Strong Reproductive Isolation between Closely Related Tropical Sea Urchins (genus *Echinometra*)[1]

Stephen R. Palumbi and Edward C. Metz
Department of Zoology and Kewalo Marine Laboratory, University of Hawaii

Morphological, mitochondrial DNA, and single-copy nuclear DNA differences show that the tropical sea urchin *Echinometra mathaei* is composed of at least four independent gene pools. Evolutionary distance between species measured with restriction-site changes (for mitochondrial DNA) and thermal renaturation (for single-copy nuclear DNA) is 1%–3% nucleotide divergence. Thus these are the most closely related sea urchin species known. Despite this genetic similarity, strong blocks to interspecific fertilization exist in this genus. Between two Hawaiian species, few eggs are fertilized in hybrid crosses, even in the presence of excess sperm. Microscopic examination of such crosses shows that sperm attachment to heterologous eggs is inhibited. Measures of genetic distance between species can help reveal the tempo of speciation and allow comparisons of morphological, biochemical, and ecological characteristics to be made in an evolutionary framework. Our results show that strong reproductive isolation can evolve by changes in egg-sperm recognition without extensive genetic divergence between species. Such mechanisms are most easily studied in free-spawning animals such as sea urchins but as well may represent an important aspect of speciation in species with internal fertilization.

Introduction

Reproductive isolation and speciation can occur quickly in behaviorally complex taxa (Diamond 1981; Dominey 1984; Kaneshiro and Boake 1987). For species with courtship, premating behavioral cues between adults frequently serve to limit interspecific hybridization (Giddings and Templeton 1983) or to produce assortative mating within species (Lande 1982; Kirkpatrick 1987). Recent research suggests that these behaviors can evolve rapidly (Carson et al. 1982). Thus, for such taxa it is not surprising to discover reproductively isolated species that differ only slightly in genetic makeup (Kornfield 1978; DeSalle and Hunt 1987).

For many marine species, such as sponges, corals, bivalves, ascidians, and echinoderms, courtship between adults does not occur before reproduction. Instead, gametes are spawned into the water column, and the most important interaction is between egg and sperm at fertilization. In these cases, reproductive isolation may arise by changes in the timing of gamete release (e.g., see Lessios 1984) or in clumping of conspecific adults (Billet and Hausen 1982). However, the behavioral components of reproductive isolation that are thought to drive rapid speciation in other taxa are largely absent. The simplicity of their spawning interaction and the wide dispersal of

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Address for correspondence and reprints: Stephen R. Palumbi, Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu, Hawaii 96822.

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planktonic eggs and larvae of broadcast spawning marine animals might help explain why reproductive isolation is thought to evolve slowly (Mayr 1954; Springer 1982) in these taxa.

Yet, even with such a simple spawning method, reproductive isolation could arise abruptly if sperm-egg interactions are disrupted. In sea urchins, sperm-egg interactions have been extensively studied, and the failure of sperm and egg to adhere and fuse between species has been called gamete incompatibility (see review in O'Rand 1988). To date, strong gamete incompatibility is known only for urchins with substantial genetic divergence. For the temperate species *Strongylocentrotus purpuratus* and *S. franciscanus,* fertilization is slight at sperm concentrations sufficient for 100% intraspecific fertilization: <5% of eggs are fertilized in hybrid crosses (Strathmann 1987; J. Minor, personal communication; S. R. Palumbi, unpublished data). However, these two species are the most distantly related sea urchin congeners known. Between them ~16% of the nucleotides in single-copy nuclear DNA (scnDNA; Hall et al. 1980) and 11% of those in mitochondrial DNA differ (mtDNA; Vawter and Brown 1986), corresponding to a divergence in the Miocene ~10–15 Mya (Hall et al. 1980). Species with more recent divergence, such as the Pliocene species *S. purpuratus* and *S. droebachiensis* (Hall et al. 1980; Vawter and Brown 1986; Palumbi and Wilson 1990), show higher cross-fertilization than occurs in the more distant *S. purpuratus–S. franciscanus* crosses (Strathmann 1987). Thus, it is possible that gamete incompatibility in this genus has arisen slowly and that it could be a consequence of long-term evolutionary divergence after reproductive isolation, rather than a cause of reproductive isolation.

A different pattern appears to dominate the tropical sea urchin genus *Echinometra.* Previous reports suggested that, in *E. mathaei,* individuals of different color varieties exhibited gamete incompatibility and might therefore be different species (Kelso 1970; Uehara and Shingaki 1985; Uehara et al. 1986). Although these results suggest that these different types represent cryptic species, genetic differentiation among types has never been measured.

To clarify the evolutionary relationships among these color types, we have measured the genetic distance between them by using mtDNA restriction sites and scnDNA thermal renaturation and have measured gamete incompatibility by using in vitro fertilization assays. Here we show that there is a significant, but small, evolutionary distance between color types and that there is strong reproductive isolation due to greatly reduced fusion between sperm and heterotypic eggs. These results show that strong gamete incompatibility and reproductive isolation may occur even in closely related species with simple mating behaviors, and it will open the door to further research on the cellular and molecular mechanisms involved.

Background and Methods

Natural History of *Echinometra*

Urchins in the genus *Echinometra* are widely distributed in shallow reef environments across the tropical Pacific and Indian Oceans. Two species from the Indo-Pacific were described originally (de Blainville 1825), but a subsequent morphological study of adults and larvae (Mortensen 1943) concluded that only one species existed. This species, *E. mathaei,* has been called the world's most abundant sea urchin, and, across its huge range from Hawaii and Tahiti to East Africa and the Persian Gulf,

many color and morphological variants have been described (Clark 1925; Russo 1977; Lawrence 1983).

Variability in *E. mathaei* has been best described in Hawaii and Okinawa. In Hawaii, two types occur. Some individuals are pastel pink or green, whereas others are jet black (Kelso 1970; Russo 1977). In Okinawa, four types of *Echinometra,* (including the two Hawaiian types) occur. Uehara et al. (1986) have documented slight color and skeletal differences that distinguish the four types (table 1).

Where types occur together, there is only slight ecological separation among them (Russo 1977). The black type is found most commonly in intertidal wave-surge areas in Okinawa and Hawaii (Kelso 1970; Russo 1977), whereas pastel individuals or those with white-tipped spines tend to occur subtidally in quieter water (Tsuchiya and Nishihira 1984, 1985, 1986). Nevertheless, there is broad spatial overlap among types. Tide pools usually contain more than one type, and individuals of different types are often adjacent (Kelso 1970; Tsuchiya and Nishihara 1984).

mtDNA Analysis

We purified mtDNA from 80 individuals from Hawaii, Tahiti, Guam, and Okinawa. Each individual was ascribed to one of four morphotypes (table 1) on the basis of the classification system of Uehara et al. (1986). Purification, restriction digestion, and data analysis were according to methods described elsewhere (Palumbi and Wilson 1990). mtDNA was analyzed from 6–32 individuals of each type by using six restriction endonucleases that recognized six-base sites. These enzymes assayed 34 inferred restriction sites. In addition, for estimations of genetic variability within localities, we used four restriction enzymes that recognize four-base sites. These enzymes generated an additional 49–53 inferred restriction sites. The percentage of nucleotide differences within and between species was calculated using Engel's (1981) maximum-likelihood method based on inferred restriction-site changes (see Palumbi and Wilson 1990).

Thermal Renaturation

scnDNA thermal stability was compared within and between types by the hydroxyapatite method as described by Britten et al. (1974, 1978), with the following modifications. Tracers were prepared from genomic DNA of the two Hawaiian *Echinometra* types (B and D; table 1) by nick translation in the presence of P^{32}-dATP. Tracers (modal size ~500 bp) were self-reacted to Cot 40 to remove repeated DNA and then were reacted to Cot 15,000 with a 10,000-fold excess of sonicated driver DNAs isolated from each *Echinometra* type (modal size ~1,000 bp). Each tracer was reacted with drivers made from each of six individuals of the two Hawaiian types and

Table 1
Distinguishing Skeletal Characteristics of Morphological Varieties of *Echinometra mathaei*

Morphotype	Spine Color	Spine and Test Color	Gonad Ossicles
A	White tipped	Pastel, purple, black	Rods
B	Unicolor	Pastel pink or green	Rods
C	Unicolor	Pastel pink	Triradiate
D	Unicolor	Black	Curls

SOURCE.—Uehara and Shingaki (1985) and Uehara et al. (1986).

from each of two individuals of the other two types (A and C). Tracer self-reaction was insignificant, as determined by reaction with calf DNA. For each reaction, the median melting temperature was determined according to the method described Hall et al. (1980).

Genetic divergence was expressed as the difference between the median melting temperature of the experimental reaction and that of homologous reactions performed with the same Cot, incubation, and assay conditions. In reporting sequence differences, we assume that a 1-degree difference in median melting temperature corresponds to 1%–1.5% nucleotide mismatch (Hall et al. 1980; Caccone et al. 1988). Because radioactive tracers were prepared only from the two Hawaiian types, we did not measure directly nuclear variability within white-tip or pastel-triradiate types (A and C). Nor did we either perform reciprocal tests between Hawaiian individuals and types A or C or measure the single-copy distance between these two types. Instead, scnDNA relationships between white-tip and pastel-triradiate types were inferred from their relationships to the other two types.

Gamete Incompatibility

Gametes were collected from urchins after inducing spawning by injection of 0.5 M KCl. Eggs were washed in 40-μm-filtered seawater (FSW) three times. Sperm were collected directly from the gonopores and were diluted with FSW to give an absorbance (Abs) reading of 0.8 at 340 nm. Absolute sperm concentrations were measured by hemocytometer counts of five independently diluted sperm samples. Under these conditions, the mean ± standard deviation (SD) sperm concentration for a solution with an absorbance reading of 0.8 is $26 \pm 2 \times 10^6$ sperm/ml. For most tests, a fourfold dilution of sperm was mixed with eggs in a 1-ml volume and was mixed every 1–2 min by inverting samples gently for 10 s. After 10 min, 120 μl of 10% glutaraldehyde in FSW was added. Percentage of fertilization was measured for each sample by scoring ⩾200 eggs for the presence of a fertilization membrane by using a light microscope. Tests that used cell division of unfixed eggs after 2 h as a criterion of fertilization gave similar results. Effects of sperm concentration were measured by fertilizing eggs with 1-, 2-, 4-, 8-, 16-, and 32-fold dilutions of sperm (Abs 0.8). For these experiments, four replicate crosses (pairwise crosses of two males and two females) were performed. Sperm-egg binding was measured on eggs that were first dejellied by passing them through a 20-guage syringe needle three times. Eggs were mixed with a sperm suspension (Abs 0.8) for 25 s by continuously inverting the samples. Glutaraldehyde [5% (v/v) in seawater] was added to make a final concentration of 1%, and the number of sperm attached around the circumference of 10 eggs was counted. For these experiments, four different replicates of each of four types of crosses (two intraspecific and two interspecific) were performed. Similar results were obtained by dejellying eggs by different methods (including treatment with calcium-magnesium-free seawater and with pH 5 seawater). Only results from mechanical dejellying are presented, because we are unsure of the effect of chemical treatments on the sperm-egg binding mechanism of *Echinometra*.

Results

Genetic Analyses

Four major mitochondrial lineages are evident in our collection, each corresponding to a single morphological category (fig. 1). Individuals in different lineages

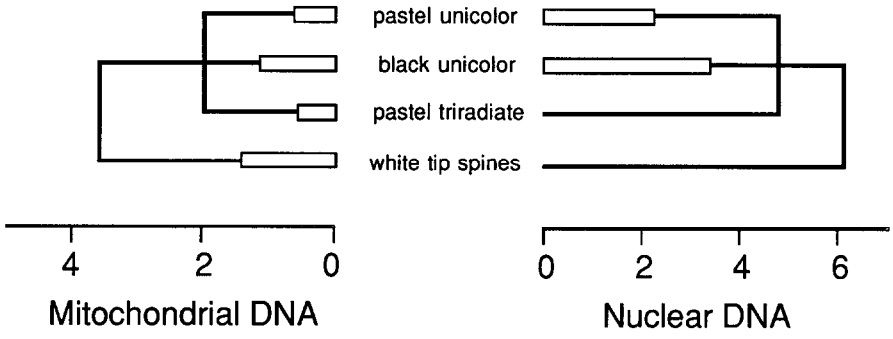


FIG. 1.—Percentage sequence difference in four morphotypes of *Echinometra mathaei* that have distinct mitochondrial and nuclear genotypes. These two trees show genetic distances between morphotypes (dark lines) and approximate genetic diversities within morphotypes, where measured (open bars at branch tips). Mitochondrial distances are based on restriction-site polymorphisms. Nuclear distances are based on thermal renaturation of scnDNA. Morphotype designations are as in table 1.

are 2%–4% different from one another (table 2). Nucleotide variation within these four mtDNA lineages was high (1%–2%) in the western Pacific; for example, every individual white-tipped urchin collected from Guam ($n = 12$) has a unique mtDNA genotype, and there appear to be two main maternal lineages ~4% different from each other. By contrast, mtDNA variation was extremely low (≤0.4%) in the isolated archipelagoes of the Hawaiian and Society Islands (table 3). All 14 Tahitian urchins were identical at 84 restriction sites. The maternal lineages present in Hawaii and Tahiti are not unique to these islands but are also found in the western Pacific. For type A, we have found the same mtDNA genotypes (assayed at 84 restriction sites) in Tahiti and Okinawa (~10,000 km distant). For type B, we have found identical genotypes (assayed at 83 restriction sites) between Hawaii and Okinawa (~5,000 km distant). Additional sampling is being performed to confirm these biogeographic patterns within morphotypes.

Table 2
Comparisons of mtDNA and scnDNA of Morphotypes of *Echinometra mathaei*

	MORPHOTYPE			
MORPHOTYPE	A	B	C	D
A	...	3.0 ± 1.1 (342)	3.8 ± 0.9 (108)	3.8 ± 0.9 (414)
B	5.5 ± 0.7 (2)	2.3 ± 0.3 (5)	1.9 ± 0.4 (114)	1.7 ± 0.5 (437)
C	...	4.9 ± 0.7 (2)	...	1.9 ± 0.7 (138)
D	6.7 ± 0.4 (2)	5.1 ± 0.5 (12)	4.6 ± 0.2 (3)	3.6 ± 0.6 (5)

NOTE.—Designations of morphotypes are as in table 1. Above the diagonal are estimates of percentage of sequence differences of mtDNA, as based on fragment analysis using restriction enzymes that recognize six-base sites; values are means and standard deviations of all possible pairwise percentage of nucleotide sequence difference estimates between individuals (numbers in parentheses are number of pairwise comparisons). Values below the diagonal are means and standard deviations of percentage of scnDNA differences, as based on thermal renaturation (numbers in parentheses are number of comparisons). Values on the diagonal are percentage of intraspecific scnDNA diversities. Ellipses indicate that comparisons were not made. scnDNA tests done on Hawaiian *Echinometra* morphotypes B and D show that mean genetic distance between types is significantly greater than that within types (Kruskal-Wallis test; $P < 0.001$). Reciprocal renaturations between morphotypes B and D gave indistinguishable results.

Table 3
mtDNA Diversity of *Echinometra* Morphotypes

LOCALITY	MORPHOTYPE A	B	C	D
Hawaii	...	0.1 ± 0.1 (12)	...	0.4 ± 0.3 (14)
Tahiti	0 (14)	...	...	...
Guam-Okinawa	1.6 ± 0.9 (18)	0.5 ± 0.4 (7)	0.4 ± 0.4 (6)	1.3 ± 0.8 (9)

NOTE.—Values are means and standard deviations of pairwise percentage of nucleotide differences between individuals of the same morphotype that were collected at each of the localities listed. Values are based on fragment analysis using restriction enzymes that recognize six-base sites. In addition, Hawaiian and Tahitian samples were surveyed with restriction enzymes that recognize four-base sites. Numbers in parentheses are number of individuals. Each value is based on $n(n-1)/2$ comparisons, where n is the number of individuals. Ellipses represent localities where particular morphotypes do not occur.

Renaturation studies showed that scnDNAs were 1°C–2°C more thermostable when individuals of the same type were compared than when individuals of different types were compared. This indicates that there is slight but significant divergence of nuclear genomes among types (Kruskal-Wallis test; $H = 17.1$, $P < 0.001$), although it is clear that there is substantial nuclear genetic variation within types.

A summary of the genetic results (fig. 1) shows that individuals of different morphotypes vary 1.5%–4% in mtDNA and 4%–7% in scnDNA. Genetic diversity within types is 0.5%–2% for mtDNA and 2%–4% for scnDNA (tables 2 and 3). To calculate evolutionary divergence (the amount of genetic change after divergence of the types), we subtract the average amount of intraspecific variation from the total differences between species (see Wilson et al. 1985). When this is done, the evolutionary divergence is 1%–3% for both types of genes.

Fertilization between Types

Individuals with both the same mitochondrial genotype and the same morphological features typically produced high levels of fertilization (mean = 95% for 26 crosses between individuals from different archipelagoes) even though they might have come from sources 10,000 km apart (Tahiti vs. Okinawa). Crosses between cooccurring individuals of different types were performed between types B and D from Hawaii ($n = 54$ crosses) and between types B and A from Guam ($n = 36$). Average percentage of fertilization was 2% (SD = 3%) for the Hawaiian crosses and 6% (SD = 8%) for the Guam crosses. Fertilization frequency did not depend on which type contributed sperm.

Gamete incompatibility was examined more closely in Hawaii through crosses between types B and D. Experiments with a 32-fold range in sperm concentration (fig. 2A) show that fertilization failure is not due to insufficient sperm. Examination of sperm and eggs by light and electron microscopy showed that sperm were activated by heterospecific eggs but failed to attach strongly to heterospecific vitelline membranes (fig. 2B). Electron microscopy showed that all sperm from type D were acrosome reacted in the presence of type B eggs ($n = 36$), whereas only 57% of sperm from type B were acrosome reacted in the presence of type D eggs ($n = 37$). Nevertheless, fertilization success in crosses between types was not enhanced by pretreatment of

sperm by egg jelly from the same type, showing that lack of fertilization was *not* due to failure of the acrosome reaction.

Attachment of sperm to eggs varied among crosses (Krukal-Wallis test; $H = 13.7$, $P < 0.01$; table 4). Planned pairwise Mann-Whitney U tests show that binding is lower in crosses between types than it was in crosses within types (experiment-wide $P < 0.05$).

Although most crosses yield only 1%–2% fertilized eggs, not all of the crosses between types had such low values. One type B female from Guam crossed equally well with type B or type A males (95% fertilization when sperm from two males of each type was used). In addition, two of 54 crosses between types from Hawaii showed 25%–50% fertilization. Thus, there is individual variation in ability to cross-fertilize among *Echinometra* types. Consistent with this observation is the occasional discovery in Hawaii of adult urchins that appear to be morphological hybrids between types. We have found four such animals, all males, in 4 years of field observations. These males produced sperm that fertilized 100% of eggs from both Hawaiian types. Kelso (1970) also reported two males that had similar morphological and fertilization characteristics. We estimate the abundance of such putative hybrids as being $<1/1{,}000$. Such a low rate of hybridization does not appear to be sufficient to homogenize nuclear or mitochondrial genetic variation among types (fig. 1).

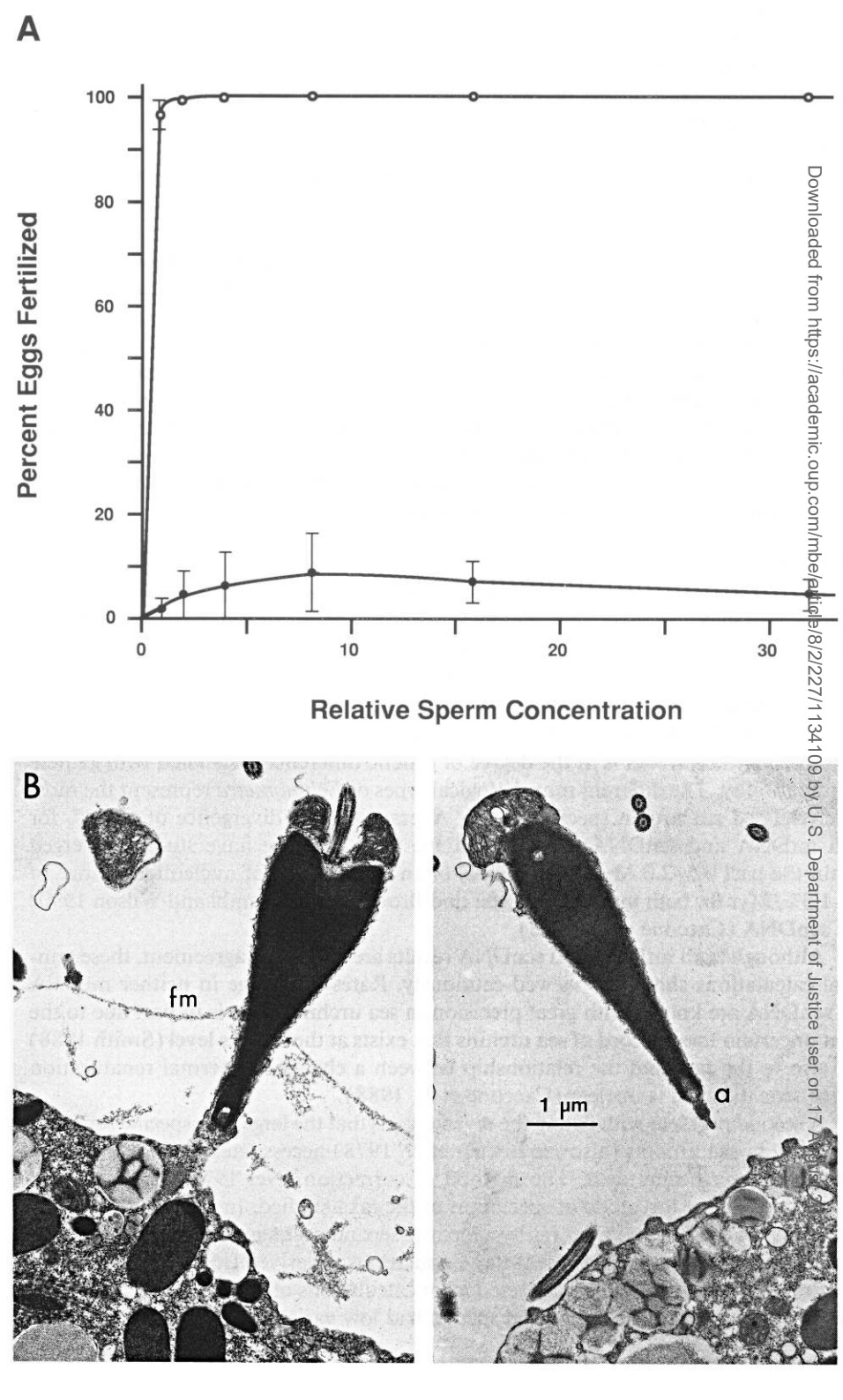
Discussion

Within the genus *Echinometra* there is a consistent association between morphology, mtDNA variation, and scnDNA variation. Thus, morphologically different individuals represent evolutionarily separate gene pools. Evolutionary distance between these gene pools is small, suggesting recent separation. Nevertheless, between these types, strong gamete incompatibility has evolved.

A major difference between the results for *Echinometra* and those for the temperate genus *Strongylocentrotus* is in the degree of genetic difference associated with gamete incompatibility. The different morphological types of *Echinometra* represent the most closely related sea urchin species known. Average genetic divergence of 1%–3% for both mtDNA and scnDNA suggests that the four types we have studied diverged within the past 0.5–2.0 Myr, on the basis of an average rate of nucleotide change of 1%–1.5%/Myr for both mtDNA (Vawter and Brown 1986; Palumbi and Wilson 1990) and scnDNA (Caccone et al. 1988).

Although both mtDNA and scnDNA results are in general agreement, these temporal calculations should be viewed cautiously. Rates of change in neither mtDNA nor scnDNA are known with great precision in sea urchins. This is in part due to the often uncertain fossil record of sea urchins that exists at the species level (Smith 1988) and also to the fact that the relationship between a change in thermal renaturation and genetic distance is unclear (Caccone et al. 1988).

A second problem with dating the divergence is that the large intraspecific scnDNA variability in sea urchins (also see Britten et al. 1978) necessitates a large correction of the raw interspecific data. The method of correction (Nei 1975) makes several assumptions about the mode of speciation of the taxa studied. In particular, the correction assumes that speciation was by allopatric separation of large populations. When the genetic variability in the present-day populations is derived from variation in the ancestral species, it should be subtracted from calculations of evolutionary divergence (Nei 1975). But if the newly formed species had low variability (perhaps because of



founder effects or selection), then correction for variation in present-day species may not always be appropriate. We have argued elsewhere (Palumbi and Wilson 1990) that the assumptions on which the correction is based are likely to be met for sea urchin nuclear DNA, but a direct test of the nature of intraspecific variation in sea urchins is needed.

Regardless of whether they are corrected by intraspecific variation, interspecific divergences among the species of *Echinometra* studied here are smaller than those in the genus *Strongylocentrotus.* The most closely related Strongylocentrotids known are *S. droebachiensis* and *S. purpuratus.* These species are 6% divergent in mtDNA and 4% divergent in scnDNA (uncorrected values are 6.5% and 7%, respectively; Hall et al. 1980; Vawter and Brown 1986; Palumbi and Wilson 1990) and probably diverged 3–5 Mya. In this species pair, interspecific crosses yield 25%–75% fertilized eggs (Strathmann 1987; S. R. Palumbi, unpublished data). Reciprocal fertilization rates as low as 1%–2% only occur between *S. purpuratus* and *S. franciscanus,* which are 11% different in mtDNA and 16% different in scnDNA (uncorrected values are 12% and 19%, respectively; Hall et al. 1980; Vawter and Brown 1986). Even in this species pair, cross-fertilization rates as high as 40%–80% can be achieved by blanketing eggs with excess sperm (Strathmann 1987). Similar treatment of *Echinometra* eggs with excess heterologous sperm does not result in high levels of cross-fertilization (fig. 2A), suggesting that the block to fertilization is stronger in *Echinometra* even though the genetic divergence in this species pair is five times less than that in *S. purpuratus* and *S. franciscanus.*

Reproductive isolation in *Echinometra* occurs at least in part because attachment of sperm to eggs—and their subsequent fusion—is curtailed between types (table 4). In sea urchins, both sperm attachment and fusion are mediated by a sperm protein called "bindin" (Vacquier and Moy 1977; O'Rand 1988). Failure of binding to attach sperm to heterologous eggs has been shown to prevent fertilization in crosses of *S. franciscanus* and *S. purpuratus* (Glabe and Vacquier 1977; Vacquier and Moy 1977). Amino acid/DNA sequencing has shown that bindin protein includes a series of repeated amino acid blocks that have been reorganized between species (Gao et al. 1986). Thus, evolution of bindin genes may involve unequal crossing-over among repetitive elements in the gene sequence. These events could cause functional evolution of the bindin protein far more quickly than occurs in those single-copy genes in which point substitution is the predominant mode of evolutionary change.

In *Echinometra,* gamete incompatibility may have evolved rapidly by changes in a few key loci such as bindin. Unequal recombination as discussed above may be a mechanism for accelerated functional evolution of bindins and might lead to rapid reproductive isolation in *Echinometra.* However, it is also possible that evolution of reproductive isolation in *Echinometra* has not been especially rapid but is merely recent. Perhaps reproductive isolation took a long time to evolve in *Echinometra* but genetic distance did not begin to build up until the process had been completed. In this case, low genetic divergence represents a short time period since the completion

FIG. 2.—A, Cross-fertilization within (○) and (●) between Hawaiian types of *Echinometra* showing low fertilization at high and low sperm concentrations. Values shown are means and standard deviations of percentage fertilization. B, Electron micrograph of egg sperm interactions showing (*left panel*) attachment and fusion in crosses within types and (*right panel*) attachment failure in crosses between types. fm = Fertilization membrane; a = acrosomal process.

Table 4
Sperm Attachment to Homologous and Heterologous Eggs

Sperm Type × Egg Type	Sperm Bound per Egg
B × B	36 ± 2
B × D	17 ± 3
D × B	9 ± 2
D × D	55 ± 3

NOTE.—Data are means and standard errors of number of sperm attached to eggs in crosses between types and within types. For each type of cross, sperm attached to 10 eggs in each of four replicate crosses were scored. Significantly fewer sperm attach to heterologous than to homologous eggs (pairwise Mann-Whitney U-tests; $P < 0.05$).

of reproductive isolation, not a short time period for the evolution of reproductive isolation.

No matter which of these scenarios is correct, our results show that, between sea urchins, strong reproductive isolation can occur without substantial genetic differentiation. Furthermore, the results of our fertilization experiments implicate egg-sperm recognition in the development of reproductive isolation. Such egg-sperm interactions are particularly easy to study in free-spawning animals such as sea urchins but also might play a role in reproductive isolation of animals that have internal fertilization.

What evolutionary processes might enhance the fixation of mutations that alter gamete recognition? In general, rapid evolution of reproductive isolation is thought to occur in at least two fundamentally different ways. First, strong selection against hybridization can lead to rapid evolution of assortative mating (Barton and Hewitt 1989). Such strong selection can be due to developmental incompatibility arising from a few genetic changes; for instance, chromosomal inversions or other alterations between species are a well-known cause of hybrid failure (e.g., see Bush 1975; White 1978) and have been associated with rapid speciation (Bush et al. 1977). Second, behavioral evolution through sexual selection may occur without large changes in genetic makeup of a species (Carson et al. 1975); for instance, in Hawaii, *Drosophila heteroneura* and *D. silvestris* have mating behaviors different enough to limit interspecific hybridization (Ahearn et al. 1974), yet they diverged within the past 500,000 years and are closely related at nuclear structural loci and in mtDNA sequences (Carson et al. 1975; DeSalle and Hunt 1987).

For the genus *Echinometra,* further research is needed to determine the factors that have led to reproductive isolation. The existence of rare fertile hybrids suggests that nuclear genetic differences between species are not large enough to cause developmental incompatibility. Artificially produced hybrids of the two Hawaiian *Echinometra* types sometimes develop with slight bilateral asymmetries after gastrulation (E. C. Metz and S. R. Palumbi, unpublished data), but it is not known whether this alters larval survival or causes selection against hybridization. Sexual selection on the basis of mate behavior appears unlikely in broadcast-spawning sea urchins. However, sexual selection could act at the gamete level in these species. Rapid evolution of bindin may disrupt egg-sperm interactions and would require compensatory changes in egg-surface receptors. Such male-female coevolution of gamete cell-surface proteins is analogous to coevolution of behavioral interactions between males and females of

species with adult courtship. Genes for interacting cell-surface proteins in eggs and sperm correspond to female preference and male trait alleles, respectively. Thus, evolutionary changes in egg-sperm interactions may lead to both gamete incompatibility and reproductive isolation through a process of sexual and natural selection. This is reminiscent of rapid evolutionary changes brought about by sexual selection in more behaviorally advanced animals (Lande 1982; Kirkpatrick 1987) and may be amenable to detailed molecular genetic analysis.

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