1.02–1.86 mya in the Pleistocene or late Pliocene indicates that in *Diadema*—as in other tropical shallow water invertebrates (Briggs 1995:173) including Pacific *Echinometra* (Palumbi 1994, 1996b)—the times of glaciation were periods of active speciation. Isolation between local populations due to decreased sea levels and the alteration of currents was possible during these times. Because there were approximately 12 major and many smaller-scale glaciations in the Pleistocene (Crowley and North 1991), it is difficult to point to a particular sea-level fluctuation as the cause of each cladogenic event. It is also possible that the divergence of *D. paucispinum,* the only form found today at Hawaii and Easter Island, was caused by the chance arrival of larvae to either island, a period of isolation, and a subsequent spread of these differentiated haplotypes to the rest of the Indo-West Pacific. The modern-day North Equatorial Current flows westward past Hawaii.

The most puzzling split of the recent polytomy is that of *D. antillarum* into eastern and western Atlantic lineages. The separation of faunas from the two coasts of the Atlantic is not unexpected, because the ''mid-Atlantic barrier'' has been identified from comparisons of other species not held in common in the two areas (Ekman 1953; Briggs 1974, p. 109). The aspect that is difficult to explain is the difference in migration patterns among sea urchin genera. Both *Eucidaris* and *Echinometra* maintain strong genetic connections between the two coasts. Yet *Diadema,* the genus in which gene flow seems to have persisted longer through other barriers, was divided by this relatively minor obstacle to migration, and its larvae from one coast are still unable to establish themselves on the other.

Two relatively recent genetic breaks exist between the West Pacific and the Indian Oceans. *D. paucispinum*-a is limited to the central and western Pacific, whereas *D. paucispinum*-b is predominantly found in the Indian Ocean. A genetic discontinuity between oceans is also indicated in *D. setosum*-a by large and significant values of F_{ST} and by the presence of one DNA site diagnostic between populations from the two oceans. The separation does not cleanly coincide with the ocean borders in either species. *Diadema paucispinum*-b may be the only *D. paucispinum* mtDNA clade in the Indian Ocean, but it is also present in New Guinea and the Philippines (Fig. 3). The haplotypes of *D. setosum* along the western coast of Australia (where we found no *D. paucispinum*) all have the Pacific, not the Indian Ocean, diagnostic site. The magnitude of DNA differences is also not the same. Divergence between the two clades of *D. paucispinum* (in the 0.6 Kb data) corresponds to an estimated date of splitting of 0.6–0.9 mya. The DNA distance between African and West Pacific populations (including western Australia) in *D. setosum* is 0.40%, suggesting a date of separation of 0.17–0.27 mya. Interestingly, the third *Diadema* species that spans the two oceans, *D. savignyi,* shows no genetic discontinuity.

Nearly all previous genetic comparisons between western Pacific and Indian Ocean populations of marine organisms reported some degree of genetic differentiation (Benzie and Stoddart 1992; Benzie et al. 1993; Lacson and Clark 1995; McMillan and Palumbi 1995; Lavery et al. 1996; Miya and Nishida 1997; Williams and Benzie 1997, 1998; Chenoweth et al. 1998; Duke et al. 1998; Benzie 1999a; Duda and Palumbi 1999; Lessios et al. 1999; Williams et al. 1999; Barber et al. 2000, but see Stepien et al. 1994). Studies of genetic structure in the sea stars *Linckia laevigata* (Williams and Benzie 1997, 1998) and *Acanthaster planci* (Benzie 1999a), in which sampling was wide ranging and western Australian populations were included, show the same pattern as ours does for *D. setosum.* Western Australian populations of both sea stars are more similar to those from the western Pacific than they are to those of the Indian Ocean. The generally accepted explanation for divergence between conspecific populations from the two oceans is that during low sea water stands in the Pleistocene (Audley-Charles 1981; Galloway and Kemp 1981), there were repeated restrictions to the passage of Pacific water between Australia and Southeast Asia that lasted long enough for differentiation to arise. The similarity of western Australian populations to Pacific ones is attributed by Williams and Benzie (1997, 1998) and by Benzie (1999a) to the Indonesian throughflow that enters the Indian Ocean north of Australia (Murray and Dharma 1988; Gordon and Fine 1996) and then continues south along the western coast as the Leeuwin Current (Phillips et al. 1991). The same scheme of a Pleistocene historical barrier with subsequent haplotype leakage could apply to the patterns we found in *D. paucispinum* and *D. setosum.* The differences between the species in haplotype distribution and divergence suggests that the isolation occurred during different sea level fluctuations. As *D. setosum* is apparently absent from islands in the central Indian Ocean and from India (Clark and Rowe 1971), an alternative explanation for differentiation in this species between western and eastern Indian Ocean populations is isolation by distance.

In the Atlantic, the genetic discontinuity evident in both the F_{ST} statistics and in the phylogeny between populations of *D. antillarum*-a from the Caribbean and from Fernando de Noronha and the coast of Brazil is mostly likely caused by the freshwater plume of the Orinoco and the Amazon Rivers (Froelich et al. 1978; Muller-Karger et al. 1988). The Amazon has been flowing into the Atlantic since the late Miocene (Hoorn et al. 1995), and the barrier created by the reduced salinity of the coastal waters has led to a sufficiently high number of endemic Brazilian species to suggest that there is a separate tropical Brazilian province (Briggs 1974, pp. 68–71). Restrictions in gene flow between the Caribbean and the coast of Brazil are also evident in *Echinometra lucunter* (McCartney et al. 2000) and in the lobster *Panulirus argus* (Sarver et al. 1998), but not in *Eucidaris tribuloides* (Lessios et al. 1999). The phylogeny indicates that the propagules of *D. antillarum*-a that colonized Ascension and St. Helena came from the coast of Brazil (Fig. 1A), but there is no modern-day genetic exchange between these islands and their source populations. Genetic isolation of populations at these islands is also present in *Eucidaris* and *Echinometra.* However, in both of these genera, the similarity between populations on

the east and west coast of the Atlantic prevent any speculation as to the source of colonization of Ascension and St. Helena.

Genetic Connections

In addition to genetic discontinuities, *Diadema* also shows some remarkable instances of high gene flow between very distant areas. In *D. savignyi,* in particular, similar haplotypes exist from the eastern Pacific to the western Indian Ocean, and indistinguishable haplotypes extend from Kiribati in the central Pacific to the East African coast, a straight line distance of 18,000 km, and spanning two open-ocean barriers in the Pacific and one in the Indian Ocean (Vermeij 1987). Other marine organisms, such as *Echinometra* (Palumbi et al. 1997), the giant clams *Tridacna maxima* (Benzie and Williams 1997), and *T. gigas* (Benzie and Williams 1995), show regional differentiation between central and western Pacific populations. In *D. paucispinum*-a, indistinguishable haplotypes are found at Hawaii and Easter Island, a distance of 7100 km, running perpendicular to the flow of both the North and the South Equatorial Currents. In *D. antillarum*-a, haplotypes are shared between Ascension and St. Helena, a distance of 1300 km. That larvae originating on one small island in the middle of the Atlantic or central Pacific can actually encounter another island so far away in sufficient numbers to establish such high rates of gene flow is remarkable. This tremendous capacity for dispersal raises the question of how historical barriers that have ceased to exist for millions of years, such as the ones we and others (reviews in Avise 1992, 2000; Palumbi 1994; Benzie 1999b) have suggested, can still leave their genetic signature on contemporary populations. The expected genetic pattern after the removal of a barrier would be the one seen on either side of Australia in both *D. setosum*-a and *D. paucispinum*-b, that is, remnants of differentiation, coupled with leakage across the line defined by the previous barrier. Given the capacity for renewed dispersal, the only explanation that can fit cases of complete allopatry between mtDNA clades without any intervening present-day barrier, such as those seen between *D. setosum*-a and *D. setosum*-b and between *D. antillarum*-a and *D. antillarum*-b, is that these mtDNA lineages represent different species, and that ecological factors (physical tolerances or competition) prevent haplotypes of each species from establishing themselves within the range of the other. This postulate requires not just the emergence of reproductive isolation during the allopatric phase, but also adaptation to the source area and ecological divergence, all of which are difficult to study among geographically separated populations.

Implications for Speciation

As mentioned in the introduction, Mayr's (1954) suggestion that the geographic overlap of *D. setosum* and *D. savignyi* was the result of ''double invasion'' appeared, in the absence of phylogenetic information, to be an ad hoc hypothesis in favor of allopatric speciation. Mayr's hypothesis to account for the sympatry of these two species may have not received support by the current data, but his main conclusion, that speciation in tropical echinoids is allopatric, holds. The phylogeny of *Diadema* is much more consistent with the expectations of an allopatric than a sympatric model. This is not because *D. setosum* and *D. savignyi* have been confirmed not to be completely sympatric, nor because we have been able to suggest a plausible geographic barrier as the cause of nearly every cladogenic event. Though the possibility of a barrier is reassuring, such suggestions are based on correlations between estimated time since divergence and geological history, and can thus only be regarded as hypotheses subject to further testing. They also incorporate the assumption, necessary in any phylogeographic reconstruction, that the present-day geographical ranges of the clades have at least some overlap with the ancestral range. The support for allopatric provided by the revealed phylogenetic relationships between species of *Diadema* comes from their fit, with one exception, to Jordan's (1908) ''law of the geminate species,'' according to which the most closely related species in a genus are allopatric, and only the more distantly related ones overlap. Indeed, the mtDNA phylogeography of *Diadema* includes all the stages expected from models of allopatric differentiation. There are anciently separated clades that now overlap in their geographic distribution (*D. setosum*-a, *D. savignyi*); clades isolated in the periphery of the genus range that have remained in the periphery (*D. setosum*-b; *D. palmeri*); clades that may have been isolated in the periphery but have since spread towards the center (*Diadema*-sp, *D. paucispinum*-a); closely related clades on either side of an existing barrier (*D. mexicanum, D. antillarum*-a, and *D. savignyi*), and closely related monophyletic entities on either side of an historical barrier that have crossed the former barrier line, but have not attained equilibrium (*D. setosum*-a, *D. paucispinum*-b).

The exception to Jordan's ''law'' is not, as Mayr had thought, *D. setosum* and *D. savignyi* (for they are distantly related), but the *D. savignyi-D. paucispinum* pair. For these two nominal species, a model of sympatric speciation would suggest that their mtDNA lineages have diverged because of the emergence of reproductive isolation somewhere in the range of the ancestral taxon. An allopatric model, on the other hand, would assume that divergence occurred during geographic isolation, and that contact between different clades now occupying the same area was secondary. The second explanation appears more likely, because isozyme data indicate that *D. paucispinum* and *D. savignyi* in Okinawa produce viable hybrids in nature (Lessios and Pearse 1996). It is easy to envision hybridization due to secondary contact between populations that did not perfect strong reproductive isolation in allopatry, but it is hard to imagine divergence of mtDNA lineages between populations that have always been sympatric and incompletely isolated reproductively. Indeed, in the absence of nuclear markers from any locality except Hawaii and Okinawa, we cannot exclude the possibility that the mtDNA lineages of *D. paucispinum* we encountered in Indo-West Pacific may all exist in descendants of hybrids with a *D. savignyi* nuclear background. Depending on the extent of introgression, *D. paucispinum* and *D. savignyi* may be one species that contains two divergent mtDNA lineages. If so, there is no question of how they speciated, but there is still a question of how these mtDNA lineages diverged. If reproductive isolation is weak or absent, this differentiation could have only happened in allopatry. Thus, despite a demonstration of tremendous capacities of dispersal in *Diadema,* the mtDNA phylogeny provides a great deal of evidence in

favor of speciation caused by geographical barriers, and little that is consistent with a model of sympatric speciation.

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