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REPRODUCTIVE ISOLATION BETWEEN SPECIES OF SEA URCHINS

H. A. Lessios

ABSTRACT

Existing knowledge on reproductive isolating barriers between sea urchin species is reviewed. Experiments involving artificial production of hybrids between congeneric echinoid species have shown that in most cases hybrids are viable and capable of backcrossing. Only species separated for > 5 million yrs show complete post-zygotic isolation. Each potential prezygotic isolating barrier appears to be incapable of completely preventing gene flow between sympatric species. Different habitat preferences exist in many, but not all, sympatric species. Annual reproductive cycles are too environmentally labile to isolate entire species. Lunar reproductive rhythms may be a form of temporal isolation in some diadematid species, but they are generally lacking in other echinoids. Gametic isolation is bidirectional and complete in a few pairs of congeneric species, but as a rule it allows one-way gene flow between congeneric species. There is no correlation between pre-zygotic isolation and the time since separation of the species. Bindin, a reproductive molecule involved in gamete incompatibility, shows evidence of strong selection in genera that contain sympatric species, but appears to be evolving neutrally in genera that do not. However, the cause of selection, where it exists, is more likely to be some form of intraspecific process, such as sexual selection, rather than reinforcement to avoid hybridization. Even though no single barrier seems to be either absolute or universal, the combination of several barriers is potentially capable of reducing the probability of hybrid production in nature, which may explain why there is little credible evidence of natural hybridization or introgression between sea urchin species.

John Pearse has made major contributions to the study of reproductive ecology of sea urchins, particularly contributions that have focused on factors that govern the timing of reproduction of these animals. The reproductive ecology of sea urchins has also received a fair amount of attention recently from the point of view of reproductive isolation, i.e., the intrinsic biological barriers that maintain species as independent gene pools. Emergence of reproductive isolation converts geographic isolates into separate species, which will then evolve independently even if they were to come into contact with each other. Before we attempt to characterize the evolution of reproductive barriers we must first know what they are. In this article, I review existing knowledge of reproductive ecology as it pertains to the emergence of reproductive isolation, and thus to speciation.

For more than a century sea urchins have been a model organism in embryology and developmental biology; the same cannot be said for their role in speciation research. As it will become obvious in this paper, we do not have the data to draw firm conclusions about the full trajectory of the evolution of reproductive isolation in any pair of sea urchin sister species. What we do know pertains to specific aspects of the biology of certain species that are relevant to speciation. With few exceptions, there is no comprehensive body of knowledge that would allow one to determine which reproductive barrier accounts for complete genetic isolation between two sympatric sea urchin species, let alone permit firm conclusions of how reproductive isolation arises in this class of echinoderms. Yet, as Mayr (1954) perceived early on, and as

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more recent treatments of speciation—such as that of Coyne and Orr (2004)—have also made clear, sea urchins do play a role in speciation theory (see also Palumbi and Lessios, 2005): They provide a test of whether ideas developed from the study of arthropods and vertebrates (for which information regarding speciation is more extensive, but far from complete) also apply to organisms with starkly different fertilization systems and reproductive ecologies. From this point of view, that fertilization in sea urchins is external and that the echinoid behavioral repertoire is limited actually represent advantages for the study of the emergence of reproductive isolation, in that they greatly simplify the possibilities of potential reproductive barriers that can isolate species.

Reproductive barriers between species are usually classified into two major categories, prezygotic and postzygotic (Mayr, 1963; Dobzhansky, 1970; Coyne and Orr, 2004). Prezygotic isolation barriers drastically reduce the ability or the opportunity of two species to mate with each other, even when their geographic ranges overlap. Postzygotic barriers consist of some aspect of the biology of hybrids that reduces their fitness, either because they survive less well than non-hybrids, or because they have a lower probability of mating successfully. Reproductive ecology of each species is obviously more closely related to prezygotic barriers, but post-zygotic barriers are not irrelevant. Low fitness of hybrids can lead to selection that will shift the reproductive ecology of the hybridizing species away from each other, the phenomenon of reproductive character displacement (Brown and Wilson, 1956), through the process of reinforcement (Dobzhansky, 1940; Butlin, 1989; Servedio and Noor, 2003). Indeed, in the view of one of the most prominent figures in speciation research, the emergence of postzygotic isolation is a stage of speciation that precedes the emergence of prezygotic isolation (Dobzhansky, 1940). I will, therefore, begin by examining what is known regarding postzygotic reproductive barriers in sea urchins.

Postzygotic Barriers in Sea Urchins

Fitness of F_1 Hybrids in the Laboratory.—Because generation time in echinoids is usually on the order of a year or more, and because sea urchin larvae and adults are not always easy to keep alive in captivity, assessments of the fitness of hybrids and their ability to back-cross to their parents are few. To my knowledge, fitness of hybrids with congeneric parental species has been determined in only five genera, *Echinometra*, *Pseudechinus, Strongylocentrotus*, *Heliocidaris*, and *Diadema* (Table 1). The most complete data, with quantified survival rates, come from the newly discovered and as yet unnamed Indo-West Pacific species of *Echinometra*. These are recently diverged species, thus it may not be surprising that their hybrids generally develop normally. The survivorship of larvae to metamorphosis in the cross between eggs of *Echinometra mathaei* (Blainville) and sperm of *Echinometra* sp. C is significantly lower than those of conspecific parents, but it is still > 60% (Rahman and Uehara, 2004), which would indicate that, by itself, this difference could not account for reproductive isolation between these two species. A slight depression in the settlement rates of hybrids between *E. mathaei* and *Echinometra* sp. A is compensated by higher growth rates of the hybrids, relative to non-hybrid offspring (Rahman et al., 2005). That the cross between *Heliocidaris tuberculata* Lamarck eggs and *Heliocidaris erythrogramma* (Valenciennes) sperm is lethal, even though these species have been isolated for only 4–5 million yrs (my), may arise from the shift

Table 1. Survivorship of hybrids in experimental crosses between congeneric species of sea urchins, relative to time since divergence.

Time* (my)		Parental species contributing: eggs		sperm	Development	Reference
1.1–1.5	*Echinometra*	*mathaei*	×	sp. C	survivorship slightly depressed	Rahman and Uehara, 2004
		sp. C	×	*mathaei*	normal	
1.1–1.3	*Echinometra*	*mathaei*	×	sp. A	survivorship slightly depressed	Rahman et al., 2005
		sp. A	×	*mathaei*	survivorship slightly depressed	
1.1–1.5	*Echinometra*	sp. A	×	sp. C	survivorship slightly depressed	Rahman et al., 2001
		sp. C	×	sp. A	normal	
1.1–1.3	*Echinometra*	sp. A	×	*E. oblonga*	normal	Aslan and Uehara, 1997
		E. oblonga	×	sp. A	normal	
7	*Pseudechinus*	*albocinctus*	×	*novaezealandiae*	normal	McClary and Sewell, 2003
		novaezealandiae	×	*albocinctus*	low settlement success	
1.6	*Pseudechinus*	*albocinctus*	×	*huttoni*	normal	McClary and Sewell, 2003
		huttoni	×	*albocinctus*	normal	
7	*Pseudechinus*	*huttoni*	×	*novaezealandiae*	dies at the 8 arm pluteus stage	McClary and Sewell, 2003
		novaezealandiae	×	*huttoni*	low settlement success	
1.2–1.4	*Strongylocentrotus*	*droebachiensis*	×	*palidus*	normal	Strathmann, 1981
		palidus	×	*droebachiensis*	normal	
4.4	*Strongylocentrotus*	*franciscanus*	×	*purpuratus*	dies at gastrulation	Newman, 1923
		purpuratus	×	*franciscanus*	normal	
4–5	*Heliocidaris*	*erythrogramma*	×	*tuberculata*	viable	Raff et al., 1999
		tuberculata	×	*erythrogramma*	dies at gastrulation	
7–14	*Diadema*	*savignyi*	×	*setosum*	viable	Uehara et al., 1990

* Approximate times are based on mitochndrial DNA data, taken from Laundry et al. (2003) for *Echinometra*, from Lee (2003) and Biermann et al. (2003) for *Strongylocentrotus*, from Jeffery et al. (2003) for *Pseudechinus*, from Zigler et al. (2003) for *Heliocidaris*, and from Lessios et al. (2001) for *Diadema*

of *H. erythrogramma* to direct development, a highly atypical evolutionary change among echinoids. However, the cross between eggs of *Strongylocentrotus franciscanus* (A. Agassiz, 1863) and sperm of *Strongylocentrotus purpuratus* (Stimpson, 1857) (both of which have planktonic larvae and split from each other at roughly the same time as the two species of *Heliocidaris*) is also lethal, which may indicate that by 5 my Dobzhansky-Muller incompatibilities (i.e., developmental difficulties arising from incompatible alleles in at least two loci of the F_1 hybrids) begin to render sea urchin hybrids inviable, even when no developmental shifts are involved. The low survivorship (in one direction) of offspring from the cross between *Pseudechinus novaezealandiae* Mortensen and *Pseudechinus albocinctus* Hutton indicates that such unidirectional developmental difficulties extend to species that have been separated for 7 my. However, the cross of *Pseudechinus huttoni* Benham and *P. novaezealandiae,* which is lethal in both directions, suggests that this is the period of time in which complete post-zygotic isolation becomes evident. One would not expect such an estimate to hold universally true in all echinoids, and indeed, data from *Diadema* appear to suggest that hybrids between *Diadema savignyi* (Michelin) and *Diadema setosum* (Leske), which diverged approximately 7–14 my ago, are completely viable in at least one direction. Uehara et al. (1990) reported producing adult hybrids with eggs of *D. savignyi* and sperm of *D. setosum*, but did not mention whether the offspring from the reciprocal cross survived. To the extent that such limited data can be generalized, it would appear that post-zygotic isolation in echinoids is absent in species that have diverged < 5 my ago, may be present in species that diverged between 5 and 10 my, but not in all cases.

Ability of F_1 Hybrids to Backcross.—Survivorship of F_1 hybrids would mean little if they were not able to backcross to the parental species. For obvious reasons, the data that could address this question are even scantier than those on survivorship (Table 2). Rahman and Uehara (2004) found that backcross fertilizations at limiting sperm concentrations of 10^{-5} are successful in all possible directions between hybrids and parentals and between all types of F_1 hybrids of *E. mathaei* and *Echinometra* sp. C. Survival rates of larvae and juveniles arising from backcrosses were as high as those of non-hybrid ones. The same result was obtained by Rahman et al. (2001) about hybrids of the cross between *Echinometra* sp. C and *Echinometra* sp. A. Aslan and Uehara (1997) found fertilization rates to be low in the backcross of sperm from one type of hybrid and eggs of *Echinometra oblonga* (Blainville), but backcrosses involving other types of hybrids proceeded at high rates. In Strathmann's (1981) study with *Strongylocentrotus*, all three hybrid individuals that survived to sexual maturity were female. These hybrids could be fertilized by sperm of *Strongylocentrotus palidus* (Sars), but not of *Strongylocentrotus droebachiensis* (O. F. Müller). Thus, these limited data would suggest that problems with backcrossing may start to arise when echinoid species have remained separated for more than a million yrs, but, even at 2–3 my, they are not sufficient by themselves to genetically isolate sympatric species.

Prezygotic Barriers to Hybridization

Habitat Separation.—It could be argued that habitat separation is a geographic, rather than reproductive, isolating barrier, but a case can also be made that habitat preferences are a barrier intrinsic to the organisms themselves, because of genetic pref-

Table 2. Rates of fertilization between hybrids, and between hybrids and pure individuals. In the designarion of the hybrids, the maternal species is listed first. Approximate times are based on mitochndrial DNA data, taken from Laundry et al. (2003).

Time (my)	Sperm from	Eggs from				Reference
1.1–1.5	*Echinometra mathaei* (Em) vs *Echinometra* sp. C (Ec)					Rahman and Uehara, 2004
		Em × Em	Em × Ec	Ec × Em	Ec × Ec	
	Em × Em	high	normal	normal	high	
	Em × Ec	normal	normal	normal	normal	
	Ec × Em	normal	normal	normal	normal	
	Ec × Ec	low	normal	normal	high	
1.1–1.3	*Echinometra oblonga* (Ed) vs *Echinometra* sp. A (Ea)					
		Ed × Ed	Ed × Ea	Ea × Ed	Ea × Ea	Aslan and Uehara, 1997
	Ed × Ed	high	high	high	low	
	Ed × Ea	low	high	high	high	
	Ea × Ed	very low	low	very low	low	
	Ea × Ea	low	low	low	high	
1.1–1.5	*Echinometra* sp. C (Ec) vs *Echinometra* sp. A (Ea)					Rahman et al., 2001
		Ea × Ea	Ea × Ec	Ec × Ea	Ec × Ec	
	Ea × Ea	high	normal	normal	high	
	Ea × Ec	normal	normal	normal	normal	
	Ec × Ea	normal	normal	normal	normal	
	Ec × Ec	low	normal	normal	high	
1.2–1.4	*Strongylocentrotus droebachiensis* (Sd) vs *Strongylocentrotus palidus* (Sp)					Strathmann, 1981
		Sd ×Sp	Sp × Sd			
	Sd × Sd	low	low			
	Sp × Sp	normal	normal			

erences for settling in a particular habitat. Either way, the evolution of such preferences will maintain isolation between sympatric species if it renders them incapable of exchanging genes because of their different ecological requirements. This is particularly true in the marine realm in which depth zonation between broadly sympatric congeneric species is frequent. How effective is this kind of barrier in echinoids?

Because there are rarely more than 10 extant species in each echinoid genus, it is not particularly common for two closely related species to inhabit the same geographic region. When such range overlap exists, congeneric species often show separation in depth, or some other aspect of preferred habitat. This is the case for *Echinometra* both in the Indo-Pacific (Tsuchiya and Nishihira, 1984; Nishihira et al., 1991; Rahman and Uehara, 2004) and in the Caribbean (Lessios et al., 1984; Hendler, et al., 1995); it is also the case for most of the sympatric species of *Strongylocentrotus* on the west coast of North America (Lillie, 1921; Newman, 1923; Pearse, 1981, 2006; Strathmann, 1981; Rogers-Bennett, 2007), for the Indo-Pacific species of *Diadema* (McClanahan, 1988; Pearse, 1998; Muthiga and McClanahan, 2007), and for the Caribbean species of *Lytechinus* (Lessios et al., 1984; Hendler et al., 1995). However, occurrence of individuals of one species in the habitat preferred by the other is not uncommon (Lessios and Cunningham, 1990; Rogers-Bennett et al., 1995; Levitan, 2002; McCartney and Lessios, 2004; Rahman and Uehara, 2004). What is more, different habitat preferences of sympatric congeneric species are not always the rule. For example, there appears to be no obvious habitat separation among the two Indo-

Pacific species of *Echinothrix* (Coppard and Campbell, 2005a), the three species of *Microcyphus*, or the two species of *Pseudoboletia* that cohabit the coasts of Australia (Miskelly, 2002). Thus, habitat separation may in some cases contribute to reproductive isolation by reducing the probability that gametes of two species will find themselves in proximity, but it is probably not an effective reproductive isolating barrier, except in combination with other kinds of barriers.

Temporal Barriers of Reproductive Isolation.—Given that most echinoids have well-defined periods of reproduction (Pearse and Cameron, 1991), even in the tropics (Pearse, 1974), it has been natural to wonder whether sympatric species remain reproductively isolated by shedding their gametes in the water at different times. The possibility that non-overlapping annual reproductive cycles may serve in that capacity has been examined in echinoids (e.g., Lessios, 1981; McClary and Barker, 1998) and rejected. Annual cycles are too environmentally labile, changing from place to place in the same species (Lessios, 1981, 1985; Lewis and Storey, 1984; Pearse and Cameron, 1991; Garrido et al., 2000) to be credible as an evolutionary mechanism for preventing genetic exchange. Moreover, annual cycles of sympatric, congeneric species often overlap (e.g., Lillie, 1921; Newman, 1923; Lessios, 1981, 1985; Pearse, 1981; Arakaki and Uehara, 1991; McClary and Barker, 1998). Lunar reproductive cycles, on the other hand, do have the potential of fulfilling this function. Coppard and Campbell (2005b) found that at Fiji *D. setosum* spawns at new moon, whereas *D. savignyi* spawns at full moon. *Echinothrix calamaris* (Pallas) spawns at new moon, but *Echinothrix diadema* (L.) spawns at full moon. Muthiga (2003) found a similar pattern in the two species of *Diadema* on the coast of Kenya, and suggested that their coexistence as separate species is made possible by these out of phase spawning rhythms. Lessios (1984) found that *Diadema mexicanum* Agassiz in the eastern Pacific and *Diadema antillarum* Philippi in the western Atlantic also spawn 15 d out of phase with each other. These two species, on the two sides of the Isthmus of Panama, have been separated for 2–3 my (Lessios et al., 2001). This finding implies that temporal isolation through lunar cycles can arise in allopatry within this period of time and convert geographic isolates into separate species that will not interbreed if they found themselves in sympatry. The assumption in proposing such cycles as reproductive isolating barriers is that moonlight affects lunar cycles directly, and that all populations of a species respond to this stimulus in the same manner. There is some evidence to support this view (Kennedy and Pearse, 1975), but the relationship is far from established (Pearse, 1968, 1990; Pearse and Cameron, 1991). For example, different studies, spaced years apart, of different populations of *D. antillarum* in the Caribbean have shown a remarkable consistency in the tendency of this species to spawn around new moon (reviews in Iliffe and Pearse, 1982; Lessios, 1984), a rhythm that persisted even after mass mortality (Lessios, 1988). This consistency, however, appears to be lacking between localities and years in the Indo-Pacific *D. setosum* and *D. savignyi* (Pearse, 1968, 1970; reviews in Muthiga, 2003; Coppard and Campbell, 2005b). Much (though not all) of the apparent variation in these species comes from comparisons of different studies, and it remains to be determined how much of it is real and how much is due to different methodologies, inconsistency in species identification of these often confused species (Pearse, 1998), or the existence of cryptic species in the Indo-Pacific (Lessios et al., 2001). Even though lunar cycles have the potential of acting as reproductive isolation barriers for diadematid sea urchins, they are not likely to serve this function in the majority of echinoids, because species in other families of sea urchins do not spawn with a lunar rhythm (Pearse, 1990; Lessios, 1991).

Gametic Isolation.—Testing sea urchin gametes for compatibility with those of other species of sea urchins is relatively easy, so there is a wealth of information on this potential isolating barrier (review in Zigler et al., 2005). Experiments in which gametic compatibility can be reduced to a common measure, so that they can be compared to each other, and for which there are data allowing an estimate of species ages are listed in Table 3. Of the 14 pairs of heterospecific fertilizations that fulfill the previous criteria, two were compatible in both directions, three were completely incompatible, and nine showed gametic isolation that was asymmetric. Thus, in the majority of cases, the eggs of a species may not permit fertilization by sperm of another, but its sperm is capable of fertilizing heterospecific eggs. It could be argued that apparent compatibility or unidirectionality is the result of the way in which such experiments have been carried out. Because there is no easy way to identify the parents of zygotes when gametes of more than one species are mixed together, gametic compatibility has been by and large determined in "no choice" experiments in which gametes of only two species are present. This could conceivably inflate the rate of heterospecific fertilization. Geyer and Palumbi (2005) found by genetically identifying larvae that gametes of *Echinometra* sp. C and *E. oblonga*, which in no-choice experiments appear to be almost perfectly compatible, can actually discriminate in a mixture between homospecific and heterospecific sperm, so that the eggs are fertilized by their own species > 50% of the time. However, when Zigler and Lessios (unpubl. data) performed the same type of choice experiments between *Lytechinus variegatus* (Lamarck) and *Lytechinus williamsi* Chesher, they found that gametes of the two species could fertilize each other indisciminantly. Given the rapid aging of sea urchin sperm in dilution and the low probability of its encountering eggs when it is not released close to a female (Pennington, 1985; Levitan, 1995), it is not clear whether the "no-choice" or the "two-way-choice" artificial fertilizations more accurately reflect what happens in nature.

It has been generally assumed that unidirectional gametic isolation is an intermediate stage on the road to complete isolation (e.g., Lessios and Cunningham, 1990; Rahman and Uehara, 2004); but the compilation of data in Table 3 does not support the hypothesis that gametic isolation is necessarily a simple function of time. There is no clear tendency for more anciently separated species to possess more incompatible gametes. It is true that *S. franciscanus* is the first extant species of *Strongylocentrotus* to have split off from the rest (Biermann et al., 2003; Lee, 2003), and it is also true that it is bidirectionally reproductively isolated from *S. purpuratus* and *S. droebachiensis*. However, *Arbacia punctulata* (Lamarck) and *Arbacia incisa* Agassiz [=*stellata* (Blainville)] have been separated from each other for even a longer time, yet their gametes are completely compatible. *Echinometra mathaei* and *Echinometra* sp. A have been separated for less than one third of that time, but they are no longer capable of fertilizing one another. There is no significant correlation between gametic compatibility and Cytochrome Oxidase I divergence (Zigler et al., 2005). As the latter is assumed to diverge linearly with time within the range of values included in these comparisons, there is also no correlation between gametic incompatibility and time. Something other than time determines whether two species of echinoids have evolved gametic incompatibility; the most likely agents are the evolutionary forces that act on the molecules that mediate recognition between sperm and egg.

Evolution of Molecules Involved in Gamete Recognition.—Attraction of sea urchin sperm to the egg, penetration of the egg jelly coat, induction of the

acrosome reaction, binding of the sperm to the egg vitelline layer, fusion of the membranes, and transfer of DNA involve many molecules (Vacquier et al., 1995; Biermann et al., 2004; Neill and Vacquier, 2004; Mah et al., 2005; Kaupp et al., 2006). Evolutionary modifications in any of them could influence the degree to which gametes of two species are capable of affecting fertilization. Two of the most important molecules in determining whether a sperm will successfully fertilize an egg are the sperm molecule bindin and its egg receptor (Vacquier et al., 1995; Swanson and Vacquier, 2002). The egg receptor has been characterized only in *S. franciscanus* and in *S. purpuratus* (Kamei and Glabe, 2003) and nothing is known about the evolution of this enormous molecule (4595 amino acids), other than that it contains regions that differ between these two species. The more manageable sperm molecule bindin, on the other hand, has been studied extensively from a number of individuals in a number of species, and thus various inferences can be made regarding its evolution.

Bindin is a protein that coats the acrosome process of the sperm after it contacts the egg jelly. It is involved in the binding of the sperm to the vitelline layer and in the fusion of the membranes of the two gametes. Its size ranges from 206 to 418 amino acid residues in the echinoid species that have been studied to date (Zigler and Lessios, 2003a). Comparisons between bindins of different echinoid species have shown that there is a core of approximately 60 amino acids in the middle part of the molecule that has remained remarkably conserved during echinoid evolution. Towards the C and the N termini of the molecule lie exons that can vary in composition and repeat structure even between alleles of the same individual. Variation in these regions is interesting from the point of view of the evolution of reproductive barriers. Though bindin is known to impart species-specificity in gamete interactions (Metz et al., 1994), it remains to be determined which parts of the molecule and what amino acid changes are responsible for this property. There are, however, several aspects that emerge from comparison of molecules from different species that are intriguing: (a) Gametic incompatibility between species within a genus is not related to the time since their speciation, but it is correlated to degree of bindin divergence (Zigler and Lessios, 2003b; Zigler et al., 2005). (b) Among the three neotropical *Echinometra* species, the majority of amino acid replacements have accumulated along the branch that leads to *Echinometra lucunter* (L.) (McCartney and Lessios, 2004), the same species in which eggs do not permit fertilization by heterospecific sperm from either a sympatric or an allopatric congener (Lessios and Cunningham, 1990; McCartney and Lessios, 2002). (c) Three genera, *Echinometra* (Metz and Palumbi, 1996; McCartney and Lessios, 2004), *Strongylocentrotus* (Biermann, 1998), and *Heliocidaris* (Zigler et al., 2003) have bindins that contain a "hot-spot" with a variable number of Glycine-rich repeats. The first exon of bindin in the same three genera shows evidence of positive selection between alleles in the form of excess amino acid replacements relative to silent substitutions. (d) Three more genera, *Arbacia* (Metz et al., 1998), *Tripneustes* (Zigler and Lessios, 2003b), and *Lytechinus* (Zigler and Lessios, 2004), have bindins that do not share these features. There are no hot-spots, and there is no evidence of selection anywhere along the length of the molecule. (e) The three genera in which bindin evolves under selection all contain sympatric species, whereas the three genera in which bindin evolves neutrally do not, except for one case of sympatry between two species with no gametic isolation in *Lytechinus*. What can these comparative facts reveal about the mode of evolution of the bindin molecule, and thus about the mode of gametic isolation in sea urchins?

That gametic isolation is correlated to bindin divergence indicates that bindin plays a role in determining whether gametes of two species can fertilize each other. That bindin in *E. lucunter*, the species with eggs protected from heterospecific fertilization, has been evolving away from that of its congeners suggests that there is selection on the male molecule to track changes that occur in the egg receptor. This, however, is not a simple lock-and-key correspondence in which a change in the female molecule results in an analogous change in the male one. Bindin in *E. lucunter* may have changed in response to changes in the receptor, but, if so, these changes have not impaired its ability to bind on the egg surfaces of *Echinometra viridis* Agassiz and *Echinometra vanbrunti* Agassiz (Table 3). This greatly complicates the understanding of the causal relationship between divergence in bindin and gametic incompatibility, and highlights the need to obtain data regarding the evolution of the egg receptor.

That there is a correlation between species range overlap and mode of bindin evolution suggests that the selective force that acts on bindin may be reinforcement, i.e., selection to modify the molecule so as to avoid investment into gametes that will result in unfit hybrids (Swanson and Vacquier, 2002). The hypothesis of reinforcement is strengthened by a pattern of character displacement in *E. oblonga* (Geyer and Palumbi, 2003). In the central Pacific, where *Echinometra* sp. C does not occur, *E. oblonga* contains alleles very similar to those of the latter species. In the western Pacific where it coexists with *Echinometra* sp. C all its bindin alleles are different from those of its closely related congener. Another pattern that fits the hypothesis of reinforcement is that bindin of *H. erythrogramma,* the species that invests most in each egg because of direct development, has accumulated the most amino acid replacements since its separation from the indirectly developing *H. tuberculata* (Zigler et al., 2003). Finally, in the neotropical species of *Echinometra*, *E. lucunter* is in higher danger of having its eggs fertilized by sperm of *E. viridis* (because the latter is occasionally found in the habitat of the former, but not vise-versa) and, once again, it is the species that possesses bindin in which amino acid changes have accumulated. On the basis of these observations, one might conclude that the reasons for differences in mode of bindin evolution between the genera have been identified. There is, however, also evidence that does not fit the reinforcement hypothesis.

Selection to avoid hybridization would produce an excess of amino acid replacements between sympatric species, but not between alleles of the same species. When one examines intra- vs inter-specific comparisons of the ratio of replacement to silent substitutions, the data from *Heliocidaris* are still consistent with the reinforcement hypothesis, even though *H. tuberculata* and *H. erythrogramma* overlap in only a small part of their range. The bindin of this genus contains 22 amino acid substitutions between species and only three within species (there are no silent substitutions) (Zigler et al., 2003). In the hotspot of three sympatric species of *Echinometra,* however, the ratio of replacement to silent substitutions is higher than unity not only between alleles of three sympatric species, but also between alleles of the same species (Metz and Palumbi, 1996). Such intraspecific positive selection cannot be explained by selection to avoid hybridization. In the hotspot of *S. franciscanus*, *S. purpuratus*, and *S. droebachiensis* (all of which are sympatric on the West Coast of North America) the ratio of replacement to silent substitutions between alleles of the same species is actually four times higher than the same ratio between alleles of different species.

Table 3. Gametic compatibility between congeneric species in "no choice" experiments. Compatibility was defined as the ratio of mean percentage of eggs ferilized in a heterospecific cross, divided by mean percentage of eggs fertilized in the homospecific crosses at a sperm concentration necessary to fertilize > 90% of eggs in the homospecific cross. * First species to be listed is the contributor of eggs. Table was modified and updated from Zigler et al. (2005), and species ages were calculated as in Table 1, assuming an approximate rate of divergence of 3% in COI per my.

		Species		Compatibility		
Time (my)	Genus	A	B	A × B*	B × A*	Reference
4.6	*Arbacia*	*punctulata*	*incisa*	1.00	1.00	Metz et al., 1998
1.1–1.3	*Echinometra*	sp. A	sp. B (*mathaei*)	0.00	0.00	Uehara et al., 1990
				0.00	0.01	Metz et al., 1994
1.1–1.3	*Echinometra*	sp. A	sp. D (*oblonga*)	0.00	0.52	Uehara et al., 1990
				0.02	0.40	Metz et al., 1994
0.8–0.9	*Echinometra*	sp. B (*mathaei*)	sp. D (*oblonga*)	0.00	0.22	Uehara et al., 1990
				0.02	0.07	Metz et al., 1994
				0.01	0.72	Rahman et al., 2004
1.1–1.5	*Echinometra*	sp. C	sp. D (*oblonga*)	0.96	0.88	Geyer and Palumbi, 2005
1.3–1.6	*Echinometra*	*lucunter*	*viridis*	0.19	0.97	Lessios and Cunningham, 1990
				0.24	0.88	McCartney and Lessios, 2002
3.1	*Echinometra*	*lucunter*	*vanbrunti*	0.09	1.00	Lessios and Cunningham, 1990
				0.12	0.89	McCartney and Lessios, 2002
3.1	*Echinometra*	*viridis*	*vanbrunti*	0.92	1.00	Lessios and Cunningham, 1990
				0.48	1.00	McCartney and Lessios, 2002
4.9	*Heliocidaris*	*erythrogramma*	*tuberculata*	0.00	0.82	Zigler et al., 2003
4.5	*Lytechinus*	*pictus*	*variegatus*	0.72	1.00	Minor et al., 1991
0.6		*variegatus*	*williamsi*	1.00	1.00	Zigler and Lessios, unpubl. data
4.0	*Strongylocentrotus*	*droebachiensis*	*franciscanus*	0.02	0.00	Levitan, 2002
1.3–1.4	*Strongylocentrotus*	*droebachiensis*	*pallidus*	0.82	0.07	Strathmann, 1981
2.4	*Strongylocentrotus*	*droebachiensis*	*purpuratus*	0.75	0.00	Levitan, 2002,
4.4	*Strongylocentrotus*	*franciscanus*	*purpuratus*	0.05	0.01	Lillie, 1921
				0.01	0.00	Minor et al., 1991
				0.00	0.00	Levitan, 2002

This intraspecific excess is not statistically significant (Debenham et al., 2000), but it certainly is not consistent with reinforcement.

Further evidence against the hypothesis that reinforcement drives the evolution of echinoid bindin comes from failures to find a pattern of character displacement in either the Atlantic *Echinometra,* or the Australian *Heliocidaris*. *Echinometra viridis* is only present in the Caribbean, whereas E. *lucunter* ranges widely in the tropical Atlantic. There is no gene flow between Caribbean and Atlantic populations of this species (McCartney et al., 2000). Yet individuals from the two regions share bindin alleles without any geographic structuring (Geyer and Lessios, unpubl. data). Similarly, *Heliocidaris erythrogramma armigera* A. Agassiz on the west coast of Australia receives no gene flow from the portion of the range of *H. erythrogramma* that overlaps with *H. tuberculata* (McMillan et al., 1992). Yet the bindin alleles of this subspecies are no different than those of *Heliocidaris erythrogramma erythrogramma* (Valenciennes) (Geyer, Zigler, Raff, and Lessios, unpubl. data). It is thus possible that the pattern of reinforcement revealed by the comparison of the mode of evolution of bindin in different genera (and possibly in the pattern of character displacement within a single species) is a secondary one. It may result not from any selective pressures created by the challenge of sympatric species; instead it may be that species with bindins that have diverged for other reasons are the only ones that are able to coexist without either merging or without one driving the other to extinction.

What are the possible forces, other than reinforcement, that could cause bindin divergence and gametic isolation between echinoid species? In a mating system, sexual selection and the different evolutionary interests of the two sexes may play a role. Sexual selection can be important in speciation if different mates are preferred by the sexes in geographically separated populations, giving rise to assortative mating that will persist in subsequent sympatry (West-Eberhard, 1983). In a system such as bindin and its receptor, frequencies of different alleles can become rapidly predominant in different populations, because of the linkage disequilibrium that will ensue when bindin alleles preferred by different egg receptors are found together with the complimentary receptor alleles in the same offspring. Assortative mating in bindin has been demonstrated in *E. mathaei.* Palumbi (1999) has shown experimentally that eggs from females carrying a particular bindin allele are preferentially fertilized by sperm from males with the same allele. Levitan and Ferrell (2006) have shown that in *S. franciscanus* eggs of females that carry rare bindin alleles are more likely to be fertilized than those carrying common alleles when sperm density is high, but that the reverse is true at limiting sperm densities. This suggests a mechanism for maintaining intraspecific polymorphism, but also sets the stage for divergent selection in populations with different point densities. It may also explain the differences seen among species and genera in the mode of bindin evolution. In both *Echinometra* (McCartney and Lessios, 2004) and *Strongylocentrotus* (Biermann, 1998; Debenham et al., 2000; Levitan and Ferrell, 2006), species that are found in shallow water in high point densities—and are thus likely to experience high sperm density during spawning events—are also species that show the strongest selection on bindin. Thus, it is possible that the selective forces that account for fast bindin evolution in some genera are sexual selection and inter-locus sexual conflict (Rice, 1998; Gavrilets, 2000), and that the pattern of reinforcement suggested by the differences between genera with sympatric and allopatric species is the result of the ability of closely related species with bindin that has diverged for other reasons to coexist in the same area.

Occurrence of Hybrids in Nature.—The frequency of hybrids in nature is direct evidence as to the effectiveness of reproductive isolating barriers between sympatric species. Reports of echinoid specimens that were considered to be hybrids because they were hard to identify are common in the literature (e.g., Shearer et al., 1914; Mortensen, 1940, 1943; Vasseur, 1952; Swan, 1953; Hagström and Lønning, 1961), but unconvincing. It is hard to know how many of these morphologically intermediate specimens are true F_1 (or later generation) hybrids, and how many come from the tail of the natural distribution of diagnostic characters in either species. Reports in which the authors have actually raised F_1 hybrids in the laboratory, so that they had a reference for identifying field-collected animals as hybrids (e.g., Strathmann, 1981; Rahman and Uehara, 2004) are much more convincing, and those that have used genetic means for hybrid identification (e.g., Lessios and Pearse, 1996; Geyer and Palumbi, 2003) are indisputable. Rahman and Uehara (2004) in Okinawa collected 600 individuals of *Echinometra* with coloration that might suggest that they were hybrids, then compared their morphology in detail with those of laboratory reared F_1 hybrids. They were unable to confirm that even a single individual was, in fact, a hybrid. Lessios and Pearse (1996) conducted an exhaustive search for hybrids of *Diadema* in Okinawa guided by examining the hybrid morphology of the cross between *D. setosum* and *D. savignyi* produced by Uehara et al. (1990), and then genotyped all suspected hybrids along with individuals of pure morphology. They discovered that out of several hundred specimens examined, one field-collected specimen was an F_1 hybrid of *D. setosum* and *D. savignyi*, and that eight other specimens were later generation hybrids of either these two species, or of one of these species and *Diadema paucispinum* Agassiz. Several specimens with intermediate morphologies turned out not to be hybrids. Significantly, the presence of diagnosticaly different electrophoretic loci between individuals of pure morphology of the three species indicated that, despite the existence of these hybrids, there were no signs of introgression. Thus, hybrids may be produced in low frequencies and they may be able to backcross to a certain degree, but descendants of such crosses are apparently at a selective disadvantage and removed from the population before they can affect transfer of genes between species.

Evidence for the rarity of hybrids in nature is also present in population genetic studies of sympatric echinoid species that would have detected hybrids, even though this was not the main aim of the study. This includes mitochondrial DNA studies, even though the molecule is maternally inherited, because they have the potential of detecting hybridization as the incorporation of a mitochondrial DNA clade into the morphology of the wrong species. Such studies, often including large sample sizes, exist for the sympatric species of *Echinometra* in the Caribbean and in the Indo-Pacific, of *Diadema* in the Indo-Pacific, and of *Strongylocentrotus* on the west coast of North America (reviewed in Palumbi and Lessios, 2005). The only report of a genetically identified hybrid in these studies is one individual out of 70 sampled that was identified as a hybrid by Geyer and Palumbi (2003). Thus, based on these studies, it would appear that natural hybrids occur at a frequency of < 1.4%, and probably much lower, in most areas. A possible exception to this pattern of little hybridization and introgression among sea urchins may be the case of the Caribbean species of *Lytechinus*. *Lytechinus variegatus variegatus* (Lamarck) and *L. williamsi* possess bindin alleles that are reciprocally monophyletic, but their mitochondrial DNA is very similar. This could be the result of introduction and subsequent spread of the

latter molecule from one species to the other through hybridization (Zigler and Lessios, 2004). There are also obvious hybrids between the subspecies *L. variegatus variegatus* and *Lytechinus variegatus carolinus* (A. Agassiz, 1863), even though these subspecies coexist only in narrow contact zones.

Conclusions

Data from different species have been used here to address the question of the existence of various classes of reproductive barriers between species of sea urchins. The data are far from conclusive, but they point to the possibility that by and large no single barrier is likely to isolate species completely. The search for reproductive isolating barriers, however, is a one-tailed process. Such barriers can take so many forms, that lack of evidence of reproductive isolation is not necessarily evidence that it is lacking. One might expect that if, as reviewed here, F_1 hybrids do not encounter developmental difficulties (except for species that have diverged for > 5 my), if habitat preferences of echinoid species are not sharply distinct, if their reproductive seasons overlap, if gametic isolation is generally unidirectional, and if molecules involved in gamete recognition are polymorphic, a substantial number of sea urchin hybrids would exist in nature in regions where congeneric species of echinoids are sympatric. These sympatric species should also show signs of introgression. This prediction comes in direct contrast with the (admittedly incomplete) evidence that hybrids are rare among sea urchins, and that introgression occurs only in exceptional cases. Although it is always possible that further study will identify reproductive barriers that are absolute, the conclusion one would reach from the existing studies is that reproductive isolation in sea urchins results from a combination of various factors, each of which makes a partial contribution. Conspecific sperm released only a few meters away from a female has a low probability of ever fertilizing her eggs (Pennington, 1985; Levitan, 1995), so any characteristic that further decreases this probability is likely to prevent the production of viable offspring in numbers large enough to be represented in the next generation. Thus, sperm of one species may rarely have the opportunity to encounter and successfully fertilize eggs of another. Habitats of sympatric species overlap, but not entirely. Annual cycles of reproduction overlap, but peaks may not coincide. Lunar spawning cycles may be shared by species in some localities, but not throughout the species ranges. Gametic incompatibility imparted by reproductive molecules may be incomplete or unidirectional, but it can reduce the frequency with which hybrids are produced. Hybrids may survive well in the laboratory, but they may be at a selective disadvantage in nature. Thus, the probability of hybridization may be low, not because it is blocked by any single factor, but simply because it is the product of many small probabilities.

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Literature Cited

Arakaki, Y. and T. Uehara. 1991. Physiological adaptations and reproduction of the four types of *Echinometra mathaei* (Blainville). Pages 105–112 *in* T. Yanagisawa, I. Yasumasu, C. Ogurao, N. Suzuki, and T. Motokawa, eds. Biology of echinodermata. Proc. 7th Int. Echinoderm Conference. A. A Balkema, Rotterdam.

Aslan, L. M. and T. Uehara. 1997. Hybridization and F_1 backcrosses between two closely related tropical species of sea urchins (genus *Echinometra*) in Okinawa. Invertebr. Reprod. Dev. 31: 319–324.

Biermann, C. H. 1998. The molecular evolution of sperm bindin in six species of sea urchins (Echinoida : Strongylocentrotidae). Mol. Biol. Evol. 15: 1761–1771.

_____, B. D. Kessing, and S. R. Palumbi. 2003. Phylogeny and development of marine model species: Strongylocentrotid sea urchins. Evol. Dev. 5: 360–371.

_____, J. A. Marks, A. C. E. S. Vilela-Silva, M. O. Castro, and P. A. S. Mourao. 2004. Carbohydrate-based species recognition in sea urchin fertilization: another avenue for speciation? Evol. Dev. 6: 353–361.

Brown, W. L. and E. O. Wilson. 1956. Character displacement. Syst. Zool 7: 49–64.

Butlin, R. 1989. Reinforcement of premating isolation. Pages 158–179 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland.

Coppard, S. E. and A. C. Campbell. 2005a. Distribution and abundance of regular sea urchins on two coral reefs in Fiji. Micronesica 37: 249–269.

_____ and _____. 2005b. Lunar periodicities of diadematid echinoids breeding in Fiji. Coral Reefs 24: 324–332.

Coyne, J. A. and H. A. Orr. 2004. Speciation. Sinauer, Sunderland. 545 p.

Debenham, P., M. A. Brzezinski, and K. R. Foltz. 2000. Evaluation of sequence variation and selection in the bindin locus of the red sea urchin, *Strongylocentrotus franciscanus.* J. Mol. Evol. 51: 481–490.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. Am. Nat. 74: 312–321.

_____. 1970. Genetics of the evolutionary process. Columbia Univ. Press, New York 505 p.

Garrido, M. J., R. J. Haroun, and H. A. Lessios. 2000. Annual reproductive periodicity of the sea urchin *Diadema antillarum* Philippi in the Canary Islands. Bull. Mar. Sci. 67: 989–996.

Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. Nature 403: 886–889.

Geyer, L. B. and S. R. Palumbi. 2003. Reproductive character displacement and the genetics of gamete recognition in tropical sea urchins. Evolution 57: 1049–1060.

_____ and _____. 2005. Conspecific sperm precedence in two species of tropical sea urchins. Evolution 59: 97–105.

Hagström, B. E. and S. Lønning. 1961. Studies on the species specificity of echinoderms. Sarsia 4: 5–19.

Hendler, G., J. E. Miller, D. L. Pawson, and P. M. Kier. 1995. Sea stars, sea urchins, and allies: echinoderms of Florida and the Caribbean. Smithsonian Institution Press, Washington.

Iliffe, T. M. and J. S. Pearse. 1982. Annual and lunar reproductive rhythms of the sea urchin, *Diadema antillarum* (Philippi) [sic] in Bermuda. Int. J. Invertebr. Reprod. 5: 139–148.

Jeffery, C. H., R. B. Emlet, and D. T. J. Littlewood. 2003. Phylogeny and evolution of developmental mode in temnopleurid echinoids. Mol. Phylogen. Evol. 28: 99–118.

Kamei, N. and C. G. Glabe. 2003. The species-specific egg receptor for sea urchin sperm adhesion is EBR1, a novel ADAMTS protein. Genes Dev. 17: 2502–2507.

Kaupp, U. B., E. Hildebrand, and I. Weyand. 2006. Sperm chemotaxis in marine invertebrates: molecules and mechanisms. J. Cell. Physiol. 208: 487–498

Kennedy, B. and J. S. Pearse. 1975. Lunar synchronization of the monthly reproductive rhythm in the sea urchin *Centrostephanus coronatus* Verill. J. Exp. Mar. Biol. Ecol. 17: 323–331.

Landry, C., L. B. Geyer, Y. Arakaki, T. Uehara, and S. R. Palumbi. 2003. Recent speciation in the Indo-West Pacific: rapid evolution of gamete recognition and sperm morphology in cryptic species of sea urchin. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 270: 1839–1847.

Lee, Y. H. 2003. Molecular phylogenies and divergence times of sea urchin species of Strongylocentrotidae, Echinoida. Mol. Biol. Evol. 20: 1211–1221.

Lessios, H. A. 1981. Reproductive periodicity of the echinoids *Diadema* and *Echinometra* on the two coasts of Panama. J. Exp. Mar. Biol. Ecol. 50: 47–61.

_____. 1984. Possible prezygotic reproductive isolation in sea urchins separated by the Isthmus of Panama. Evolution 38: 1144–1148.

_____. 1985. Annual reproductive periodicity in eight echinoid species on the Caribbean coast of Panama. Pages 303–311 *in* B. F. Keegan and B. D. O'Connor, eds. Echinodermata. Proc. 5th Int. Echinoderm Conference, Galway. A. A. Balkema, Rotterdam.

_____. 1988. Population dynamics of *Diadema antillarum* (Echinodermata: Echinoidea) following mass mortality in Panama. Mar. Biol. 99: 515–526.

_____. 1991. Presence and absence of monthly reproductive rhythms among eight Caribbean echinoids off the coast of Panama. J. Exp. Mar. Biol. Ecol. 153: 27–47.

_____ and C. W. Cunningham. 1990. Gametic incompatibility between species of the sea urchin *Echinometra* on the two sides of the Isthmus of Panama. Evolution 44: 933–941.

_____ and J. S. Pearse. 1996. Hybridization and introgression between Indo-Pacific species of *Diadema.* Mar. Biol. 126: 715–723.

_____, B. D. Kessing, and J. S. Pearse. 2001. Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema.* Evolution 55: 955–975.

_____, J. D. Cubit, D. R. Robertson, M. J. Shulman, M. R. Parker, S. D. Garrity, and S. C. Levings. 1984. Mass mortality of *Diadema antillarum* on the Caribbean coast of Panama. Coral Reefs 3: 173–182.

Levitan, D. R. 1995. The ecology of fertilization in free-spawning invertebrates. Pages 123–156 *in* L. R. McEdward, ed. Ecology of marine invertebrate larvae. CRC Press Inc, Boca Raton.

_____. 2002. The relationship between conspecific fertilization success and reproductive isolation among three congeneric sea urchins. Evolution 56: 1599–1609.

_____ and D. L. Ferrell. 2006. Selection on gamete recognition proteins depends on sex, density, and genotype frequency. Science 312: 267–269.

Lewis, J. B. and G. S. Storey. 1984. Differences in morphology and life history traits of the echinoid *Echinometra lucunter* from different habitats. Mar. Ecol. Prog. Ser. 15: 207–211.

Lillie, F. R. 1921. On the measure of specificity in fertilization between two associated species of the sea-urchin genus *Strongylocentrotus*. Biol. Bull. 40: 1–22.

Mah, S. A., W. J. Swanson, and V. D. Vacquier. 2005. Positive selection in the carbohydrate recognition domains of sea urchin sperm receptor for egg jelly (SuREJ) proteins. Mol. Biol. Evol. 22: 533–541.

Mayr, E. 1954. Geographic speciation in tropical echinoids. Evolution 8: 1–18.

_____. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge.

McCartney, M. A. and H. A. Lessios. 2002. Quantitative analysis of gametic incompatibility between closely related species of neotropical sea urchins. Biol. Bull. 202: 166–181.

_____ and _____. 2004. Adaptive evolution of sperm bindin tracks egg incompatibility in neotropical sea urchins of the genus *Echinometra.* Mol. Biol. Evol. 21: 732–745.

_____, G. Keller, and H. A. Lessios. 2000. Dispersal barriers in tropical oceans and speciation of Atlantic and eastern Pacific *Echinometra* sea urchins. Mol. Ecol. 9: 1391–1400.

McClanahan, T. R. 1988. Coexistence in a sea urchin guild and its implications to coral reef diversity and degradation. Oecologia 77: 210–218.

McClary, D. and M. Barker. 1998. Reproductive isolation? interannual variability in the timing of reproduction in sympatric sea urchins, genus *Pseudechinus.* Invertebr. Biol. 117: 75–93.

_____ and M. A. Sewell. 2003. Hybridization in the sea: gametic and developmental constraints on fertilization in sympatric species of *Pseudechinus* (Echinodermata: Echinoidea). J. Exp. Mar. Biol. Ecol. 284: 51–70.

McMillan, W. O., R. A. Raff, and S. R. Palumbi. 1992. Population genetic consequences of developmental evolution in sea urchins (genus *Heliocidaris*). Evolution 46: 1299–1312.

Metz, E. C. and S. R. Palumbi. 1996. Positive selection and sequence rearrangements generate extensive polymorphism in the gamete recognition protein bindin. Mol. Biol. Evol. 13: 397–406.

_____, G. Gómez-Gutiérrez, and D. Vacquier. 1998. Mitochondrial DNA and bindin gene sequence evolution among allopatric species of the sea urchin genus *Arbacia.* Mol. Biol. Evol. 15: 185–195.

_____, R. E. Kane, H. Yanagimachi, and S. R. Palumbi. 1994. Fertilization between closely related sea urchins is blocked by incompatibilities during sperm-egg attachment and early stages of fusion. Biol. Bull. 187: 23–34.

Minor, J. E., D. R. Fromson, R. J. Britten, and E. H. Davidson. 1991. Comparison of the bindin proteins of *Strongylocentrotus franciscanus*, *S. purpuratus*, and *Lytechinus variegatus*: sequences involved in the species specificity of fertilization. Mol. Biol. Evol. 8: 781–795.

Miskelly, A. 2002. Sea urchins of Australia and the Indo-Pacific. Capricornica Press, Sydney. 180 p.

Mortensen, T. 1940. A monograph of the Echinoidea. v. III_1. Aulodonta. C. A. Reitzel, Copenhagen.

_____. 1943. A monograph of the Echinoidea vol. III_3. Camarodonta, II. C. A. Reitzel, Copenhagen

Muthiga, N. A. 2003. Coexistence and reproductive isolation of the sympatric echinoids *Diadema savignyi* Michelin and *Diadema setosum* (Leske) on Kenyan coral reefs. Mar. Biol. 143: 669–677.

Muthiga, N. A. and T. R. McClanahan. 2007. Ecology of *Diadema*. Pages 205–225 *in* J. M. Lawrence, ed. Edible sea urchins: biology and ecology. Elsevier, Amsterdam.

Neill, A. T. and V. D. Vacquier. 2004. Ligands and receptors mediating signal transduction in sea urchin spermatozoa. Reproduction 127: 141–149.

Newman, H. H. 1923. Hybrid vigor, hybrid weakness, and the chromosome theory of heredity. J. Exp. Zool. 37: 168–205.

Nishihira, M., Y. Sato, Y. Arakaki, and M. Tsuchiya. 1991. Ecological distribution and habitat preference of four types of the sea urchin *Echinometra mathaei* on the Okinawan coral reefs. Pages 91–104 *in* T. Yanagisawa, I. Yasumasu, C. Oguro, N. Suzuki, and T. Motokawa, eds. Biology of echinodermata. Balkema, Rotterdam.

Palumbi, S. R. 1999. All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. Proc. Natl. Acad. Sci. USA 96: 12632–12637.

_____ and H. A. Lessios. 2005. Evolutionary animation: How do molecular phylogenies compare to Mayr's reconstruction of speciation patterns in the sea? Proc. Natl. Acad. Sci. USA 102, Suppl. 1: 6566–6572.

Pearse, J. S. 1968. Patterns of reproduction in four species of Indo-Pacific echinoderms. Proc. Ind. Acad. Sci. Ser. B 67: 247–279.

_____. 1970. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. III. The echinoid *Diadema setosum* (Leske). Bull. Mar. Sci. 20: 697–720.

_____. 1974. Reproductive patterns of tropical reef animals: three species of sea urchins. Proc. 2nd Int. Symp. Coral Reefs 1: 235–240.

_____. 1981. Synchronization of gametogenesis in the sea urchins *Strongylocentrotus purpuratus* and *S. franciscanus*. Pages 53–68 *in* W. H. Clark and T. S. Adams, eds. Advances in invertebrate reproduction. Elsevier, Amsterdam.

_____. 1990. Lunar reproductive rhythms in marine invertebrates: maximizing fertilization? Pages 311–316 *in* M. Hoshi and O. Yamashita, eds. Advances in invertebrate reproduction. Elsevier, New York.

_____. 1998. Distribution of *Diadema savignyi* and *D. setosum* in the tropical Pacific. Pages 777–782 *in* R. Mooi and M. Telford, eds. Echinoderms: San Francisco. Balkema, Rotterdam.

_____. 2006. Ecological role of purple sea urchins. Science 314: 940–941.

_____ and R. A. Cameron. 1991. Echinodermata: Echinoidea. Pages 513–662 *in* A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Reproduction of marine invertebrates. Boxwood Press, Pacific Grove.

Pennington, J. T. 1985. The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. Biol. Bull. 169: 417–430.

Raff, E. C., E. M. Popodi, B. J. Sly, F. R. Turner, J. T. Villinski, and R. A. Raff. 1999. A novel ontogenetic pathway in hybrid embryos between species with different modes of development. Development 126: 1937–1945.

Rahman, M. A. and T. Uehara. 2004. Interspecific hybridization and backcrosses between two sibling species of Pacific sea urchins (Genus *Echinometra*) on Okinawan intertidal reefs. Zool. Stud. 43: 93–111.

_____, T. Uehara, and J. S. Pearse. 2001. Hybrids of two closely related tropical sea urchins (Genus *Echinometra*): evidence against postzygotic isolating mechanisms. Biol. Bull. 200: 97–106.

_____, T. Uehara, and J. M. Lawrence. 2005. Growth and heterosis of hybrids of two closely related species of Pacific sea urchins (Genus *Echinometra*) in Okinawa. Aquaculture 245: 121–133.

Rice, W. R. 1998. Intergenomic conflict, interlocus antagonistic coevolution, and evolution of reproductive isolation. Pages 261–270 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms. Species and speciation. Oxford University Press, New York.

Rogers-Bennett, L. 2007. The ecology of *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus*. Pages 393–425 *in* J. M. Lawrence, ed. Edible sea urchins: biology and ecology. Elsevier, Amsterdam.

_____, W. A. Bennett, H. C. Fastenau, and C. M. Dewees. 1995. Spatial variation in red sea urchin reproduction and morphology: implications for harvest refugia. Ecol. Appl. 5: 1171–1180.

Servedio, M. R. and M. A. F. Noor. 2003. The role of reinforcement in speciation: theory and data. Annu. Rev. Ecol., Evol. Syst. 34: 339–364.

Shearer, C., W. de Morgan, and H. M. Fuchs. 1914. On the experimental hybridization of echinoids. Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci. 204: 255–362.

Strathmann, R. R. 1981. On barriers to hybridization between *Strongylocentrotus droebachiensis* (O.F. Muller) and *S. pallidus* (G.O. Sars). J. Exp. Mar. Biol. Ecol. 55: 39–47.

Swan, E. F. 1953. The Strongylocentrotidae (Echinoidea) of the Northeast Pacific. Evolution 7: 269–273.

Swanson, W. J. and V. D. Vacquier. 2002. Reproductive protein evolution. Annu. Rev. Ecol., Syst. 33: 161–179.

Tsuchiya, M., and M. Nishihira. 1984. Ecological distribution of two types of the sea-urchin, *Echinometra mathaei* (Blainville), on Okinawan reef flat. Galexea 3: 131–143.

Uehara, T., H. Asakura, and Y. Arakaki. 1990. Fertilization blockage and hybridization among species of sea urchins. Pages 305–310 *in* M. Hoshi and O. Yamashita, eds. Advances in invertebrate reproduction. Elsevier, Amsterdam.

Vacquier, V. D., W. J. Swanson, and M. E. Hellberg. 1995. What have we learned about sea urchin sperm bindin? Dev. Growth Differ. 37: 1–10.

Vasseur, E. 1952. Biographic variation in the Norwegian sea urchins, *Strongylocentrotus droebachiensis* and *S. pallidus.* Evolution 6: 87–100.

West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. Q. Rev. Biol. 58: 155–183.

Zigler, K. S. and H. A. Lessios. 2003a. 250 million years of bindin evolution. Biol. Bull. 205: 8–15.

_____ and _____. 2003b. Evolution of bindin in the pantropical sea urchin *Tripneustes*: comparisons to bindin of other genera. Mol. Biol. Evol. 20: 220–231.

_____ and _____. 2004. Speciation on the coasts of the new world: phylogeography and the evolution of bindin in the sea urchin genus *Lytechinus.* Evolution 58: 1225–1241.

_____, M. A. McCartney, D. R. Levitan, and H. A. Lessios. 2005. Sea urchin bindin divergence predicts gamete compatibility. Evolution 59: 2399–2404.

_____, E. C. Raff, E. Popodi, R. A. Raff, and H. A. Lessios. 2003. Adaptive evolution of bindin in the genus *Heliocidaris* is correlated with the shift to direct development. Evolution 57: 2293–2302.

Address: (H.A.L.) *Smithsonian Tropical Research Institute, Box 0843-03092, Balboa, Panama. Telephone: 703-487-3770 x 8708, E-mail: <LessiosH@post.harvard.edu>.*