

ENDLESS FORMS

Species and Speciation

EDITED BY

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New York Oxford

Oxford University Press

1998

The First Stage of Speciation as Seen in Organisms Separated by the Isthmus of Panama

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The study of speciation, like the study of many other dynamic historical processes, consists of assessing the status of fixed stages and placing them in a chronological series, so that a trajectory can be reconstructed. Mayr (1954a) has pointed out that in this respect the methodology of evolutionary biology is not unlike that of cytology or any other process-oriented branch of science. In this idealized view, populations at various stages of speciation are analogous to results of natural experiments allowed to run for different lengths of time. In practice, however, the study of speciation is more similar to an experiment in which most traces of materials and methods have been lost and need to be reconstructed from the results themselves. Thus, the nature, efficacy, and timing of an extrinsic barrier thought to have resulted in geographic speciation are rarely self-evident and must be deduced from present-day distributions and patterns of divergence. Such reconstructions by necessity have to rest on many assumptions. It is therefore not surprising that critics of Mayr's insistence on the primacy of geographic isolation (e.g., Bush 1975, 1994; Bush and Howard 1986; White 1978; Tauber and Tauber 1989; King 1993) often complain that many speciation studies shoehorn their interpretation of the mode of speciation into a preconceived geographic model.

One setting in which the barrier that split populations can be identified with confidence consists of the tropical marine species on the two sides of Central America. Geological (Woodring 1966; Emiliani et al. 1972; Saito 1976; Keigwin 1978, 1982; Jones and Hasson 1985; Keller et al. 1989; Duque-Caro 1990; Coates et al. 1992; Coates and Obando 1996) as well as biogeographic (Jordan 1908; Ekman 1953; Briggs 1974; Vermeij 1978) evidence makes it clear that the tropical Atlantic and Pacific oceans were connected until the Pliocene. As the Central American land barrier emerged, it removed any potential for gene exchange between populations of tropical marine organisms on either side and allowed for the independent evolution in allopatry considered necessary

for geographic speciation. Successive sedimentological (Duque-Caro 1990; Coates et al. 1992) and paleontological studies of both marine (Woodring 1966; Saito 1976; Keigwin 1978, 1982; Jones and Hasson 1985; Coates et al. 1992) and terrestrial (Marshall et al. 1979, 1982; Marshall 1985) organisms have narrowed the estimates of the timing of the final isthmus completion to 3.0–3.5 million years (but see Crouch and Poag [1979] and Keller et al. [1989] for estimates of more recent closure). Thus, this particular natural experiment is one in which materials and methods have not been completely lost, or rather one in which they can be reconstructed from independent evidence. It should therefore have a great deal to tell us about the allopatric stage of geographic speciation. The isolates on the two sides of the isthmus, known as “gemmates”—a term coined by Jordan (1908) and equally useful whether or not Atlantic and Pacific populations are recognized as separate species—should be helpful in shedding light on the factors that affect rates of divergence and the emergence of reproductive isolation, information that is difficult to gather in other marine or terrestrial organisms.

In this chapter I first give a general overview of the advantages of studying speciation on the two sides of the Isthmus of Panama, a brief historical account of the studies that have used the isthmus as a backdrop for such studies, and a list of the possible pitfalls that can cause misinterpretation of the results. I then discuss the existing data on divergence and reproductive isolation between marine populations from the Caribbean and the eastern Pacific and their relevance to understanding allopatric speciation.

Why Study Speciation on the Two Coasts of Central America?

As pointed out above, the very fact that an identified and fairly well-dated barrier to gene flow exists to the present

day is sufficient motive for conducting studies of geographic speciation on the two coasts of tropical America. As Vermeij (1993) has put it, "tropical America will continue to be perhaps the finest laboratory in which to answer the big questions about what controls biological diversity" (p. 1604). However, there are additional reasons that bring this particular setting closer to the ideal for providing information on the first stage of geographic speciation.

(1) The time scale is right. The elapsed time since the final completion of the isthmus is not only well defined geologically but also sufficient for the accumulation of measurable interpopulational differentiation. Yet the vicariant event is not so ancient as to lead to the emergence of higher taxa and major uncertainties about the relationships of the species in question.

(2) There is no question about the efficacy of the barrier. Unlike most obstacles to migration that restrict, but do not completely sever, gene flow, the presence of a strip of land, interrupted only by the recently completed (1914) freshwater Panama Canal, ensures that nearly all contemporary populations do not exchange propagules.

(3) Populations of organisms belonging to many phyla were separated by the same barrier. The presumably simultaneous separation of populations from many taxa, with different life histories, population densities, generation times, fertilization systems, and dispersal abilities, provides ample opportunities for comparisons that could help tease apart the contribution of each of these factors to the probability of differentiation and speciation.

(4) There are well-defined differences in the environments on the two sides of the isthmus. Neotropical Atlantic and Pacific environments differ both in physical (Glynn 1972) and biotic (Glynn 1982) parameters. Most of these dissimilarities owe their existence to the presence of the isthmus itself, and have thus been in place for the last 3 million years (Cronin 1985; Jackson et al. 1993; Cronin and Dowsett 1996). Thus, comparisons between sister species can provide information on how adaptation to specific environmental differences can affect genetic divergence and speciation.

A Brief History of Studies Conducted with Geminant Species

For more than a century, evolutionary biologists have recognized the advantages of the isthmus for evolutionary studies and have employed the geminate species of Panama as examples of vicariant separation and geographic speciation (table 14.1). Günther (1868) was the first to note the faunal similarities of fishes on the two sides of the Isthmus of Panama. Based on this evidence, he proposed a prior connection between the Caribbean and the eastern Pacific. Following this study, the initial work in each taxonomic group consisted of recognizing and enumerating sister species in the two oceans (Jordan

1885; Meek and Hildebrand 1923–1928; Mortensen 1928–1950; de Laubenfels 1936; Hedgpeth 1948; Bayer et al. 1970; Chesher 1972; Vermeij 1978; Thomson et al. 1979; Voight 1988). Then came generalizations regarding factors responsible for divergence and speciation through the examination of distributions of geminate species among taxonomic lines (Rosenblatt 1963, 1967) or ecological habitats (Ekman 1953; Rosenblatt 1963; Vermeij 1978). Quantitative estimates of morphological divergence started early (Rubinoff 1963) but—considering the ease with which they can be conducted—are still limited in number (Lessios 1981a; Weinberg and Starczak 1988, 1989; Lessios and Weinberg 1994). Estimates of genetic divergence through protein (Gorman et al. 1976; Gorman and Kim 1977; Lessios 1979a,b, 1981a; Vawter et al. 1980; West 1980; Laguna 1987; Bermingham and Lessios 1993; Knowlton et al. 1993; Lessios and Weinberg 1994) or mitochondrial DNA (mtDNA) comparisons (Collins 1989; Bermingham and Lessios 1993; Knowlton et al. 1993) appeared as these techniques became available. With the exception of the pioneering work of Rubinoff and Rubinoff (1971), studies of the emergence of reproductive isolation between the geminates (Lessios 1984; Lessios and Cunningham 1990; Knowlton et al. 1993; Lessios and Weinberg 1993) were late in coming—despite their importance for understanding speciation—probably because they required the permanent establishment of evolutionary biologists in the tropics. Studies of adaptation of each member of a geminate pair to its respective environment (Graham 1971; Lessios 1979a, 1981b; Graves et al. 1983; Lessios 1990), though not intended to address questions of speciation *per se*, provided information on some of the factors responsible for divergence in particular traits. The possibility of a saltwater sea-level canal, taken seriously in the late 1960s and early 1970s, was the catalyst of many speculative articles regarding the biological effects of renewed faunal exchange between the oceans (Menzies 1968; Briggs 1969; Topp 1969; Rubinoff 1968, 1970; Glynn 1982), but of little original research. Though not directly addressing speciation issues, several works (e.g., Bowen et al. 1991; Martin et al. 1992; Cunningham and Collins 1994; Lessios et al. 1995; Shulman and Bermingham 1995; Collins 1996) have taken advantage of the well-defined times of splitting between geminate species to calibrate rates of molecular divergence.

Potential Problems

Although the geminate species hold many advantages as model organisms for the study of the first stage of speciation, the interpretation of observed patterns still encounters problems. As with any other reconstruction of evolutionary processes, one has to consider various possible pathways through which the present-day patterns of distribution and divergence have come to be. The in-

Table 14.1. Studies of genetic divergence and speciation using the setting of the Central American isthmus.

Reference	Emphasis of Study	Study Organisms
Günther (1868)	Biogeography	Fishes
Jordan (1908)	Mode of speciation	Fishes
Mayr (1954a)	Mode of speciation	Sea urchins
Rosenblatt (1963)	Rates of divergence	Fishes
Rubinoff (1963)	Morphological divergence	Fishes
Rubinoff and Rubinoff (1971)	Reproductive isolation	Fishes
Gorman et al. (1976)	Molecular (protein) divergence	Fishes
Gorman and Kim (1977)	Molecular (protein) divergence	Fishes
Vermeij (1978)	Morphological adaptation	Gastropods
Lessios (1979a)	Molecular, morphological, and ecological divergence	Sea urchins
Lessios (1979b)	Molecular (protein) divergence	Sea urchins
Vawter et al. (1980)	Molecular (protein) divergence	Fishes
West (1980)	Molecular (protein) divergence	Crabs
Lessios (1981a)	Molecular (protein) and morphological divergence	Sea urchins
Lessios (1984)	Temporal reproductive isolation	Sea urchins
Cronin (1985)	Rates of speciation	Ostracodes
Collins (1989)	Molecular (mtDNA) divergence	Gastropods
Weinberg and Starczak (1989)	Morphological divergence	Isopods
Lessios and Cunningham (1990)	Gametic reproductive isolation	Sea urchins
Birmingham and Lessios (1993)	Molecular (protein, mtDNA) divergence	Sea urchins
Knowlton et al. (1993)	Molecular (protein, mtDNA) and behavioral divergence	Alpheid shrimp
Lessios and Weinberg (1993)	Reproductive isolation	Isopods
Lessios and Weinberg (1994)	Genetic (protein) and morphological divergence	Isopods

formation we have is pairs of species, apparently closely related, with each member of the pair on one side of the isthmus. The most parsimonious explanation is that they were created by the splitting of an ancestral range by the isthmus (I will call this the "true geminate" model), but other possibilities exist. There is no doubt that the final closure of the Isthmus of Panama separated many species at roughly the same time, but any given pair of alleged geminate species may not represent a "typical" case. The potential problems of incorrectly assuming that present-day patterns have resulted from the true geminate model are obvious and have already been considered on an ad hoc basis in the literature (e.g., Gorman et al. 1976; Lessios 1979b, 1981a; Vawter et al. 1980; Selander 1982; Weinberg and Starczak 1989; Lessios and Cunningham 1990; Knowlton et al. 1993; Lessios and Weinberg 1994; Collins 1996). However, it may be advantageous to mention them all in one place and to examine the degree to which they can affect conclusions.

The first problem is one of identifying which of many potential candidates in each ocean are geminate species. This requires evidence supporting not just their status as sister species, but also their splitting through the erection of the Central American isthmus. Traditionally both the phylogenetic relationships and the assumption about the timing of the split have rested on morphology (e.g., Meek and Hildebrand 1923–1928; Mortensen 1928–1950; Hedgpeth 1948; Rubinoff 1963; Chesher 1972; Vermeij 1978; Thomson et al. 1979). However, lacking a "mor-

phological clock," we have no means of estimating the amount of morphological change expected to occur in 3 million years. Thus, lists of geminate pairs prepared by various authors (e.g. Jordan 1908; Mayr 1954a; Bayer et al. 1970; Chesher 1972; Thomson et al. 1979) do not always include the same species. In groups such as sea urchins, in which most of the genera have only one species on each side of the isthmus (Chesher 1972), the potential for confusion of true sister species relationships (figure 14.1A) is minimized. The danger of inclusion of a species into the wrong pair increases as the gaps between lineages leading to the supposed geminates become smaller. Thus, in genera such as the fish *Abudefduf* or *Kyphosus*, with at least two species on each coast, and in the much more speciose ones such as the shrimp genus *Alpheus*, with a minimum of 23 species in the eastern Pacific (Kim and Abele 1988) and 22 in the Caribbean (Chace 1972), or the gastropod genus *Conus*, with 22 proposed geminate pairs (Vermeij 1978), the probability of error in presumed sister pair affiliation is considerably higher.

The second possible source of error involves the assumption of simultaneous splitting in all pairs. Species on either side of the isthmus may have been separated by the same barrier, but the final interruption of gene flow may not have occurred at the same time (figure 14.1B). As Ekman (1953) concluded on biogeographic considerations alone, deep water species were probably split much earlier than shallow water ones. The shoaling of

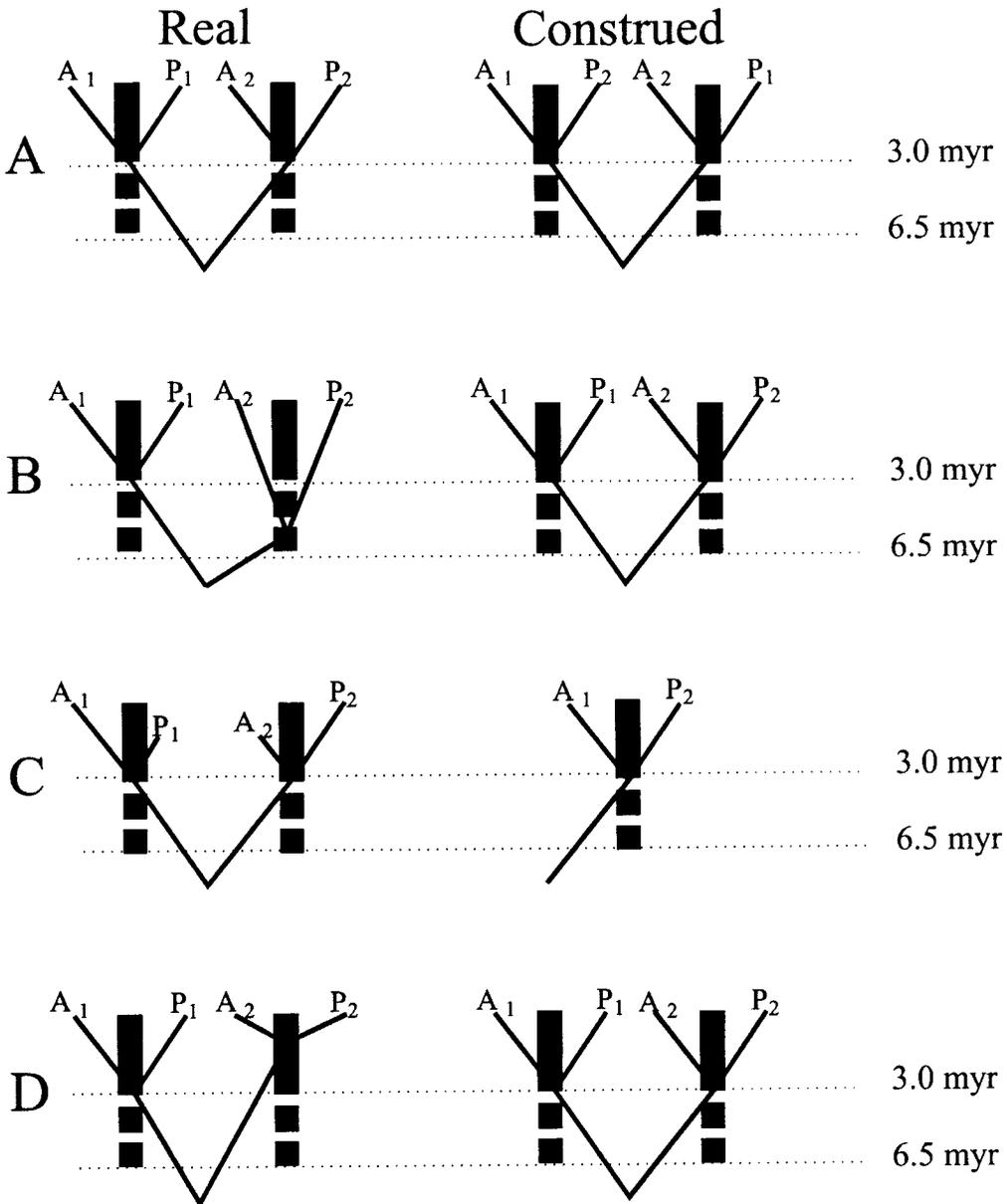


Figure 14.1. Possible mistakes in interpretation arising from making wrong assumptions regarding phylogeny and times of splitting of organisms that appear, on the basis of present-day distribution and divergence, to be geminate species. Cladograms on the left depict real phylogenies; cladograms on the right, phylogenies as they may be misconstrued. Letters with the same subscript indicate true members of geminate pairs. Vertical bar indicates the Central American isthmus. A, species in the Atlantic; P, species in the Pacific.

the isthmus is thought to have proceeded for a period longer than the length of time between the final closure and the present (for reviews, see Jones and Hasson 1985; Coates and Obando 1996). In the Late Miocene (6–7 mya) the channels connecting the Caribbean to the Pacific were 150 meters deep. The restrictions to circulation presum-

ably increased with time, finally becoming complete in the late Pliocene, approximately 3 mya (Holcombe and Moore 1977; Coates and Obando 1996). Thus, the possibility of different times of final interruption of gene flow, because of differences in mode of dispersal, habitat preferences, physiological tolerances, and vagility of

adults and larvae, is one that needs to be considered, even for shallow water species.

Erroneous times of separation may also arise from replacement of a species in one of the two oceans by another species invading the region from somewhere else. This is a more likely possibility for the eastern Pacific, where many species of clear Indo-Pacific origin are found (Rosenblatt et al. 1972; Glynn and Wellington 1983; Vermeij 1991; Lessios et al. 1996). Indeed, extinctions and incomplete sampling of species can cause many errors in presumed phylogenies. The most extreme case would occur if members of two different geminate pairs in opposite oceans became extinct (or were not sampled), and if the clades leading to each pair were not so dissimilar as to preclude assumptions of geminate relationships between the remaining species (figure 14.1C). Then both present-day distributions and divergence would lead one to think that species that had speciated before the rise of the isthmus for other reasons and coexisted in the same ocean during part of their history are members of a geminate pair.

The final alternative to the true geminate model is that there was gene flow between members of the geminate species after the completion (or near-completion) of the Central American Isthmus (figure 14.1D). There are various ways this could have happened. For example, it is possible that with sea level fluctuations occurring over the last 3 million years (Golik 1968; Haq et al. 1987), the lowest parts of the isthmus were breached by salt water incursions (Coates and Obando 1996; Cronin and Dowsett 1996). Even though such breaches must have been limited in extent and duration (otherwise they would have obliterated the biogeographic signature of the geminate species), they may have resulted in reticulate evolution in some species pairs. Limited foraminiferan evidence suggests that "incipient littoral-neritic leakage" may have occurred across the Panama Isthmus as recently as 1.8 mya (Crouch and Poag 1979; Keller et al. 1989). More recent introductions of lineages of euryhaline species into the wrong ocean may have occurred through the fresh waters of the Panama Canal (Hildebrand 1939; Rubinoff and Rubinoff 1969; McCosker and Dawson 1975). Ballast water taken by ships transiting the canal may transport planktonic organisms and larvae (Chesher 1968; Carlton and Geller 1993). A similar possibility of anthropogenic conveyance exists for fouling organisms attached to ship hulls (Spivey 1976). Finally, cosmopolitan species may maintain gene flow between the two coasts of America via circumglobal dispersal.

Do these potential confounding factors negate the advantages of geminate species as objects of speciation studies? I think not. Although it is dangerous to assume, without any corroborating evidence, that any two species on either side of the isthmus are *ipso facto* geminates separated for 3 million years, there are solutions to most of these problems. The greatest advantage of the historical setting of the Isthmus of Panama for evolutionary studies is that it separated marine organisms of a vast

array of taxa. Many of the possible errors in inference can be avoided through the judicious choice of organisms with characteristics tailored to the question of a particular study. Thus, it would be foolhardy to use holoplanktonic or fouling organisms to calibrate rates of genetic divergence under the assumption that they have not exchanged genes for 3 million years. However, it is easy to find species for which the assumption of lack of passage through the Panama Canal is likely to hold true. There are many marine invertebrates with larvae unlikely to survive a trip through pump impellers and the effects of antifouling paint in sufficient numbers to have a measurable probability of reaching sexual maturity and thus of transporting genes from one ocean to the other (Rubinoff 1970). Similarly, choice of species with similar modes of dispersal can increase the possibility of simultaneous times of splitting, when this assumption is crucial (Lessios 1979b). What is more, most of the erroneous reconstructions of phylogenetic topology depicted in figure 14.1 can be avoided by adequate sampling of species and characters. The misidentification of geminate pairs depicted in figure 14.1A will disappear through simple pairwise comparisons of divergence when any phylogenetically informative character is measured, as long as all closely related species on each side of the isthmus are sampled. Differences in separation times (figure 14.1A–14.1D) can also be detected through the use of multiple character sets to estimate divergence (e.g., Lessios 1981a; Bermingham and Lessios 1993; Knowlton et al. 1993). It is likely that species pairs that were split earlier than the rest will show more differentiation between their members in several unrelated sets of characters even if these characters do not evolve in a clocklike manner. Such pairs can be excluded from comparisons requiring the assumption of simultaneous separation. Conversely, each additional character set showing equivalent degrees of divergence between members of several pairs increases confidence that they conform to the true geminate model. For example, general agreement in mtDNA, protein, and behavioral compatibility divergence values in alpheid shrimp on the two sides of the Panama isthmus (Knowlton et al. 1993) has produced compelling evidence that three presumed species pairs were split at various times (and possibly due to different causes) before the closure of the isthmus, but it has also produced evidence that four additional species pairs, which cluster around a central value for each measure of intrapair divergence, were split at roughly the same time (Cunningham and Collins 1994). Future studies of geminate alpheid shrimp that need to compare evolution between species split simultaneously will have the benefit of knowing which species to use and which to avoid. Problems will undoubtedly remain. For example, when various lines of evidence suggest different times of splitting between species on either side of Central America (e.g., Knowlton et al. 1993), it is still not possible to distinguish whether this is due to the gradual shoaling of the Isthmus (figure 14.1B), or

whether it might be due to incorrect assumptions regarding sister species relationships (i.e., figure 14.1A,C). Only extensive fossil evidence (such as that presented in Jackson et al. [1993] for strombinid gastropods) can help with the complications arising from extinctions. However, the important point is that the geminate species provide the means with which to verify the assumptions on which conclusions will rest.

What Questions about Speciation Have the Geminate Species Helped Answer?

The first (and possibly most important) conceptual advance to which geminate species contributed was support for geographic speciation. Using the sister species of Panama along with other examples, D. S. Jordan (1908) proposed the "law of geminate species":

Given any species in any region, the nearest related species is not to be found in the same region nor in a remote region, but in a neighboring district separated from the first by a barrier of some sort or at least by a belt of country, the breadth of which gives the effect of a barrier. (p. 73)

In today's intellectual climate in which the debate has shifted toward the question of whether sympatric speciation is even possible (Bush 1975; Bush and Howard 1986), Jordan's (1905, 1908) advocacy of Wagner's (1868) hypothesis of geographic speciation may not seem a great insight, but coming at a time in which DeVries's (1901) ideas about saltational emergence of new species through macromutations reigned supreme among experimental biologists, this was an important step in the history of speciation research (Mayr 1963, p. 487).

Of course, if support for the geographic speciation model was all that geminate species had to offer to speciation research, there would be little need to write about them today, except as a note in the history of science. However, there are few questions in speciation that have been closed, and thus many hypotheses for which the geminate species can provide pertinent evidence. The rest of this paper is devoted to exploring the relevance of data from geminate species to the two major components of speciation: the accumulation of overall genomic divergence and the emergence and perfection of reproductive isolation. How the two are related to each other and what factors affect the evolution of each are questions central to the study of speciation.

Divergence and Reproductive Isolation in Geminate Species

Because both the magnitude of genetic divergence and the probability of emergence of reproductive isolation between allopatric populations are related to the time two

isolates have remained separate, they are often correlated with each other (Coyne and Orr 1989, 1997). For this reason it is difficult to determine whether divergence has caused reproductive isolation, as most models of geographic isolation tacitly assume (Wright 1940, 1982a,b; Muller 1942; Mayr 1954b, 1963, 1982; Carson 1968, 1975, 1982, 1985; Templeton 1980, 1982; Carson and Templeton 1984; Barton and Charlesworth 1984) or whether reproductive isolation can appear independently of major genetic restructuring, as a minority has postulated (Lewontin 1974; Bush 1975; Nei et al. 1983; Wu 1985). The geminate species pairs that were split simultaneously can provide information on this relation between reproductive isolation and genetic distance by eliminating the confounding effect of time from the comparisons. Because sister species are allopatric but some of them are in the same ocean as other congeners, they can also help evaluate the importance of reinforcement (Dobzhansky 1940, 1970; Butlin 1989) in perfecting prezygotic reproductive isolation. Finally, because the species pairs that have been compared across the Isthmus of Panama differ in dispersal ability, and because informed guesses can be made about the past ranges of some of them, they can help evaluate the importance of vagility and of restrictions in effective population size in determining whether geographically separated populations will speciate.

Table 14.2 presents data on divergence between members of geminate pairs of sea urchins, fish, shrimp, and isopods in which reproductive isolation has been assessed or can be reasonably inferred. Three species pairs of shrimp from the Knowlton et al. (1993) comparisons have been omitted, because both isozymes and mtDNA indicate that they may have been split earlier than the rest. To avoid circularity, it is necessary to use at least one character set as an external clock that would indicate whether the assumption of simultaneous splitting holds. Mitochondrial DNA appears to fit this requirement for the three species of sea urchins studied by Bermingham and Lessios (1993), and for four species of shrimp studied by Knowlton et al. (1993) (though not for all presumed geminate species; see Lessios et al. [1995] for a much smaller value of mtDNA percentage dissimilarity in a species pair of the fish genus *Abudefduf*). The uniformity of mtDNA divergence values even holds fairly well across phyla and assaying techniques. Though the estimates of percentage dissimilarity in this molecule come from restriction fragment length polymorphisms (RFLPs) in sea urchins and from sequencing of 681 base pairs in the Cytochrome Oxidase I (COI) region in shrimp, their range is rather narrow (5.3–8.5% dissimilarity), thus suggesting that it is reasonable to assume that the timing of bifurcation in all of these independent lineages is (on a geologic time scale) comparable. Preliminary data from approximately 620 base pairs in the COI region in sea urchins (Gonzalez and Lessios, unpublished) suggest that *Diadema* (4.7% sequence dissimilarity between *D. antillarum* and *D. mexicanum*) may have diverged more re-

Table 14.2. Morphological, isozyme, and mtDNA divergence between geminate species of Panama in which reproductive isolation was assessed or can be inferred.

Ocean		Morphology		Isozymes		mtDNA		Reproductive Isolation	
Atlantic	Pacific	Within Species	Between Species	Within Species	Between Species	Within Species	Between Species	Prezygotic	Postzygotic
Sea urchins									
<i>Diadema antillarum</i>	<i>D. mexicanum</i>	8.041 ¹	7.827 ¹	0.013 ³	0.051 ³	0.0044 ⁴	0.053 ⁴	Temporal, bidirectional	—
<i>Echinometra lucunter</i>	<i>E. vanbrunti</i>	5.325 ¹	6.412 ¹	0.023 ³	0.341 ³	0.003 ⁴	0.061 ⁴	Gametic, unidirectional	—
<i>Echinometra viridis</i>	<i>E. vanbrunti</i>	4.788 ¹	12.913 ¹	0.015 ¹	0.524 ³	0.006 ⁴	0.081 ⁴	None known	—
<i>E. lucunter</i> – <i>E. viridis</i>		4.803 ¹	11.746 ¹	0.021 ³	0.201 ³	0.004 ⁴	0.065 ⁴	Gametic, unidirectional	Complete
Fish									
<i>Bathygobius soporator</i>	<i>B. ramosus</i>	—	6.515 ²	—	0.420 ³	—	—	Behavioral, incomplete	—
<i>Bathygobius soporator</i>	<i>B. andrei</i>	—	8.402²	—	0.146³	—	—	Behavioral, incomplete	—
	<i>B. andrei</i> – <i>B. ramosus</i>	—	7.980 ²	—	0.418 ³	—	—	Behavioral, complete	Complete
Shrimp									
<i>Alpheus paracrinitus</i> sp. b	<i>A. rostratus</i>	—	—	—	0.028 ³	—	0.077 ⁵	Behavioral, incomplete	—
<i>Alpheus paracrinitus</i> sp. a	<i>A. paracrinitus</i>	—	—	—	0.114 ³	—	0.066 ⁵	Behavioral, incomplete	—
<i>Alpheus formosus</i> sp. a	<i>A. panamensis</i>	—	—	—	0.109 ³	—	0.077 ⁵	Behavioral, incomplete	—
<i>Alpheus cylindricus</i>	<i>A. cylindricus</i>	—	—	—	0.121 ³	—	0.085 ⁵	Behavioral, incomplete	—
Isopods									
<i>Exciorolana braziliensis</i> morph C	<i>E. braziliensis</i> morph C'	0.778¹	2.263¹	0.059³	0.215³	—	—	?	Complete?
<i>Exciorolana braziliensis</i> morph C	<i>E. braziliensis</i> morph P	0.649 ¹	7.035 ¹	0.122 ³	0.763 ³	—	—	?	Complete?
	<i>E. braziliensis</i> morph C'–morph P	0.650 ¹	8.134 ¹	0.125 ³	0.665 ³	—	—	?	Almost complete

Where more than one value of divergence is available because of multiple intraspecific sampling, the mean is shown. Where there is ambiguity of geminate relationship, rows in boldface indicate most likely geminate pair. The column labeled "postzygotic" includes information on whether genetic data indicate the absence of introgression, even though it is not known whether this is due to truly postzygotic isolation or an unstudied mechanism of prezygotic isolation. Data from Rubinoff and Rubinoff (1971), Gorman et al. (1976), Lessios (1981a, 1984), Lessios and Cunningham (1990), Bermingham and Lessios (1993), Knowlton et al. (1993), Lessios and Weinberg (1994), Bermingham and Lessios (unpublished).

¹Mahalanobis's (1936) $\sqrt{D^2}$

²Coefficient of difference (Mayr et al. 1953).

³Nei's (1987) D.

⁴Nei and Miller's (1990) D_{xy} .

⁵Kimura's (1980) corrected percentage sequence divergence.

cently than *Echinometra* (9.8% dissimilarity between *E. lucunter* and *E. vanbrunti*), but if true, this would only serve to strengthen the inferences from geminate comparisons regarding the role of genetic divergence in the emergence of reproductive isolation (see below). Because no mtDNA data exist for the isopod *Exciorolana braziliensis*, the question of whether the Caribbean morph C and the eastern Pacific morph C', which are actually different species (see Lessios and Weinberg 1993, 1994), split at the same time as the sea urchin and shrimp geminates must be regarded as open. However, the extensive sampling of *Exciorolana* on both coasts of Central America by Weinberg and Starczak (1988, 1989), Lessios et al. (1994), and Lessios and Weinberg (1993, 1994) suggests that the only other extant possible geminate is a morph called P' on the Atlantic coast of Brazil, which is likely to be a sister species of the Pacific P morph (Weinberg and Starczak 1989). Thus, the possibility that an unsampled species might result in confusion between members of different geminate pairs in *Exciorolana* is remote. Weinberg and Starczak (1988) and Lessios and Weinberg (1994) considered the possibility that the C-C' pair may be the result of a recent introduction through the Panama Canal, but rejected it in part due to the distributions of the two forms.

Information on reproductive isolation and genetic divergence from additional species, congeneric and sympatric with one of the geminates, is also included in table 14.2 when available. For sympatric species, genetic data can indicate whether or not they exchange genes. Thus, two allozyme loci fixed for different alleles (Birmingham and Lessios, unpublished) indicate that the sympatric sea urchin species *Echinometra lucunter* and *E. viridis* are not hybridizing in nature, even though they show only unidirectional gametic isolation in the laboratory (Lessios and Cunningham 1990). Lack of introgression is likely to be the result of postzygotic isolation, because the annual reproductive cycles of these species overlap (Lessios 1981b, 1985a) and because they show no lunar cycles in their spawning (Lessios 1991). Similarly, the sympatric species of the goby *Bathygobius* will not hybridize in captivity (Rubinoff and Rubinoff 1971), and genetic data indicate that they do not exchange genes in nature, because they show no shared alleles at five loci (Gorman et al. 1976). Evidence for reproductive isolation in *Exciorolana* comes only from the genetics of natural populations (Lessios and Weinberg 1993, 1994), and can thus not be conclusive in the case of allopatric morphs. However, reproductive isolation in this species complex, as measured by the excess of migration over gene flow, is present even between adjacent populations of the same morph (Lessios and Weinberg 1993). It would therefore be very surprising if the allopatric and divergent C and C' morphs hybridized freely if they were to find themselves in the same ocean.

What can these eight species pairs tell us about factors that affect genetic divergence and reproductive isolation in allopatry? The first question they can help answer

is whether overall genetic divergence is a requirement for the emergence of reproductive isolation. It should be remembered that even though speciation is the emergence of reproductive isolation, most models of speciation only deal with genomic divergence. This is because it is implicitly assumed that reproductive isolation is the product of small effects of many loci (Mayr 1963; Barton and Charlesworth 1984; Paterson 1985), and can thus come about only as the result of a general overhaul of the genome. In part, this general belief seems to have its origins on the overreliance of evolutionary biology on evidence from terrestrial vertebrates and insects. In these groups complicated courtship rituals and the exchange of multiple behavioral cues, the necessary prerequisites to successful mating, are likely to have a polygenic basis. Change in such traits usually (but not always) may require genetic substitutions in many loci (see Zouros 1991; Coyne 1992), or else may be controlled by relatively few loci but with major developmental effects (Lewontin 1974; Templeton 1981; Bush and Howard 1986). The evidence from the geminate species, though still very limited, indicates that species that depend on courtship show a correlation between divergence and premating reproductive isolation, whereas species lacking courtship may show prezygotic reproductive isolation that is independent of divergence. In shrimp, for which behavioral interactions are important, the pair consisting of *Alpheus paracrinatus* sp. b and *A. rostratus*, which has the aberrantly smallest value of Nei's D (table 14.2), is also the pair in which laboratory experiments show the highest compatibility between members (Knowlton et al. 1993). However, among free-spawning sea urchins, complete reproductive isolation through gamete release at different lunar phases has evolved among the geminates of *Diadema*, the genus in which both isozyme and morphological transisthmian differentiation is not substantially larger than intraspecific variability (Lessios 1984). The geminate species of *Echinometra*, on the other hand, *E. lucunter* and *E. vanbrunti*, even though they are one order of magnitude more divergent in allozymes than the species of *Diadema* (and somewhat more divergent in morphology and mtDNA as well), show only unidirectional gametic isolation. Even this partial mechanism is absent between the allopatric *E. viridis* and *E. vanbrunti*, the most differentiated pair in the trio. Although other, as yet undiscovered, mechanisms of reproductive isolation may exist in *Echinometra* (Lessios and Cunningham 1990, 1993), the evidence at hand suggests that in these organisms with external fertilization the emergence of reproductive isolation may be unrelated to the amount of accumulated genetic divergence. Such decoupling is easy to understand if gametic recognition depends on mutations in only two loci, those controlling the gamete recognition molecules bindin (Palumbi and Metz 1991) and bindin receptor (Foltz et al. 1993), or if spawning time is under simple genetic control. Lack of correlation between genetic divergence and reproductive isolation in

organisms with simple genetic control of reproduction does not necessarily mean that speciation will be either frequent or rapid. Because there will be strong selection against mutant genotypes that produce incompatible gametes or spawn asynchronously with the rest of the population, the rise of new species through such mechanisms requires the convergence of many events of low probability to occur (Lessios and Cunningham 1990).

A similar dichotomy between species with and without courtship is suggested when one considers the role of reinforcement of reproductive isolation through selection to avoid hybridization in sympatry. In the fish *Bathygobius*, single-pair no-choice experiments in aquaria suggested that mating discrimination between the two geminates, and between each of them and the more distantly related *B. ramosus*, was complete. However, when males of a second species were introduced in a tank containing both sexes of another, some heterospecific spawnings occurred between allopatric species, but not between sympatric ones. This led Rubinoff and Rubinoff (1971) to conclude that reinforcement had perfected reproductive isolation in sympatry. This conclusion remains robust if one considers the possible effects of time, because the outgroup species, *B. ramosus*, is equidistant in both morphology and isozymes from the two members of the geminate pair (table 14.2). These results from fish contrast with results from sea urchins. In *Echinometra*, eggs of *E. lucunter* are less likely to be fertilized by sperm of the allopatric *E. vanbrunti* than by sperm of the sympatric *E. viridis* (Lessios and Cunningham 1990), the opposite of what the reinforcement hypothesis would expect. Time cannot be factored out in this comparison. Even though the RFLP data shown in table 14.2 cannot resolve the phylogeny of the three species of *Echinometra*, sequencing of the COI region of mtDNA (Gonzalez and Lessios, unpublished) indicates that the two sympatric species split from a common stock that had been already separated from the Pacific *E. vanbrunti*. There was, therefore, less time for *E. lucunter* to evolve reproductive isolation toward the sympatric *E. viridis* than toward *E. vanbrunti*. However, if gametic isolation depended on time alone, one would expect not just *E. lucunter* but also *E. viridis* to show gametic incompatibility with *E. vanbrunti*, and this is not so (Lessios and Cunningham 1990). Thus, reinforcement seems to have been important in the evolution of prezygotic isolation of fish, but not of sea urchins, suggesting that in the latter the emergence of reproductive isolation is more a matter of chance appearance in a lineage than of selection to avoid wasting gametes in inferior hybrids.

The Importance of Population Size in Divergence and Reproductive Isolation

The second question about which geminate species can provide relevant data concerns the importance of popu-

lation size restrictions in causing rapid speciation. Most speciation models which assume that reproductive isolation results from overall genetic divergence also postulate passage through small population size as a mechanism of rapid genetic change. Authors of such models envision different ways in which population bottlenecks will destabilize the genome, but they all believe that the consequences will be drastic; they have accordingly coined appropriately radical terms for these events. Thus, there is "genetic revolution" (Mayr 1954b, 1963), "founder-flush cycles" (Carson 1975), and "genetic trasilience" (Templeton 1980). Although none of these authors deny that, given enough time, separation of large populations can also cause speciation, the assumption is that divergence in the absence of bottlenecks will proceed at much slower rates and that large isolates have a much higher chance of fusing if the geographic barrier is removed (e.g., Grant 1963; Carson 1982). A necessary consequence is the belief that most species are the products of peripatric speciation, that is, speciation that involves small isolates at the periphery of the species range (see Bush 1975, 1994; Mayr 1982; Lynch 1989). However, Barton and Charlesworth (1984) and Barton (1989), even though they also believe that reproductive isolation requires genomic divergence, have argued that restrictions in population size are unimportant in speciation, because passage from one adaptive peak to another can be achieved even in large populations.

Coyne and Orr (1989) have concluded from a review of extensive data on electrophoretic differentiation and reproductive isolation that speciation in *Drosophila* requires 1.5–3.5 million years to be completed, and Coyne and Orr (1997) have estimated that allopatric species in this genus require 2.7 million years to speciate. If small population size is important in the completion of reproductive isolation, most speciation in *Drosophila* presumably has involved restrictions in population size. Geographic isolation in the geminate species of Panama, however, is more likely to conform to what has come to be known as the dumbbell (Mayr 1982), vicariance (Lynch 1989), or dichopatric (Bush 1994) model, that is, one that involves populations sufficiently large to exclude inbreeding as a factor, and would thus be expected to show few, if any, isolating mechanisms. Mayr (1967) has speculated the bisection of the Panamic biogeographic province must have left "two colossal gene pools" on either side of the isthmus, and that, for this reason, "differences are still either nonexistent or they are so slight that one doesn't really like to rank these as species" (p. 49).

Mayr's estimate of the size of gene pools was probably correct. Fractionation of large populations is suggested by the present-day ranges of geminate species. If the completion of the isthmus separated only a peripheral deme of each genus in one of the oceans, is 3 million years enough time for all such demes to have expanded their populations, such that they range in the entire

tropical portion of each ocean as most of them do? Even if this is a possibility for other groups, fossil evidence of echinoids—despite this group's propensity to fossilize poorly (Kier 1977; Gordon 1991)—suggests that the ranges of both isolates of various genera that include presumed geminates (Chesher 1972) have remained wide from the Miocene to the Recent (figure 14.2).

Genetic variability in extant populations also provides evidence for a long history of large population size. Both isozyme heterozygosity (18.1–27.8%, Lessios 1979b) and intraspecific mtDNA diversity (0.237–0.614%, Birmingham and Lessios, unpublished) in *Diadema* and *Echinometra* are high and approximately equal in all species. Heterozygosities in shrimp (1.6–8.0%, Knowlton et al. 1993) and in *Bathygobius* (2.7–6.9%, Gorman et al. 1976) are lower than in sea urchins, but still not suggestive of bottlenecks in either ocean. Despite the apparent lack of drastic reductions in history of population size, the allopatric species of *Diadema*, *Alpheus*, and *Bathygobius* show premating reproductive isolation, thus giving credence to Barton and Charlesworth's (1984) claim that speciation does not require small demes.

Another piece of evidence suggesting that the importance of population bottlenecks as a cause of divergence may have been overemphasized comes from a catastrophe that befell one of the geminate species. *Diadema antillarum* suffered mass mortality in 1983, which reduced populations throughout the western Atlantic by more than 97% (Lessios et al. 1984a,b; Lessios 1988a,b). This drastic reduction had no effects on the average heterozygosity, number of alleles, or gene frequencies of *D. antillarum* populations (Lessios 1985b). In 1993 the populations in Panama were still at less than 3.5% of their premortality levels (Lessios 1995a), but genetic variability and gene frequencies remained unaltered despite the prolonged reduction in population size (Lessios 1995b).

In addition to historical fluctuations in population size, genetic divergence and the emergence of reproductive isolation should theoretically be affected by the mode of dispersal of the organisms in question. Though he gave no quantitative estimates, Rosenblatt (1963) stated that there are more recognized geminate species among families of large fish with a long-lived planktonic larval stage than among families of small fish that lead a sedentary

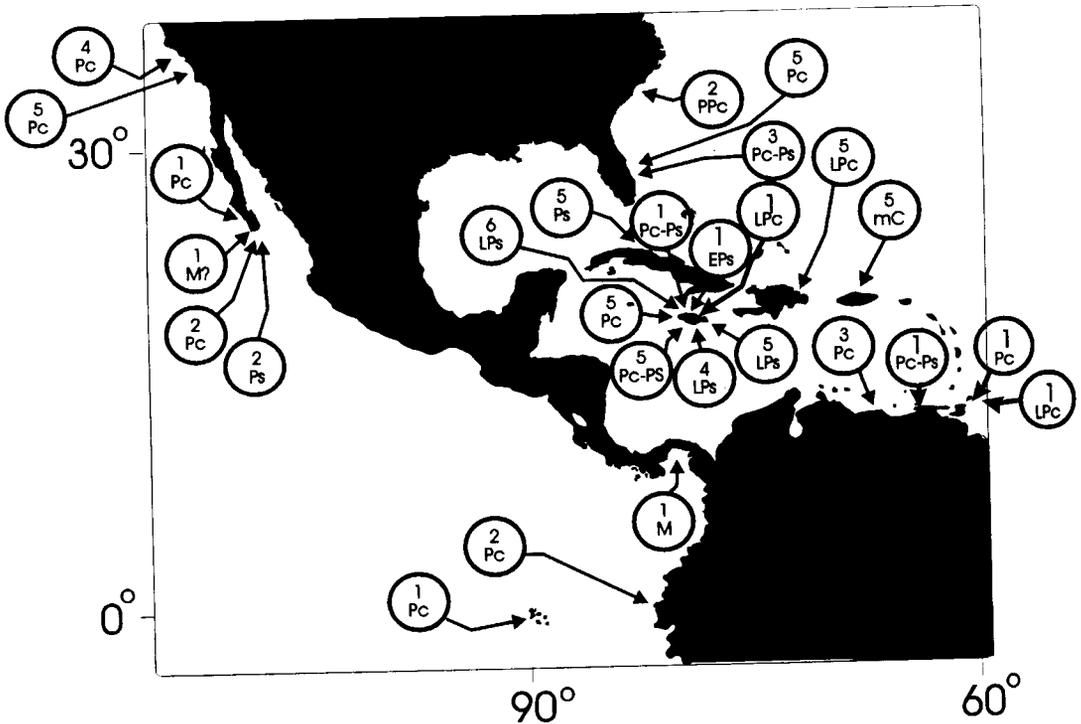


Figure 14.2. Localities and epochs in which sea urchin fossils of species considered to be members of geminate pairs have been found. Compiled from Mortensen (1928–1950), Grant and Hertlein (1938), Kier (1963), Cooke (1961), Gordon (1991), Gordon and Donovan (1992), Donovan and Gordon (1993), Donovan and Embden (1996). Genera: 1, *Eucidaris*; 2, *Arbacia*; 3, *Lytechinus*; 4, *Triploneustes*; 5, *Echinometra*; 6, *Diadema* or *Astropyga*. Epochs: M, Miocene; Pc, Pliocene; Lpc, late Pliocene; Ps, Pleistocene; EPs, early Pleistocene; LPs, late Pleistocene; mC, mid-Cenozoic; Ppc, post-Pliocene.

existence as adults and have a short-lived larval stage. He suggested that the pattern is due to accelerated rates of divergence among the latter, produced by lower levels of intraoceanic gene flow and more pronounced genetic structuring. However, more recent genetic data from other geminate species, with much more pronounced differences in vagility than large and small fish, have failed to support this view. Sea urchins, shrimp, and fish have planktonic larvae that can disperse over wide ranges. Isopods, on the other hand, are brooders with no dispersal phase other than possible rafting of adults. This sedentary habit of all life stages, along with infrequent extinctions and colonizations of intertidal environments from a single source (Lessios et al. 1994), has resulted in populations of *Excirolana braziliensis* that are highly structured genetically. Populations of the same morph less than 1 km apart are fixed for alternate alleles (Lessios and Weinberg 1994) and show incipient reproductive isolation (Lessios and Weinberg 1993). Despite these high levels of intraoceanic genetic divergence, however, the allozyme transisthmian divergence between geminate morphs of isopods is no larger than that of most sea urchins, shrimp, or fish (table 14.2), possibly because ancestral alleles are preserved in low frequencies in each ocean by occasional hybridization between populations and morphs (Lessios and Weinberg 1994). Thus, the expectation that organisms with low dispersal would tend to have accelerated rates of divergence and higher probability of speciation is not fulfilled. Whether this is a general result, or whether it is the consequence of the peculiarities of the population genetics of *Excirolana*, remains to be seen.

Conclusions

The geminate species on the two sides of Central America have had a long history of contributing evidence relevant to speciation, though they have yet to be used to their full potential. From eight species pairs for which there are multiple measures of genetic divergence and assessments of reproductive isolation, I have concluded that in organisms lacking copulation and courtship, reproductive isolation in allopatry may arise even in the absence of major reorganization of the genome, and that reinforcement is not important in perfecting reproductive isolation. It is obvious that comparisons between eight species pairs, no matter how well their history of splitting is known, cannot provide definitive answers to questions that have not been answered in 100 years of speciation research. It is entirely possible, indeed it is likely, that the next study using geminate species may generate evidence that would lead to exactly the opposite conclusions from the ones reached here. A result that may remain unaltered, on the other hand, is that Atlantic and Pacific populations in each pair have acquired varying degrees of reproductive isolation despite the absence of bottlenecks, which suggests

that restrictions in population size may not be of primary importance in speciation. No matter how future studies turn out, if this chapter has provided a flavor of the kinds of questions geminate species can help answer and convinced some evolutionary biologists that they are a very underutilized tool for understanding vicariant speciation, it will have served its purpose. As Jones and Hasson (1985) have put it, "The evolutionary experiment begun in the late Pliocene by the uplift of Central America has been performed; more investigators are needed to interpret the data" (p. 351).

Acknowledgments I thank M. McCartney, W. G. Eberhard, B. R. Grant, D. J. Howard, B. Kessing, I. Rubinoff, M.-J. West-Eberhard, and an anonymous reviewer for comments on the manuscript, and D. Howard and S. Berlocher for the invitation to participate in the "Endless Forms" conference. The Smithsonian Tropical Research Institute has supported 18 out of the 20 post-1954 studies listed in table 14.1, so it is accurate to state that this chapter would not have been written without it.

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