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SPECIATION AND POPULATION GENETIC STRUCTURE IN TROPICAL PACIFIC SEA URCHINS

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Abstract.—Unlike populations of many terrestrial species, marine populations often are not separated by obvious, permanent barriers to gene flow. When species have high dispersal potential and few barriers to gene flow, allopatric divergence is slow. Nevertheless, many marine species are of recent origin, even in taxa with high dispersal potential. To understand the relationship between genetic structure and recent species formation in high dispersal taxa, we examined population genetic structure among four species of sea urchins in the tropical Indo-West Pacific that have speciated within the past one to three million years. Despite high potential for gene flow, mtDNA sequence variation among 200 individuals of four species in the urchin genus *Echinometra* shows a signal of strong geographic effects. These effects include (1) substantial population heterogeneity; (2) lower genetic variation in peripheral populations; and (3) isolation by distance. These geographic patterns are especially strong across scales of 5000–10,000 km, and are weaker over scales of 2500–5000 km. As a result, strong geographic patterns would not have been readily visible except over the wide expanse of the tropical Pacific. Surface currents in the Pacific do not explain patterns of gene flow any better than do patterns of simple spatial proximity. Finally, populations of each species tend to group into large mtDNA regions with similar mtDNA haplotypes, but these regional boundaries are not concordant in different species. These results show that all four species have accumulated mtDNA differences over similar spatial and temporal scales but that the precise geographic pattern of genetic differentiation varies for each species. These geographic patterns appear much less deterministic than in other well-known coastal marine systems and may be driven by chance and historical accident.

Key words.—Gene flow, marine, mtDNA, sea urchins, speciation, population structure.

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Determinants of population structure vary widely between habitats and between different taxa. Among terrestrial animals and plants, habitat fragmentation, topography, soil types, watershed characteristics, and a host of other factors are known to affect species distributions and gene flow within species ranges (reviewed in Avise 1994). These boundaries are sometimes stable over long periods, and allow the build-up of substantial genetic differentiation. Conspecific freshwater fish in different watersheds, for example, show striking genetic differences (Avise 1992). Similarly, many terrestrial animal populations show genetic discontinuities where gene flow is interrupted by geographic boundaries (Avise et al. 1979; Patton and Smith 1989). By contrast, genetic boundaries among populations of marine animals and plants are often more difficult to discern, and the physical and biological factors that determine gene flow patterns among marine populations are poorly understood.

Robust physical barriers to migration are rare in marine systems (Vermeij 1978). Even species that are restricted to specialized ecosystems (like estuaries or hydrothermal vents) often have larvae that can easily move between habitat patches (France et al. 1992). High dispersal potential across barrier-free habitats tends to lead to poorly differentiated populations across large geographic scales. Large population sizes (as occur in many marine species) slow both genetic drift and any approach to genetic equilibrium (Birky et al. 1983). Nevertheless, many marine taxa with high dispersal potential that once were thought to represent single species are actually sibling species complexes with very low genetic distances, implying recent species formation (Knowlton 1993). The great biodiversity of many shallow and deep-water habitats (e.g., Grassle and Maciolek 1992) is due in part to recent speciation (Palumbi 1997). What leads to high rates of speciation in high dispersal marine taxa?

Some marine species may have strong genetic structure despite high dispersal potential. Selection, natal homing, stable migration routes, and larval behavioral patterns are just a few of the mechanisms by which population genetic structure can arise in species with the potential to move large distances (Burton and Feldman 1982; Bowen et al. 1989; Baker et al. 1993; Burton and Lee 1994; Palumbi 1996a). It is possible that recent species formation happens to be in groups that tend to have strong geographic structure despite high potential for gene flow. It is also possible that even high dispersal species have a practical dispersal limit, and that the ranges of these species are large enough for isolation by distance to play a role in population differentiation and speciation.

To understand the mechanisms underlying differentiation of marine species undergoing rapid speciation over large spatial scales, we compared mitochondrial DNA (mtDNA) sequences from four species of sea urchins in the genus *Echinometra* collected from populations scattered throughout the tropical Pacific. These species diverged from one another over the past one to three million years (Palumbi 1996b) and represent a good test case for exploring the relationship between species formation and geographic structure. In addition, the tropical Pacific is one of the largest continuous marine habitats. The geographic extent of this habitat may reveal patterns of gene flow, especially over long distances, that are

TABLE 1. COI sequence heterogeneity within and between populations of four Pacific species of *Echinometra*. Along the diagonals are within-population, average pairwise sequence diversities. Above the diagonal are between-population values. Below the diagonal, in boldface, are estimates of F_{ST} from pairwise population comparisons.

E. oblonga							
$F_{ST} = 0.306$, $P < 0.01$							
	1	2	3	4			
1. Okinawa ($n = 5$)	0.80	0.76	0.62	0.76			
2. Niue ($n = 8$)	**0.37**	0.16	0.54	0.16			
3. Papua New Guinea ($n = 10$)	**0.03**	**0.49**	0.40	0.53			
4. Hawaii ($n = 14$)	**0.38**	**0.06**	**0.49**	0.14			
E. mathaei							
$F_{ST} = 0.389$, $P < 0.001$							
	1	2	3	4	5	6	7
1. Guam ($n = 11$)	0.80	0.68	0.72	1.10	0.59	0.92	1.06
2. Niue ($n = 6$)	**0.23**	0.25	0.95	1.23	0.20	0.44	1.16
3. Hawaii ($n = 16$)	**0.43**	**0.87**	0.00	0.42	0.71	1.17	0.85
4. Bali ($n = 8$)	**0.27**	**0.58**	**0.07**	0.78	0.89	1.50	1.10
5. Tahiti ($n = 4$)	**0.31**	**0.48**	**1.00**	**0.56**	0.00	0.57	0.98
6. Australia ($n = 8$)	**0.21**	**0.03**	**0.62**	**0.53**	**0.43**	0.66	1.37
7. Okinawa ($n = 7$)	**0.03**	**0.35**	**0.27**	**0.09**	**0.36**	**0.31**	1.25
E. sp. nov. C							
$F_{ST} = 0.145$, $P < 0.02$							
	1	2	3	4	5		
1. Fiji ($n = 12$)	0.45	0.71	0.80	0.67	0.20		
2. Palau ($n = 14$)	**0.25**	0.62	0.90	0.80	0.58		
3. Okinawa ($n = 4$)	**0.03**	**0.04**	1.10	1.00	0.60		
4. Papua New Guinea ($n = 10$)	**0.06**	**0.11**	**0.05**	0.80	0.48		
:)	**0.00**	**0.46**	**0.80**	**0.17**	0.00		
' < 0.001							
	1	2	3	4	5	6	7
21)	1.05	2.10	2.20	1.50	2.30	1.90	2.20
)	**0.44**	1.30	1.10	1.80	1.10	1.70	1.10
7)	**0.64**	**0.19**	0.50	1.80	0.90	1.60	0.80
4. Papua New Guinea ($n = 10$)	**0.08**	**0.17**	**0.39**	1.70	1.80	1.90	1.80
5. Okinawa ($n = 4$)	**0.60**	**0.05**	**0.28**	**0.31**	0.80	1.60	0.80
6. Bali ($n = 6$)	**0.30**	**0.15**	**0.34**	**0.13**	**0.25**	1.60	1.60
7. Great Barrier Reef ($n = 3$)	**0.53**	**0.00**	**0.06**	**0.25**	**0.11**	**0.18**	1.00

not apparent in more restricted bodies of water. Finally, because *Echinometra* species appear to have similar larval dispersal abilities, tracking mtDNA variants throughout the Pacific for all four species allows four independent views of the evolution of population structure.

METHODS AND MATERIALS

Nomenclature

Valid names for these species have been debated (Mortensen 1943), but recent morphological and genetic studies all agree that the genus *Echinometra* is represented by at least four species in the Indo-West Pacific (IWP; Uehara et al. 1986; Matsuoka and Hatanaka 1991; Palumbi and Metz 1991). We follow Edmondson's usage of the names *E. mathaei* and *E. oblonga* for the species found in Hawaii (Edmondson 1935), which are the same as types B and D, respectively, in Okinawa (Motokawa 1991; Nishihira et al. 1991). We refer to the other two species as *Echinometra sp. nov.* A and *Echinometra sp. nov.* C, corresponding to Okinawan types A and C, respectively.

Collections, DNA Amplification and Sequencing

Collection localities are listed in Table 1 and in Palumbi (1996b). Animals were collected and preserved whole in 70% ethanol at room temperature and shipped to Hawaii. Genomic DNA was isolated and a 601-bp part of the Cytochrome Oxidase I gene (corresponding to positions 6439–7039 in the mitochondrial genome of *Strongylocentrotus purpuratus*) was amplified using primers COI-f long 5′ (TTTCTTGACCCTGCAGGAGGAGGAGAYCC) and COI-d 3′ (GAACATGATGAAGAAGTGCACCTTCCC). PCR amplification chemistry was previously described (Palumbi 1996c). Forty amplification cycles were performed with the following profile: 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec. The COI-f primer was biotinylated by the manufacturer (Operon, Inc.), and used in solid phase sequencing (Palumbi 1996a).

Data Analysis

For each individual, we analyzed a 450-bp region of sequence corresponding to positions 6503–6952 in the mitochondrial genome of *S. purpuratus* (Jacobs et al. 1988). Se-

quences (GENBANK Accession Nos. AF018673-AF018885) were aligned by eye for phylogenetic analysis. Unique sequences were determined using MacClade 3.0, and used to construct maximum-likelihood trees for each species using FastDNAML (Olsen et al. 1994). The maximum likelihood topology was imported into PAUP 3.1.1 and the intraspecific tree length was estimated. This length was compared with the length of the most parsimonious trees found by performing 100 heuristic searches using random input order, holding five trees per search.

Patterns of molecular evolution within the protein coding regions and overall levels of genetic diversity within and between populations were analyzed with HEAP BIG, a Macintosh-based program written by and available from SRP. Population-level differentiation was estimated using the F_{ST} approach of Hudson et al. (1992). Statistical significance of F_{ST}-values was determined by 500 Monte Carlo randomizations of mtDNA sequences among populations using HEAP BIG.

Results

Phylogenetic Relationships among Sequences

Cytochrome Oxidase sequences were obtained from 200 individual sea urchins of the four IWP *Echinometra* species and for the outgroup species *E. vanbrunti* (E. Pacific) and *E. lucunter* (Caribbean; both gifts of H. Lessios). Although this region of the mitochondrial genome is highly conserved at the amino acid level (Jacobs et al. 1988), there are substantial numbers of silent substitutions within and between species.

Among the 200 individual COI sequences, 116 were different and fell into five phylogenetic clades. Bootstrap parsimony analysis using a 10:1 weighting scheme for transversions:transitions and using *E. vanbrunti* and *E. lucunter* as outgroup taxa suggest that the most ancestral clade is that of *E. sp. nov.* C, followed by *E. oblonga*, and then a cluster of three clades from *E. mathaei* and *E. sp. nov.* A (Fig. 1). Analysis by genetic distance methods (using Jukes-Cantor, Kimura two-parameter, or Nei and Tamura multiple hit algorithms) and neighbor-joining trees gave similar topologies.

Mitochondrial sequence and morphology are highly congruent in this set of species. In 198 of 200 cases, species designations based on morphology (spine color and gonad spicules, see Palumbi and Metz 1991) were the same as designations based on mtDNA sequence. Of the two exceptions, one was an individual from Okinawa (OKtm39) that had triradiate gonad spicules and pastel-colored spines (placing it in the species *E. sp. nov.* C), but had the mtDNA of *E. mathaei*. The other was a dusky pink individual from Bali (Bm5) with rod-shaped gonad spicules (placing it in the species *E. mathaei*), that had the mtDNA of *E. oblonga*. These two individuals were removed from population-level analyses.

Intraspecific Phylogenetic Patterns

Within species, phylogenetic relationships were estimated using maximum-likelihood trees (FastDNAML) constructed with the unique sequences, and rooted by a sequence of the Eastern Pacific species *E. vanbrunti*. In three of four species,

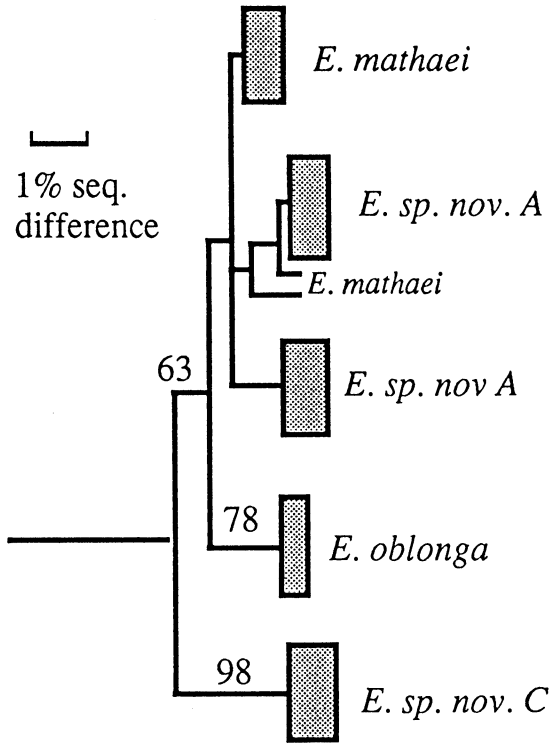

FIG. 1. Neighbor-joining tree showing the phylogenetic relationships among five clades of COI sequences from 200 individuals of Indo-West Pacific *Echinometra* species. Intraspecific variation is represented by the depth of the box drawn for each clade. Bootstrap values are shown above branches.

the maximum-likelihood (ML) tree had the same length as the set of minimal-length trees found by parsimony analysis. In *E. mathaei*, the ML tree was two steps longer than the minimal-length trees, and differed from the minimum-length tree in the placement of one long branch. The topologies of these intraspecific phylogenetic relationships are most easily represented as networks because in many cases sequences in the dataset are hypothesized to reside at nodes of the phylogenetic tree. Instead of drawing such trees with many zero length branches, it is more informative to draw the stepwise evolutionary relationships among existing sequences (Fig. 2A–D). Also drawn onto these trees are the names of identical sequences. In many cases, these identical sequences sit deep within the structure of the phylogenetic tree, suggesting that these common sequences are old enough to have given rise to many derived mtDNA types. For example, a widespread sequence in *E. sp. nov.* A was found in Papua New Guinea, Fiji, Okinawa, and the Great Barrier Reef (Fig. 2A). This sequence is hypothesized to have given rise to all the sequences we obtained in Tahiti, plus derived sequences in Papua New Guinea, Fiji, Okinawa, and Guam. In general, the most common sequences in all four species are found within phylogenetic networks, and only occasionally do these sequences represent terminal twigs on the trees. This is apparent from the ML trees (Fig. 2), as well as from inspection of random subsets of minimal-length trees generated by PAUP. For example, of 50 equally parsimonious trees for *E. mathaei* sequences, all showed the most widespread haplo-

type (found in Hawaii, Okinawa, Guam and Bali) at an interior node.

These phylogenetic reconstructions also suggest strong geographic patterns of mtDNA sequence variation in all four species. For example, sequences from *E. mathaei* (Fig. 2B) fall into two clades, one of which is common in Hawaii and Bali, and the other of which is common in Guam, Australia, Niue, and Tahiti. Likewise, sequences from *E. oblonga* from Papua New Guinea and Okinawa cluster together in a derived clade (Fig. 2D), whereas sequences from the isolated islands of Hawaii and Niue tend to occur near the base of the tree.

Variation of COI Sequence and Population Structure within Species

Within populations, COI sequence diversity varies between 0% and 1.7% (Table 1), consistent with prior reports of mtDNA RFLP variation within these urchins (Palumbi and Metz 1991). Among the four species, within-population variation averages about 0.5%, except for *E. sp. nov.* A, in which populations display average variation above 1.0%. This difference is due to the presence in *E. sp. nov.* A of two distinct mitochondrial clades that are about 2.0% different from each other (Fig. 2A). Taking each clade separately, individuals differ by about 0.5% within populations, just as in comparisons of other species.

Variation between populations tends to be higher than variation within populations (Table 1). Differences range from 0.16% to 2.3% with an average between population difference of 1.6% for *E. sp. nov.* A and 0.56–0.89% for the other three species. If populations were genetically homogeneous, there would be the same degree of genetic variation within populations as between them. Hudson et al. (1992) suggested that the ratio of between-population sequence variation to within-population variation could be used to estimate Wright's F_{ST}, which can then be related to gene flow. This approach shows that F_{ST}s are high in Pacific *Echinometra* species. The highest average values are for *E. mathaei*, *E. sp. nov.* A and *E. oblonga*, (0.389, 0.366, and 0.306, respectively); *E. sp. nov.* C shows the lowest value at 0.145. Even this low value is significantly higher than would be expected in a panmictic population ($P < 0.02$, as estimated from 500 Monte Carlo simulations). Thus, all species of *Echinometra* show significant population structure throughout the Pacific. Average female gene flow per generation (*Nm*) among all populations ranges from a high of 3.0 for *E. sp. nov.* C to a low of 0.7 for *E. mathaei*.

The data in Table 1 can also be used to estimate gene flow among pairs of populations (Hudson et al. 1992; Slatkin 1994). These results show substantial heterogeneity from population to population. In all four species, some population comparisons show low F_{ST} and high gene flow, whereas others show the opposite pattern. In *E. sp. nov.* C, most pairs of populations show relatively high gene flow (F_{ST}s < 0.05). Only the Palau population shows substantially different mtDNA haplotype frequencies. By contrast, in the other three species, low gene flow and high F_{ST}s between populations are the rule. In *E. oblonga*, *E. mathaei*, and *E. sp. nov.* A, most pairs of populations show $F_{ST} > 0.20$ (Table 1).

These results reinforce the conclusion that gene flow between most populations of these species is uncommon. However high gene flow is inferred for some population comparisons of all species. For example, *E. oblonga* from Okinawa and Papua New Guinea show a gene flow value of about 16 female migrants per generation. Phylogenetic reconstruction of the mtDNA sequences shows that most Okinawa and Papua New Guinea individuals fall into a unique clade defined by a transition change at position 189. In this case, high gene flow is inferred from the similarity of sequences across broad spatial scales.

In three cases, however, high inferred gene flow is an artifact of the equations used to estimate *Nm* (Hudson et al. 1992). For example, Bali and Hawaii show high gene flow (and a low F_{ST} of 0.07) because the average pairwise sequence difference between these two localities (0.42%) is similar to the average within-population sequence difference (0.39%). However the within-population average is the average of a very low intrapopulation diversity in Hawaii (0%) and a higher diversity in Bali (0.78%). Wright's island model, upon which the link between F_{ST} and *Nm* is based (Hudson et al. 1992), assumes all populations have equal size and equal average diversity. When these assumptions are violated, *Nm* estimates based on F_{ST}-values may be incorrect. Thus the similarity of percent nucleotide variation within and between populations in Hawaii and Bali does not necessarily imply high gene flow. In fact, Hawaii is dominated by a single COI sequence that only appears in three of nine individuals in Bali. Bali and Hawaii populations are very different genetically despite the apparent low F_{ST}-value between them.

Geographic Isolation and Intraspecific Genetic Variation

Geographic position is highly correlated with the amount of mtDNA variation. The lowest mtDNA diversities were observed in populations of each species most distant from the Indonesian Archipelago (considered to be the center of the Indo-West Pacific biogeographic province; Fig. 3). In contrast, the populations with the greatest mtDNA diversities tend to be from the center of the Indo-West Pacific in Bali or Papua New Guinea. For each species, we compared the geographic position of populations that are more diverse than the mean with those that are less diverse than the mean. Populations more diverse than the mean tend to be closer to the Indonesian Archipelago (average 4300 km) than those less diverse than the mean (average 9400 km; $P = 0.005$ Mann Whitney *U*-test).

Because of these diversity clines within species, the assumptions of Wright's island model of gene flow are violated (see above). Moreover, the conclusion that these four species of *Echinometra* show population structure might largely be due to the presence of peripheral populations with low genetic variability. To test this, we reanalyzed gene flow patterns within each species after removing data from peripheral populations with low variability. For *E. mathaei* and *E. sp. nov.* A, populations with high variability were genetically distinct (populations G, B, AUS, OK: $F_{ST} = 0.212$, $P < 0.002$ for *E. mathaei*; populations G, B, F, PNG: $F_{ST} = 0.206$, $P < 0.002$, for *E. sp. nov.* A). For *E. oblonga*, the only high variation populations we surveyed (Okinawa and Papua New Guinea) are not distinct ($F_{ST} = 0.01$, $P > 0.25$). High di-

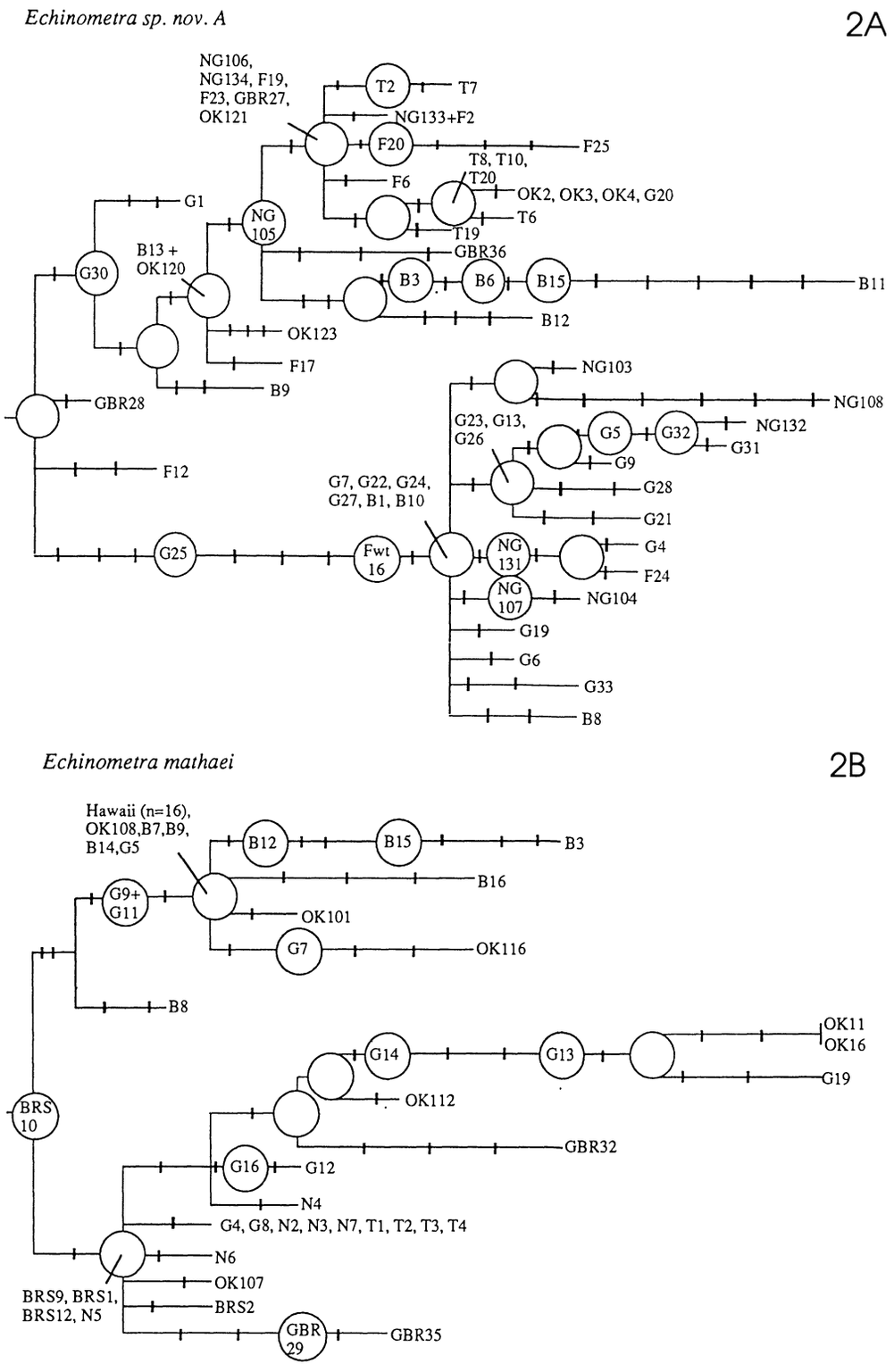

FIG. 2 Phylogenetic networks depicting the relationships among COI sequences within each of the four sea urchin species. All trees are maximum-likelihood trees rooted to the eastern Pacific species *Echinometra vanbrunti*. All but the tree for *E. mathaei* (Fig. 2B) are also shortest length trees. The tree for *E. mathaei* is two steps longer than a shortest length tree. Vertical tick marks represent nucleotide substitutions. Ancestral nodes are represented by circles. Empty circles are hypothesized nodes we have not seen in our collections. For sequences that have been seen multiple times, each occurrence is listed. Each sequence name consists of a locality designator in capitals followed by an individual ID number. Locality abbreviations are: GBR: Great Barrier Reef; B: Bali; BRS: Brisbane; F: Fiji; G: Guam; H: Hawaii; N: Niue; NG: Papua New Guinea; OK: Okinawa; P: Palau; T: Tahiti.

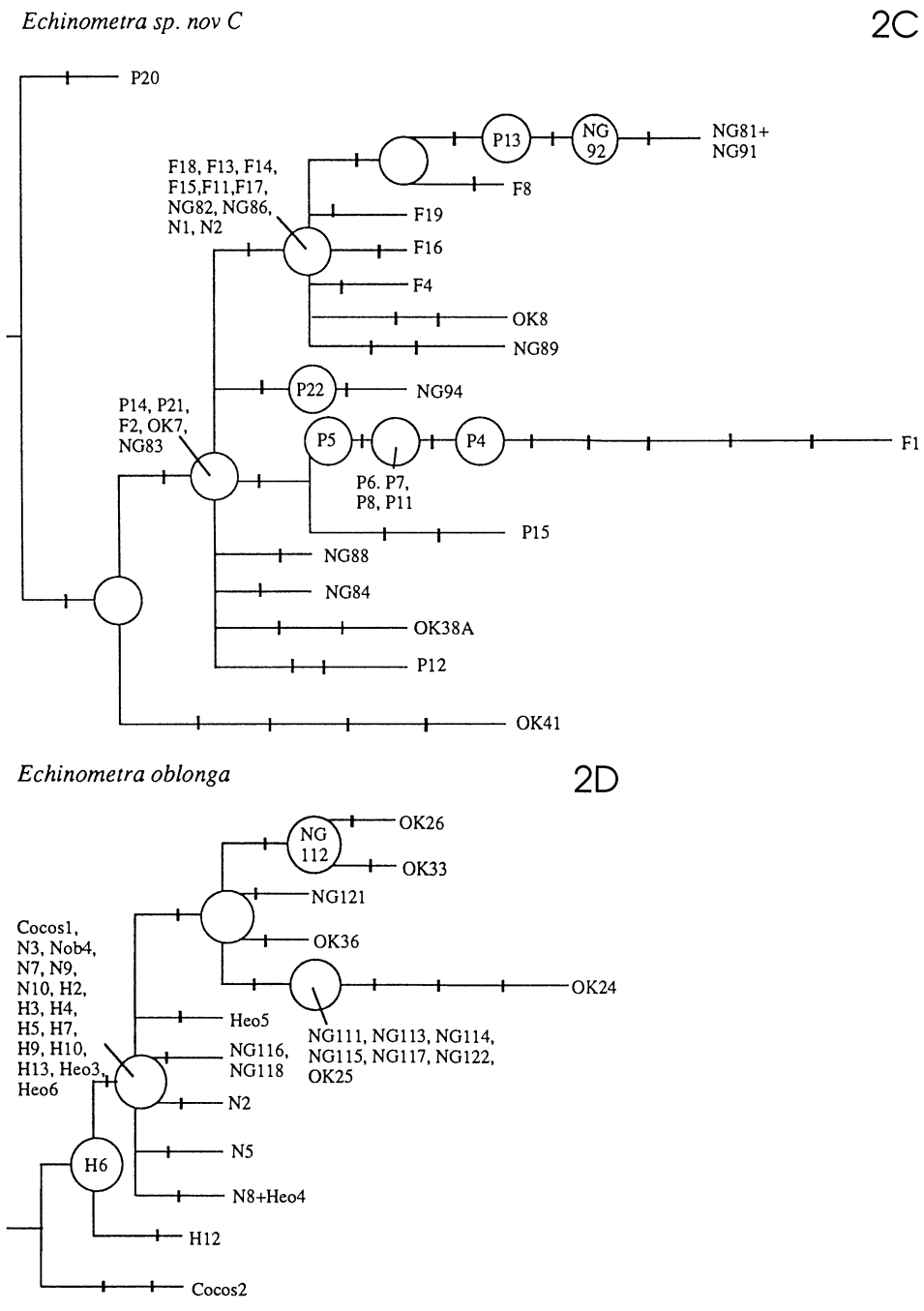

FIG. 2. Continued.

versity populations of *E. sp. nov.* C in Palau, Okinawa, and Papua New Guinea are genetically differentiated, but Monte Carlo simulations suggest this difference is slight (F_{ST} = 0.21, P = 0.05). These results suggest that peripheral populations with low mtDNA variability have a strong impact on population structure. Nevertheless, high variability populations are not panmictic, and can add significantly to overall population differentiation.

Gene Flow, Isolation by Distance and Current Paths

Isolation by distance can be detected by a negative relationship between geographic distance and gene flow (Slatkin 1994; for a marine example, see also Hellberg 1994). Across the tropical Pacific, straight-line distance between localities is negatively related to gene flow (Fig. 4A, Mantel tests for separate species, $P < 0.01$) suggesting strong isolation by distance. Localities separated by 1500–3000 km generally had *Nm* larger than three females per generation. By contrast, localities separated by 5000–10,000 km usually (13 of 23 cases) showed *Nm* less than one. Only two of these comparisons showed *Nm* greater than five. Note, however, that there is substantial scatter in the relationship of geographic and genetic distances. Also note that comparisons across 5000–10,000 km usually involve populations that differ in

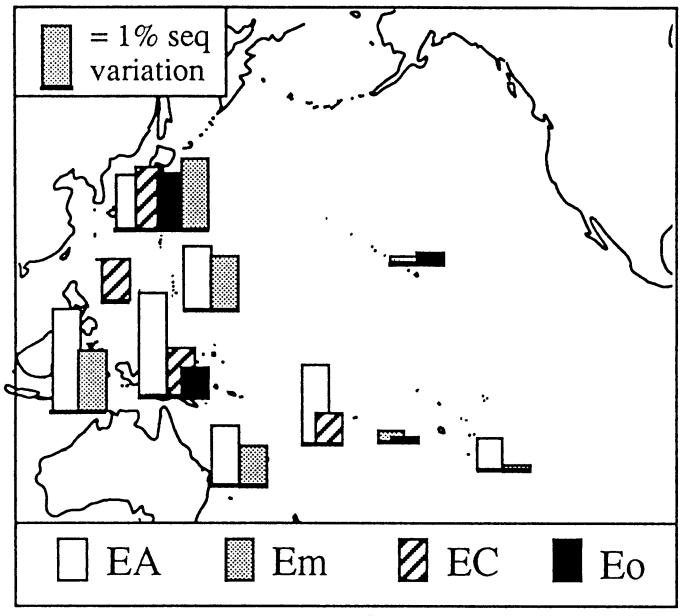

FIG. 3. Population variability in COI sequences in different Pacific archipelagoes. Average pairwise sequence variation is plotted for all four species in each collection locality.

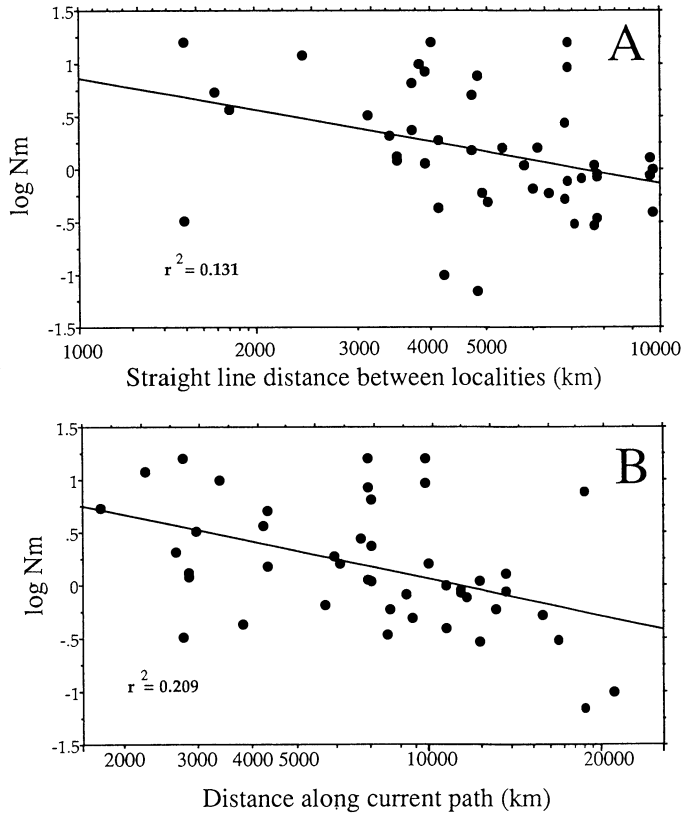

FIG. 4. Relationship between estimated gene flow (log *Nm*) and (A) straight-line geographic distance between populations; or (B) the distance between localities traced along current paths in the Pacific. Straight-line geographic distance was estimated from a Lambert Azimuthal Equal Area Projection map of the Pacific. Distance along current paths was estimated by tracing the most likely pathway between two islands based on compilations of Pacific surface currents by NOAA (see htlp://www.aoml.noaa.gov./phod/dac/dacdata.html). Because the points plotted are not all independent, the standard correlation coefficients shown must be interpreted conservatively based on $n - 1$ degrees of freedom where n is the number of populations compared (not the number of pairwise comparisons; Hellberg 1994). Mantel tests show significant relationships between gene flow and distance for each species.

within-population variability (Table 1), and that subsequent estimates of gene flow may be inaccurate.

To understand if "current-path" distance is a better descriptor of gene flow patterns in the Pacific than straight geographic distance, we estimated current paths between all localities using a recent compilation of data from drifting buoys (data from NOAA, see Fig. 4 legend), and used these paths to measure the route a marine larva might take to drift between localities. *Nm* declined significantly with increasing current-path distances (Fig. 4B, Mantel tests, $P < 0.05$), but this relationship has no better explanatory power than does straight-line distances between islands. Population pairs whose current-path distances are especially great compared with the average (i.e., whose points fall above a regression of current-path distance on straight-line distance) do not have especially low gene flow between them (i.e., they do not tend to fall below a regression line relating gene flow to straight distance, $P > 0.05$.) This result (Fig. 5) is similar in principle to a multiple regression of straight-line distance and current-path distance on gene flow, but it allows us to adjust the number of degrees of freedom to be the number of different populations examined instead of the number of pairs of populations compared (Hellberg 1994).

DISCUSSION

Genetic Structure in the Tropical Pacific

Our mtDNA data from four species of Pacific sea urchins show three strong geographic patterns. First, we found significant population genetic structure for all four urchin species across the Pacific. Second, there is a gradient of decreasing genetic diversity from the "center" to the "periphery" of the tropical Pacific. Third, genetic differentiation among island populations shows strong isolation by distance. Oceanic currents explain this isolation no better than does the spatial distribution of islands.

Most populations sampled from localities more than 5000 km apart showed significant differences in mtDNA sequence. Estimated gene flow between them was less than one female per generation in 13 of 23 comparisons, and only twice did we estimate *Nm* to be greater than five. In contrast, populations 1500–5000 km apart tended to have more similar mtDNA sequences. In 18 of 23 such comparisons, estimated gene flow was more than one female per generation, and in nine cases, gene flow was estimated to be higher than five females per generation (Table 1).

In most cases where relatively close populations have moderate gene flow (one to five females per generation), these moderate levels of gene flow do not appear sufficient to completely homogenize mtDNA haplotype frequencies (see also Lavery et al. 1996). For example, populations of *E. sp. nov.* A sampled from Guam, Indonesia, and Fiji have estimated gene flow of about two females per generation, yet there are

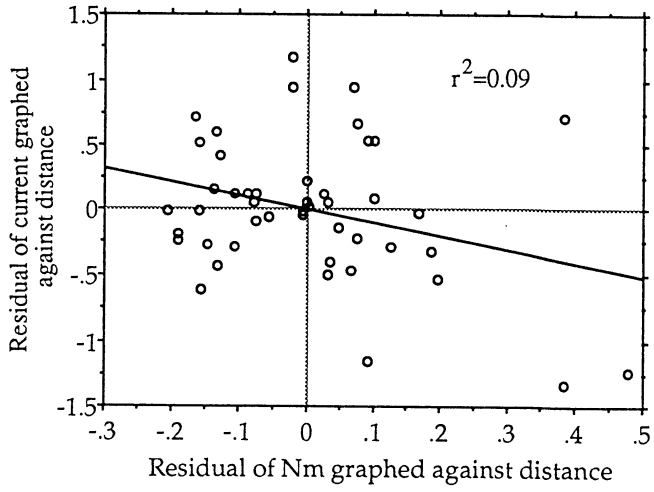

FIG. 5. Gene flow versus straight-line and current-path distance in the Pacific. Some localities are much further away by currents than by linear distance. To test whether these localities tend to show lower than average *Nm*-values, we plot the residuals of a graph of current versus linear distance against the residuals of a graph of *Nm* versus linear distance. Points much further away by currents (above zero on the y-axis) have slightly lower *Nm*-values (below zero on the x-axis), but this relationship is weak and nonsignificant.

significant differences in the mtDNA types present at these localities (randomized chi-square, $P < 0.01$; Roff and Bentzen 1989). In such cases, moderate amounts of gene flow have not prevented the divergence of mtDNA frequencies. Wright (1969) pointed out that substantial variation in gene frequencies can occur between demes despite low F_{ST}, and Slatkin and Barton (1989, p. 1351) suggest that *Nm*-values slightly above 1.0 act mainly to prevent "near fixation of alternative alleles." Thus, moderate levels of gene flow are consistent with gene frequency differences among populations, especially if these populations have not attained an equilibrium between gene flow and genetic drift (Takahata 1983).

That *Echinometra* have not yet attained this genetic equilibrium is suggested by the occurrence of identical sequences across most of the Pacific despite significant population structure. For example, the only COI sequence found in *E. mathaei* in Hawaii is also found over 6000 km away in Guam and 12,000 km away in Bali. In *E. sp. nov.* C, the dominant sequence occurred in 10 of 42 individuals, and was found in Papua New Guinea, Fiji, and Niue. Rapid spread of haplotypes over vast distances suggests high gene flow, but differences in frequencies of these sequence types in different populations point to infrequent genetic exchange. These results might be reconciled by periods of strong gene flow when mtDNA types spread across the Pacific followed by periods when gene flow and larval exchange throughout the Pacific is low. Recently, Lessios et al. (1996) have identified sea urchins of west Pacific origin in the fauna of islands of the Eastern Pacific, and suggested that events like the El Niño–Southern Oscillation may periodically increase the likelihood of pan-Pacific larval transport. In addition, mtDNA data suggest that the coconut crab has shown a recent expansion in the tropical Pacific, perhaps due to an expansion of the number of available islands during low sea level stands in the Pleistocene (Lavery et al. 1996). Slight genetic differentiation in modern coconut crab populations suggests that this ecological expansion was followed by a slowing of gene flow. For *Echinometra*, we find a much stronger pattern of geographic structure. This may be because contemporary gene flow is lower in sea urchins than coconut crabs, or because different archipelagoes in the Pacific have been colonized, by chance, by different mtDNA haplotypes.

Other evidence for lack of genetic equilibrium may be found in the highly variable relationship between gene flow and distance. For populations separated by 3000–6000 km, *Nm*-values range from 0.1 to 16.0. In addition, when we compare results for multiple species collected from the same set of islands, we often observe different patterns of gene flow. For example, Okinawa and Papua New Guinea populations show high gene flow for comparisons of *E. oblonga* and *E. sp. nov.* C ($Nm = 16$ and 8.5, respectively), but low gene flow for comparisons of populations of *E. sp. nov.* A ($Nm = 0.31$).

In contrast to the low-to-moderate gene flow that we found among *Echinometra* populations, previous studies have found high mtDNA exchange among temperate sea urchin populations (Palumbi and Wilson 1990; Palumbi and Kessing 1991; McMillan et al. 1992). Other studies of marine species with similarly high dispersal potential (reviewed in Avise 1994; Palumbi 1992, 1994) have also observed low genetic differentiation among populations. However, this association is by no means perfect (Burton and Feldman 1982; Palumbi 1994), and there are a growing number of examples of fine-scale genetic differentiation in marine species with high dispersal potential (Watts et al. 1990; Hare and Avise 1996). Even in previously studied temperate urchins, there may be genetic differences between populations on a scale small enough to suggest patchy recruitment by related larvae (Edmands et al. 1996). Whether such fine-scale genetic variation is the rule and how it relates to long-distance genetic exchange remain to be discovered.

In the tropical Pacific, allozyme studies tend to show low levels of population differentiation across large distances (Winans 1980; Benzie and Stoddart 1992; Macaranas et al. 1992; Williams and Benzie 1993, 1996; Lavery et al. 1995). Information from mtDNA tends to show more population structure, often with a distinct pattern of isolation by distance (Lavery et al. 1996; Williams and Benzie 1997), and sometimes lower mtDNA variation at peripheral populations (Williams and Benzie, unpubl. data; but see Lavery et al. 1996). More studies of species widely scattered across the Indo-West Pacific are needed before any generalizations about normal levels of gene flow are made, but the distances between archipelagoes may be large enough for the build-up of significant population structure even in high dispersal marine species.

Mitochondrial DNA Variation in Isolated Populations

We found high mtDNA diversity in most populations near the center of the Indonesian Archipelago. By contrast, populations at the periphery of the Indo-West Pacific populations had low mtDNA diversity (Fig. 3). Williams and Benzie (1996) also reported low mtDNA diversity for peripheral pop-

ulations of the tropical Pacific starfish *Linkia laevigata*. These differences in mtDNA diversity suggest that isolated populations are the result of recent colonization by few immigrants. Low allozyme variation has also been found in some isolated island populations (Nishida and Lucas 1988; Macaranas et al. 1992) suggesting our mtDNA results could be generalizable to the rest of the genome (but see Williams and Benzie 1996). Mitochondrial DNA is more susceptible to genetic drift (Avise 1994), and may be a more sensitive indicator of genetic bottlenecks in island populations.

Low dispersal to islands in the central Pacific has long been suspected because of the lower number of marine species and higher number of endemic taxa on these isolated reefs (Kay and Palumbi 1987; Rosen 1988). These biogeographic patterns, however, reveal little about dispersal of the species that succeed in colonizing isolated islands. These species may be specialists for long-distance dispersal and may have high genetic connection to the rest of the Pacific. Alternatively, these species may represent random, long-distance colonizations. Low genetic diversity of isolated populations of widespread urchins (Fig. 3) suggests that the latter explanation is likely to be true, and that the species inhabiting isolated reefs may be a random mix of possible colonists from the Indo-West Pacific. These genetic results plus the patchwork biogeography of *Echinometra* species throughout the Pacific (Palumbi 1996b) and the abrupt appearance and disappearance of fossil species from Pacific reefs (Kohn 1981) suggest that the population genetics and perhaps community composition of isolated reef assemblages is affected strongly by random dispersal events. Whether these random events lead to ecological indeterminacy or to changes in community stability of isolated reefs has yet to be explored.

Oceanic Determinants of Genetic Differentiation

Including current-path data in our genetic analysis did not substantially improve the correlation between genetic and geographic distance (Fig. 4A,B). The Pacific is dominated by two major east-to-west currents, which will tend to move larvae toward Guam and the northern Philippines in the northern hemisphere, and toward Australia in the southern hemisphere. These surface currents may not completely explain gene flow in the Pacific for several reasons. First, marine larvae may not be passive particles: they may be able to affect long-distance transport through behavioral responses to currents (Burton and Feldman 1982; Doherty et al. 1995). Second, current patterns are variable in space and time and may lead to high gene flow at some times but not others. The arrival at the Galapagos Islands of larvae from the Indo-West Pacific during El niño events (Richmond 1990) suggests a pervasive effect of short-term climate change on gene flow patterns. Third, oceanic circulation is sensitive to global climate, and has probably shifted dramatically since the last glacial maximum 12,000 years ago (Pollock 1992). Given the large population size of many marine species, this period of time might be shorter than the time necessary to attain genetic equilibrium. Fourth, current-path and straight-line distance measures are highly correlated and it is difficult to separate the effects of current from those of spatial proximity. One way to do this is to compare genetic exchange across major current paths. We did this only three times with our data because of the patchwork occurrence of *Echinometra* in the Indo-West Pacific (Palumbi 1996b). Of these three comparisons, two showed very low genetic exchange across current paths (see one example below), whereas the third showed high genetic exchange.

Tahiti and Hawaii are separated only by about 4200 km, but straight-line distance between Tahiti and Hawaii crosses virtually all of the east-west currents in the Pacific. Travel along current paths from Tahiti to Hawaii requires movement toward Indonesia, transport north to Okinawa, and then drift along the strong North Pacific current to Hawaii, a distance of about 20,000 km. Our data show that populations of *E. mathaei* have very different mtDNA sequences in Hawaii and Tahiti, despite their spatial proximity, consistent with strong effects of current patterns on gene flow.

In contrast, Niue and Hawaii populations of *E. oblonga* have similar mtDNA sequences (Nm = 7.8). In this case, genetic distance seems more in line with the close spatial proximity (4800 km) of these archipelagoes rather than the long current-path distance between them (ca. 18,000 km). Genetic similarity of Niue and Hawaiian *E. oblonga* is due to the domination of a single mtDNA sequence in both localities. This sequence is also found elsewhere in the Pacific and could represent independent colonization of Hawaii and Niue by separate propagules from the Indo-West Pacific. Alternatively, this mtDNA type may have been transported across currents by unusual oceanographic conditions.

Mitochondrial DNA Regionalization among Pacific Sea Urchins

Gene flow values for adjacent island groups are shown in Figure 6, and suggest that mtDNA haplotype frequencies on island archipelagoes tend to fall into broad geographic regions. For example, *E. oblonga* consists of two mtDNA clusters: one in the central Pacific (Hawaii-Niue) and one in the western Pacific (Papua New Guinea–Okinawa). Mitochondrial DNA regions can be drawn for all four species based on geographic patterns of inferred gene flow (Fig. 6). Comparison of these region for different species, however, shows little concordance. Unlike the broad similarity of mtDNA regions for marine and terrestrial species along the southeastern coast of North America (Avise 1994), the *Echinometra* species we studied have developed strong but independent geographic structure in the Pacific. The implication of concordance in southeastern North America is that similar population processes determine genetic structure (Avise 1994). Lack of concordance in the Pacific, even though genetic structure is strong, might result from the chance effects of gene flow due to colonization of peripheral habitats by a few founder individuals. Alternatively, it is possible that these different genetic patterns are due to deterministic effects (such as current patterns) that act differently on the different species, despite their overall similarity.

Private Clades and the Opportunity for Divergence

Mitochondrial DNA differences between populations tend to be due to differences in frequency of widespread haplotypes. However, there are also many haplotypes with small

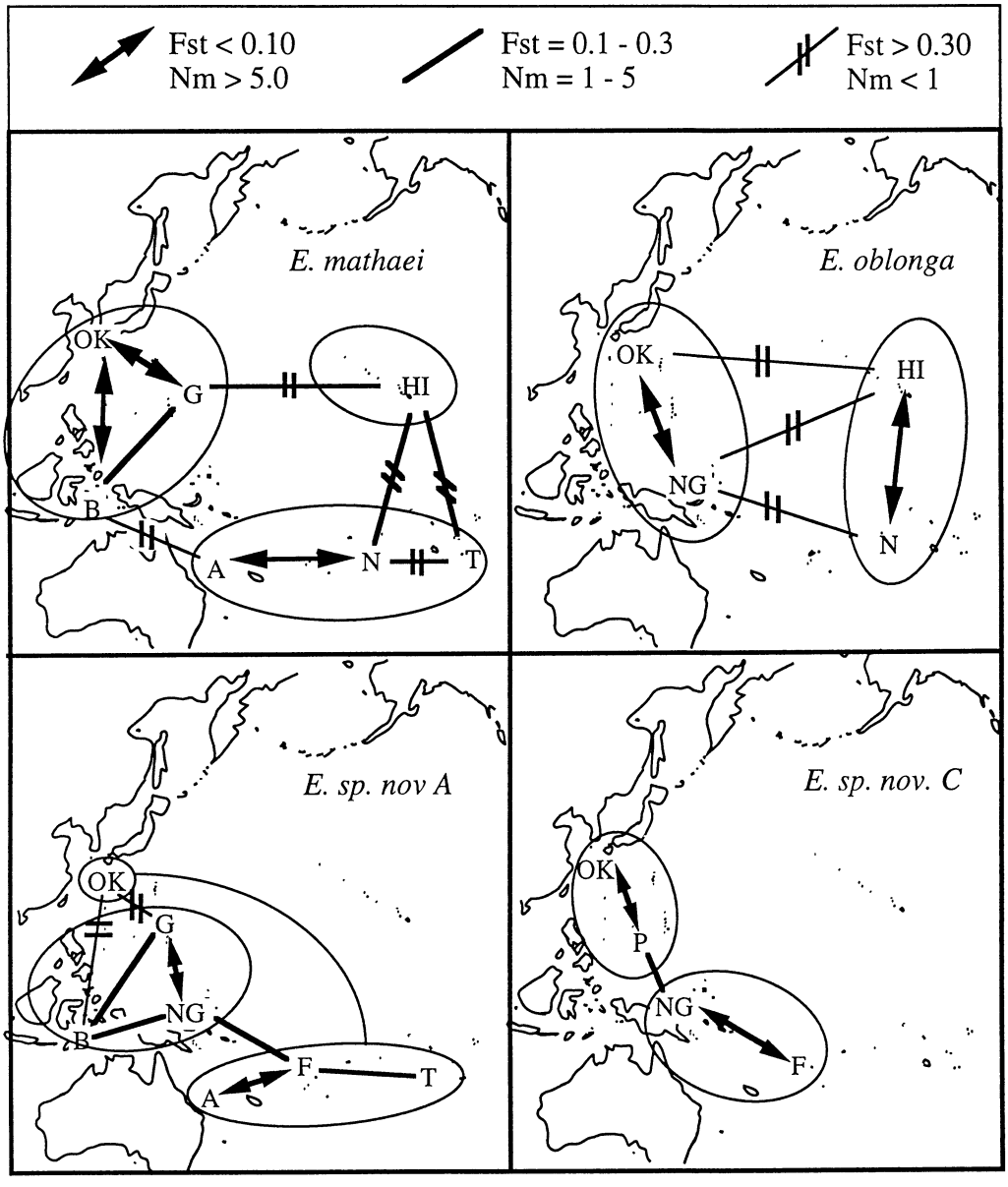

FIG. 6. Regionalization of mtDNA gene flow for four species of Pacific *Echinometra*. Gene flow among adjacent localities is drawn using three different scales: high gene flow (double headed arrow), moderate gene flow (unadorned line), and low gene flow (line broken by double hash marks). For each species, populations with similar mtDNA frequencies are circled: these circles represent mtDNA regions. Regions for the four species are very different from one another, suggesting poor concordance in the processes generating population differentiation.

geographic range, and the positions of these haplotypes may give clues about the first stages of genetic divergence. Where in the Pacific can unique haplotypes arise and accumulate?

We define a private haplotype or clade as a mtDNA sequence or a clade of sequences (identified by significant branch length on an ML tree) that occurs in multiple individuals but in only a single collecting locality. Private haplotypes and private clades (Fig. 7) are distributed broadly throughout the Pacific. These results suggest that unique mtDNA types can evolve and accumulate virtually anywhere in the Pacific: even islands in close proximity in the center of the Indo-West Pacific can harbor unique mtDNA sequences. Genetic variants at other loci might also arise and accumulate on these islands, and this might set the stage for species diversification. Although mtDNA sequences are not a perfect proxy for new species, the accumulation of private haplotypes and clades in many places in the Pacific suggests that species (as a more advanced stage of genetic differentiation) might form anywhere. Indeed, the differences in regionalization of mtDNA types among species (Fig. 6) might be taken as the second stage in spread of private clades from their point of origin. If species tend to arise within mtDNA regions, then species boundaries in Pacific *Echinometra* will form in widely different ways in different instances. This scenario is very different from most views of Pacific biogeography, in which species are thought to originate pre-

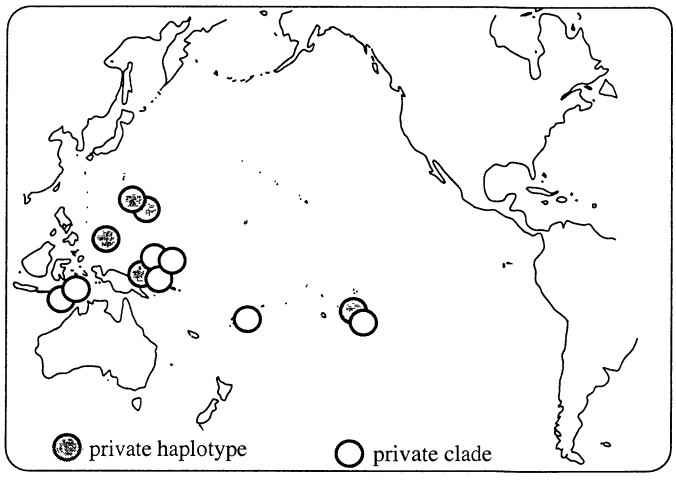

FIG. 7. Private mtDNA haplotypes and private mtDNA clades in *Echinometra* are scattered throughout the Pacific, suggesting unique mtDNA types can arise and accumulate in most populations.

dominantly in the center of the Indo-West Pacific (Ekman 1953; Briggs 1974) or on peripheral islands (Ladd 1960; Kay 1980).

Conclusions

Patterns of gene flow and genetic diversity vary greatly in *Echinometra* species across the tropical Pacific, but are not a simple reflection of island position in oceanic currents. Instead, our results suggest that the degree of genetic differentiation among populations is affected by a combination of island position, oceanic currents, and random long-distance dispersal events. Only moderate structure would have been visible for these species except over distances exceeding 5000 km, but the vast extent of the tropical Pacific allows significant population differentiation. Low mtDNA diversity in isolated archipelagoes suggests that colonists to these island groups are rare, even for marine species with high dispersal potential, and also suggests that recruitment from local populations is the rule rather than large-scale immigration from other island groups.

These species have formed recently, yet all show substantial genetic differentiation across the Pacific. In this case, recent speciation is associated with distinct population structure and isolation by distance over scales of 5000 km or more. The overall pattern suggests semi-isolated populations connected by low or sporadic gene flow. Such scenarios are common among terrestrial and freshwater species (Avise 1994), but those geographic scales of differentiation are one to two orders of magnitude smaller than seen here.

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