Speciation in sea urchins

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ABSTRACT: Data relevant to processes that give rise to new species of echinoids are reviewed. Phylogeographic information from mitochondrial DNA is used to ask whether speciation in sea urchins fits the allopatric model, which predicts that if reproductive isolation accumulates steadily with time, then young, closely related species would tend to occur on two sides of a geographic barrier. The conclusion of this analysis is that most genera show a strong signature of allopatric speciation, but that *Echinometra*, *Lytechinus* and *Strongylocentrotus* also contain recently diverged sister species that are sympatric. The reason for these exceptions is probably not that sympatric speciation has occurred, but rather that reproductive isolation is not a function of divergence time alone. Although postzygotic isolation (lower hybrid fitness) in echinoids is correlated with divergence time, prezygotic isolation seems to arise due to additional factors, which are not necessarily related to the time that species have remained separate. There is no correlation between gametic incompatibility and time; bindin, one of the molecules responsible for gametic isolation, evolves under strong selection in some genera, but neutrally in others. Even though there is some evidence for reinforcement as a selective force on bindin, the differences in intensity of selection between the genera are more likely caused by intraspecific processes, such as variation in local sperm density. If so, age of species would not be a good predictor of geographic overlap, because young species may be reproductively isolated, while old species may be compatible, regardless of whether they arose sympatrically or allopatrically. The paucity of extant species in echinoids indicates that even though gametic reproductive isolation can arise rapidly, such events, leading to speciation, do not happen often.

What are the processes that give rise to new species of sea urchins? One might assume that echinoids conform to general principles that hold for all sexually reproducing organisms, but what are the data that support this assumption? And given that many of these principles are still the subject of debate, which views do the echinoid data support? In this paper I attempt to summarize what is known about speciation in sea urchins and how data obtained from the class Echinoidea can address general questions of speciation.

The last century has seen the development of general principles of speciation meant to apply to all sexually reproducing animals (Mayr 1942 1963 1970, Dobzhansky 1937 1970, Otte & Endler 1989, Howard & Berlocher 1998), an effort that is continuing to the present day, (Coyne & Orr 2004, Gavrilets 2004). Very briefly, new species arise when gene flow is interrupted by a geological or oceanographic barrier. If during this period of isolation they diverge in traits important for their development or reproduction (so they are no longer able to interbreed even when the barrier is lifted), they are converted to different biological species. This is the model of allopatric speciation that arose from the "New Synthesis", and was supported persuasively by Mayr and Dobzhansky. Evolution of reproductive isolation in the presence of gene flow (sympatric speciation) is considered by these authors as improbable, but according to various models (reviewed by Coyne & Orr 2004, Gavrilets 2004), it can occur if some very restrictive conditions obtain. The data on which these speciation principles are based have come primarily from insects and vertebrates. In speciation research, echinoids have played a small supporting role, but one that is not without importance. Perusal of the latest compendium (Coyne & Orr 2004) indicates that data on echinoids are used to address questions of allopatric vs. sympatric modes of speciation (p. 94), of temporal (p. 61) and gametic (pp. 63, 226, 235) reproductive isolation, of the extent of hybridization (p. 70), and of natural selection to avoid wastage of gametes in hybrids (pp. 243, 359). The importance of the echinoid data, limited as they

are, is that they provide a test as to whether ideas developed from the study of arthropods and vertebrates also apply to organisms with different fertilization systems and reproductive ecology. The two main questions to which data from echinoids have contributed are the spatial mode of speciation and the processes that give rise to reproductive isolation.

1 SPATIAL MODE OF ECHINOID SPECIATION

Do sea urchins speciate only allopatrically, or is there evidence for sympatric speciation as well? The existence of biogeographic provinces in the ocean (Ekman 1953, Briggs1974) leaves no doubt that major obstacles to gene flow can cause speciation in marine organisms. Sympatric speciation can occur anywhere in a species range, independently for each event. Thus, if all speciation were sympatric, there should be no congruence in species ranges of different organisms. The question that remains open is whether some sympatric speciation might also occur. Because species ranges change with time, the question is not easily resolved by simply examining modern species distributions, but, if one assumes that recently formed species are more likely to be present where they appeared, extensive overlap between sister species would be evidence for sympatric speciation. This is the well-known "Jordan's rule". In 1905 D.S. Jordan wrote: "Given any species in a region, the nearest related species is not found in the same region nor in a remote region, but in the neighboring district separated from the first by a barrier of some sort..." (Jordan 1905).

Jordan's rule was applied to tropical echinoid genera by Mayr (1954). Mayr used the monograph of Mortensen on echinoid systematics (Mortensen 1928-1951) to illustrate that sea urchins in the sea, like birds on land, tend to speciate allopatrically. His approach was to plot the range of each species in 16 tropical shallow water sea urchin genera and ask whether there was evidence that recently separated species tended to have non-overlapping ranges. Mayr was aware that echinoid systematics were still at the stage of alpha taxonomy, and that the only available documentation of specific status was morphological. Lacking phylogenetic information, he was forced to deduce which genera contained young and which contained old species in part by species distributions, which led to a certain degree of circularity. For example, he assumed what he intended to prove when he proposed that *Diadema savignyi* and *D. setosum*, which at the time were considered to have identical geographic distributions, (and the phylogeny of which was unknown) must be old species, one of which invaded the range of the other after a long period in allopatry. Molecular phylogenies have since provided the opportunity to reconstruct robust phylogenies of many of these genera and to provide approximate times of splitting between the species. Palumbi & Lessios (2005) have reviewed the degree to which these more recently obtained data support Mayr's conclusions. In this section, I present brief summaries of these phylogenies and

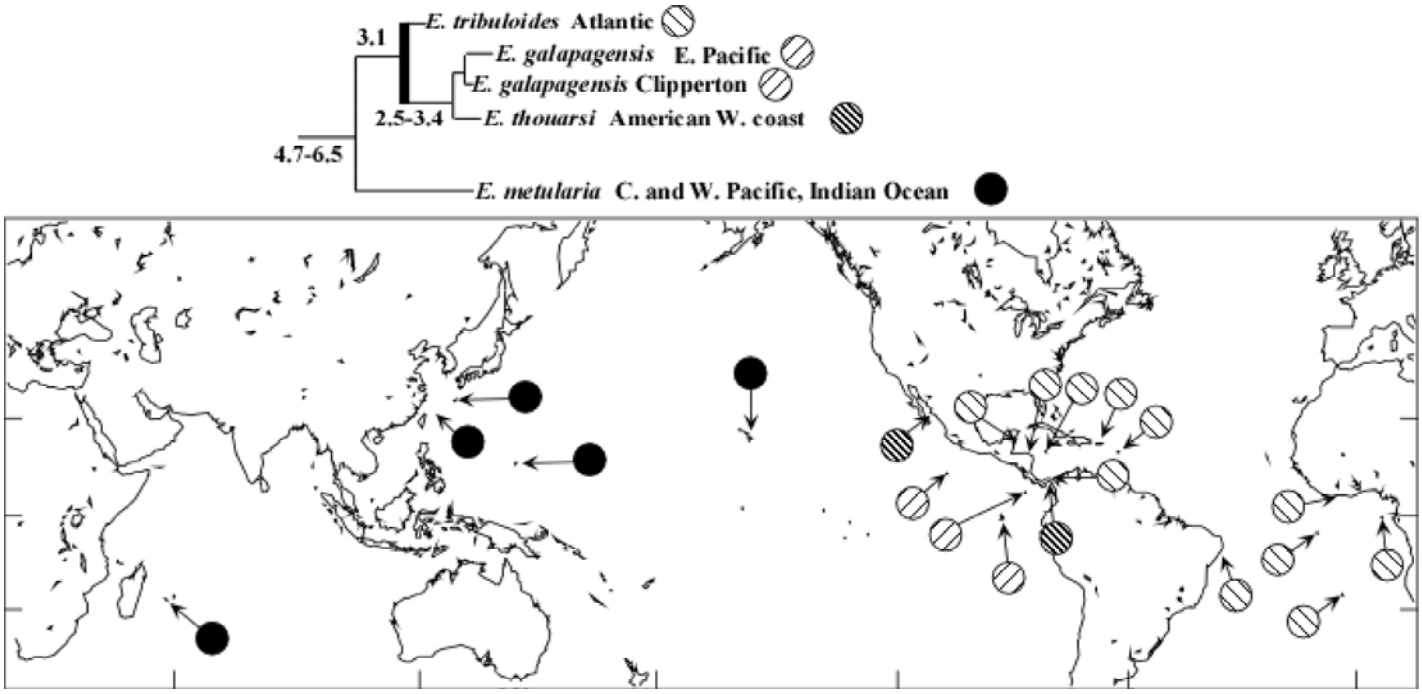

Figure 1. Phylogeny of the extant species of *Eucidaris*, based on sequences from the Cytochrome Oxidase I (COI) region of mitochondrial DNA, and geographic distribution of the clades (Lessios et. al. 1999). The thick bar indicates the presumed break arising from the emergence of the Isthmus of Panama, 3.1 million years (my) ago. Numbers next to the nodes indicate approximate dates (in my) of most recent common ancestor, based on a calibration of COI divergence by the emergence of the Isthmus. Actual locations in which the samples were collected are shown on the map.

the information they can provide as to the actual barriers that caused speciation events in each genus.

1.1 *Eucidaris*

In the genus *Eucidaris* all extant species are allopatric, so there was never any question as to whether they speciated sympatrically. The mitochondrial DNA (mtDNA) phylogeny of Lessios et al. (1999) permits examination of whether the species as defined by Mortensen (1928-1951) on the basis of morphology are valid, and--by placing time constraints on the nodes--the likely barriers that resulted in their existence (Fig. 1). The oldest speciation event between extant species of this genus was caused by the Eastern Pacific Barrier, the long stretch of deep water without stepping stones between the eastern and the central Pacific, which presumably isolated *E. metularia* from the eastern Pacific clade of this genus. At some earlier point, the Benguela cold water upwelling in SW Africa was also operating, so that Atlantic and Indian Ocean populations could not exchange genes with each other. Then came separation of the Atlantic *E. tribuloides* from an eastern Pacific clade, as the result of the rise of the Isthmus of Panama, approximately 3 million years (my) ago. The outer islands in this region, Galapagos, Isla del Coco, and Clipperton, together harbor a clade of mtDNA that is reciprocally monophyletic from the coastal *E. thouarsi*, indicating that even relatively short ocean distances can act as barriers to gene flow and be implicated in speciation. The alternative, that *E. thouarsi* and *E. galapagensis* speciated sympatrically and that the two clades sorted themselves out in different areas seems improbable, given the occurrence of a number of other endemic echinoderm species in the Galapagos (Maluf 1991), which attests to the potential isolation of this Archipelago from the mainland. There are no phylogenetic breaks in the Atlantic between populations of *Eucidaris* from the Caribbean, Brazil, Ascension, St. Helena, and the African coast, but high F_{ST} values indicate that *Eucidaris clavata* in the central Atlantic islands is isolated from coastal populations of *E. tribuloides*. All other populations exchange genes with each other at high rates. Thus, all speciation events in *Eucidaris* conform to a model of allopatric speciation, and the phylogenetic breaks coincide with major phylogeographic barriers as defined on the basis of faunal provinces from a variety of tropical shallow water organisms (Ekman 1953, Briggs 1974).

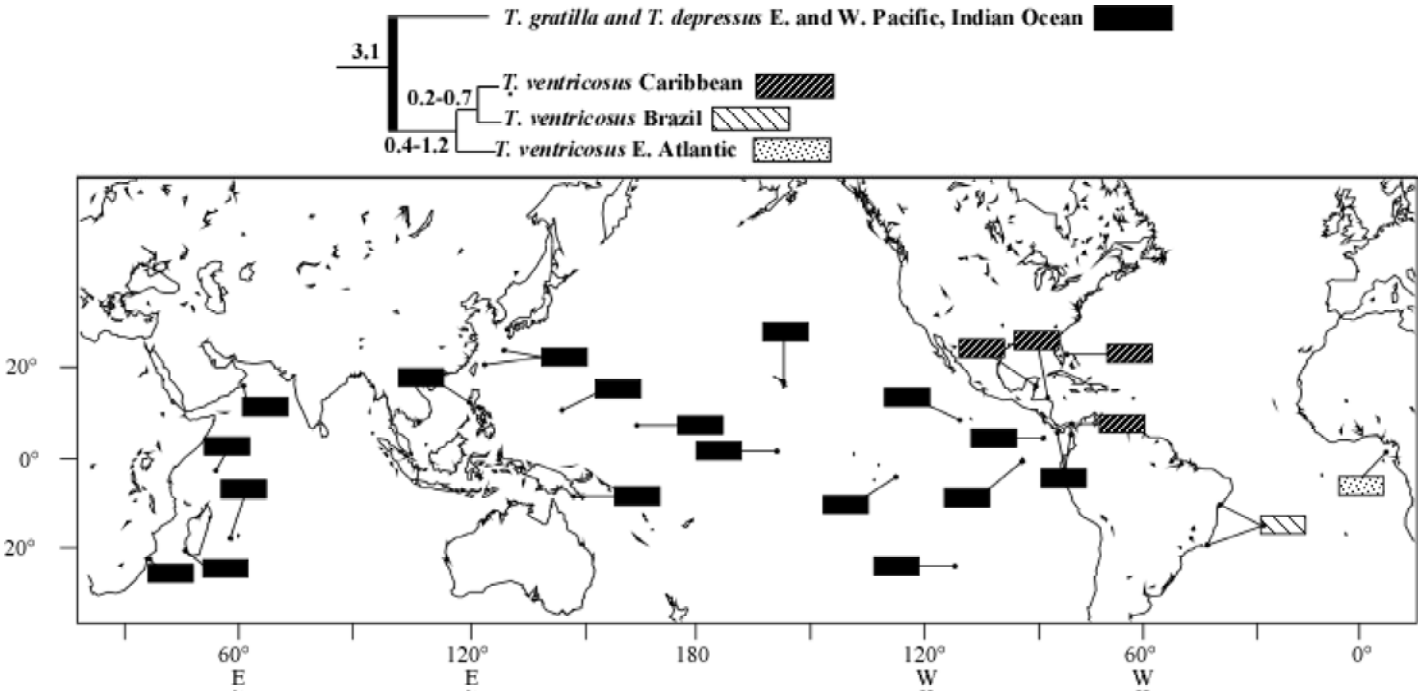

Figure 2. Phylogeny of the extant species of *Tripneustes*, based on sequences from the Cytochrome Oxidase I (COI) region of mitochondrial DNA, and geographic distribution of the clades (Lessios et. al. 2003). The thick bar indicates the presumed break arising from the emergence of the Isthmus of Panama, 3.1 million years (my) ago. Numbers next to the nodes indicate approximate dates (in my) of most recent common ancestor, based on a calibration of COI divergence by the emergence of the Isthmus. Actual locations in which the samples were collected are shown on the map.

1.2 *Tripneustes*

Tripneustes is another genus of shallow water tropical echinoid in which all the species are allopatric. The mtDNA phylogeny of Lessios et al. (2003) indicates that, unlike larvae of *Eucidaris*, those of *Tripneustes* are able to cross the Eastern Pacific Barrier

on a regular basis (Fig. 2). There are identical mitochondrial haplotypes of *Tripneustes* spread from the west coast of America to the east coast of Africa, and there is no phylogenetic distinction between the Cytochrome Oxidase I (COI) of the presumed separate species *T. gratilla* from the Indo-West Pacific and *T. depressus* from the eastern Pacific. Phylogeny of the nuclear locus bindin is consistent with the mtDNA phylogeny (Zigler & Lessios 2003). In contrast to the ease with which Pacific populations of *Tripneustes* appear to maintain connections between Pacific regions, and in contrast to the facility with which genes of Atlantic populations of *Eucidaris* spread from the American to the African coast, the Atlantic populations of *Tripneustes* appear to encounter major obstacles to dispersal. Caribbean and Brazilian populations have been separated by the inhospitable habitat created by the plume of the Orinoco and the Amazon for a period long enough to have developed reciprocally monophyletic mtDNA haplotypes. The American clade is also distinct from the African one, suggesting that larvae of this genus are unable to cross the mid-Atlantic barrier. Thus, all speciation events in *Tripneustes*, like those in *Eucidaris*, can be explained with an allopatric model, but the two genera only share the Isthmus of Panama and the Benguela upwelling as common barriers to gene flow.

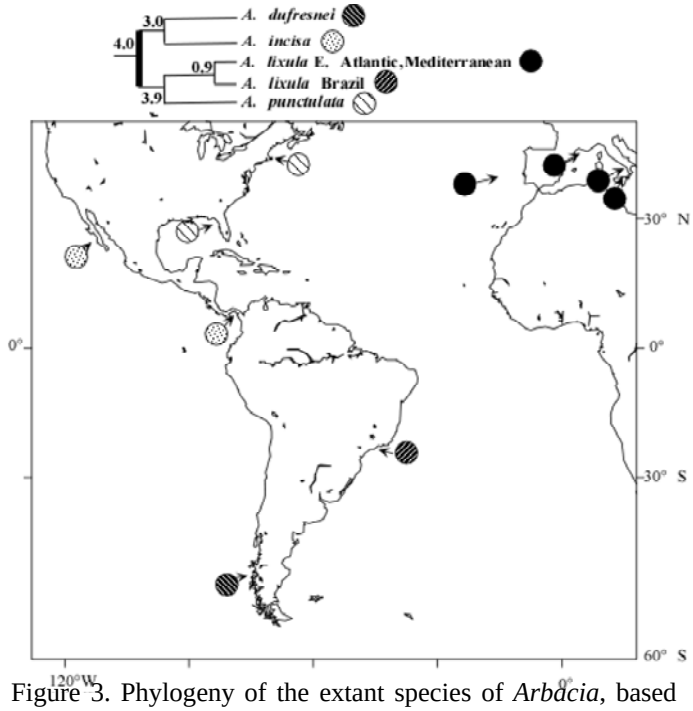

Figure 3. Phylogeny of the extant species of *Arbacia*, based on sequences from the Cytochrome Oxidase I (COI) region of mitochondrial DNA, and geographic distribution of the clades (Metz et. al. 1998). The thick bar indicates the presumed break arising from the emergence of the Isthmus of Panama, 3.1 million years (my) ago. Numbers next to the nodes indicate approximate dates (in my) of most recent common ancestor, based on a calibration of COI divergence by the emergence of the Isthmus. Actual locations in which the samples were collected are shown on the map

1.3 *Arbacia*

Arbacia is yet another genus in which all extant species (except for some range overlap between *A. stellata (= A. incisa*), *A. spatuligera*, and *A. dufresni* on the W. coast of S. America) are allopatric. This genus is found only in the Atlantic and the eastern Pacific. Mayr (1954) considered the species of *Arbacia* to be so old, that the present-day ranges give no indication of where the speciation events occurred, but the COI and bindin phylogeny of Metz et al. (1998) provides little justification for this view (Fig. 3). The ancestral population of all extant species in this genus appears to have been divided by the emergence of the Isthmus of Panama, though the split between the Atlantic and the Pacific clades may have occurred before the complete closure of the portals connecting the two oceans. The Atlantic branch was then divided by the mid-Atlantic barrier to the eastern Atlantic-Mediterranean *A. lixula* and the western Atlantic *A. punctulata*. *A. lixula* then crossed the Atlantic to establish a branch in Brazil, which has remained cut off from its parental population for a sufficiently long time to be reciprocally monophyletic. Metz et al. (1998) did not include *A. spatuligera* in their phylogeny, but this species, ranging from Ecuador to S. Chile, is sister to *A. dufresni* (Lessios, unpubl.). Thus, in the eastern Pacific there was first a speciation event separating the more northern *A. incisa* from the southern clade, which subsequently split into *A. dufresni* and *A. spatuligera*. Although the barriers that caused speciation events in the eastern Pacific are not obvious (and certainly do not seem to impede gene flow in *Eucidaris thouarsi* or in *Tripneustes depressus*), there is little reason to suspect sympatric speciation in this genus, given their present-day ranges.

1.4 *Diadema*

The genus *Diadema*, with more species than the previous two genera, presents a more complicated picture. MtDNA and isozyme phylogenies (Lessios et al. 2001) indicate that *Diadema setosum* split first from all other species of *Diadema*, probably during the initiation of wide fluctuations in global sea levels in the Miocene (Fig. 4). This *D. setosum* clade then split 3–6 million years ago into two clades, one in the northern Indian Ocean, and the other in the southern Indian Ocean and the West Pacific. There is no obvious barrier that might have isolated the two clades, but they remain allopatric. On the lineage leading to the other species of *Diadema*, there was an early branching event leading to the New Zealand and SE Australia endemic *D. palmeri*, coinciding with the time of cooling of the climate of New Zealand that lead to the extinction of other

tropical echinoids in this region. The next lineage to separate was composed of a currently undescribed species of *Diadema* found at Japan and also at the Marshall Islands. The barrier that caused this split is also not obvious; this undescribed species exists sympatrically with *D. savignyi* at both locations, but as it split from it 4-5 my ago, it represents no challenge to the allopatric model. The Eastern Pacific Barrier caused the next cladogenic event that separated *D. mexicanum* in the eastern Pacific, from which the Atlantic *D. antillarum* was subsequently split by the Isthmus of Panama. Within the Atlantic, there is a biogeographic barrier between the Caribbean and Brazil (Lessios et al. 2001). *Diadema antillarum* populations of the central Atlantic islands of Ascension and St. Helena are genetically isolated and phylogenetically derived from those of Brazil (Lessios et al. 2001). A completely separate clade in the E. Atlantic, suggests that the mid-Atlantic barrier has been effective in this genus. Apparently, the Indo-Pacific *D. paucispinum* and *D. savignyi* maintained genetic contact with *D. antillarum* around the southern tip of Africa for some time after the Isthmus of Panama was complete, but ceased to do so at the onset of Pleistocene. It is not clear how these two species separated from each other in the Pleistocene, but one possibility is that *D. paucispinum* was isolated in Hawaii or Easter Island, where it speciated, then invaded the rest of the central and Indo-West Pacific, as well as the Indian Ocean. *D. paucispinum* actually contains two lineages: One clade of this species is the only representative of *Diadema* in Easter Island and Pitcairn, but is also found in Okinawa in sympatry with two other species. A second mitochondrial clade of *D. paucispinum* extends from East Africa and Arabia to the Philippines and New Guinea. Presumably, these two clades are the result of water flow restrictions in the straits between northern Australia and Southeast Asia during Pleistocene episodes of low sea level, and the subsequent leakage of the Indian Ocean clade into the fringes of the western Pacific. Thus, the mtDNA phylogeography of *Diadema* indicates that all stages expected from a model of allopatric differentiation are present in this genus. There are anciently separated clades that now overlap in their geographic distribution, clades isolated in the periphery of the genus range that have remained in the periphery, clades that may have been isolated in the periphery but have since spread towards the center, closely related clades on either side of an existing barrier, and closely related monophyletic entities on either side of an historical barrier that have subsequently crossed the former barrier line. Except for *D. paucispinum* and *D. savignyi*, in which hybridization may have lodged mtDNA from one species into the genome of the other, closely related clades are always allopatric. Thus, the phylogenetic history and

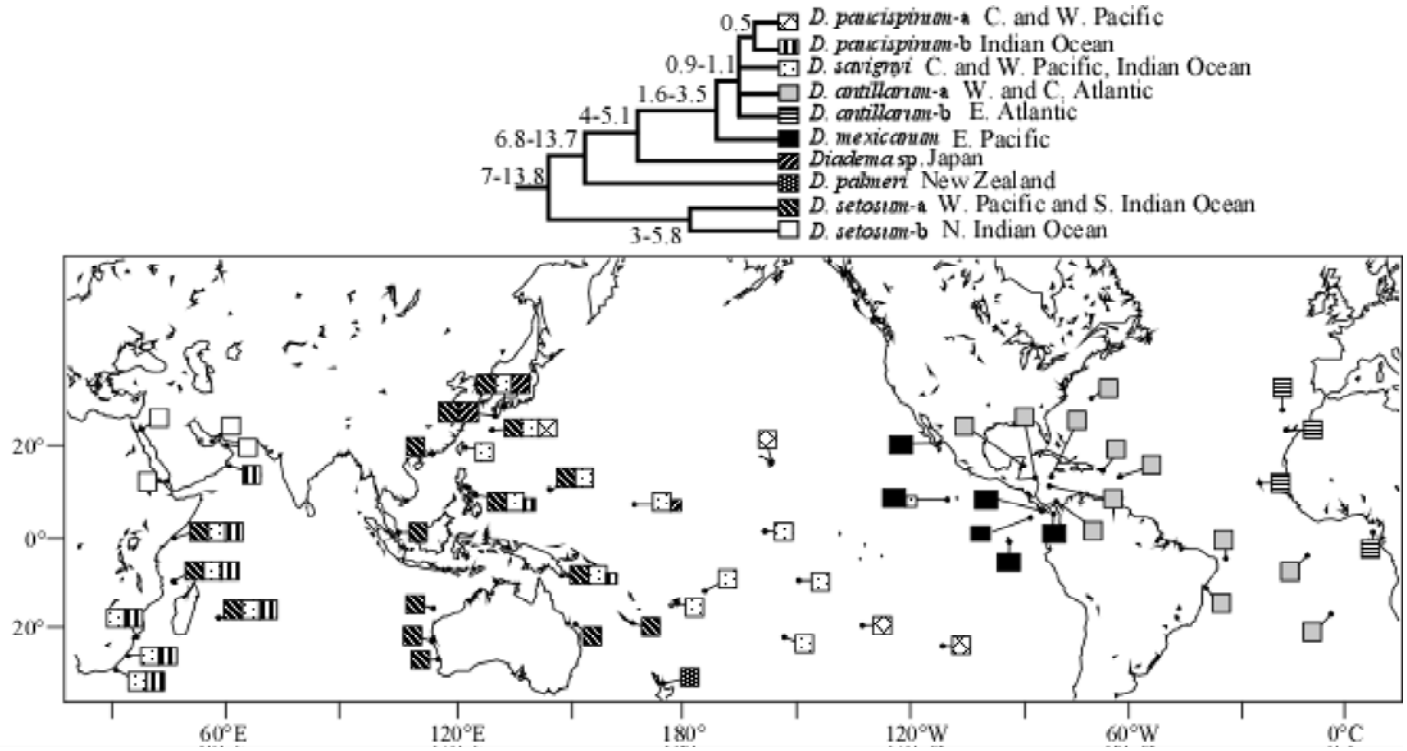

Figure 4. Phylogeny of the extant species of *Diadema*, based on sequences from the Cytochrome Oxidase I (COI) and the ATPase regions of mitochondrial DNA, and geographic distribution of the clades (Lessios et. al. 2001). The thick bar indicates the presumed break arising from the emergence of the Isthmus of Panama. Numbers next to the nodes indicate approximate dates (in my) of most recent common ancestor, based on a calibration of COI divergence by the emergence of the Isthmus. Actual locations in which the samples were collected are shown on the map. Smaller squares indicate that only occasional haplotypes of a clade inhabit a particular area.

distribution of extant species of *Diadema* is generally (though not completely) consistent with allopatric speciation.

1.5 *Lytechinus*

Another genus in which the distributions of extant clades are mostly, but not entirely, consistent with a model of allopatric speciation is *Lytechinus*. (Fig. 5). This genus is confined to the coasts of America, with only one species at the Cape Verde Islands. A combined mtDNA and bindin phylogeny by Zigler & Lessios (2004) (which does not include the Cape

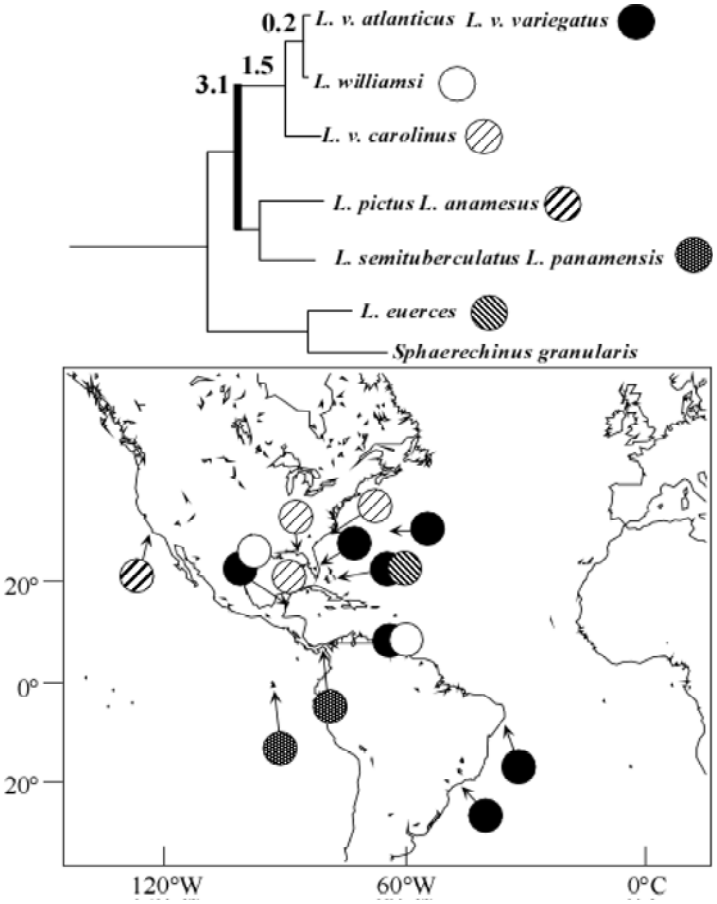

Figure 5. Phylogeny of the extant species of *Lytechinus*, based on sequences from the Cytochrome Oxidase I (COI) region of mitochondrial DNA and from bindin, and geographic distribution of the clades (Zigler & Lessios 2004). The thick bar indicates the presumed break arising from the emergence of the Isthmus of Panama, 3.1 million years (my) ago. Numbers next to the nodes indicate approximate dates (in my) of most recent common ancestor, based on a calibration of COI divergence by the emergence of the Isthmus. Actual locations in which the samples were collected are shown on the map.

Verde species) has found that the deep water *L. euerces* is distantly related to the other species of *Lytechinus* and is best considered as a member of a different genus. The first split among the remaining species was probably due to the Isthmus of Panama. The eastern Pacific clade was subsequently divided into north and south clades, most likely as the result of the muddy inhospitable habitat stretching between S. Mexico and Costa Rica. There is no distinction in either COI or bindin between *L. anamesus* and *L. pictus* in the North, nor between *L. panamensis* and *L. semitubreculatus* in the South. The former pair has also been shown to represent different ecotypes of the same species by other evidence (Clark 1940, Cameron 1984). The status of *L. panamensis* as a separate species cannot be determined until the coast of Ecuador has been sampled (Lessios 2005). The Atlantic clade of *Lytechinus* challenges the allopatric model of speciation. The COI of *L. williamsi* and *L. variegatus variegatus* is very similar, and so are their isozymes; their bindin alleles, however, are reciprocally monophyletic, even though they differ in only 4 amino acids (Zigler & Lessios 2004). Thus, in a phylogeny of these taxa based on both bindin and COI, *L. williamsi* is actually nested within *L. variegatus*, *with L. variegatus carolinus* as an outgroup (Fig. 5), whereas in a phylogeny based on bindin alone it is sister to the entire *L. variegatus* complex (Zigler & Lessios 2004). The monophyly of bindin, the existence of diagnostic morphological characters (Chesher 1968, Lessios, unpubl.) and the marked difference in adult size of the two species indicate that *L. williamsi* is not a juvenile form of *L. variegatus*, but a separate species. The difficulty for the allopatric speciation model, when Jordan's rule is applied, is that these two closely related sister species are sympatric. It is possible that they speciated in allopatry, and their genetic similarities accumulated after secondary contact through hybridization. Although they occupy different habitats (table 4 in Lessios et al. 1984), the distance between them is not so large as to prevent cross-fertilization. However, hybridization should have resulted in exchange of bindin alleles, because there is no evidence that this molecule is under selection in *Lytechinus* (Zigler & Lessios 2004). Thus, the possibility of sympatric speciation cannot be excluded on the basis of phylogeography in the case of the two Caribbean sister species of *Lytechinus*.

1.6 *Echinometra*

Echinometra is a genus in which Jordan's rule breaks down completely as a means of deducing the spatial mode of speciation. COI phylogenies by Palumbi et al. (1997) and by Landry et al. (2003) for the Indo-West Pacific newly discovered and as yet unnamed species and by McCartney et al. (2000) for the eastern Pacific and Atlantic species indicate a great deal of spatial overlap between sister species (Fig. 6). Bindin phylogenies (Metz and Palumbi 1996, Geyer & Palumbi 2003, McCartney & Lessios 2004) are generally congruent with the mtDNA phylogenies. *E. mathaei* coexists with one of the two clades of *E. oblonga* from which it is separated for about one my and *E. viridis* coexists with *E.*

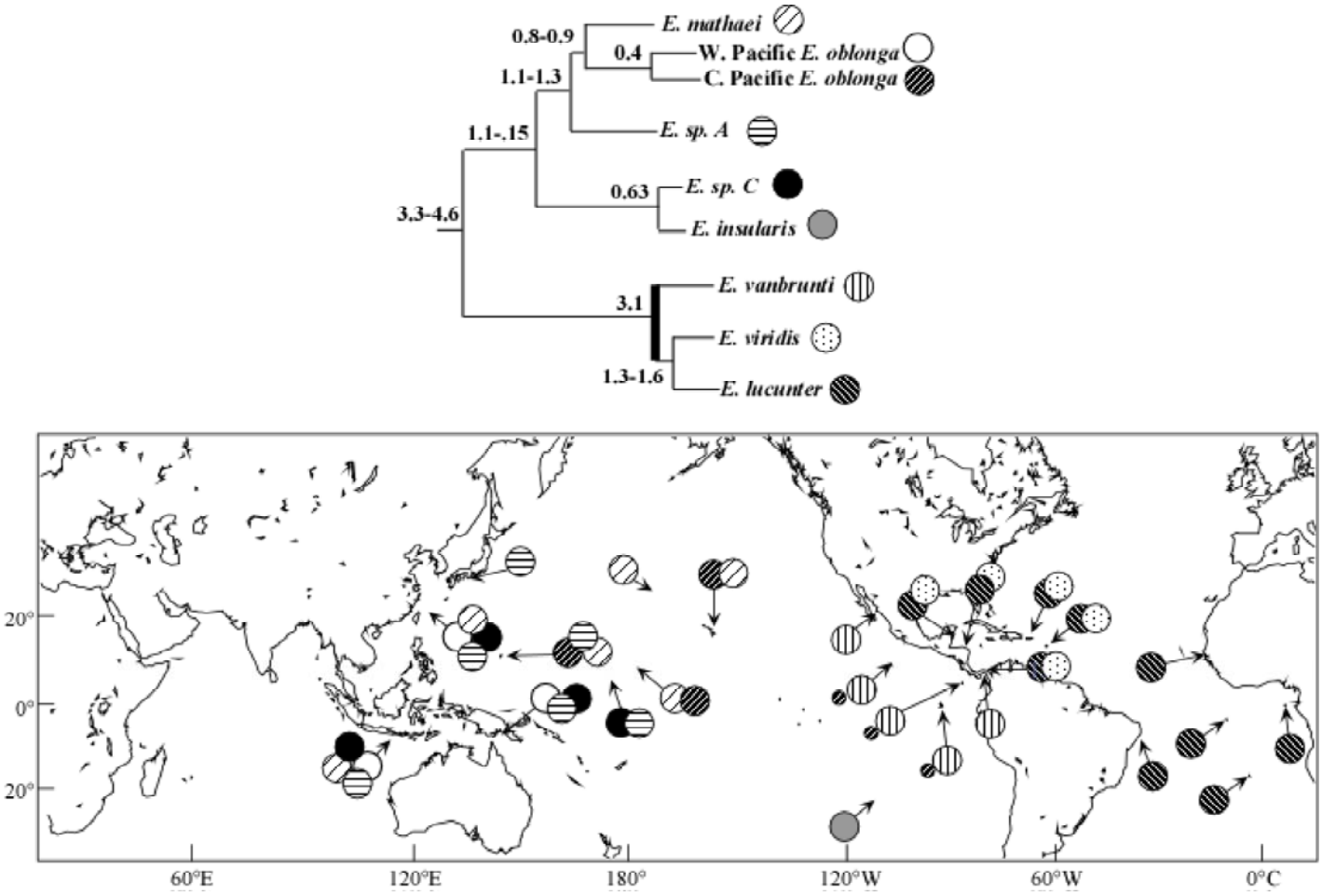

Figure 6. Phylogeny of the extant species of *Echinometra*, based on sequences from the Cytochrome Oxidase I (COI), and geographic distribution of the clades (McCartney et al. 2000, Landry et al. 2003). The thick bar indicates the presumed break arising from the emergence of the Isthmus of Panama, 3.1 million years (my) ago. Numbers next to the nodes indicate approximate dates (in my) of most recent common ancestor, based on a calibration of COI divergence by the emergence of the Isthmus. Actual locations in which the samples were collected are shown on the map. Smaller circles indicate that only occasional haplotypes of a clade inhabit a particular area.

lucunter from which it diverged approximately 1.5 my ago. *E. sp. A*, which has been a separate species from *E. mathaei* and *E. oblonga* for about 1.2 my, is found with one of these species in just about every locality. In both the Indo-West Pacific (Tsuchiya & Nishihira 1984, Nishihira et al. 1991, Rahman & Uehara 2004) and in the Caribbean (McCartney & Lessios 2004) there is habitat separation between the sympatric species, but this separation could not be the barrier that caused interruption of gene flow and speciation, because habitat preference is an intrinsic characteristic of each species, subject to evolution, rather than an obstacle presented by the environment. What is more, the only geographic barriers that can be deduced from the phylogeny are the Eastern Pacific Barrier, which accounts for the first split in the genus; the Isthmus of Panama, which separated *E. vanbrunti* from the two Atlantic species; and the geographic isolation of Easter Island, which presumably accounts for the speciation of the endemic *E. insularis*. Pleistocene sea-level fluctuations may have caused the separation of the rest of the clades, but it would be impossible to specify exactly how, where, or when.

1.7 *Strongylocentrotus*

Yet another genus in which sympatric speciation cannot be excluded on the basis of phylogeography alone (and the only example from higher latitudes) is *Strongylocentrotus* (Fig. 7). MtDNA phylogenies by Biermann et al. (2003) and by Lee (2003), as well as a bindin phylogeny by Biermann (1998), indicate that this genus should also include *Hemicentrotus pulcherrimus* and *Allocentrotus fragilis*. There is a deep phylogenetic break in this genus, presumably caused by distance, between species that are limited to the western Pacific and species that are found in the eastern Pacific and the Atlantic. The geographical distribution of the species within each of these major clades shows that sister species are not always allopatric. Specifically the sister species *H. pulcherrimus* and *S. intermedius*, both inhabiting shallow water, are sympatric in the Sea of Japan; *S. purpuratus*, *A. fragilis*, *S. pallidus* and *S. droebachiensis* overlap in the eastern Pacific. *S. pallidus* and *S. droebachiensis* are also sympatric on both shores of the Atlantic. It can be argued that sister species

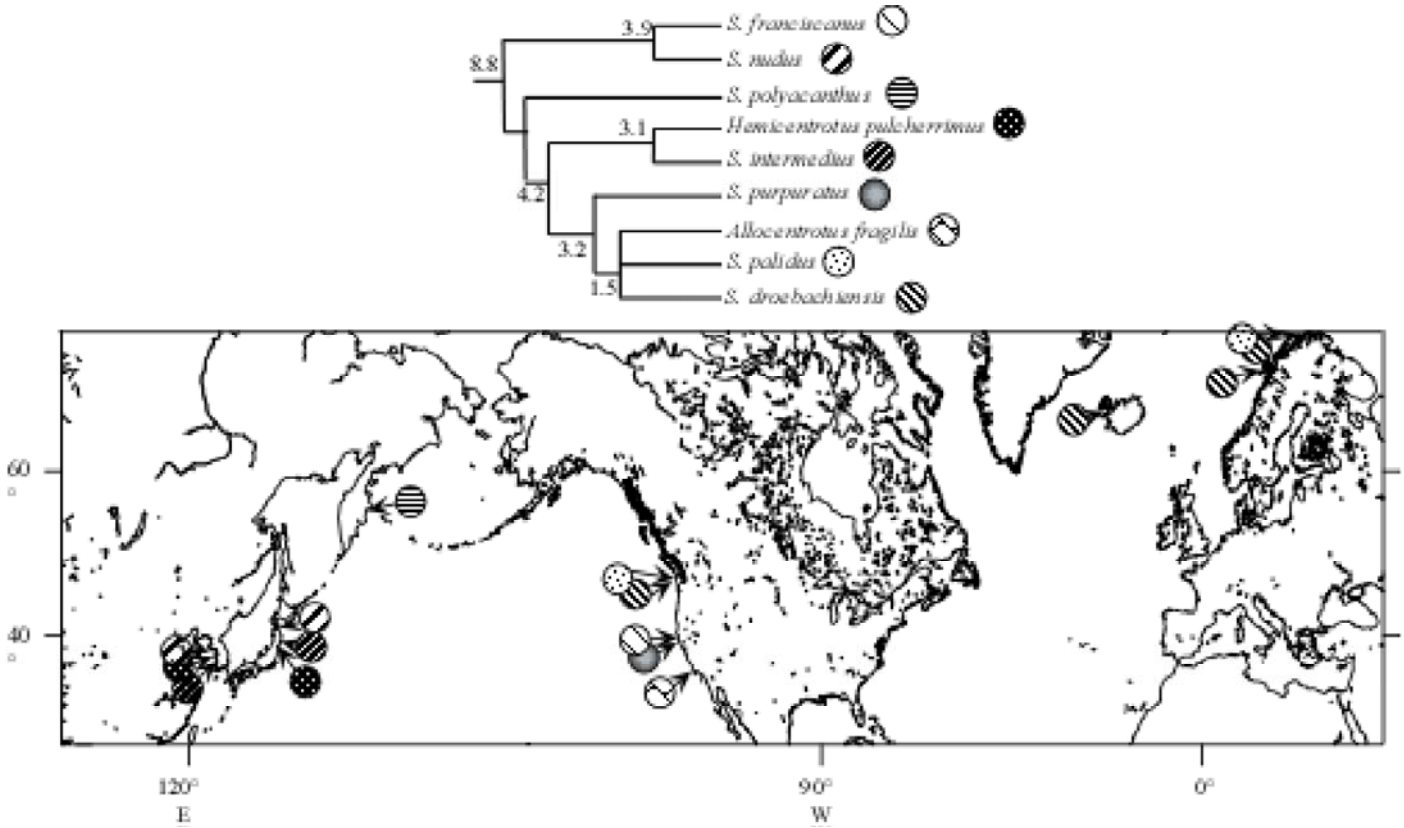

Figure 7. Phylogeny of the extant species of *Strongylocentrotus*, based on sequences from the Cytochrome Oxidase I (COI), and geographic distribution of the clades (Biermann et al. 2003). Numbers next to the nodes indicate approximate dates (in my) of most recent common ancestor, based on a calibration of COI divergence by the emergence of the Isthmus of Panama in other genera. Actual locations in which the samples were collected are shown on the map.

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status is not of importance, as unknown extinctions can cause extant species to appear as sister clades, when in fact they did not speciate from each other, and that determinations on whether Jordan's rule supports allopatric speciation should be made on the basis of divergence time alone. By this criterion, the *H. pulcherrimus*-*S. intermedius* pair does not falsify the rule, because the timing of the split exceeds 3 my. Similarly, the relationship of *S. purpuratus* to the tritomy of three species with which it is partially sympatric can also be excluded on the basis of the antiquity of the split. It is harder to claim, however, that phylogeography rejects the hypothesis of sympatric speciation among *A. fragilis*, *S. pallidus* and *S. droebachiensis*. It is true that the three species show only minimal overlap in their bathymetric distributions, but, as argued previously for habitat preference in *Echinometra*, depth zonation alone cannot be considered a barrier, because one still needs to ask how each species evolved to be adapted to a particular depth zone.

Thus, the combination of phylogeny and geographic distribution of species in each genus suggests that many speciation events in echinoids are the result of allopatric speciation, but leaves some cases in *Lytechinus*, *Echinometra* and *Strongylocentrotus* in which sympatric speciation is a possibility. Echinoids thus illustrate the limits of Jordan's rule and of phylogeography in helping deduce the spatial mode of speciation events. An important problem with Jordan's rule is that it is based on the assumption that reproductive isolation accumulates gradually over time, so older species will be better isolated than young ones. But does this assumption hold in echinoids?

2 REPRODUCTIVE ISOLATION

Jordan (1905) and Mayr (1954) tacitly assumed that reproductive isolation is the product of many small changes in many loci, and thus that speciation requires extended periods of geographic isolation to be completed. If, however, reproductive isolation can arise "accidentally" independently of divergence time, then recently separated species can become reproductively incompatible in either allopatry or sympatry, and anciently separated species may remain compatible. What do we know about the relationship between time of separation and the emergence of reproductive isolation in echinoids? The possible reproductive barriers between species of sea urchins have been reviewed by Lessios (2007). One conclusion of this paper is that there are two reproductive isolation barriers in echinoids for which the relationship between species isolation and diver-

gence time can be examined: (a) Postzygotic isolation in the form of reduced survivorship of hybrids or inability to back-cross to their parental species, and (b) prezygotic isolation in the form of gamete incompatibility.

Figure 8 presents the relationship between time since speciation and postzygotic isolation, as determined by tabulating data about hybrid crosses from different genera, and estimating their divergence times from COI differentiation. Because different studies present hybrid survival information differently (only the studies on *Echinometra* include quantitative data), it was necessary, for the purposes of summary depiction, to construct an arbitrary "index of hybrid fitness", as a composite measure of the survivorship of hybrid larvae produced from two crosses (one with eggs of one species and sperm

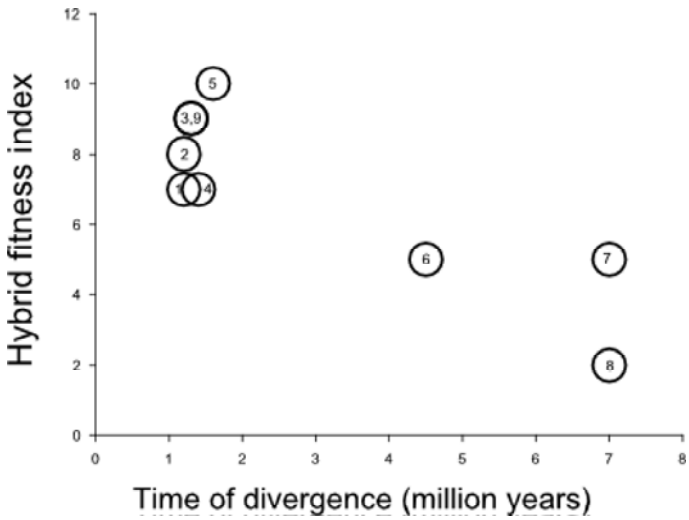

Figure 8. Relationship between fitness of interspecific hybrids and time since parental species split from each other. The hybrid fitness index is an arbitrary value, based on information provided in the original publications regarding the survivorship of F_1 hybrids and their ability to back-cross to the parents. See Lessios (2007) for a tabulation of the data, and for the sources of COI differentiation, used here to estimate divergence time. Explanation of symbols: 1: *Echinometra Sp. A* vs. *E. oblonga* (Aslan & Uehara, 1997); 2: *E. mathaei* vs. *E. sp. A* (Rahman et al. 2005); 3: *E. sp. C* vs. *E. mathaei* (Rahman & Uehara 2004); 4: *Strongylocentrotus pallidus* vs. *S. droebachiensis* (Strathmann 1981); 5: *Pseudechinus huttoni* vs. *P. albocinctus* (McClary & Sewell 2003); 6: *Heliocidaris tuberculata* vs. *H. erythrogramma* (Raff et al. 1999); 7: *Pseudechinus novaezealandiae* vs. *P. albocinctus*; 8: *P. novaezealandiae* vs. *P. huttoni* (McClary & Sewell 2003); 9: *Echinometra sp. A* vs. *E. sp. C* (Rahman et al. 2001).

from a second one, the other from the reverse cross), as well as the ability of the hybrids to produce viable offspring when back-crossed to the parentals. The data come from only four genera, *Echinometra*, *Strongylocentrotus*, *Heliocidaris*, and *Pseudechinus*. The divergence times mainly fall in two clusters: Those from the Indo-Pacific species of *Echinometra* and from the cross between *Pseudechinus huttoni* with *P. albocinctus*, with estimated ages of 1-2 my, and those of *Pseudechinus*, in which one species, *P. novaezealandiae*, split from the other two congeners, approximately 7 my ago. Only *Heliocidaris* has an intermediate divergence time. Because of multiple comparisons, the points are also not independent from each other, which precludes statistical inferences. Thus the available evidence is far from incontrovertible, but it does support the idea that time is a good predictor of the degree to which species will develop developmental incompatibilities. Older species are better isolated than younger ones, and several million years are required for post-zygotic isolation that is measurable in captive animals and would prevent gene flow between sympatric species

Prezygotic isolation, on the other hand, does not obey the same rules as hybrid inviability. Zigler et al. (2005) compiled data from 15 comparisons of congeneric sea urchin species for which data were available on gametic compatibility, COI divergence, and bindin divergence. They found that there is no correlation between compatibility among gametes of different species and COI divergence. Thus--assuming a molecular clock--preference of gametes to combine with those of their own species does not evolve as a function of time alone. Young species, such as *Echinometra mathaei* and *E. sp. A*, separated for slightly more than a million years, have gametes incapable of fertilizing each other, whereas much older species, such as *Arbacia punctulata* and *A. incisa*, separated for more than 4 million years have gametes that can fertilize each other at the same rates as gametes of their own species.

Zigler et al. (2005) also found that gametic incompatibility was correlated with divergence in bindin. Bindin is a molecule that covers the acrosome process of the sperm, binds with a receptor on the vitelline layer, then fuses with the egg membrane to permit transfer of sperm DNA into the egg. Although it is not yet clear what modifications of the bindin and the bindin receptor molecules are necessary to confer incompatibility between gametes, the correlation with time indicates that study of these molecules can shed light on some of the factors involved in the evolution of pre-zygotic isolation between sea urchin species. Very little is known about the bindin receptor, because it is a large molecule difficult to sequence (Kamei & Glabe 2003), but there is a modest accumulation of comparative facts about bindin. These are reviewed in Lessios (2007) and will be presented here only briefly.

In addition to the correlation between gametic incompatibility and bindin divergence, we also know that in *Echinometra* most amino acid replacements accumulate in the species with eggs which do not permit fertilization by heterospecific sperm, and that three genera with sympatric species have bindins that show evidence of positive selection, whereas

three other genera with no sympatric species have bindins that do not share these features (Metz & Palumbi 1996, Biermann 1998, Metz et al. 1998, Zigler et al. 2003, Zigler & Lessios 2003 2004, McCartney & Lessios, 2004). There is, therefore, selection on bindin to track changes that occur in the egg receptor, and one may well think that this selection is avoidance of hybrid production (reinforcement). Corroborating this hypothesis is the geographical pattern of character displacement found by Geyer & Palumbi (2003) in *Echinometra oblonga*, which has bindin alleles different than those of *E. sp. C* where the two species coexist, but similar to those of *E. sp. C* where it found alone. Not all evidence, however, is consistent with the hypothesis that the selective force acting on bindin is reinforcement.

In both *Echinometra* and *Strongylocentrotus* an excess of amino acid replacement over silent substitutions between alleles of the same species suggests that there is positive selection not just for divergence between species, but also for intraspecific polymorphism. Selection to avoid hybridization with other species cannot generate adaptive differences between alleles of the same species. 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* (Lessios 2007). In both cases, bindin alleles of populations that are not sympatric with a congener and do not receive gene flow from the area of overlap are similar to the those in the area of overlap. It is thus possible that the pattern of reinforcement suggested by the comparison of the mode of evolution of bindin in different genera does not result from selective pressures created by the challenge of sympatric species. Instead, it may be a secondary effect of bindin divergence that has accumulated between species by intraspecific forces. If only species with bindins that have diverged for other reasons are able to coexist sympatrically without merging, then we would also expect to see a pattern of correlation between range overlap and bindin divergence.

The intraspecific forces of selection that may drive bindins to diverge in some species but not others are likely to be related to sexual selection (West-Eberhard 1983) and inter-locus sexual conflict (Rice 1998, Gavrilets 2000). In a mating system such as bindin and its receptor, frequencies of different alleles can become rapidly predominant in different populations, because of linkage disequilibrium. When bindin alleles are preferred by different egg receptor alleles in each generation, there will be an over-representation of offspring in which male and female alleles are matched. If different combinations become predominant in different geographically isolated populations, they will lead to reproductive isolation. Assortative mating in bindin has been demonstrated experimentally in *Echinometra mathaei* by Palumbi (1999). Levitan and Ferrell (2006) have shown in *Strongylocentrotus franciscanus* that reproductive success of bindin alleles depends on local sperm density. 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 the reverse is true at limiting sperm densities. If there is linkage disequilibrium between bindin and receptor loci, different combinations of alleles will predominate in each species, depending on sperm density, which is affected by habitat specialization and prevailing oceanographic conditions. In *Echinometra* (McCartney & Lessios 2004) and also in *Strongylocentrotus* (Biermann 1998, Debenham et al. 2000, Levitan & 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. We do not know enough about other molecules that may be involved in species recognition between sperm and egg (Biermann et al. 2004, Neill & Vacquier 2004, Mah et al. 2005, Kaupp et al. 2006) to determine whether similar selection forces as those of bindin may be acting on them. But if what we do know about bindin is indicative of how gametic isolation evolves in sea urchins, it is not surprising that prezygotic isolation between species is not a simple function of time.

3 CONCLUSIONS

How do new species of sea urchins arise? The existing data are not adequate to provide a complete answer to this question (a statement that holds true for any group of organisms), but we do know a great deal more than what was available to Mayr in 1954. The combined information of phylogeny and species distribution has made it possible to ask whether young species tend to be still separated by extrinsic barriers, as the allopatric model would predict if reproductive isolation requires long periods of time to develop. We have phylogeographic data for only shallow water species and mostly for tropical ones, but the answers they have provided will likely be applicable to all species of echinoids: The majority of speciation events appear to be allopatric, but there are some cases of closely related sympatric sister species that raise the possibility of sympatric speciation. Indeed, if reproductive isolation evolved only as a function of time, the young sympatric species of *Echinometra*, *Strongylocentrotus* and *Lytechinus* would be evidence that sympatric speciation does occur in echinoids. Data on postzygotic isolation limited as they are, suggest that developmental in-

compatibilities in hybrids between species increase steadily with the time that the parental species have been separated. The same, however, is not true for prezygotic isolation. Gametic compatibility and bindin divergence can appear rapidly in some species and can be absent in others, despite much longer times spent in allopatry. This makes Jordan's rule an inadequate tool for assessing the possibility of sympatric speciation, because it does not allow the falsification of the null hypothesis of allopatric speciation. There is, however, good reason for doubting that new species can appear in sympatry through mutations that will alter gametic compatibility. Individuals with gametes that cannot combine with those of the opposite sex will be rapidly eliminated from a population, unless an extrinsic barrier happens to isolate them in a small population in which some other deviants are present. Only under very special conditions, stipulated by models reviewed in Coyne & Orr (2004) and Gavrilets (2004), can different species arise sympatrically through sexual selection in the absence of a barrier. That the emergence of prezygotic isolation can be rapid in geographically separated populations due to conditions such as sperm density, does not necessarily mean that it will also happen often. There are, after all, less than 900 described extant species in the entire class Echinoidea, fewer than in many families of more speciose groups, despite the very solid foundation of echinoid alpha taxonomy. Barring an unknown reason for extraordinary rates of echinoid extinction, the paucity of extant sea urchin species suggests that speciation is a rare event in this class of animals.

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