



Indo-Pacific echinoids in the tropical eastern Pacific

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Abstract. The existing literature reports that only one species of Indo-Pacific echinoid (*Echinometra oblonga*), occurs in the eastern Pacific. In this study we confirm the presence of this species at Islas Revillagigedo and also report the presence of two species of *Echinothrix* (a genus hitherto unknown outside the Indo-Pacific) at Isla del Coco and at Clipperton Island. We also present evidence from isozymes and from mitochondrial DNA sequences indicating that at least one individual of *Diadema* at Clipperton may belong to a maternal lineage characteristic of the west Pacific species *D. savignyi*. These data are consistent with the hypothesis that the observed populations of Indo-Pacific echinoid species are recent arrivals to the eastern Pacific, as opposed to the view that they are relicts of Tethyan pan-tropical distributions. *Echinothrix diadema*, in particular, may have arrived at Isla del Coco during the 1982–1983 El Niño. In addition to Indo-Pacific species, Clipperton, Isla del Coco and the Revillagigedos contain a complement of eastern Pacific echinoids. The echinoid faunas of these islands should, therefore, be regarded as mixtures of two biogeographic provinces. Though none of the Indo-Pacific species are known to have reached the coast of the American mainland, their presence at the offshore islands of the eastern Pacific suggests that, for some echinoids, the East Pacific Barrier is not as formidable an obstacle to migration as was previously thought.

Introduction

The large expanse of deep water between the Line Islands in the central Pacific and Clipperton Atoll in the eastern Pacific is habitat inhospitable to adults of shallow water animals. Because this 5400 km stretch has existed for

a long time, possibly since the beginning of the Cenozoic (Grigg and Hey 1992), it has resulted in distinct biogeographic differences between the two regions. The “East Pacific Barrier”, as it has been named, is held to constitute the greatest marine obstacle to the dispersal of organisms that live in the sea (Ekman 1953; Briggs 1961; Grigg and Hey 1992). Though most taxa show very small degrees of faunal similarity on the two sides of the East Pacific Barrier (Ekman 1953; Vermeij 1978, 1987; Emerson 1967, 1978, 1982), there are species with “amphipacific” distributions among the Mollusca (Emerson 1967, 1978, 1982, 1991; Vermeij 1978, 1987; Zinsmeister and Emerson 1979), Crustacea (Chase 1942; Garth 1965, 1974; Holthuis and Loesch 1967), Asteroidea (Nishida and Lucas 1988), and particularly corals (Dana 1975; Glynn and Wellington 1983; Grigg and Hey 1992) and fishes (Rosenblatt et al. 1972; Briggs 1974; Leis 1983). These transpacific species have been interpreted either as resulting from transport of larvae across the barrier (Dana 1975; Glynn and Wellington 1983; Rosenblatt and Waples 1986; Vermeij 1987; Richmond 1990; Grigg and Hey 1992) or as the vicariantly separated remnants of previously continuous Tethyan distributions (McCoy and Heck 1976; Heck and McCoy 1978; Rowe 1985; Emerson 1991).

On the basis of the existing literature, shallow water echinoids appear to conform to Darwin’s (1872:361) notion that the East Pacific Barrier is “impassable”. A compilation of tropical echinoid species ranges by Mayr (1954), used to deduce patterns of speciation in the group, shows not a single transpacific species, leading him to conclude that “of the existing barriers, by far the most potent has been that between Polynesia and the American west coast”. In a more recent exhaustive compilation of the range of eastern Pacific echinoderms, Maluf (1988) shows only one Indo-Pacific species of regular echinoid, *Echinometra oblonga*, as being present in the eastern Pacific. Ziesenhenné (1937) has reported *E. oblonga* from Clarión; Clark (1948) lists it as occurring at Clarión, Socorro and the Galapagos Islands, but states that “it intergrades . . . in a perplexing way” with the eastern Pacific *E. vanbrunti*. Rowe (1985), in a review of the records of echinoderms

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with reported amphipacific distributions, concluded that reported transpacific species in this phylum are either the result of inadequate taxonomic study or Tethyan remnants of morphologically conservative self-sustaining separate populations.

Here we present morphological and molecular evidence from specimens collected in recent expeditions which shows that the echinoid fauna of a number of the eastern Pacific islands is a mixture of east and west Pacific species. We confirm the existence of *Echinometra oblonga* in the eastern Pacific and report that both species of the Indo-Pacific genus *Echinothrix* (*diadema* and *calamaris*) have arrived in the eastern Pacific (possibly recently). We also present molecular evidence indicating that *Diadema* genotypes at these islands are represented not just by the eastern Pacific *D. mexicanum*, but also by the Indo-west Pacific *D. savignyi*.

Materials and methods

Material examined

We collected specimens and made observations during cruises of the R/V Benjamin to Isla del Coco (5° 32' N, 87° 04' W) on 2–7 December 1987, of the R/V Gyre to the same island 7–9 November 1990, and of the M/V Royal Star between 11 April and 3 May 1994 to the Islas Revillagigedo [Clarión (18° 22' N, 114° 44' W), Roca Partida (19° 01' N, 112° 02' W), San Benedicto (19° 18' N, 110° 49' W)] and to Clipperton (10° 17' N, 109° 13' W). On the Gyre cruise we made quantitative determinations of the density of echinoids in three transects placed at depths of 3–5 m in areas where at least one haphazardly chosen individual of *Echinothrix diadema* was observed. Three diadematoid specimens collected during the Royal Star cruise by R. van Syoc and deposited in the California Academy of Sciences were also examined; regions of mitochondrial DNA were amplified and sequenced from one of them (see later). We additionally identified the diadematoid collections of the Los Angeles County Museum, obtained at Isla Socorro and Isla del Coco between 23 April 1988 and 25 March 1989. Finally, we examined all diadematoids from the eastern Pacific (identified and unidentified) from the US National Museum of Natural History.

Electrophoresis of proteins

No good morphological characters exist to differentiate between Indo-Pacific and eastern Pacific species of the genus *Diadema*. We, therefore, resorted to molecular characters in an effort to determine whether individuals of this genus at Isla del Coco and Clipperton belong to the eastern Pacific *D. mexicanum*, the western Pacific *D. setosum* and *D. savignyi*, or the predominantly Hawaiian *D. paucispinum*. We froze (in liquid nitrogen) both the gut and Aristotle's lantern muscle tissues of *Diadema* at Isla del Coco during the R/V Benjamin cruise, and the muscle of *Diadema* at Clipperton during the M/S Royal Star cruise. These specimens were assayed for the following enzymes (abbreviations in parentheses):

Clipperton. acid phosphatase (ACPH), glucose-6-phosphate-dehydrogenase (G6PDH), aspartate aminotransferase (AAT), isocitrate dehydrogenase (IDH), mannose-6-phosphate isomerase (M6PI), malate dehydrogenase (MDH), L-leucyl-L-tyrosine peptidase (PEPLT), phosphoglucose isomerase (PGI) phosphoglucomutase (PGM), triosephosphate isomerase (TPI).

Isla del Coco. ACPH, G6PDH, AAT, IDH, M6PI, MDH, octanol dehydrogenase (ODH), PEPLT, PGI, PGM, superoxide dismutase

(SOD), TPI, and xanthine dehydrogenase (XDH). Running buffers and staining recipes are listed in Lessios (1981) and Lessios and Pearse (in press 1996). To compare electrophoretic patterns of *Diadema* from Clipperton and Coco to those of other species of the genus, we used data from Bermingham and Lessios (1993) for the eastern Pacific *D. mexicanum* and from Lessios and Pearse (in press 1996) for the three species of the western Pacific. They are based on collections of *D. mexicanum* from Panama and from Guymas, Mexico ($n = 17$ –53 per locality), of *D. paucispinum* from Honolulu, Hawaii ($n = 21$), and of *D. savignyi* ($n = 23$) and *D. setosum* ($n = 28$) from the Ryukyu Islands. Allozymes were standardized by including individuals from the known species in gels containing specimens from Clipperton and Isla del Coco.

Mitochondrial DNA sequencing. Electrophoretic assays of proteins permit many samples to be processed with relative ease and provide information on both the maternal and the paternal lineages of each specimen. However, while the electrophoretic results excluded the possibility that any of the *Diadema* specimens from the eastern Pacific belonged to *D. setosum*, they proved to be less powerful in differentiating between *D. mexicanum*, *D. savignyi*, and *D. paucispinum* (see results). For this reason, we sequenced 585 nucleotides of mitochondrial DNA (mtDNA) in 5 individuals of *Diadema* from Clipperton and 3 individuals from Coco. Seven samples consisted of muscle from the Aristotle's lantern from individuals that were also included in the electrophoretic survey. One sample was muscle from the base of primary spines of an individual collected by R. van Syoc (California Academy of Sciences Catalog Number 100751), which had been preserved in 75% ethanol. We used *Diadema savignyi* from the Ryukyus ($n = 2$), Samoa ($n = 3$) and Guam ($n = 4$), *D. paucispinum* from Hawaii ($n = 8$) and *D. mexicanum* from Panama ($n = 3$) and Mexico ($n = 2$) as references for the expected DNA sequences from individuals with known species affiliations.

Genomic DNA was isolated by lysing 10–30 µg of muscle or gonad tissue at 65 °C for 1–3 hours in 60 µL of proteinase K preparation [50 mM Tris (pH 9.0), 20 mM ammonium sulfate, 1.5 mM magnesium chloride, 1 µg/ml proteinase K, 1% NP-40]. Then, the proteinase was inactivated by heating the sample to 95 °C for 5–15 min, the lysate was diluted with 140 µL of H₂O, and centrifuged at 16 000 g for 5 min. The supernatant was stored at –20 °C. Genomic DNA of one *D. paucispinum* specimen was extracted by the CTAB procedure described in Lessios et al. (1995). In one individual of *D. mexicanum*, purified mtDNA was obtained from cesium chloride gradient centrifugation (Lansman et al. 1981) instead of genomic extractions.

Primers for the polymerase chain reaction (PCR) (Saiki et al. 1988) were designed to amplify 626 base pairs (of which 585 were sequenced) starting at position 8364 within the cytochrome oxidase II protein coding region, spanning the lysine t-RNA, and ATPase 8 regions, and ending at position 8990 of the ATPase 6 region of sea urchin mtDNA [positions refer to *Strongylocentrotus purpuratus* sequence of Jacobs et al. (1988)]. The two primers were CO2b: 5'-GAATCTGTTCTTTCTCTAC-3', and ATP6b: 5'-GCCAGGTAGAACCCGAGAAT-3'. PCR amplification was performed in 50 µL of PCR reaction mixture [0.5 units of Tfl thermostable DNA polymerase (Epicentre Technologies), 1 µM of each primer, 0.8 mM dNTPs, 50 mM Tris (pH 9.0), 20 mM ammonium sulfate, 1.5 mM MgCl₂]. The samples were cycled 40 times through 95 °C for 30 s, 50–55 °C for 30 s, 72 °C for 60 s. The PCR products were purified by running them on 2% agarose gels, removing the band, and recovering the DNA with the GeneClean kit (BIO 101, Inc.). Cleaned PCR products were resuspended in 21 µL of H₂O. 7 µL of the suspension were cycle-sequenced using one of the PCR primers and the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit from Applied Biosystems. The products of the sequencing reactions were run on a 373 A automatic sequencing machine from Applied Biosystems. All individuals were sequenced at least twice from separate amplifications to minimize the possibility of artifacts due to contamination. Thirty-three percent of the bases were further verified through the sequencing of both complementary strands.

Table 1. Shallow-water regular echinoids observed by at least one of the authors at each of the eastern Pacific islands, or found in museum collections. + : present.

+ + : abundant, ? : genus was present, but specimens were not identified to species. Abbreviations: OO, own observations; LACM, specimens at LA County Museum; CAS, specimens at California Academy of Sciences; NMNH, specimens at the National Museum of Natural History. All identifications are by the authors

	Isla del Coco	Clipperton	Revillagigedos	Documentation
Indo-Pacific species				
<i>Echinothrix diadema</i>	++	++	++	OO, LACM
<i>Echinothrix calamaris</i>	+	++	++	OO, CAS, NMNH
<i>Diadema setosum</i>	-	-	-	OO
<i>Diadema savignyi</i>	-	+	?	CAS (OO mtDNA id)
<i>Diadema paucispinum</i>	-	-	-	OO
<i>Echinometra oblonga</i>	-	-	++	OO; Clark (1984)
Eastern Pacific species				
<i>Eucidaris thouarsi</i>	++	++	++	OO; Clark (1948)
<i>Diadema mexicanum</i>	++	++	++	OO; Clark (1948)
<i>Tripneustes depressus</i>	++	++	++	OO; Clark (1948)
<i>Echinometra vanbrunti</i>	++	++	++	OO; Clark (1948)

Table 2. Density (N/m²) of echinoids in three transects at Chatham Bay

Transect number	Depth (m)	Area (m ²)	<i>Echinothrix diadema</i>	<i>Diadema sp.</i>
1	5	1.8	16.1	5.6
2	4	11.4	0.6	6.1
3	3	3.3	3.0	1.0

Results

Our observations regarding the existence of Indo-Pacific and east Pacific species of echinoids at the offshore islands of the eastern Pacific are summarized in Table 1. The shallow water regular echinoid fauna of Isla del Coco, Clipperton and the Revillagigedos includes species from both sides of the East Pacific Barrier. Most of the west Pacific species are represented by a large number of individuals. *Echinothrix diadema* was abundant in both Bahia Wafer and Bahia Chatham at Isla del Coco in 1987. Our quantitative survey at Bahia Chatham in 1989 revealed that in water 3–5 m deep, it had densities equivalent to those of *Diadema mexicanum* (Table 2). The Los Angeles County Museum also contains specimens of *E. diadema* from Bahia Wafer and Bahia Weston at Isla del Coco. All specimens of this species from Isla del Coco have dates of collection more recent than 1987. The same is true for *E. calamaris*, with one exception. We found one unidentified specimen of this species from Isla del Coco in the National Museum of Natural History (Cat. No. G00431). It was collected by W. L. Schmitt on 24 April 1941 at a depth of “25 fathoms”, which, if accurate, is close to the lower known limit of this species (Mortensen 1940:290). *Echinometra oblonga*, on the other hand, is stated by Clark (1948) to have been common at Sulphur Bay, Clarión Island and at Braithwaite Bay, Socorro Island during the Velero-III expeditions (1931–1941). Unlike *Echinothrix*, which we have never seen at the Galapagos, *Echinometra oblonga* was also present in three islands of this archipelago (Clark 1948). Our own specimens of this species come from Roca Partida, Revillagigedos. The pronounced elongate oval and high test, the long stout aboral primary spines, the jet-black coloration, and the presence of only 4 pore-pairs per arc aborally, leave no doubt that they

belong to *E. oblonga* rather than to the east Pacific *E. vanbrunti*. Though Mortensen (1943:392) considered *E. oblonga* as a variety of *E. mathaei*, recent molecular evidence (Palumbi and Metz 1991) has shown conclusively that it is a separate species. The characters listed and the absence of any hint of white rings at the base of primary spines place the specimens from Roca Partida in *E. oblonga*.

Though *Echinothrix* and *Echinometra oblonga* cannot be mistaken for eastern Pacific species, *Diadema mexicanum* is very hard to tell apart from two of the three western Pacific species of the same genus. The large notched tridentate pedicellariae said by Mortensen (1940:256) to be characteristic of this species are often absent and unreliable as a specific character (Chesher 1972, and own observations). Blue lines running down the test, a character used to distinguish between Indo-Pacific species, are sometimes present and sometimes absent in *D. mexicanum*. The only diagnostic morphological feature in the specimens we examined from Coco and Clipperton, was that none had the elongate tridentate pedicellariae and orange anal ring usually found in *D. setosum*.

The gene frequencies of *Diadema* from Isla del Coco, and from Clipperton Island, as well as those from the reference collections of each of the known species of *Diadema* found in the Pacific Ocean; are given in Table 3. There were two diagnostic loci, *Sod* and *Tpi*, distinguishing *D. setosum* from all other Pacific species of *Diadema*. The specimens from Isla del Coco could be said with certainty not to belong to this species, because at both of these loci they carried alleles not found in *D. setosum*. Similarly, the specimens from Clipperton could be distinguished from *D. setosum* by their *Tpi* genotype. However, none of the loci turned out to be diagnostic between *D. mexicanum*, *D. paucispinum*, and *D. savignyi*, so it was necessary to rely on

Table 3. Sample size (number of individuals) and gene frequencies of *Diadema* at Clipperton and Isla del Coco, and for each of four Pacific species of the genus. Gene frequencies of *D. mexicanum* from Mexico and Panama have been pooled. See text for locus abbreviations : no data

Locus	Allele	<i>Diadema</i> Clipperton	<i>Diadema</i> Coco	<i>D. mexicanum</i> Mexico + Panama	<i>D. paucispinum</i> Hawaii	<i>D. savignyi</i> Ryukyus	<i>D. setosum</i> Ryukyus
<i>AcpH</i>	N	21	27	57	21	23	28
	90	0.000	0.000	0.000	0.000	0.022	0.446
	100	0.595	0.574	0.658	0.881	0.565	0.482
	200	0.405	0.426	0.342	0.119	0.413	0.071
<i>G6pdh</i>	N	20	30	43	21	23	28
	100	1.000	1.000	1.000	1.000	1.000	1.000
<i>Aat-1</i>	N	21	27	58	21	23	28
	100	1.000	1.000	1.000	1.000	1.000	1.000
<i>Idh</i>	N	21	29	44	21	21	28
	90	0.000	0.000	0.080	0.000	0.000	0.000
	100	1.000	1.000	0.921	1.000	1.000	1.000
<i>M6pi</i>	N	21	29	38	21	23	28
	95	0.024	0.000	0.026	0.000	0.000	0.000
	98	0.191	0.172	0.224	0.071	0.022	0.054
	100	0.548	0.586	0.382	0.452	0.087	0.107
	105	0.095	0.190	0.145	0.214	0.065	0.143
	106	0.071	0.035	0.171	0.095	0.217	0.536
	110	0.071	0.017	0.040	0.048	0.370	0.089
	111	0.000	0.000	0.000	0.071	0.196	0.018
	112	0.000	0.000	0.013	0.048	0.044	0.054
<i>Mdh-1</i>	N	21	30	55	21	23	28
	98	0.000	0.017	0.000	0.000	0.000	0.000
	100	1.000	0.983	0.982	0.643	0.935	0.982
	103	0.000	0.000	0.018	0.357	0.065	0.018
<i>Mdh-2</i>	N	20	21	50	21	23	27
	100	0.975	1.000	1.000	1.000	1.000	1.000
	103	0.025	0.000	0.000	0.000	0.000	0.000
<i>Odh</i>	N	–	30	49	20	23	28
	100	–	1.000	1.000	1.000	1.000	1.000
<i>Pept-1</i>	N	21	30	57	21	23	28
	90	0.000	0.000	0.000	0.476	0.000	0.000
	95	0.000	0.000	0.000	0.381	0.000	0.000
	100	1.000	0.983	0.895	0.143	1.000	1.000
	102	0.000	0.000	0.070	0.000	0.000	0.000
<i>Pept-2</i>	N	21	30	59	21	23	28
	98	0.000	0.000	0.085	0.000	0.000	0.000
	100	0.714	0.983	0.814	1.000	1.000	1.000
	102	0.286	0.017	0.102	0.000	0.000	0.000
<i>Pgi</i>	N	21	30	54	21	23	28
	80	0.000	0.000	0.000	0.000	0.000	0.018
	95	0.000	0.000	0.000	0.071	0.109	0.250
	98	0.000	0.067	0.083	0.024	0.109	0.107
	99	0.000	0.000	0.000	0.000	0.044	0.000
	100	0.952	0.850	0.852	0.714	0.609	0.536
	104	0.048	0.083	0.065	0.167	0.065	0.071
	105	0.000	0.000	0.000	0.024	0.022	0.018
	106	0.000	0.000	0.000	0.000	0.044	0.000
<i>Pgm-1</i>	N	21	30	53	21	23	28
	80	0.000	0.000	0.000	0.000	0.000	0.018
	88	0.000	0.000	0.094	0.000	0.000	0.000
	96	0.000	0.000	0.000	0.024	0.044	0.179
	98	0.071	0.133	0.236	0.286	0.087	0.357
	100	0.452	0.700	0.453	0.333	0.500	0.339
	106	0.000	0.000	0.000	0.238	0.152	0.018
	108	0.405	0.150	0.170	0.119	0.109	0.071
	110	0.071	0.017	0.047	0.000	0.109	0.018

Table 3. (Continued)

Locus	Allele	<i>Diadema</i> Clipperton	<i>Diadema</i> Coco	<i>D. mexicanum</i> Mexico + Panama	<i>D. paucispinum</i> Hawaii	<i>D. savignyi</i> Ryukyus	<i>D. setosum</i> Ryukyus
Sod	N	—	30	47	21	23	28
	100	—	1.000	1.000	0.929	1.000	0.000
	110	—	0.000	0.000	0.071	0.000	1.000
<i>Tpi</i>	N	20	29	43	21	23	28
	80	0.000	0.000	0.012	0.000	0.000	0.000
	90	0.100	0.017	0.174	0.000	0.000	0.000
	100	0.900	0.983	0.814	1.000	1.000	0.000
	105	0.000	0.000	0.000	0.000	0.000	1.000
<i>Xdh</i>	N	—	30	49	21	23	28
	100	—	1.000	1.000	1.000	1.000	1.000

composite genotypes to identify specimens from the eastern Pacific islands. For this analysis, we assigned each individual a score of 0 if it did not carry a particular allele, a score of 1 if it carried the allele in the heterozygous condition, and a score of 2 if it carried it in the homozygous condition. This created a matrix of 52 variables, one for each allele observed in every locus. Two separate discriminant functions were calculated, one for loci assayed for specimens from Isla del Coco and one for those assayed from Clipperton. The discriminant functions were based on specimens of “known” species affiliation (i.e., specimens from the eastern Pacific offshore islands were excluded). Because allele frequencies alone eliminated the possibility that our “unknown” specimens belonged to *D. setosum*, this species was not included in the multivariate analysis. Calculated discriminant functions were then used to classify specimens from Isla del Coco and from Clipperton.

The results of the multivariate analyses are shown in Fig. 1. The discriminant functions are capable of separating specimens of known species on the basis of their composite genotypes, with one function misclassifying only one and the other function only two specimens of *D. paucispinum* as *D. savignyi* (Table 4). When specimens from the eastern Pacific Islands were classified by these functions, six specimens from Isla del Coco were identified as belonging to *D. savignyi*, three of them with marginal (0.54–0.57) probability. These three are almost certainly artifacts of the multivariate analysis. However, the other three specimens from Coco were assigned to *D. savignyi* with high (0.95–0.99) probabilities. One specimen from Clipperton fell squarely into the *D. savignyi* cluster with an assignment probability of 1 (Fig. 1). To further investigate whether these four specimens belonged to *D. savignyi* we sequenced part of their mtDNA.

The mtDNA sequences proved to be highly species-specific. Though some sites (not shown here) were variable within each species, there were 23 nucleotide positions that were diagnostic between *D. savignyi*, *D. paucispinum* and *D. mexicanum* (Table 5). There were six diagnostic amino acid replacements (positions 8539, 8606, 8611, 8644, 8633, and 8873–4). Three specimens of *Diadema* from Coco and four from Clipperton, including those identified by isozymes as *D. savignyi*, proved to possess mtDNA sequences that did not differ in any diagnostic position from those of *D.*

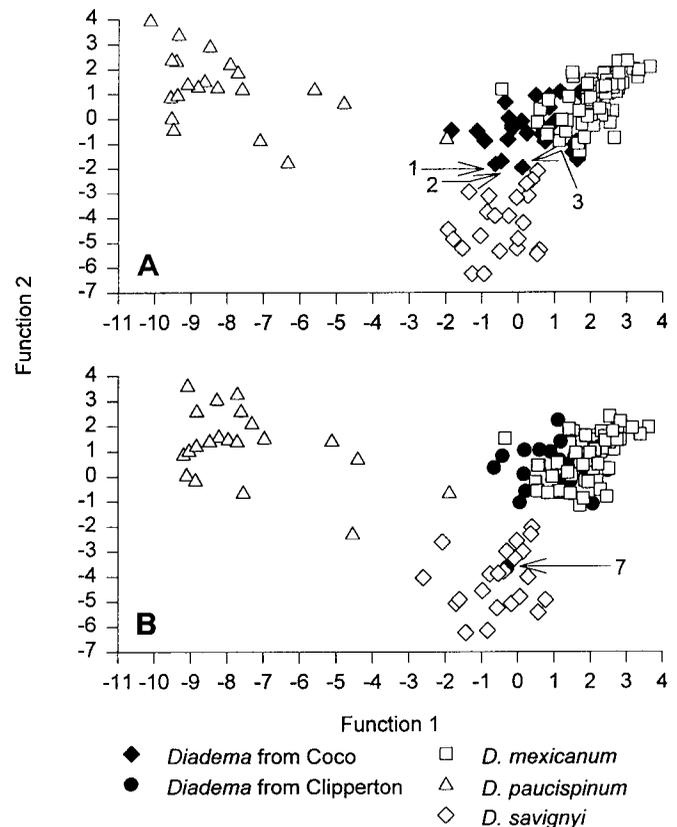


Fig. 1A, B. Position of individuals of known species affiliation and of unknown specimens from Isla del Coco and Clipperton Island in space defined by the first two discriminant functions calculated from composite isozyme genotypes. **A** functions calculated from individuals of known species affiliation assayed for the same loci as those of *Diadema* from Isla del Coco; **B** functions calculated from individuals of known species affiliation assayed for the same loci as those of *Diadema* from Clipperton. Numbers at the ends of arrows identify individuals that were classified by the discriminant functions as *Diadema savignyi* with a probability ≥ 0.95 . Mitochondrial DNA from these individuals was sequenced (see Table 5)

mexicanum. However, the fifth specimen from Clipperton (*Diadema*-8), for which there are no isozyme data because it had been preserved in alcohol, had an mtDNA sequence that was very similar to that of *D. savignyi*, differing only in

never before been reported from the east Pacific. Nor are the specimens from Coco and Clipperton likely to belong to new species, because we have been unable to find any characters in either our own or in museum specimens of *Echinothrix diadema* or *E. calamaris* from the eastern Pacific that differ from those of specimens of the respective two species from Hawaii. Though Clark (1948) writes of intergradation between *Echinometra oblonga* and *E. vanbrunti*, our specimens from the Revillagigedos are typical of *E. oblonga* from the west Pacific. It would also be unreasonable to suggest that the mtDNA sequence of individual *Diadema*-8 might belong to a new, undescribed species of *Diadema*, just because it differs in one third-codon diagnostic position from the typical sequence of *D. savignyi* from the Ryukyus and Guam. We, therefore, think that our data leave little doubt that Indo-Pacific species of regular echinoids are present in the east Pacific offshore islands. It is obvious from our own quantitative data of *Echinothrix diadema*, from our subjective evaluations of the abundance of *Echinothrix calamaris* from Clipperton, and from Clark's (1948) report of 85 specimens of *Echinometra oblonga* in the *Velero* collections that these species have substantial population densities in the eastern Pacific. It is, therefore, possible to regard these species as established on the offshore islands of the eastern Pacific, rather than as temporary invasions. Typical eastern Pacific species are also present on the offshore islands, often side-by-side with the Indo-Pacific ones. Thus, one is justified in considering Isla del Coco, Clipperton and the Revillagigedos as containing an echinoid fauna that is a mixture of two biogeographic provinces.

Our effort to identify the species of *Diadema* from the east Pacific islands by molecular means may seem excessive. However, the question of the exact limits of the ranges of *D. setosum*, *D. savignyi*, and *D. paucispinum* and the question of whether any of them is sympatric with *D. mexicanum* is crucial for understanding patterns of speciation and phylogeny in the genus. Because described species of *Diadema* are morphologically so similar, the working practice of systematists (including ourselves) has been to assign specimens of this genus to species on the basis of the locality from which they came. Indeed, some of the species descriptions are based entirely on this criterion (see Mortensen 1940:254; Mayr 1954). The result has been continuing confusion about the biogeography of this genus. For example, the reported presence of *D. mexicanum* at Easter Island is said by Fell (1974) to be the result of misidentification of specimens of *D. savignyi* from this area. Though in biogeographic treatments reports of absence of a taxon from an area must always be regarded as subject to revision, our samples from Coco and Clipperton indicate that neither the western Pacific *D. setosum* nor the predominantly Hawaiian *D. paucispinum* are likely to be present in these islands. However, the data also show that the range of *D. savignyi* may well extend to the eastern Pacific. Because mtDNA is maternally inherited, and because this specimen was preserved in alcohol (which prevented isozyme assaying), it is not possible to say whether the specimen from Clipperton found to possess *D. savignyi* mtDNA is the offspring of a *D. savignyi* or a *D. mexicanum* father. There is also no way of knowing whether any of the

specimens assigned by the discriminant analysis to *D. savignyi* on the basis of their nuclear genotypes might be hybrids, or artifacts of the discriminant analysis. That all of the specimens that the discriminant analysis assigned to *D. savignyi* with high probability turned out, by mtDNA analysis, to have a *D. mexicanum* mother suggests that the limitations of the discriminant analysis are responsible for the identification of some specimens as having Indo-Pacific origins. The misclassification by the discriminant functions of two specimens of *D. paucispinum* as *D. savignyi* would also favor this interpretation. However, even if all isozyme multilocus identifications of *D. savignyi* in the eastern Pacific are wrong, the mtDNA haplotype of specimen *Diadema*-8 indicates that representatives of *D. savignyi* have been present in the eastern Pacific in modern times.

Echinoids are not the only taxon in which Indo-Pacific species have been reported in the eastern Pacific. In addition to corals (Dana 1975; Glynn and Wellington 1983; Grigg and Hey 1992) and fishes (Rosenblatt et al. 1972; Briggs 1974; Leis 1983, Robertson & Allen 1996), reliable reports of transspecific species include those for mollusks (Hertlein 1938, 1963; Emerson 1967, 1978, 1982, 1991; Robertson 1976, 1979; Vermeij 1978, 1987, 1991; Zinsmeister and Emerson 1979; Shasky 1983a, b, 1984, 1985), Crustacea (Chase 1942; Hertlein 1963; Garth 1965, 1974; Holthuis and Loesch 1967), Asteroidea (Nishida and Lucas 1988) and Ophiuroidea (Ekman 1953). For groups with small degrees of faunal similarity on the two sides of the East Pacific Barrier it is generally assumed that such anomalous distributions are the result of recent breaching of the barrier (Hertlein 1938; Clark 1948; Ekman 1953; Emerson 1967, 1978, 1982; Vermeij 1978, 1987; but see Emerson 1991). However, groups such as corals and fishes, in which a relatively large proportion of eastern Pacific species are of west Pacific origin, have been the focus of continuing biogeographic debate regarding the interpretation of their disjunct distributions. "Center-of-origin" biogeographers (Dana 1975; Glynn and Wellington 1983; Rosenblatt and Waples 1986; Richmond 1990; Grigg and Hey 1992) have explained the presence of transspecific corals and fishes as the result of larval dispersal across the East Pacific Barrier, made easier since the late Pleistocene by the movement of the Line Islands into the path of equatorial countercurrents. "Vicariance" biogeographers (McCoy and Heck 1976; Heck and McCoy 1978; Rowe 1985), on the other hand, view these distributions as the relicts of previously continuous Tethyan distributions, separated by the appearance of the East Pacific Barrier and extinction of intervening populations. A central argument of the vicariance view is that currents flowing from the west to the east Pacific are too slow to transport larvae within their competent life-time. The possible time of arrival of *Echinothrix* in the eastern Pacific is relevant to this question.

Echinothrix diadema was first found at Isla del Coco in 1987. Given that specimens in the Los Angeles County Museum were also taken after 1987, there is no evidence that this species was present in the eastern Pacific before the last decade. Isla del Coco had been visited by several zoological expeditions between 1891 and 1941 (see Maluf 1988 for summary). The Zaca 1937–1938 and the *Velero*-

III 1931–1941 expeditions, in particular, concentrated on shallow water stations, and their echinoderm collections were described by H. L. Clark (1940, 1948). Clark (1948) reports 191 specimens of *D. mexicanum* (which he calls *Centrochinus mexicanus*) from 38 stations all over the east Pacific, including Coco. Yet, he makes no mention of *Echinothrix*. The Allan Hancock Foundation echinoderm collection of the Los Angeles County Museum, in which the Velero collections were deposited, includes no *Echinothrix*. It is difficult to believe that, if *E. diadema* were as abundant in Coco as we found it to be in 1987 and 1989, it would have been missed by these expeditions. It is, therefore, likely that this species was either absent or rare in the eastern Pacific until recently. If a small number of *E. diadema* have always been present at Coco, one needs to ask why its populations have recently expanded. One possible explanation is that the extensive coral mortality at this island during the 1983 El Niño event (Guzmán and Cortés 1992) has altered the habitat in a way favorable towards echinoid population growth. However, the preferred habitat of *E. diadema* is subtidal boulder areas, down to a depth of 5 m, an area that had little coral cover, even before 1983. It is also difficult to explain why the El Niño event, or any other factor, would have favored *E. diadema* disproportionately, so that its densities in shallow water would come to equal those of *Diadema*. An alternate hypothesis is that the recent expansion of *E. diadema* populations at Isla del Coco is the result of larval influx from the Central Pacific. This hypothesis would explain not only the absence of this species from the collections of previous expeditions, but also the lack of any report of its presence on the mainland coast. Isla del Coco is situated directly in the path of the North Equatorial Counter-current, the primary proposed avenue of transport of tropical larvae from west to east Pacific (e.g., Richmond 1990; Emerson 1991; Grigg and Hey 1992; Glynn et al. 1996). In periods of normal circulation, it takes 100–155 days for parcels of water carried by this current to traverse the distance from the Line Islands to the American Coast (Wyrteki et al. 1981; Richmond 1990). However, during years of strong El Niño flow, this time is reduced to 50–81 days (Firing et al. 1983; Wyrteki 1985; Richmond 1990; Glynn et al. 1996). Search for propagules within the barrier itself has shown that larvae of other taxa are capable of crossing it at least part of the way (Johnson 1974; Leis 1983, 1984; Scheltema 1988). The length of larval life of *Echinothrix* is unknown, but that of the related *Diadema* exceeds six weeks in the laboratory (Mortensen 1937). It is, therefore, possible that such larvae could cross the East Pacific Barrier during strong El Niño years; they may have done so in 1982–1983. After the same El Niño event, five species of Indo-Pacific fishes, previously unreported from the eastern Pacific, were found in the Galapagos, and another four in Isla del Coco [J. S. Grove and R. J. Lavenberg, cited in Grigg and Hey (1992)]. Interestingly, the previous very strong El Niño event was in 1925–1926 (Quinn et al. 1987; Glynn 1988). However, transportation of larvae depends on various stochastic factors, so there is no reason to expect that those of a given species will successfully traverse extremely long distances at every instance in which the currents are favorable. It is, however,

possible that this, or previous, strong El Niños brought *Echinometra oblonga* and *Echinothrix calamaris* into the eastern Pacific. Genetic comparisons between central and eastern Pacific populations can reveal whether the populations are connected by larval exchange or whether they have been isolated since the Pleistocene. Rosenblatt and Waples (1986) have found that allozyme divergence of Hawaiian and eastern Pacific populations of 12 species of shore fishes is very slight. Similar results were obtained by Huber (1985) for the coral crab *Trapezia* and by Nishida and Lucas (1988) for the starfish *Acanthaster planci*. Preliminary isozyme and mtDNA results in our laboratory also indicate that *Echinothrix diadema* from Coco and Hawaii are genetically very similar. Had all of these populations been divided since the Pleistocene, one would expect much higher levels of divergence.

After consideration of the coral faunas of Clipperton and Coco Islands, Durham (1963) stated that “the influence of (the East Pacific) barrier is not as great as previously thought”. The evidence we present suggests that this statement applies to echinoids as well.

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