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Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*

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Abstract

***Echinometra* is a pantropical sea urchin made famous through studies of phylogeny, speciation, and genetic structure of the Indo-West Pacific (IWP) species. We sequenced 630 bp of the cytochrome oxidase I (COI) mitochondrial gene to provide comparable information on the eastern Pacific and Atlantic species, using divergence between those separated by closure of the Isthmus of Panama 3.1 million years ago (Ma) to estimate dates for cladogenic events. Most recently (1.27–1.62 Ma), the Atlantic species *E. lucunter* and *E. viridis* diverged from each other, at a time in the Pleistocene that sea levels fell and Caribbean coral speciation and extinction rates were high. An earlier split, assumed to have been coincident with the completion of the Isthmus of Panama, separated the eastern Pacific *E. vanbrunti* from the Atlantic common ancestor. Transisthmian COI divergence similar to that in the sea urchin genus *Eucidaris* supports this assumption. The most ancient split in *Echinometra* occurred between the IWP and the neotropical clades, due to cessation of larval exchange around South Africa or across the Eastern Pacific Barrier. Gene flow within species is generally high; however, there are restrictions to genetic exchange between *E. lucunter* populations from the Caribbean and those from the rest of the Atlantic. Correlation between cladogenic and vicariant events supports E. Mayr's contention that marine species, despite their high dispersal potential, form by means of geographical separation. That sympatric, nonhybridizing *E. lucunter* and *E. viridis* were split so recently suggests, however, that perfection of reproductive barriers between marine species with large populations can occur in less than 1.6 million years (Myr).**

Keywords: gene flow, Isthmus of Panama, marine speciation, mitochondrial DNA, phylogeny, phylogeography

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Introduction

The study of speciation of marine organisms is made particularly challenging by the high dispersal potential conferred by planktonic larvae, coupled with the difficulty of determining barriers to gene flow in the ocean (Palumbi 1992, 1994). Often evidence for marine geographical speciation must be evaluated through geographical studies of genetic and morphological differences among populations and between species. One of the early papers dealing with speciation of marine organisms was by Mayr (1954). Extracting taxonomic and distributional information from Mortensen's (1928–51) monograph on the Echinoidea, Mayr concluded that, in addition to the emergence of the central American land bridge, oceanic barriers consisting of deep or cold water were likely causes of speciation in several tropical shallow water genera of sea urchins. He interpreted morphological differentiation between geographical populations, sometimes enough to warrant subspecific status, to be a sign of the early stages of allopatric speciation. Because Mayr viewed reproductive isolation as the product of divergence at many loci scattered throughout the genome, he assumed that it would be

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slow to emerge in large populations. For this reason, he concluded that sympatric sea urchin species must have spent a long time in allopatry before invading the same area without fusing.

Mayr noted that both the early phase of geographical speciation (divergence between allopatric populations), and the late phase (highly differentiated species occurring sympatrically), were evident in the genus *Echinometra*. These are pantropical sea urchins with a fossil record going back to the Palaeocene (Fell & Pawson 1966: U433). Mayr followed Mortensen in the belief that only one (or at the most two) species of this genus occurred in the Indo-West Pacific (IWP). Recent studies of morphology (Arakaki *et al*. 1998), allozymes (Matsuoka & Hatanaka 1991), gamete compatibility (Uehara *et al*. 1990; Palumbi & Metz 1991; Metz *et al*. 1994; Metz & Palumbi 1996; Aslan & Uehara 1997), DNA-DNA hybridization (Palumbi & Metz 1991), mitochondrial DNA (mtDNA) (Palumbi & Metz 1991; Palumbi 1996; Palumbi *et al*. 1997), and the loci coding for gamete recognition molecules (Metz & Palumbi 1996) showed that there are four species of *Echinometra* in the IWP. Molecular phylogenies indicate that these species split from each other in the last 3 Myr (Palumbi 1996). An additional species is endemic on Easter Island in the central Pacific (Fell 1974); mtDNA sequence comparisons suggest that it is an outgroup to all IWP species (S. R. Palumbi, personal communication).

The molecular phylogenetic relationships and genetic structure of IWP species of *Echinometra* have been studied more extensively than those of any other genus of sea urchin. *Echinometra*, however, is not confined to the IWP. Individuals of the IWP species *E. oblonga* are occasionally found at the eastern Pacific islands of Galapagos, Isla del Coco, and Revillagigedos (Clark 1948; Lessios *et al*. 1996; Palumbi 1997). Much more common and widespread in the eastern Pacific is *E. vanbrunti*, which ranges along the coast of the Americas from the Gulf of California to Peru. Two additional species are found in the tropical Atlantic: *E. lucunter* occurs from Bermuda south to Brazil and east to the Atlantic coast of tropical Africa, and *E. viridis* is restricted to the Caribbean. Different sets of characters produce conflicting phylogenies of the three neotropical species of *Echinometra*. *E. viridis* is morphologically most distinct from the other two species (Mortensen 1928–51; Lessios 1981a), but in isozymes it is most closely allied with its sympatric *E. lucunter* (Lessios 1979, 1981a; Bermingham & Lessios 1993). Restriction fragment length polymorphisms (RFLPs) of mtDNA (Bermingham & Lessios 1993) produce a tritomy. Thus, it is not known whether the completion of the Isthmus of Panama 3.1 Ma (Coates & Obando 1996) split *E. vanbrunti* from one of the two species that now occur in the Atlantic or from their common ancestor, which subsequently underwent a speciation event. These phylogenetic ambiguities have made it difficult to draw conclusions regarding the point at which prezygotic reproductive isolation between *E. lucunter* and the other two species arose (Lessios & Cunningham 1990).

We sequenced part of the cytochrome oxidase I (COI) mtDNA gene in species of eastern Pacific and Atlantic *Echinometra* and compared our data with those from IWP species previously published by Palumbi *et al*. (1997). We set out to answer the following questions: (i) Does neotropical and east Atlantic *Echinometra* parallel the IWP in containing undetected sibling species? (ii) What are the phylogenetic relationships between the neotropical species, and between them and the IWP species? (iii) What are the possible barriers that might have led to speciation in *Echinometra*, and are they consistent with those found in the tropical sea urchin genus *Eucidaris*, which has also been studied in a similar manner through sequencing of its COI (Lessios *et al*. 1999)? (iv) Does the timing of speciation events suggest that a long allopatric stage is necessary before species can become sympatric and still retain their separate genetic identities? And (v) are there restrictions to gene flow among conspecific populations that produce the kind of geographical variation that Mayr considers to be a stage preliminary to allopatric speciation?

Materials and methods

We sampled a total of 73 individuals of *Echinometra vanbrunti*, *E. lucunter*, and *E. viridis* from 15 locations in the eastern Pacific, Caribbean, and the central and eastern Atlantic (see Fig. 1). We also sequenced two individuals of the IWP species *E. oblonga*, which we found at Isla del Coco (eastern Pacific) and a single specimen of *E. mathaei* from Hawaii. To determine the relationship of our study species to those from the IWP, we haphazardly selected for comparison a total of 18 sequences of *E. mathaei, E. oblonga, Echinometra type A*, and *Echinometra type C* deposited in GenBank by Palumbi *et al*. (1997).

We amplified and sequenced 630 bp of the COI region lying between positions 6469 and 7098 of the mitochondrial genome of *Strongylocentrotus purpuratus* (Jacobs *et al*. 1988). Palumbi *et al*. (1997) sequences of the IWP species encompass a fragment between positions 6503–6952. Methods used in genomic DNA isolation, polymerase chain reaction (PCR) amplification, PCR product purification, and automated DNA sequencing are described in Lessios *et al*. (1999). Sequences have been deposited in GenBank under accession nos AF255468–AF255540.

Maximum-likelihood trees were constructed by the quartet-puzzling method of Strimmer & von Haeseler (1996) using their program PUZZLE 4.0. PUZZLE also calculates quartet-puzzling reliability values that indicate support for internal nodes in a manner analogous to bootstrap resampling. Neighbour-joining (Saitou & Nei 1987), minimum evolution (Cavalli-Sforza & Edwards 1967), and

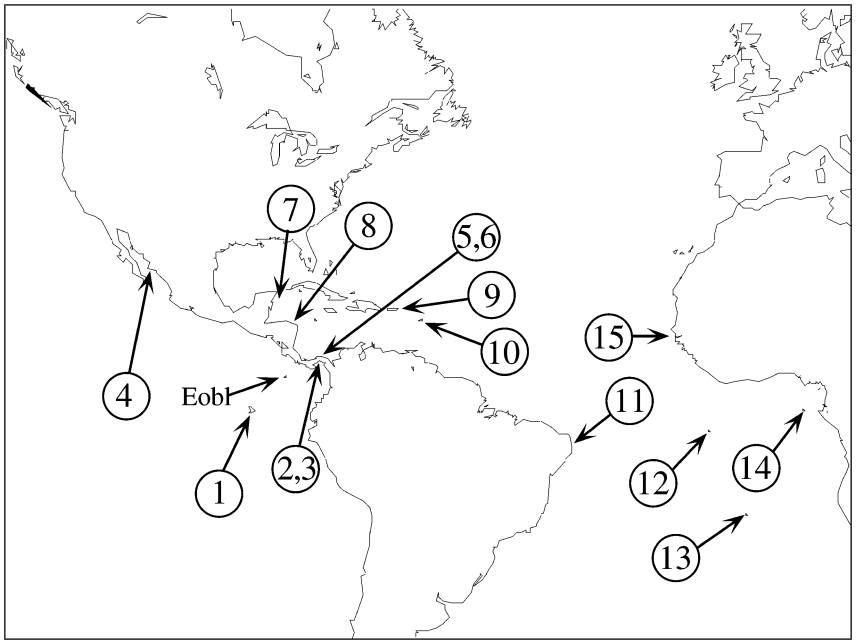

Fig. 1 Localities at which specimens were collected and sample sizes (*n*). **1**: Galápagos (Isla Santiago; *n* = 3 *Echinometra vanbrunti*), **2**: Panamá (Isla Taboguilla; *n* = 3 *E. vanbrunti*), **3**: Panamá (Punta Paitilla; *n* = 3 *E. vanbrunti*), eobl: Isla del Coco (Costa Rica; *n* = 2 *E. oblonga*), **4**: Mexico (Guaymas; *n* = 5 *E. vanbrunti*), **5**: Panamá (Portobelo; *n* = 3 *E. lucunter*, *n* = 3 *E. viridis*), **6**: Panamá (San Blas; *n* = 1 *E. lucunter*, *n* = 3 *E. viridis*), **7**: Belize (Carrie Bow Cay; *n* = 5 *E. lucunter*), **8**: Honduras (Cayos Cochinos; *n* = 6 *E. lucunter*, *n* = 1 *E. viridis*), **9**: Puerto Rico (several sites; *n* = 6 *E. lucunter*, *n* = 7 *E. viridis*), **10**: US Virgin Islands (St. John; *n* = 2 *E. lucunter*, *n* = 2 *E. viridis*), **11**: Brazil (Recife; *n* = 5 *E. lucunter*), **12**: Ascension Island (*n* = 3 *E. lucunter*), **13**: St. Helena (*n* = 7 *E. lucunter*), **14**: Gulf of Guinea (São Tome; *n* = 2 *E. lucunter*), **15**: Senegal, Africa (Dakar; *n* = 3 *E. lucunter*).

maximum parsimony phylogenetic analyses were performed using test version 4.0d64 of PAUP*, written by David Swofford and used with his permission.

We calculated the number of synonymous nucleotide substitutions per synonymous site (K_s) using a modified version of the methods of Pamilo & Bianchi (1993) and Li (1993) available in SEQUENCER, an Apple MacIntosh program written by B. D. Kessing. SEQUENCER estimates K_s by calculating the actual numbers of nucleotide substitutions at codon positions between pairs of sequences, then employs the equations of Pamilo & Bianchi (1993) to correct for multiple hits. We also used SEQUENCER to estimate gene flow among conspecific populations by calculating nucleotide sequence analogue of F_{ST} (Hudson *et al*. 1992). Whether observed values of F_{ST} could be due to chance was evaluated by randomly reshuffling individuals among populations 500 times. Nucleotide diversity (π) and haplotype diversity were calculated using the equations provided by Nei (1987), using DnaSP 3.0 (Rozas & Rozas 1999).

Results

Phylogenetic relationships

The maximum-likelihood tree for our 73 COI sequences and the 18 sequences of Palumbi *et al*. (1997), using the homologous region of *Strongylocentrotus purpuratus* (Jacobs *et al*. 1988) as an outgroup, shows four distinct clades, each with very high support (> 98% of all quartet puzzling steps; see Fig. 2). One clade is comprised of all individuals from the four IWP species. Each of the three neotropical species belong to a separate subclade, and these subclades are joined as a sister lineage to the IWP clade. The *Echinometra lucunter*, *E. viridis*, and *E. vanbrunti* clades are reciprocally monophyletic, each containing only individuals judged to be conspecific by traditional morphological criteria. The tree topology shows that the most recent cladogenic event was the split between the two Atlantic species, *E. lucunter* and *E. viridis*. The immediately previous split was between the eastern Pacific *E. vanbrunti* and the ancestor of the Atlantic species. The most ancient split was between the IWP clade and the ancestor of the eastern Pacific and Atlantic species.

Nearly identical topologies were generated by neighbour-joining, minimum evolution, and maximum parsimony reconstructions (the latter weighting transversions:transitions 3:1), except for minor differences among terminal clades. Support for the four major clades was > 95% in 1000 bootstrap resamplings of trees produced by each distance method; maximum parsimony also produced > 95% support for all clades, except for that of *E. lucunter*, which was supported at 80%. In the quartet-puzzling tree there was also moderate support for monophyly of a geographically distinct subclade within *E. lucunter*, composed of all Caribbean sequences. Parsimony, minimum evolution, and neighbour joining methods also generated this geographical grouping, but with bootstrap support always < 50%. The tendency for quartet-puzzling reliabilities to exceed bootstrap values when nodes are separated by small distances has been noted by Cao *et al*. (1998).

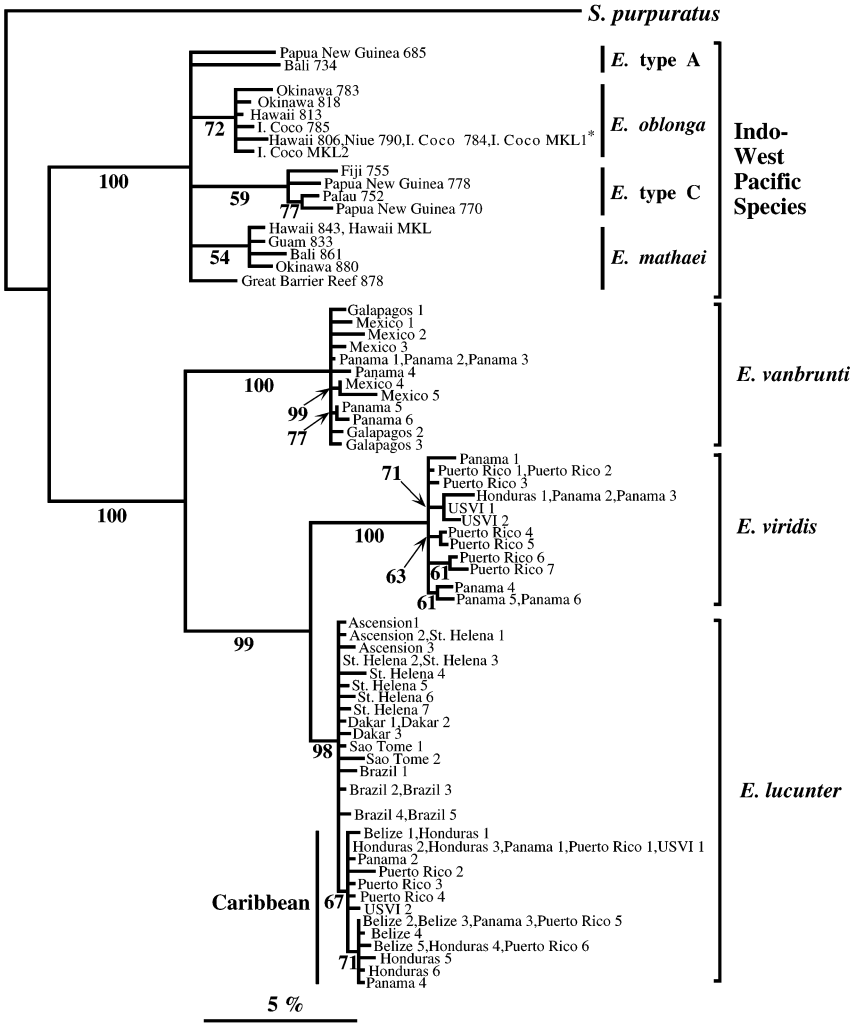

Fig. 2 Maximum-likelihood quartet puzzling phylogeny of *Echinometra* COI sequences. Branch lengths are proportional to maximum-likelihood distances between nodes, corrected by Hasegawa's model of base substitution (Hasegawa *et al*. 1985), with evolutionary rates free to vary. Numbers next to nodes denote reliability indices (% of quartet puzzling steps generating the given bifurcation). Only nodes with > 50% support are labelled; boldface is solely for emphasis. Vertical bars mark major clades. Termini are labelled with source locality of individuals, numbered sequentially in neotropical species; identical sequences are separated with a comma. For labels of Indo-West Pacific (IWP) samples, the number refers to the last three digits of the corresponding GenBank accession number AF018xxx. *MKL indicates the three Indo-West Pacific individuals sequenced in the present study.

Dating of cladogenic events

The accuracy of our estimated dates for splitting events obviously depends on whether molecular divergence has been constant. To examine rate constancy of COI in *Echinometra*, we compared the log likelihood of the quartet puzzling tree on which the assumption of constant rates was imposed, to that of the tree in which rates were allowed to vary. The tree with variable rates fit the data significantly better (log likelihood ratio test statistic = 99.68, d.f. = 70, $P < 0.01$). Thus, time estimates obtained from COI divergence need to take rate variation into account. To calculate upper and lower bounds for time estimates that incorporate variable rates, we used the differences between branch lengths that would have been equal if a strict molecular clock held true. Under an assumption of a molecular clock, Kimura (1980) two-parameter (K_2) distances between the IWP species and all other *Echinometra* would be equivalent, as would divergence between *E. vanbrunti* and each of the Atlantic species. Among these comparisons (see Table 1), the largest difference is between the average genetic distance separating *E. vanbrunti* from *E. lucunter* ($K_2 = 10.2\%$) compared to the distance separating *E. vanbrunti* from *E. viridis* ($K_2 = 12.6\%$). Divergence rates for all nucleotide sites can therefore vary by a maximum factor of 1.24 across lineages. Synonymous site (K_s) divergence values (see Table 1) vary by a factor of 1.28.

To calibrate rate of molecular divergence we assumed that the last date of genetic exchange between eastern Pacific and Atlantic populations coincided with the closure of the Isthmus of Panama, which independent geological evidence has placed at 3.1 Ma (Coates & Obando 1996). By dividing the average pairwise divergence of eastern Pacific *E. vanbrunti* sequences from all Atlantic sequences (both *E. lucunter* and *E. viridis*), we derived a rate estimate of 10.83/3.1 = 3.49% Kimura-corrected sequence divergence per million years. With a range that spans 24% of this mean, the estimated dates of cladogenic events shown by our data

Table 1 Average pairwise differences between *Echinometra* cytochrome oxidase (COI) sequences. Per cent nucleotide differences at all sites (K_2), corrected by Kimura (1980) two-parameter model are given in the lower left half of the matrix (in bold). The upper right half shows values for per cent differences at synonymous sites (K_s), estimated using Pamilo & Bianchi's (1993) method with modification as described in the text. On the diagonal are average K_2 and K_s values within species. IWP = Indo-West Pacific, CAR = Caribbean, and CEAB = Central and Eastern Atlantic + Brazil

	Strongylocentrotus purpuratus	*Echinometra* IWP	*E. vanbrunti*	*E. lucunter* CAR	*E. lucunter* CEAB	*E. viridis*
Strongylocentrotus purpuratus	—	73.49	99.26	96.83	100.65	92.39
Echinometra IWP		9.94	38.80	40.71	42.43	47.40
	21.24	**3.19**				
E. vanbrunti			1.72	34.83	34.61	44.54
	24.28	**12.42**	**0.84**			
E. lucunter CAR				1.40	2.09	14.88
	23.93	**12.40**	**10.17**	**0.55**		
E. lucunter CEAB					1.14	14.56
	24.21	**12.81**	**10.18**	**0.78**	**0.45**	
E. viridis						1.79
	23.09	**13.98**	**12.58**	**5.00**	**4.97**	**0.91**

are as follows: *E. lucunter* split from *E. viridis* 1.27–1.62 Ma. By definition *E. vanbrunti* split from the Atlantic species 3.1 Ma. The IWP lineage separated from the lineage leading to all neotropical species 3.30–4.18 Ma, a date considerably more recent than the 5.5–6.6 Ma estimate obtained previously (Palumbi 1996). This previous estimate used the same assumptions we make here, but relied on comparisons to single sequences each of *E. vanbrunti* and *E. lucunter,* which are not included in the present data set.

Gene flow between conspecific populations

Wright's model links F_{ST} with migration rate (Wright 1951). Assuming an island model and equilibrium between migration and drift, F_{ST} values smaller than 0.20 indicate migration of more than one individual per generation (Avise 1994; p. 208). Values of F_{ST} are useful as indicators of relative gene flow, even when genetic equilibrium has not been reached and the island model is not strictly applicable (Neigel 1997). Hence our estimates of gene flow, while admittedly based on small sample sizes for some populations (Table 2), are a useful means for contrasting patterns of population genetic structure between the three neotropical species of *Echinometra*.

Each of the three species showed a different pattern. The eastern Pacific *E. vanbrunti* showed high gene flow over long distances. Average genetic differences across the three sampled geographical populations of *E. vanbrunti* were quite small ($F_{ST} = 0.057$), and showed a high probability of being generated by chance (Table 2). Mexico and Panama populations of *E. vanbrunti* are linked by high levels of genetic exchange (over a distance along some 4700 km of coastline), as are mainland and Galápagos island populations.

Unlike *E. vanbrunti*, *E. lucunter* showed moderate genetic structure among populations spread across much of its tropical Atlantic range (Table 2). Restrictions to gene flow between Caribbean populations and those in the rest of the Atlantic account for the bulk of this intraspecific differentiation. If the Caribbean is considered as one population and the rest of the Atlantic (including Brazil) as another, gene flow estimated between them is restricted ($F_{ST} = 0.368$, $P < 0.001$). Comparisons of nucleotide subsitutions also indicate that Caribbean populations do not exchange genes with those in the rest of the Atlantic. All individuals we sampled from outside the Caribbean share a substitution at a single site in COI that distinguishes them from Caribbean individuals.

E. viridis occurs only in the Caribbean. Yet even within this small stretch of water dotted by islands, gene flow appears to be limited (Table 2). Given the small sample sizes, it is unwarranted to point at any locality as being more isolated from the others.

Discussion

Systematics

In contrast to the case in the IWP, mtDNA data from the eastern Pacific and Atlantic species of *Echinometra* do not suggest the presence of new, undescribed species. Earlier designations of variants of both *E. vanbrunti* and *E. lucunter* as new species, which were subsequently dismissed on the basis of morphology (see Mortensen 1928–51), are also not supported by our mtDNA data.

The only taxonomic concern relates to the systematic status of *E. lucunter* populations from the Caribbean vs. those from the rest of the Atlantic. Our results from COI

Table 2 Polymorphism and genetic structure of populations of *Echinometra* based on cytochrome oxidase (COI) sequences. Nucleotide and haplotype diversity measures were calculated from the data using DnaSP 3.0 (Rozas & Rozas 1999), according to the equations provided by Nei (1987); NA = estimate not available due to small sample size. Nucleotide diversity is Nei's π multiplied by 100, with ambiguous or missing nucleotide sites deleted in affected pairwise comparisons. F_{ST} was calculated as in Hudson *et al*. (1992), and its probability of being due to chance (*P*) was determined from 500 reshufflings of haplotypes among populations; F_{ST} values yielding $P < 0.05$ are bolded. *n* = number of individuals, CAR = Caribbean, CEAB = Central and Eastern Atlantic + Brazil, and EP = Eastern Pacific

				Polymorphism		Population structure		
Species	Location	Region	*n*	Nucleotide diversity ($\pi \times 10^2$)	Haplotype diversity	Comparison	F_{ST}	*P*
E. lucunter	Ascension I.	CEAB	3	0.429	1.000	Within CEAB	0.146	0.072
	St. Helena	CEAB	7	0.533	0.857			
	Dakar	CEAB	3	0.054	0.533			
	São Tome	CEAB	2	NA	NA			
	Brazil	CEAB	5	0.449	0.800			
	Belize	CAR	5	0.389	0.900	Within CAR	−0.096	0.888
	Honduras	CAR	6	0.550	0.933			
	Panamá	CAR	4	0.378	1.000			
	Puerto Rico	CAR	6	0.506	0.933			
	St. John	CAR	2	NA	NA	Between CEAB & CAR	**0.368**	< 0.001
Total			43	0.634	0.946	Among all populations	**0.261**	< 0.001
E. viridis	Panamá	CAR	6	0.698	0.867			
	Puerto Rico	CAR	7	0.620	0.905			
	St. John	CAR	2	NA	NA			
	Honduras	CAR	1	NA	NA			
Total			16	0.903	0.908	Among all populations	**0.361**	< 0.001
E. vanbrunti	Galápagos	EP	3	0.698	1.000			
	México	EP	5	1.44	1.000			
	Panamá	EP	6	0.406	0.800			
Total			14	0.839	0.890	Among all populations	0.057	0.300

agree with those of Pawson (1978), who on the basis of morphological differences, assigned specimens from the central Atlantic islands of Ascension and St. Helena to a new subspecies, *E. lucunter polypora*. However, they disagree with his placement of African populations in *E. l. lucunter*, the subspecies also found in the Caribbean. By mtDNA similarity, eastern Atlantic populations are closer to central Atlantic ($K_2 = 0.62$) and Brazilian ($K_2 = 0.61$) populations; they are more distinct from those in the Caribbean ($K_2 = 0.84$). All Caribbean individuals, moreover, lack the fixed informative substitution in COI that is characteristic of Atlantic sequences from outside the Caribbean. If the division into subspecies is maintained, the mtDNA data suggest that only Caribbean populations should fall into *E. l. lucunter*, whereas the rest (including the ones from Brazil) should be placed into *E. l. polypora*. Mortensen (1928–51; III_3, 366) remarked on morphological similarities between St. Helena and Brazilian varieties.

Gene flow within species

The overall picture presented by the three species of neotropical *Echinometra* suggests that, as expected, gene flow in sea urchins is possible over very large distances. However, Mayr's view that echinoid species are not panmictic throughout their range — and thus show the potential for allopatric speciation — is also supported by the mtDNA data. That populations of *E. vanbrunti* distributed along the west American coast constantly exchange genes with each other is not surprising. That gene flow is also high between mainland and eastern Pacific Galápagos populations can be easily explained by the prevalence of currents that could transfer larvae from West to East (Wyrtki 1965, 1966, 1967; Abbott 1966). However, *Echinometra* does differ in this respect from *Eucidaris*, in which COI data indicated that different, geographically separated, species inhabit the eastern Pacific islands and the west coast of Central America (Lessios *et al*. 1999).

Gene flow patterns within the Caribbean differ between the two sympatric species. Negative F_{ST} values among *E. lucunter* populations within the Caribbean indicate that they all belong to the same genetic population, and the phylogeny also shows no evidence of phylogeographic structure between Caribbean locales of this species. High

gene flow within this small sea is to be expected. Larval transport can easily occur via the main Caribbean current, flowing to the northwest until deflected by the Yucatan peninsula, then travelling northeast to become the Gulf Stream (Lessios 1984; Roberts 1997). With this in mind, it is noteworthy that *E. viridis* showed a value of F_{ST} indicative of differentiation among Caribbean populations. As the present analysis is based on a small sample size of *E. viridis* from a limited number of sites, it should be interpreted with caution, and bears further examination.

The clearest pattern of genetic structure occurs among *E. lucunter* populations in the equatorial portion of the Atlantic. In *Eucidaris*, Caribbean, Brazilian, and west African populations are virtually panmictic (Lessios *et al*. 1999), while in *Echinometra* there are genetic subdivisions between populations from the Caribbean and from the rest of the Atlantic. It is tempting to attribute these differences to different lengths of larval life. *Eucidaris* larvae settle in the laboratory in 25–30 days (Emlet 1988), whereas *E. lucunter* do so in as little as 19 days (Mortensen 1921). However, the COI differences between Ascension, St. Helena, and the African coast populations of the two genera show that length of larval life cannot be the only explanation of why the two genera perceive the same potential barriers in a dissimilar manner. Despite their longer larval life, populations of *Eucidaris* from the two central Atlantic islands are much more differentiated from each other and from populations at São Tome than are *Echinometra* populations from the same locations.

It may be that the lack of a pattern common to the two sea urchins is due to the highly stochastic nature of events in which larvae successfully cross large distances. Certain island populations of *Echinometra* in the IWP have low or no nucleotide diversity, presumably due to founder effects (Palumbi *et al*. 1997). *Eucidaris* has low mtDNA diversity at St. Helena, which also suggests fairly recent events of successful colonization by extant mtDNA lineages (Lessios *et al*. 1999). *Echinometra*, on the other hand, both at Ascension and at St. Helena, shows nucleotide diversity that is indistinguishable from populations on the mainland (Table 2). In *Echinometra*, the population with apparently little genetic diversity is at Dakar. This, however, does not mean that *Echinometra* has only recently established itself on the African coast, because *E. lucunter* is known as a fossil from the Pleistocene of Angola (Dartevelle 1953). Edwards & Lubbock (1983) found *Eucidaris* at St. Paul's Rocks, 2000 km from Ascension and 960 km from Brazil, but despite the presence of apparently suitable habitat, they reported no specimens of *Echinometra* on this potential stepping stone. Thus, the haphazard nature of the East Pacific Barrier (Lessios *et al*. 1996, 1998) seems to be repeated in the Atlantic; larvae of some species transit occasionally, sometimes in large enough numbers to homogenize populations. However, when large distances and few stepping stones are involved, there is no way of predicting which species will transit which barrier and how often. If we were to conclude that no exchange presently occurs between Caribbean and eastern/central Atlantic populations, then their mtDNA divergence would date their split at 199,000–253 000 years ago.

Cladogenic events between species, their timing and their causes

The most recent cladogenic event between recognized species of *Echinometra* separated *E. lucunter* from *E. viridis* 1.27–1.62 Ma during the Pleistocene. This coincides with the first Pleistocene sea-level drop due to northern hemisphere glaciation (Haq *et al*. 1987), and falls within the period of high rates of Caribbean coral species extinction and origination (Frost 1977; Johnson *et al*. 1995; Budd *et al*. 1996). Palumbi (1996) has suggested that restrictions to gene flow resulting from fluctuations in sea level, temperature and current shifts may account for the emergence of the extant species of IWP *Echinometra*. The speciation of *E. lucunter* and *E. viridis* in the Atlantic is roughly contemporaneous with speciation in the IWP, and it may be due to the same global changes.

We have followed the assumption of all previous workers (e.g. Mortensen 1928–51; Lessios 1979, 1981a; Lessios & Cunningham 1990; Palumbi 1996) in placing the immediately previous cladogenic event in the history of neotropical *Echinometra*, the separation of eastern Pacific and Atlantic species, at the completion of the Isthmus of Panama. Our calibration of the COI molecular clock depends on this assumption. One of us has pointed out elsewhere the potential pitfalls of making such an assumption without corroborating evidence (Lessios 1998). Knowlton & Weigt (1998) presented molecular divergence data for snapping shrimp indicating that some morphologically similar species on either side of the Isthmus may have speciated prior to its completion. In the present case, the hypothesis of speciation at the time of Isthmus closure is supported by a comparison to another sea urchin. Average COI divergence of *E. vanbrunti* from its two Atlantic congeners ($K_2 = 10.83\%$) is similar to that found between eastern Pacific and Atlantic species of *Eucidaris* ($K_2 = 9.52\%$). Data from nuclear introns also show similar degrees of transisthmian divergence in the two genera (P. Gonzalez & H. A. Lessios, unpublished), and so do isozyme and RFLP-determined mtDNA differences (Bermingham & Lessios 1993). This evidence points to a contemporaneous split between transisthmian lineages in these two genera, which suggests a common vicariant event as the cause.

Separation of Atlantic and eastern Pacific species at the time of the closure of the Isthmus is the most parsimonious hypothesis. The alternative — that speciation preceded Isthmus closure — would also require the extinction of

one taxon in each ocean to occur, following closure, in order to produce the phylogeographic pattern we observe today. Obviously, there is some error associated with setting the split between Atlantic and Pacific *Echinometra* lineages at the precise date of final closure. Small inaccuracies in our calibration, however, are unlikely to alter any of our primary conclusions. Ranges on estimated divergence times already incorporate substantial variation in COI evolutionary rates.

The most ancient splitting event among the extant species of *Echinometra* was the separation of the Indo-Pacific and American/Atlantic lineages from one another, 3.30–4.18 Ma, not all that long before the rise of the Isthmus of Panama. The corresponding cladogenic event in *Eucidaris* is dated at approximately 2 Myr earlier (Lessios *et al*. 1999). This split is much too recent to have been caused by the separation of Atlantic and Indian Oceans by the closure of the Tethyan sea, some 12–20 Ma (Por 1989). There are two possible post-Tethyan routes through which populations of *Echinometra* from the IWP could have maintained genetic contact with the Atlantic and the eastern Pacific: from east to west around the tip of South Africa or from west to east through the eastern Pacific Barrier. The route around South Africa cannot be ignored. The cold water Benguela upwelling system off the south-west African coast first appeared in the Miocene (Diester-Haass & Schrader 1979; Siesser 1980), but did not become a permanent feature until the late Pliocene (Shannon 1985). It still allows occasional migration of tropical organisms (Briggs 1974; Bowen *et al*. 1994).

We think it more likely, however, that the final separation between IWP *Echinometra* populations and those from the seas bordering the Americas was by the eastern Pacific Barrier. This major biogeographic barrier for sea urchins and many other groups behaves as a haphazard filter, allowing sporadic pulses of larvae to pass through (Lessios *et al*. 1996; Lessios *et al*. 1998). It has, however, kept such migration from occurring for periods of time long enough to cause speciation in many marine groups (Mayr 1954; Vermeij 1987). Our reason for favouring this as the last route of genetic exchange in *Echinometra* comes from the fact that *E. oblonga* occurs in the eastern Pacific islands. The average corrected per cent dissimilarity between *E. oblonga* sequences we obtained at Isla del Coco and those obtained by Palumbi *et al*. (1997) from the IWP is 0.47%, less than the difference between Isla del Coco specimens (0.78%). This low amount of divergence across the Eastern Pacific Barrier suggests that *E. oblonga*, after being separated from the American populations long enough to become a new species, has been able to make the crossing once again. Apparently, the hiatus in larval transport that caused speciation (or the colonization event that established the progenitors of the American species) occurred 3.30–4.18 Ma.

Reproductive isolation in light of phylogeny

Our well-resolved *Echinometra* phylogeny allows us to determine the point at which reproductive isolation emerged between neotropical members of the genus. Among these three species, only *E. lucunter* has eggs that are fertilized poorly by heterospecific sperm. Mapping the emergence of its gametic incompatibility onto an independent phylogeny was previously difficult (Lessios & Cunningham 1990). As gametic incompatibility is likely to be irreversible, it has evolved once, in the lineage leading to *E. lucunter* (Lessios & Cunningham 1990, 1993). Our mtDNA phylogeny demonstrates that this event occurred after the *E. lucunter*/*E. viridis* split i.e. within the last 1.5 Myr. Otherwise, the fertilization block would be shared with *E. viridis*. Though the block in *E. lucunter* also functions towards allopatric *E. vanbrunti* sperm, its evolution does not depend on time alone, because *E. viridis* and *E. vanbrunti* are completely interfertile.

Though gametic isolation between the two Caribbean species is only unidirectional, there is no hybridization between *E. lucunter* and *E. viridis*. This was evident from isozyme data that included loci fixed for alternate alleles (Lessios 1998), and is now confirmed by the absence of any paraphyletic mtDNA lineages. Unlike another sea urchin, *Diadema*, the species of which spawn at different phases of the moon (Lessios 1984), *Echinometra* has no lunar periodicity in reproduction (Lessios 1991). The annual reproductive cycles of *E. lucunter* and *E. viridis* overlap (Lessios 1981b, 1985). As there are few other means of prezygotic reproductive isolation known for free-spawning organisms, complete reproductive isolation of the two sympatric species is likely to be maintained by a postzygotic mechanism. It is therefore unlikely that models of sympatric speciation through gametic incompatibility promoted by sexual selection (e.g. Nei *et al*. 1983; Wu 1985) apply to the formation of these two species. This holds despite the fact that divergence of the male gamete recognition molecule of *E. lucunter* itself may have been driven by sexual selection (M.A. McCartney & H.A. Lessios, in preparation). The coincidence of the estimated time of this speciation event with glaciation-induced sea level drops also suggests the possibility that some populations may have been physically isolated from the rest. We therefore agree with Mayr's (1954) conclusion that allopatric speciation and subsequent geographical overlap best account for the present-day distribution of *E. lucunter* and *E. viridis*. The recency of this cladogenic event, however, also suggests that the allopatric stage, during which reproductive isolation was acquired, could not have lasted for more than about 1.5 million years. Either one of the species underwent a population bottleneck, or reproductive isolation can evolve rapidly, even in large populations connected by high gene flow (Barton & Charlesworth 1984).

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