



What can molecular genetics contribute to marine biogeography? An urchin's tale

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Abstract

The high marine species diversity in the Indo-West Pacific declines sharply to the east and west of the Indonesian Archipelago. This biogeographic pattern has attracted attention for over a century, but the mechanisms generating such diversity gradients remain uncertain. Molecular genetic data can help address biogeographic mechanisms in at least 4 ways. They can provide (1) independent evidence for genetic boundaries between cryptic species, (2) a temporal framework for species divergence times, (3) phylogenetic reconstructions of species divergence patterns, and (4) information about the geography of allelic variants within species. Data from mitochondrial DNA variation in Pacific sea urchins show that (1) congeneric species are of recent origin, having diverged within the Pleistocene, (2) populations are genetically heterogeneous despite high dispersal potential, and (3) clines of mtDNA variation within species are similar to diversity clines in the entire fauna. Phylogenetic analysis of mtDNA variants does not support the center-of-overlap or center-of-accumulation models for biogeographic patterns in the Indo-West Pacific, but instead suggests that genetic differentiation and perhaps species formation can occur throughout the Indo-West Pacific.

Keywords: Biogeography; mtDNA, sea urchin; Speciation; Population structure

1. Introduction

The Indo-West Pacific has long been known as a center of marine biodiversity. Thousands of marine species co-exist on the reefs of the tropical Pacific, making this ecosystem one of the most diverse among marine habitats. It has also been long appreciated that the scattered reefs of the wide tropical Pacific and Indian Oceans do not possess identical levels of species diversity (Briggs, 1974; Ekman, 1953). Instead, there is a gradient of diversity at the species and generic levels that extends from the high diversity center of the Indo-West Pacific to the outlying archipelagoes. This gradient has

been described for many taxa including shore fish, corals, molluscs, and sea grasses (Kay, 1980, 1984; Stoddart, 1992; Vermeij, 1987a).

Many theories developed to explain the strong biogeographic patterns observed in the Pacific have been proposed throughout the last century. Rosen (1988b) summarized these ideas into 13 theories which fall into several major groups. Focal centers-of-origin theories were the first to gain wide acceptance (e.g. Ekman, 1953; Stehli and Wells, 1971). In this view, regions with high species diversity, like the Indonesian center of the Indo-West Pacific, are also areas in which speciation takes place. The gradient of species diversities seen in the Pacific is ascribed to diffusion of species out from the center or to vicariant processes that operate over extended time scales. In contrast to this idea, Ladd (1960) proposed that speciation took place largely in the isolated archipelagoes of the Pacific basin, and that species originating on the periphery were transported to the Indo-West Pacific via prevailing currents that flow from east to west across the tropical Pacific. In this view, high diversity at the core of the Indo-West Pacific occurs because this area is a center-of-accumulation. A third possibility is that the Indo-West Pacific consists of several biogeographic provinces defined by oceanic conditions and past geological events. If such provinces (e.g. Pacific Ocean, Indian Ocean, and Indonesian, Ekman, 1953) overlap in the Indonesian archipelago, this would create high species diversity. In this view, the gradient in species diversity observed is an artifact of the overlap of three otherwise separate biogeographic provinces.

These three major ideas about Pacific biogeographic patterns (center-of-origins, center-of-accumulation, and center-of-overlap) have been examined many times for particular taxa like corals, sea grasses, and molluscs (see Rosen, 1988a; Kay, 1990; Wallace et al., 1991; Jokieli and Martinelli, 1992; Mukai, 1992; Pandolfi, 1992; Stoddart, 1992; Vermeij, 1987a; Veron, 1995). Although most of these studies provide support for one or other mechanistic model, there has been no consensus about which model may be more appropriate, or, more importantly, in which circumstances different models generally apply.

1.1. Impact of molecular genetics on biogeography

Molecular genetics can contribute in at least four ways to studies of marine biogeography. First, genetic data can help distinguish and define cryptic species. Even well studied marine taxa harbor cryptic species (Knowlton, 1993), and genetic data are a powerful means to assess the boundaries of evolutionarily independent gene pools (Palumbi and Metz, 1991). Correct assessment of species boundaries is fundamental to biogeographic hypothesis testing.

Second, genetic data can help establish the approximate timing of species divergence. Although this requires a taxonomically-appropriate calibration (Martin et al., 1992), genetic distance information can sometimes test between alternative biogeographic mechanisms. For example, Springer (1982) emphasizes tectonic events in the determination of Indo-Pacific species patterns. These events (like the collision of the Indian subcontinent with Asia, or the movement of the Australian plate towards Indonesia) require tens of millions of years to operate. By contrast, other biogeographers (Potts, 1983; Rosen, 1988a; Rosen and Smith, 1988) emphasize Pleistocene sea level

changes as important vicariant events. The order of magnitude time difference between these mechanisms should lead to detectable differences in genetic distance between species separated by Miocene vs. Pleistocene events (Cipriano, 1996).

The third use of molecular data is in understanding the phylogenetic relationships among species. Rooted phylogenies allow the identification of derived species. The geographic locality of such species in relation to phylogenetically basal taxa can be used to test biogeographic theories (e.g. that derived species are found in isolated localities, Pandolfi, 1992), and to further our understanding of the series of evolutionary events leading to biogeographic patterns.

Fourth, genetic data on allelic variation within species can be used to track the movement and patterns of origination of alleles within species. Do patterns of allele endemism and species endemism agree? Do patterns of genetic diversity match patterns of species diversity? Where in the species' range do derived alleles occur? Answers to such questions can illuminate the mechanisms that act within populations to distribute alleles. Concordance of intraspecific and interspecific biogeographic patterns (Avice, 1992) can be an important tool in elucidating evolutionary mechanisms. High resolution genetic studies of the distribution of alleles within species across their ranges may help us understand the distribution of species within oceanic basins. For example, Avice and co-workers (see Avice, 1994 for review) have found that the biogeographic boundary along the SE coast of North America (which was defined on the basis of species distributions) can be a boundary for transport of alleles within species that are distributed widely along this coast. Such concordance of species-level and allele-level biogeographic patterns suggests that processes determining species distribution are similar to those determining gene flow. Such parallel patterns have yet to be examined comprehensively in any other region.

1.2. Pacific sea urchins as a evolutionary model

To address biogeographic theories using molecular data, we are examining the molecular genetics of a suite of four closely related sea urchin species in the genus *Echinometra*. The Pacific and Indian Oceans were thought by Mortensen (1943) to be inhabited by a single species in this genus. Subsequently, four species of these reef burrowers have been recognized in the central and western Pacific on the basis of slight morphological and genetic differences (Uehara et al., 1986; Matsuoka and Hatanaka, 1991; Palumbi and Metz, 1991). The species are widespread, as expected for taxa with planktonic larvae that can disperse for 4–8 weeks before settlement. However, because previous workers combined them all under one (Mortensen, 1943) or two names (Edmondson, 1935), the ranges of the recently recognized forms are poorly known.

When they occur in the same archipelagos, *Echinometra* species occur on the same limestone benches and back reefs, with slight habitat differences (e.g. Russo, 1977; Nishihira et al., 1991). Species remain reproductively isolated because egg and sperm from different species cross fertilize poorly (Metz et al., 1994; Palumbi and Metz, 1991). The small genetic and morphologic differences among species coupled with their strong reproductive isolation makes them a valuable group for studies of marine speciation.

2. Methods and materials

2.1. Nomenclature

Although the four types of *Echinometra* discussed here are recognized as four distinct species on the basis of morphology (Uehara et al., 1986) and genetics (Palumbi and Metz, 1991), valid names for these species have been debated (Mortensen, 1943). I follow Edmondson's usage of the names *E. mathaei* and *E. oblonga* for the species found in Hawaii (Edmondson, 1935). These species are the same as types B and D respectively in Okinawa (Motokawa, 1991; Nishihira et al., 1991). The other two species will be called *E. sp. nov. A* and *E. sp. nov. C*, and correspond to Okinawan types A and C, respectively.

2.2. Collections, DNA amplification and sequencing

Collection localities for *E. sp. nov. A* ($n = 65$) were: Tahiti, Society Islands; Fiji; Madang, Papua New Guinea; Great Barrier Reef (GBR), Townsville, Australia; Bali, Indonesia; Piti Reef, Guam; and Okinawa, Japan. For the other three species, additional localities for *E. mathaei* ($n = 51$) were: Oahu and Midway, Hawaiian Islands; Piti Reef, Guam; Bali, Indonesia; Great Barrier Reef, Townsville, Australia; Brisbane, Australia; and Niue. For *E. oblonga* ($n = 39$), Oahu and Midway, Hawaiian Islands; Okinawa, Japan; Madang, Papua New Guinea; and Niue. For *E. sp. nov. C* ($n = 43$): Okinawa, Japan; Palau; Madang, Papua New Guinea; and Fiji. Two individuals of *E. sp. nov. C* were collected in Niue.

Urchins were collected and preserved whole in 70% ethanol at room temperature. They were shipped to Hawaii, and genomic DNAs were isolated by the differential centrifugation method in (Palumbi et al., 1991). A part of the Cytochrome Oxidase I gene of the mtDNA was amplified using primers COI-f 5' [TTCTTTGACCCTGCAGGAGGAGGAGAYCC] and COI-d 3' [GAACATGATGAAGAAGTGCACCTTCCC]. In some cases, an internal primer (COI-w 3' [GANCCCTGRAGNGTTGCCATTCA] or COI-x 5' [TGAGCCCACCATATGTTTACAGT]) was used to amplify or sequence DNAs from particular individuals. COI-f and COI-d amplify a 588 bp section of the COI gene from position 6451 to 7039 in the *S. purpuratus* mitochondrial genome (Jacobs et al., 1988). PCR amplification cocktails were as described in (Palumbi et al., 1991). Forty amplification cycles were performed with the following profile: 94°C for 30 s, 55°C for 30 s, 72°C for 30 s. The COI-f and COI-x primers were biotinylated by the manufacturer (Operon), and used in solid phase sequencing (Palumbi, 1996).

3. Results

3.1. Pacific distribution of *Echinometra* species

There is usually more than one species of the genus *Echinometra* found on reefs of the Pacific Fig. 1. All four species (*sensu* Uehara and Shingaki, 1984) occur on reefs in

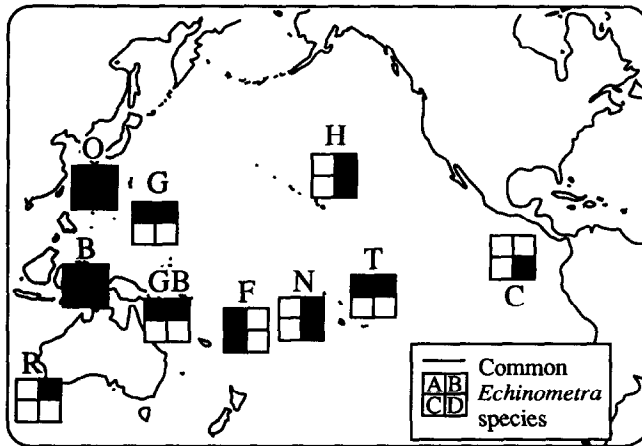


Fig. 1. Species of *Echinometra* common on Pacific reefs. Species are identified by the criteria of Uehara (1991), and given letter designations according to the published scheme (see also Palumbi and Metz, 1991). Localities are C: Isla del Coco, Costa Rica; H: Hawaiian Islands; G: Guam; O: Okinawa; B: Bali; GB: Great Barrier Reef, R: Rottneest Is.; F: Fiji; N: Niue; T: Tahiti.

Okinawa and Indonesia. It is more common, however, to find only two sympatric species in island archipelagoes of the central/western Pacific. At the edges of the tropical Pacific, *Echinometra* species are often found alone (e.g. *E. oblonga* in Cocos Is., Costa Rica, and *E. mathaei* at Rottneest Is., SW Australia).

The identity of the species found can change over surprisingly short geographic scales. For example, *E. mathaei* and *E. oblonga* are found together in Hawaii and on Niue. In Fiji, 1300 km to the west of Niue, there are also two species of *Echinometra*, but *E. mathaei* and *E. oblonga* do not occur. Instead *E. sp. nov. A* and *E. sp. nov. C* are common. To the east of Niue, in the Society Islands, only *E. mathaei* and *E. sp. nov. A* are common. As a second example, *E. mathaei* and *E. sp. nov. A* are the common calm-water species in Guam and Papua New Guinea. In Palau (1300 km SW of Guam) *E. sp. nov. C* is the commonest *Echinometra* with *E. sp. nov. A* occurring rarely. Our understanding of species distributions are likely to change as more detailed surveys are conducted. However, even with our currently incomplete sampling, it is clear that discontinuities of species ranges are common for Pacific *Echinometra* (Fig. 1). The result is a patchwork distribution of species.

3.2. Genetic relationships among *Echinometra* species

Sequences from the COI gene region of *Echinometra* mtDNA show small differences among central/western Pacific species, but large differences to congeners from the eastern Pacific (Fig. 2). Across all positions in the COI gene fragment, *E. lucunter* (from the Caribbean) and *E. vanbrunti* (from the Eastern Pacific) differ from the western species by 11–13%. This divergence is very high considering that the amino acid

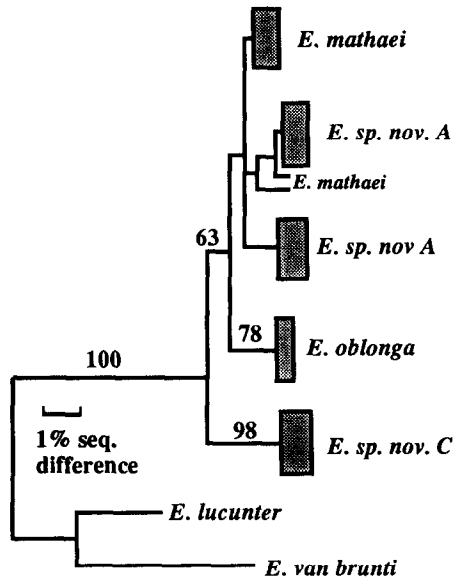


Fig. 2. Genetic differences in sequences of the Cytochrome Oxidase I gene within and between species of *Echinometra*. For the Eastern Pacific/Caribbean species (*E. vanbrunti* and *E. lucunter*), mtDNA from only one individual was sequenced. For the others, mtDNAs from 39–65 individuals were sequenced. Shaded boxes give estimates of the amount of sequence diversity within these species. Numbers refer to bootstrap estimates of branch reliability based on phylogenetic analysis using PAUP. Branch lengths are based on a Neighbor-joining tree produced by PHYLIP 3.4.

sequence of the COI gene is strongly conserved. Because of this, virtually all of the sequence differences occur at silent positions, which show 44–53% divergence.

These data also suggest that the central/western Pacific cluster of species have diversified more recently than the split between *E. lucunter* and *E. vanbrunti*. For substitutions at silent sites (calculated using the K_s statistic of Wu and Li, 1985), comparison of *E. vanbrunti* to *E. lucunter* show differences of 28% (Table 1). This corresponds to an approximate divergence rate of about 8% per million years if we take the final rise of the Isthmus of Panama 3.5 million years ago as the divergence date of these species (Bermingham and Lessios, 1993; Knowlton et al., 1993).

In the central/western Pacific, genetic distance (for all substitutions at silent positions) is 17–25% between *E. sp. nov. C* and the other central Pacific species. Among the three remaining species divergence ranges from 8–17%. These values suggest that *E. sp. nov. C* diverged about 3 million years ago, whereas the other three species diverged about 1–2 million years ago. It is important to emphasize that these estimates are based on only 450 bp of sequence, and that they are likely to be accurate to only $\pm 50\%$. The current data merely indicate that the Pacific species of *Echinometra* are of late Pliocene–Pleistocene origin. Thus, these species are not the products of divergence that took place deep in the Pliocene or Miocene, as is the case for the well-studied,

Table 1
Silent site differences between representatives of 6 *Echinometra* species

	<i>E. sp.C</i>	<i>E. oblonga</i>	<i>E. sp.A(OK)</i>	<i>E. sp.A(FJ)</i>	<i>E. mathaei</i>	<i>E. lucunter</i>	<i>E. vanbrunti</i>
<i>E. sp. C</i> (NG82)	–	18.17	25.19	23.54	17.30	55.35	43.93
<i>E. oblonga</i> (HI03)	1	–	17.29	13.10	8.63	55.69	47.13
<i>E. sp. A</i> (OK03)	0	1	–	12.08	12.22	76.78	52.53
<i>E. sp. A</i> (FJ24)	1	2	1	–	9.90	63.70	52.67
<i>E. mathaei</i> (HI03)	2	3	2	1	–	62.49	49.28
<i>E. lucunter</i>	10	12	11	12	13	–	27.89
<i>E. van brunti</i>	10	12	11	12	12	2	–

Values above the diagonal are fractions of silent sites that differ between sequences (based on the K_s statistic of Wu and Li, 1985) calculated from a portion of the COI gene. Below the diagonal are listed the number of transversions at four-fold degenerate sites in this region of mtDNA. For one of the species (*sp. A*), individuals from two distinct clades are included. For the central Pacific species, collection localities are - NG: Madang, Papua New Guinea; HI: Oahu, Hawaii; FJ: Fiji; OK: Okinawa. Samples of *E. lucunter* and *E. vanbrunti* (from the Caribbean and Eastern Pacific, respectively) were gifts of H. Lessios.

temperate, urchin genus *Strongylocentrotus* (Smith, 1988; Palumbi and Wilson, 1989; Palumbi and Kessing, 1991).

3.3. Monophyly of central/western Pacific mtDNA sequences

Bootstrap analyses of the full mtDNA sequence data set based on parsimony or on Neighbor-joining shows that two of the central/western Pacific species (*E. sp. nov. C* and *E. oblonga*) are each monophyletic with respect to mtDNA (Fig. 2). The branch leading to *E. sp. nov. C* is supported in over 90% of bootstrap replicates. The branch leading to *E. oblonga* is supported 78% of the time in parsimony analyses and 97% of the time in Neighbor-joining analyses.

By contrast, mtDNA sequences of the other two species are not monophyletic. For *E. sp. nov. A*, there are two distinct mtDNA clades (Fig. 2) that are as different from one another as they each are from *E. mathaei*. Thus these clades have probably persisted since the divergence of this species from *E. mathaei*. By contrast, the sequences from *E. mathaei* tend to occur in a single clade and would be monophyletic except for the rare occurrence of outlying sequences that do not cluster clearly with any clade. These sequences (two simple lines in Fig. 2) both branch from the base of one of the *E. sp. A* clusters and are most likely to be relicts of the mtDNA diversity in the ancestral species.

3.4. Population structure

Analysis of the geographic patterns in our full data set will be presented elsewhere (Palumbi et al., in preparation). Here, I examine patterns in one of the species, *E. sp. nov. A*, in order to illustrate the use of molecular data to examine biogeographic hypotheses.

Sequences from 65 individuals show marked variation within and between populations. As described above, there are two distinct mtDNA clades exhibited in this species

(Figs. 2 and 3), and these clades are distributed widely across the Pacific. Both clades were found in all localities, except in Okinawa and Tahiti where only one (denoted clade WTB for white-tip, clade B, see Fig. 3) has been found to date. Average pairwise sequence difference within all populations is 1.0%. By contrast, sequence heterogeneity tends to be much greater between individuals from different localities. Overall, individuals from different localities differ by 1.64%. Using $(H_b - H_w) / H_b$ to estimate F_{st}

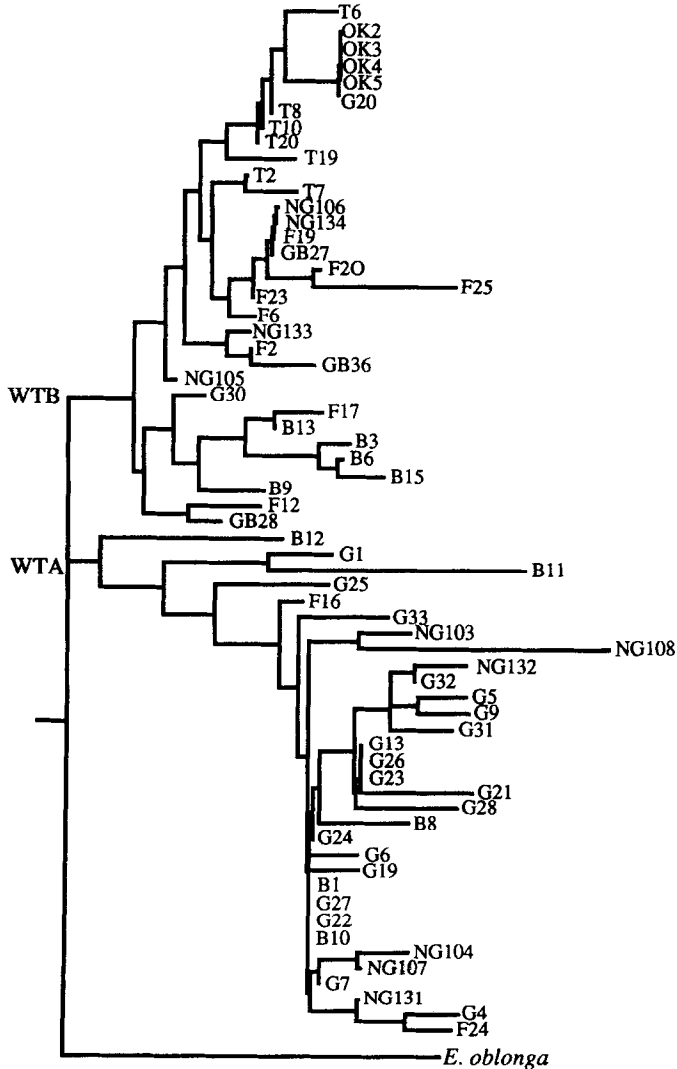


Fig. 3. Neighbor-joining tree describing the relationships among 65 COI sequences from *E. sp. nov.* A. One sequence from *E. oblonga* is used as an outgroup. The mtDNA haplotypes fall into two clades (also depicted in Fig. 2). The upper clade is denoted WTA whereas the lower clade is called WTB.

(where H_b is the average pairwise nucleotide variation between localities, and H_w is the average variation within localities, Hudson et al., 1992) yields overall estimates of geographic partitioning of genetic variation for all individuals of $F_{st} = 0.39$. On the basis of a Monte Carlo simulation, this value is significantly greater than zero, and would be expected to occur by chance alone less than 1% of the time. This result shows there is significant population structure in this species across the range we sampled.

These results combine analysis of both sequence clades within this species. If the analysis is done separately, average sequence variation among individuals from the same population is 0.93% and 0.66% for the WTA and WTB clades, respectively. Between localities, clade WTA individuals differ by 0.93% whereas WTB individuals differ between localities by 0.98%. These separate analyses show no significant population structure for clade WTA ($F_{st} = 0.01$, $p > 0.5$), but strong structure for individuals in clade WTB ($F_{st} = 0.31$, $p < 0.001$). These disparate results could be because WTA tends to dominate in western Pacific locations like Bali, Papua New Guinea and Guam, but does not occur as often in more isolated islands like the Society Islands and Okinawa.

The significant population differentiation among Bali, Papua New Guinea and Guam is largely due to differences in the proportion of WTA and WTB in these populations (Palumbi, 1996). Okinawa and Tahiti show only clade WTB (although sample size for Okinawa is currently small). By contrast, Guam (about 2600 km from Okinawa) shows mostly (80%) clade WTA. Bali and Papua New Guinea show about equal frequencies of the two clades. On the basis of a randomized chi-square test (Roff and Bentzen, 1989), these differences are greater than those expected by chance, showing that the distribution of major clades is heterogeneous throughout the Pacific (Palumbi, 1996).

3.5. Genetic diversity gradients

Within these *Echinometra* species, there are strong differences between populations in average sequence heterogeneity. The populations sampled from the center of the Indo-West Pacific (Bali and Papua New Guinea) show high sequence heterogeneity (1.6–1.7%) whereas to the north and to the east, heterogeneity declines (Fig. 4). Okinawa and Tahiti are the most isolated populations from which we have data for *E. sp. nov. A*. These localities show 0% and 0.5% sequence heterogeneity, respectively (Table 2). This trend remains apparent if WTA or WTB are analyzed separately, although differences in diversity between populations are smaller. In addition, other species in our data set show lower mtDNA variability in isolated archipelagoes in the central Pacific (Palumbi and Metz, 1991; Palumbi et al., in preparation).

4. Discussion

The mtDNA data we have collected on Echinometrid sea urchins can be used to illustrate a genetic approach to biogeographic questions. The four research areas suggested in the Introduction (species boundaries, timing of divergence, species divergence patterns, and allele biogeography) are not the only uses for genetic data, but

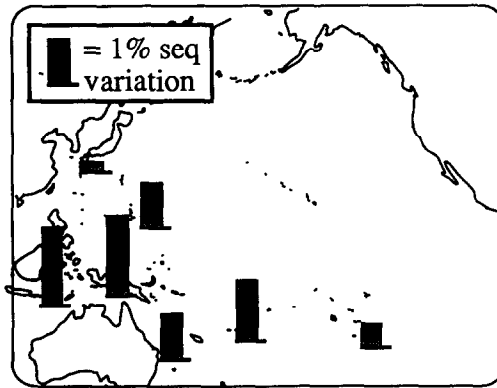


Fig. 4. Sequence variation within populations of *E. sp. nov.* A. Average nucleotide variation was calculated by averaging the percent sequence difference between individuals of a population compared pairwise. Sample sizes are given in Table 2.

represent a common way in which genetic data might be used across diverse taxonomic groups and geographical settings.

4.1. Species boundaries and timing of divergence

Species of *Echinometra* have only been recently recognized (Uehara et al., 1986) and can be distinguished only on the basis of minor differences in gonad spicules and spine color. Evidence from mtDNA and nuclear genes, however, shows that these slight morphological differences correspond well with genetic differences: morphotypes show differences in mtDNA, single copy nuclear DNA, and in specific nuclear genes like those involved in gamete interaction (Palumbi and Metz, 1991; Metz and Palumbi, 1996; Palumbi, 1996).

Recent divergence of species is suggested by a variety of genetic tools. Average genetic distance between species is low for allozymes (Matsuoka and Hatanaka, 1991),

Table 2

Average pairwise nucleotide differences within and between populations of *E. sp. nov.* A

	Guam <i>n</i> = 21	Fiji <i>n</i> = 10	Tahiti <i>n</i> = 7	PNG <i>n</i> = 10	OK <i>n</i> = 5	Bali <i>n</i> = 10	GBR <i>n</i> = 3
Guam	1.1	2.1	2.2	1.5	2.5	1.9	2.2
Fiji		1.3	1.1	1.8	1.3	1.7	1.1
Tahiti			0.5	1.8	0.6	1.6	0.8
Papua NG				1.7	2.2	1.9	1.8
Okinawa					0	1.7	1.0
Bali						1.6	1.6
Great Barrier Reef							1.0

Above the diagonal are average percent substitutions between individuals from different collection localities. Along the diagonal are average percent nucleotide differences between individuals from the same collection locality.

sequences of the mitochondrial COI gene and for RFLPs of whole mtDNAs (Palumbi and Metz, 1991). Similarly, there is low divergence of single copy nuclear genes and of introns of conserved genes (Metz and Palumbi, 1996). These genetic results suggest that the central/western Pacific species of *Echinometra* diverged during late Pliocene–Pleistocene.

Is the recent divergence of *Echinometra* a general feature of Pacific genera, or is this an exceptional genus? To date, there are few comparable genetic data with which to examine the timing of species diversification in the Indo-Pacific. However, the pattern we observe in *Echinometra* is also apparent in the butterfly fish genus *Chaetodon* (McMillan and Palumbi, 1995). Sequences from the cytochrome *b* gene of species from the sub-genus *Exonator* showed the Pacific species to be of very recent origin with average genetic distances much less than 0.5% (McMillan and Palumbi, 1995). Thus, these species groups probably diversified within the past million years.

Although genetic data are not yet available, the fossil record suggests explosive recent diversification of other Pacific genera. For example, the gastropod genus *Conus* shows an exponential increase in the number of recognized species in the Pleistocene (Kohn, 1990). Among the Cowries (genus *Cypraea*), several genera show distinct Pleistocene species radiations in the Pacific (Kay, 1995). However, this pattern may not be true of all speciose Pacific taxa. For example, ostracodes collected from sediments of Micronesian atolls are very diverse, and may include many species that have persisted since the Miocene (Weissleder et al., 1989). In this case, as well in most cases of the Pacific fauna, the importance of sibling (or cryptic) species to patterns of diversification are unknown. Advent of rigorous but simple molecular genetic techniques may make the elucidation of sister species relationships in the Pacific more tractable.

4.2. Divergence patterns of *Echinometra*

Phylogenetic reconstruction of the genus *Echinometra* is incomplete, but the information we have to date suggests two interesting patterns. First, the relationship between the central/western Pacific species and the Eastern Pacific and Caribbean species is an ancient one. The large genetic distances between these two clades suggests that they diverged in the early to middle Miocene. Following this split, there was a long period without any species formation in the Pacific. This quiescent period was followed by divergence of at least 4 species in the central/western Pacific and 3 species in the Eastern Pacific and Caribbean in the late Pliocene and early Pleistocene. Do these patterns indicate pulses of speciation? Lack of species formation from the mid-Miocene to the Pliocene may be because speciation rates were low during this time. Alternatively, it may be that extinction rates were very high at the end of the Pliocene.

Changes in speciation or extinction rates are commonly thought to be associated with the acceleration of glacial cycles in the late Pliocene and Pleistocene. Glacial activity increased markedly after about 2.7 million years ago (Duplessy, 1982; Jansen and Sjøholm, 1991), with concomitant sea level changes of up to 100 m that caused great changes in coral reef habitats throughout the Pacific (Paulay, 1990). In addition, there were probably periodic sea surface temperature changes of as much as 2–6 degrees

(Lyle et al., 1992; Guilderson et al., 1994), as well as fluctuations in upwelling, current strength (Lyle et al., 1992) and wind patterns (Jansen and Sjøholm, 1991).

A number of careful paleontological studies have shown that the late Pliocene events were associated with rapid extinctions of large numbers of regional species. For planktonic foraminifera, molluscs in the Caribbean and Atlantic, and Caribbean corals, there was a pulse of extinction associated with the onset of glaciation in the Northern Atlantic (Jackson, 1994). In some cases (Allmon et al., 1993) high extinction was associated with high speciation and the result is a fauna dominated by very young species (Jackson, 1994).

It is possible that *Echinometra* has experienced a similar pulse of extinction and speciation. The long time period with no divergence shown in Fig. 2 may reflect extinction of currently unknown Pliocene *Echinometra* species in the Pacific. If a single species survived this extinction, and subsequently diversified, then the phylogenetic pattern shown in Fig. 2 would result. However, the tropical Pacific may have been far less affected by widespread extinction events in the Pliocene than other oceanic basins (Vermeij, 1987a). Thus, there may have been only one central Pacific *Echinometra* species during the late Miocene and Pliocene, which subsequently diversified to form the species we see now. Unfortunately, the fossil record of *Echinometra* is limited because of poor preservation of reef echinoids, and so these alternatives are impossible to test with current information. In particular, we do not know whether speciation has been continuous with fluctuations in extinction, if speciation rate has fluctuated but extinction rate has been constant, or if both speciation and extinction have varied significantly over the past few million years.

4.3. Population structure and allele biogeography

Species of *Echinometra* have feeding larvae that probably spend weeks or months in the plankton before metamorphosis (Uehara and Shingaki, 1984), and thus have high dispersal potential. High realized dispersal in the Pacific is suggested by the discovery of indistinguishable mtDNA sequences in individuals collected from distant localities. For the *E. sp. nov.* A individuals examined here, we have seen identical sequences from Guam and Bali (4000 km distant), Fiji and Papua New Guinea (4500 km), and Okinawa and Guam (2600 km). The observation of indistinguishable mtDNA sequences in geographically distant localities is common for marine species with high dispersal potential (Avise, 1994; Palumbi, 1994; Shulman and Bermingham, 1995).

If a reef can support populations of several species of *Echinometra*, and *Echinometra* species have high dispersal potential from reef to reef, why are not all four species found in all available reef habitats? One potential explanation for the heterogeneous distribution of species in the central Pacific is that local populations are founded on archipelagoes largely by chance. If these founder events are relatively rare, there may not have been enough time since these species formed for the colonization of all available reefs in the Pacific by all species. Micronesian ostracodes also show this pattern (Weissleder et al., 1989), with distinct heterogeneity between atolls in species composition, but no clear relationship between species similarity and distance between atolls.

Geographic patterns of mtDNA haplotypes parallel patterns of species ranges: in both cases geographic ranges (of species and haplotypes) are large but distributions are heterogeneous. In *E. sp. nov. A*, some islands that are separated by 2600 km show very different frequencies of mitochondrial haplotypes (e.g. Guam and Okinawa) despite sharing individual haplotypes. However, this geographic sub-division is not always observed. In some cases, very similar gene frequencies are found at localities separated by 4500 km (e.g. Fiji and Papua New Guinea). Thus, the high dispersal potential of *Echinometra* is reflected in wide distribution of mtDNA genotypes and high similarity between some populations, but this potential does not result in genetic homogeneity of populations across even moderate spatial scales.

Some of the patchwork of genetic variation in the Pacific might be due to variable gene flow between some localities. In the tropical Pacific, such variation in gene flow might be mediated by current patterns that link some archipelagoes more tightly than others. In the central Pacific, major currents flow along the equator whereas in the western Pacific, major currents flow from the north coast of Papua New Guinea to the Philippines and toward Japan (Mukai, 1992; Semina and Levashova, 1993). When localities showing high levels of genetic similarity in *E. sp. nov. A* are connected on a map, a similar pattern emerges: high connectivity along the equatorial islands of the central Pacific, and populations along the New Guinea-Japan corridor. This relationship must be interpreted cautiously however, and suggests that the relationship between long distance dispersal and major oceanic current tracks should be tested further.

A second source of variation in gene flow among populations could be due to temporal changes in current flow patterns. Especially during temporary climate changes like the El Niño/Southern Oscillation, gene flow may shift dramatically in direction and magnitude. Together, temporal and spatial variation in gene flow patterns may be important sources of heterogeneity in the marine fauna of the Pacific.

4.4. Implications for biogeographic models

That marine species can be of recent origins in the Pacific suggests that the geological events of the deep past can not explain all current biogeographic patterns. Events in the Eocene like the fragmentation of the Tethys Sea or the mid-Miocene collision between Australia and SE Asia (Pandolfi, 1992) have probably affected some aspects of Pacific biogeography, but not the distributions of species which evolved later. Instead, ecological, geological and evolutionary processes that have occurred throughout the late Pliocene and Pleistocene are probably important in determining the distributions and abundance of sibling species.

If these processes have acted throughout the recent past, we might also see their imprint on patterns of genetic variation within species. The results presented here show that biogeographic patterns at the species level in one genus of sea urchins are similar to geographic intraspecific patterns (i.e. phylogeography) of mtDNA haplotypes in three ways. First, recently evolved species have broad geographic ranges, and identical mitochondrial haplotypes are spread over a vast distance in the Pacific. Second, species and mtDNA haplotypes within species have a patchwork distribution across the Pacific.

Third, genetic diversity is highest near Indonesia where species level diversity is also highest.

These similarities suggest that patterns of species distribution and patterns of allele distribution share some of their underlying mechanisms. Although this type of concordance is not likely to be true for groups of Pacific ocean species whose divergence is ancient, for recently diverged species complexes like *Echinometra*, allele biogeography and species biogeography might have basic similarities. Avise has suggested a similar concordance between biogeographic provinces (defined at the species level) and genetic breaks of widely distributed species (defined at the genetic level), Avise (1992), (1994). More comparisons are needed to test this generalization.

The patterns of species and allele distribution listed above combine to suggest that movement of haplotypes and species around the Pacific is rapid but sporadic. Long distance dispersal evidently occurs, but not often enough to homogenize mtDNA haplotypes or species distributions. Instead, archipelagoes may represent more or less isolated gene pools, whose genetic composition (for mtDNAs) depends on founder events and local genetic drift. The frequency of importation of new alleles from outside an archipelago may determine local genetic diversity. Thus, isolated archipelagoes like the Hawaiian Islands and Society Islands have low mtDNA diversity despite large population sizes for the species that inhabit these island chains.

These isolated gene pools may be prime candidates for species formation. In Hawaii, for example, about 30% of marine species are endemic (Kay and Palumbi, 1987). Our emerging population genetic results suggest, however, that genetic isolation can occur within the Indo-West Pacific as well as on its periphery. For example, populations in Guam and Papua New Guinea are different genetically for both of the *Echinometra* species shared in these two localities. Thus, species formation may not be limited to peripheral or to central localities, but may be possible throughout the Indo-West Pacific even for taxa with high dispersal potential.

4.5. Genetic tests of biogeographic models

The strong, parallel gradients in species diversity and genetic diversity are not predicted by the center-of-overlap model of Pacific biogeography. If high Indo-West Pacific diversity is only the result of overlap of independent faunas, then genetic diversity in individual species should have no relationship to the high species diversity in the overlap zone.

By contrast, existence of a genetic diversity gradient is predicted by both center-of-origin and center-of-accumulation models and cannot be used to distinguish between them. This is because alleles (like species) might either originate or accumulate in the Indo-West Pacific to give rise to the observed pattern. However, these two models differ in their predictions of the phylogeography of alleles within species in a manner that may allow hypothesis testing.

A phylogeographic prediction of the center-of-origin model is that movement to the periphery of the Indo-West Pacific is uncommon and is from larger populations in the Indo-West Pacific center. The alleles that arrive at outlying localities are a small random

sample of those available. If they arrive at peripheral locations by a stepping stone process, invading intervening habitats, then alleles at peripheral locations should be highly derived phylogenetically, appearing at branch tips. Ancient allele clades (those branching at the base of phylogenetic trees) should occur in the center. If gene flow to the periphery is sudden, then there is no predicted relationship between alleles of outlying archipelagos and those at the center of the Indo-West Pacific.

By contrast, the center-of-accumulation model predicts that species coalesce at the periphery of the Indo-West Pacific and diversify there. Migration from the periphery establishes the new species at the center. If alleles in the new species are monophyletic and diverged at the peripheral locality, then the most ancient allele clades should exist in peripheral localities.

The current data can be used to test these hypotheses only preliminarily because we have only one outlying population to examine for *E. sp. nov. A*. This population, Tahiti, shows a cluster of strongly derived alleles in clade WTB. Furthermore, the alleles present in Fiji, the locality one step closer than Tahiti to the center of the Indo-West Pacific, include some that appear basal to the cluster of Tahitian alleles. Thus a pattern of sequentially derived mtDNA sequences appears on a transect from west to east, suggesting a center-of-origins model for this species.

However, an mtDNA clade in the Tahitian allele cluster is found in Okinawa and Guam (Fig. 3). Whether this is the result of rare one-step dispersal, human mediated gene flow via ships, or parallel dispersal from some unknown intermediate locality is unknown. More comparisons of Pacific allele phylogenies and geography are needed to test center-of-origin and center-of-accumulation models.

5. Conclusions

The genetic evidence reported here suggests that the central Pacific species of *Echinometra* are of recent origin, and currently are distributed heterogeneously across their ranges. Recent speciation has contributed to biogeographic patterns in the Pacific and these patterns cannot be completely explained by ancient vicariant events. Geographic patterns in genetic diversity and in the mtDNA haplotypes found, suggest that isolation by distance occurs in the Indo-West Pacific, with archipelagos that are more distant from the center of the region having lower genetic diversity. This might favor peripheral speciation. However, genetic differences across moderate spatial scales in the central Indo-West Pacific suggest this region could also give rise to new species.

If species form throughout the Indo-West Pacific, what generates the clear species diversity gradient? One possible answer is that species extinction varies geographically, being more common in peripheral archipelagos (Vermeij, 1987b, pp. 103–104). If such extinctions are mediated by wide fluctuations in population size in outlying archipelagos, this could also explain low genetic variation in isolated populations. Such potential links between demography and species diversity patterns have been suggested for latitudinal diversity gradients (e.g. Pianka, 1988), and need to be explored for the Indo-West Pacific as well.

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