

POSSIBLE PREZYGOTIC REPRODUCTIVE ISOLATION IN SEA URCHINS  
SEPARATED BY THE ISTHMUS OF PANAMA

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New species are formed when populations acquire reproductive isolation from other, previously conspecific, populations. How and when in the process of speciation prezygotic reproductive isolation arises has yet to be established. Two views are predominant (Littlejohn, 1969; Dobzhansky, 1970; Mayr, 1970; Dobzhansky et al., 1977). The "sympatric hypothesis" or "speciation by reinforcement" holds that populations, after they have been separated by a geographic barrier, diverge genetically, and if they should come in contact again, produce hybrids of lower fitness due to the incompatibility of their genomes. Prezygotic reproductive isolation, according to this view, appears in sympatry through natural selection to prevent investment of gametes in standard hybrids (Dobzhansky, 1940, 1970). The "allopatric hypothesis," on the other hand, asserts that prezygotic isolation mechanisms can arise in allopatry either as the result of overall genetic divergence stemming from adaptation to local environments (Moore, 1957; Mayr, 1970; Paterson, 1978; Templeton, 1981), or due to a slight amount of genetic change in a few loci that control characteristics of the organism vital for reproductive isolation (Muller, 1942; Lewontin, 1974).

Though speciation by reinforcement is believed by some authors to be unsupported by either theory or evidence (Moore, 1957; Paterson 1978; Templeton, 1981; Futuyma, 1983), it is still accepted by many evolutionary biologists as a reasonable hypothesis for the evolution of prezygotic reproductive isolation (Dobzhansky et al., 1977; also see Futuyma, 1983). Obtaining evidence either in favor or against it from natural populations has been difficult, because the history of geographic barriers to gene flow is usually unknown. Observed cases of prezygotic reproductive isolation between allopatric populations, which otherwise would constitute evidence against the sympatric hypothesis, have thus been explained as the result of previous (and unknown) invasions by one isolate into the range of the other (Dobzhansky, 1940; Dobzhansky et al., 1968). To distinguish between predictions of the sympatric and allopatric hypotheses one has to find populations separated by a known geographic barrier that appeared at a known time and has remained continuously in place to the present day. The Isthmus of Panama is a geographic feature that fulfills these conditions. Connections between the

tropical Atlantic and Pacific oceans were severed in the Pliocene, 3.1 to 3.5 million years ago (Saito, 1976; Keigwin, 1978, 1982). The separation between previously continuous marine populations has resulted in a host of "geminant species," i.e., closely related species, one on each side of Central America (Jordan, 1908; Ekman, 1953).

One genus containing a geminate pair is the sea urchin *Diadema*. *Diadema antillarum* occurs throughout the tropical Atlantic, while *D. mexicanum* is found along the Pacific coast of Central America. Multivariate morphometrics have shown that populations from the two sides of the Isthmus of Panama are on the average more similar to each other than to populations from the same coast (Lessios, 1981a), and echinoid taxonomists have recognized that if *D. antillarum* and *D. mexicanum* had not been spatially separated, they would not have been described as different species (Mortensen, 1940; Mayr, 1954). Other pairs of echinoid geminate species exhibit transisthmian morphological divergence, which, though still small, is larger than that of *Diadema* (Lessios, 1981a). Electrophoresis has revealed a great deal of biochemical similarity between the geminate species of *Diadema* (Lessios, 1979, 1981a). Enzymatic proteins of Atlantic and Pacific populations are as similar to each other as those of populations from the same ocean. Other echinoid genera assayed with the same techniques displayed transisthmian molecular differentiation up to 20 times greater than that of *Diadema*. The similarity in *Diadema*, therefore, is not an artifact of the limited resolving power of electrophoresis. Thus, *D. antillarum* and *D. mexicanum* both by the standard of intra- versus inter-specific differentiation and by comparison to other echinoid geminate species are genetically very similar. Whether they have acquired prezygotic reproductive isolation in the three million years that they have been separated is important for our understanding of the process of geographic speciation.

One possible form of prezygotic reproductive isolation between populations is asynchronous reproductive cycles (Littlejohn, 1969; Dobzhansky, 1970; Mayr, 1970). The annual reproductive cycles of *D. antillarum* and *D. mexicanum* were studied during 1976-1977 and found to be unlikely candidates as mechanisms of reproductive isolation, because they overlap (Lessios, 1981b). Many diadematoïd sea urchins, however, also have a lunar spawning cycle

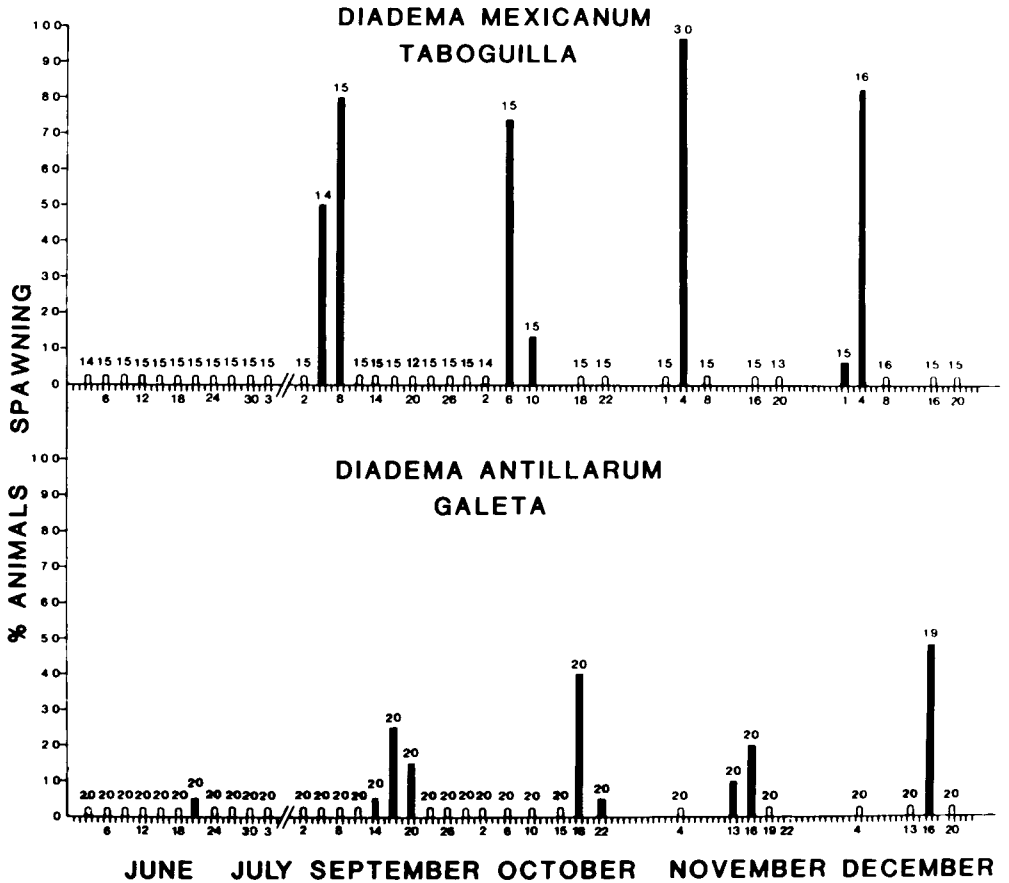


Fig. 1. Percent of individuals in each sample that spawned copiously after injections with isotonic KCl at Taboguilla Island, Bay of Panama (*Diadema mexicanum*) and at Galeta Point, Caribbean coast of Panama (*D. antillarum*). Numbers above bars indicate sample size. ◻: days in which samples were taken, but no animal spawned.

superimposed on their annual cycles of gonadal growth (Pearse, 1975).

I studied the lunar spawning cycles of the geminate species of *Diadema* between June and December 1982 to determine whether they might constitute a potential mechanism of prezygotic reproductive isolation. *Diadema mexicanum* was sampled at the Island of Taboguilla, Bay of Panama; *D. antillarum* was studied at Galeta Point, near the Atlantic entrance of the Panama Canal. Additional data for *D. antillarum* were obtained from the Island of Nalunega, San Blas Archipelago. Animals larger than the size of first sexual maturity were isolated in separate containers and injected with 5 ml of .5 M KCl each. KCl induces spawning in ripe sea urchins (Hinegardner, 1975). The spawning re-

sponse was classified in one of four categories, according to the amount of gametes produced. Female genital products were preserved in formalin and examined microscopically. Animals in category 2 (little spawning) as a rule produced mostly oocytes, those in category 3 (some spawning) produced a mixture of oocytes and ova, while animals in category 4 (copious spawning) produced only ova. Animals that spawned copiously in response to KCl are the ones most likely to be ready to spawn naturally, while those that responded mildly to KCl may be at the beginning of their gametogenic cycle, or they may have already spawned. The percentage of animals in category 4 is, therefore, the best index of readiness of the population to spawn on any given day.

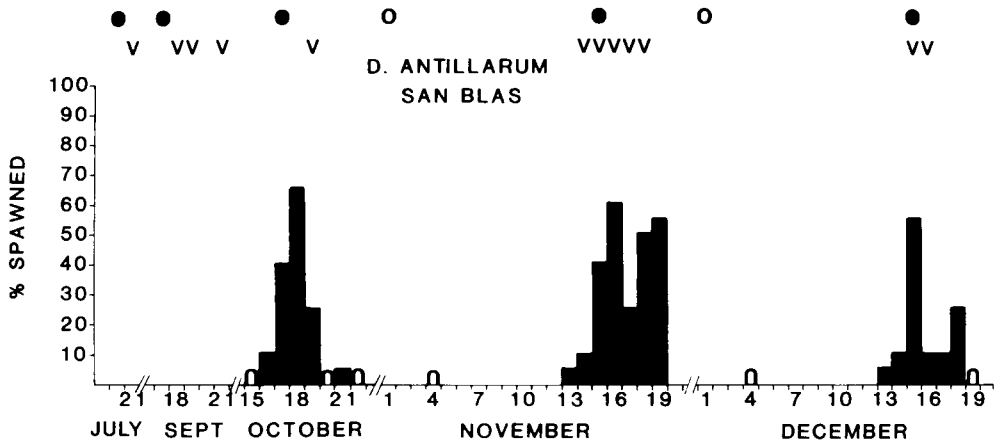


FIG. 2. Percent of individuals of *Diadema antillarum* that spawned copiously after injections with isotonic KCl, and observations of natural spawning at the Island of Nalunega, San Blas Archipelago. Each daily sample consisted of 20 individuals. ○: days in which samples were taken, but no animal spawned. V: days in which natural spawning was observed on the reef.

At Taboguilla and Galeta, samples were taken every third day during June and September. Results of these two months (Fig. 1) suggest that in 1982, as in 1976–1977 (Lessios, 1981b), the two species are ready to spawn in September but not in June. The September data also suggest that *D. mexicanum* spawns during the third lunar quarter, with a peak 3–4 days after full moon, while *D. antillarum* spawns in the first lunar quarter, with a peak 1–2 days after new moon. To verify that this pattern is consistent between months I sampled the same populations from the beginning of October to the end of December 1982 under the following regime: one sample was taken before the start of the suspected spawning cycle, one during peak spawning, one at the end of the spawning period, and one or two during the peak spawning of the species on the opposite coast of the Isthmus (Fig. 1). The results of these months are consistent with the pattern obtained in September. The percentage of animals spawning each month is lower in *D. antillarum* than in *D. mexicanum*, because annual reproductive periodicity of the former is not as well-defined as that of the latter (Lessios, 1981b). *Diadema antillarum* was also sampled with KCl injections in the San Blas Archipelago; daily samples were taken between three days before and four days after new moon, (i.e., from beginning to end of the spawning cycle as suggested by data from Galeta) during October, November and December 1982. An additional sample each month was taken two days after full moon, at the peak spawning of *D. mexicanum*. Good underwater visibility in some days also permitted observations of natural spawning on the reef. Although the San Blas data (Fig. 2) do not give an indication of whether the animals are reproductively active in the days that they were not sampled

or seen to spawn, they are consistent with the more extensive results from Galeta. They show that *D. antillarum* spawns during the first lunar quarter in the San Blas as well, and that it releases no gametes on the day that *D. mexicanum* reaches its spawning peak on the Pacific side. Thus, *D. antillarum* and *D. mexicanum* both spawn with a lunar cycle but with peaks roughly 15 days out of phase, and with no overlap.

The non-overlapping spawning cycles of *D. antillarum* and *D. mexicanum* can be a form of reproductive isolation if they stay constant throughout the range of each species, and if they can be expected not to change should one species invade the range of the other. Lunar spawning in *D. antillarum* has been studied in Florida, Bermuda and the Virgin Islands, in addition to Galeta Point and San Blas Islands (Iliffe and Pearse, 1982). Despite the fact that animals from these areas were studied at different years, and despite differences between localities in environmental fluctuations and in annual reproductive cycles of the animals, *D. antillarum* in all cases was found to spawn during the first lunar quarter. It seems likely, therefore, that the lunar cycle of *D. antillarum* stays constant in space and time. No data exist about reproductive cycles of *D. mexicanum* in areas other than Panama. Pearse (1968) has found that in another species of *Diadema*, the Indo-Pacific *D. setosum*, different populations are not in reproductive synchrony with each other. Whether *D. mexicanum* is more similar to *D. antillarum* or to *D. setosum* in geographic constancy of lunar spawning remains to be seen.

The second requirement for showing reproductive isolation, i.e., demonstrating that the two species will maintain their respective cycles even if they were to invade each other's ranges, is difficult to

meet, because a ban exists on the experimental transfer of animals between the coasts of Panama to avoid the risk of liberating gametes, larvae, or parasites into the wrong ocean. However, even though little is known about how lunar spawning cycles are maintained in sea urchins (Pearse, 1975), there are some indications that they may be governed by moonlight directly. The constancy of the spawning cycles of *D. antillarum* between areas that have little in common except moonlight periodicity (Iliffe and Pearse, 1982) suggests such a relationship. Kennedy and Pearse's (1975) study of reproductive cycles in the diadematoïd sea urchin *Centrostephanus coronatus* showed that spawning followed fluctuations in moonlight, not tide. *Diadema*, like *Centrostephanus*, is sensitive to light, even at low intensities, (Millot, 1955), so it is possible that its lunar cycles are also mediated by moonlight. Even if an endogenous, genetically fixed cycle is not present, a different response by *D. antillarum* and *D. mexicanum* to an environmental factor fluctuating in concert in both oceans (such as moonlight), would cause individuals that may some day cross the Isthmus of Panama (perhaps through the proposed sea-level canal) to continue spawning out of phase with the resident population on the other side. Sea urchin sperm diluted in sea water remains active only for an hour (Hinegardner, 1975). Asynchrony in spawning would, therefore, ensure that descendants of Atlantic and Pacific populations would exist in sympatry as separate species whether or not their gametes are capable of fertilizing one another.

Thus, it is possible that the geminate species of *Diadema* may have acquired a prezygotic isolating mechanism while still in allopatry. If so, the virtual certainty that they have never come in contact after their initial separation indicates that such reproductive isolation need not be the product of natural selection against hybridization, and casts doubt on the universality of the "speciation by reinforcement" hypothesis. Furthermore, the fact that *D. antillarum* and *D. mexicanum* are so similar by all measures of genetic divergence that have been applied to them supports the view that reproductive isolation can be acquired in allopatry even in the absence of major genetic differentiation (Lewontin, 1974).

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