GENETIC DIVERGENCE, REPRODUCTIVE ISOLATION, AND MARINE SPECIATION

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Abstract

In marine species, high dispersal is often associated with only mild genetic differentiation over large spatial scales. Despite this generalization, there are numerous reasons for the accumulation of genetic differences between large, semi-isolated marine populations. A suite of well-known evolutionary mechanisms can operate within and between populations to result in genetic divergence, and these mechanisms may well be augmented by newly discovered genetic processes.

This variety of mechanisms for genetic divergence is paralleled by great diversity in the types of reproductive isolation shown by recently diverged marine species. Differences in spawning time, mate recognition, environmental tolerance, and gamete compatibility have all been implicated in marine speciation events. There is substantial evidence for rapid evolution of reproductive isolation in strictly allopatric populations (e.g. across the Isthmus of Panama). Evidence for the action of selection in increasing reproductive isolation in sympatric populations is fragmentary.

Although a great deal of information is available on population genetics, reproductive isolation, and cryptic or sibling species in marine environments, the influence of particular genetic changes on reproductive isolation is poorly understood for marine (or terrestrial) taxa. For a few systems, like the co-evolution of gamete recognition proteins, changes in a small number of genes may give rise to reproductive isolation. Such studies show how a focus on the physiology, ecology, or sensory biology of reproductive isolation can help uncover the

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genetic changes associated with speciation and can also help provide a link between the genetics of population divergence and the speciation process.

INTRODUCTION

The formation of species has long represented one of the most central, yet also one of the most elusive, subjects in evolutionary biology. Darwin (28) sought out the mechanisms and implications of natural selection in order to explain the origins of species. Later, both Dobzhansky (29) and Mayr (88) would use speciation as a pivot around which to spin their divergent yet complementary views of the evolutionary process. They called their works *Genetics and the Origin of Species* and *Systematics and the Origin of Species,* perhaps to emphasize that they were using genetics and systematics primarily to advance understanding of the speciation process (45).

As a result of these efforts, and the series of papers that developed and used the new synthesis, a basic model of speciation arose. Now termed *allopatric speciation,* the basic scenario is familiar to virtually all evolutionary biologists: A large, continuous population is broken up into smaller units by extrinsic barriers; genetic exchange between these separated populations ceases, and genetic divergence takes place between them; the build-up of genetic differences leads to intrinsic barriers to reproduction. If the separated populations (now separate species) reconnect with one another through the breakdown of the original extrinsic barriers, they will remain reproductively isolated and selection for increased reproductive isolation may occur (30).

Much of the early evidence for this process was based on discovery of species groups at the range of stages predicted by the above scenario (88). Some species have broad distributions, often with local variants. Other species are easily divided into allopatric subspecies whose taxonomic rank is debated. In other cases, two similar but slightly different species inhabit the same region, yet are distinguished by mating preferences or habitat differences that limit hybridization between them.

Even though Mayr (89) could identify this series in marine species, there have been relatively few attempts to examine patterns and processes of speciation in marine habitats. This is unfortunate because marine species often represent a serious challenge to the idea of allopatric speciation, especially in marine taxa with high fecundity and larvae that can disperse long distances. These life history traits result in species that have large geographic ranges, high population sizes, and high rates of gene flow between distant localities.

Such attributes might be expected to limit the division of a species' range into allopatric populations. Few absolute barriers to gene flow exist in oceans, and as a result, even widely separated regions may be connected genetically. Furthermore, marine populations tend to be large, which can slow genetic divergence between populations. Population genetics has shown that many

species with these life history traits have little genetic population structure and appear to act as large, panmictic units (101). For these species, allopatric speciation events may be infrequent and slow (89).

Yet, speciation in these taxa is common enough that marine species with these life history traits dominate important marine groups like echinoderms (33) and fish (17, 58). Furthermore, some types of marine habitats like coral reefs and the soft sediments of the deep sea have a huge number of species (46, 47, 74, 113, 149), some of which appear to be closely related (71, 101). Thus, the generalization that speciation must be rare in marine taxa with high dispersal appears to be incorrect.

In fact, a number of factors affect the chance of speciation through allopatric mechanisms in the sea. Like most useful generalizations, the process of allopatric speciation as described above includes a wide range of exceptions. What mechanisms are there that might enhance population subdivision and promote genetic divergence in species with high dispersal? How does reproductive isolation evolve in recently diverged species? What aspects of marine speciation have attracted the most research, and where are the future opportunities? To answer some of these questions (at least partly), and to arrange these topics in a manageable way, I have separated them into (i) opportunities for population subdivision, (ii) mechanisms of genetic differentiation, and (iii) reproductive isolation in closely related species. Together, these sections highlight the success of research into marine speciation, but they point out the existence of a major gap in our understanding.

OPPORTUNITIES FOR POPULATION SUBDIVISION

Population genetic studies of marine species have shown that, especially along continental margins, high dispersal potential is often associated with only mild genetic differentiation over large scales (101). These results suggest high levels of gene flow between populations, but there may often be limits to the actual dispersal of marine species with high dispersal potential (122). These limits vary widely with species, habitat, local ocean conditions, and recent history, and they may create ample opportunity for genetic divergence. Although such limits may seldom create absolute barriers to gene flow, they may often limit gene flow in some directions or at some times. Thus, partially isolated populations may occur quite commonly in marine systems. Throughout this section, the main focus is on mechanisms by which marine species with high dispersal may become at least partially isolated. The goal is to summarize ways in which these populations can diverge genetically despite their potential for gene exchange. Species with low dispersal often show interesting and unexpected biogeographic patterns (e.g. 63) or remarkable levels of genetic distinction over mere meters (138a), but in general it is no mystery how genetic barriers in low dispersal species arise (49).

Invisible Barriers

Even if larvae were simply passive planktonic particles, drifting helplessly in ocean currents (5, but see next section), gene flow across the world's oceans would be neither continuous nor random. The physics of a liquid ocean on a spinning globe, heated differentially at the poles and the equator, will always provide complex oceanic circulation (124). Today, these patterns include a prevailing westward-flowing equatorial current and two large circulation centers in the northern and southern hemispheres in both the Pacific and Atlantic Oceans. Schopf (124) suggested that these basic patterns also occurred in the past, and that biogeographic boundaries—the defining limits of biogeographic provinces—are typically set by these physical forces (see also 61, 133).

If most planktonic dispersal follows these currents, then movement from one circulation center to the others might be infrequent. Data on the distribution and abundance of fish (60), planktonic copepods (90), and other zooplankton (87) show that even the open ocean is a fragmented habitat. Across a large geographic scale, species composition of planktonic communities may be determined by currents such as gyres and mesoscale eddies (122). Although few data exist on the influence of these currents on species formation, gene flow across the oceans is probably constrained and directed by such circulation patterns.

Smaller geographic features also influence oceanic circulation, and probably gene flow as well. On the east coast of North America, Cape Hatteras and Cape Cod define biogeographic boundaries set by near-shore currents and a steep temperature gradient (124). Along this coast, genetic variation seems to be over a far shorter geographic scale than those predicted by gene flow estimates based on larval biology and current patterns (1, 11, 108, 120). Similarly, on the west coast of North America, Point Conception is a focus for the range endpoints of many species (61, 143). The Indonesian Archipelago is also a biogeographic indicator, separating Indian Ocean from Malayan provinces (124, 143). Several studies have shown that this complex of islands represents a barrier to gene flow within species (8) as well as separating closely related species (91).

A different type of pattern has been seen in the central Pacific (67). Here, the fish and gastropods of the islands of the Pacific tectonic plate are sometimes very different from those of archipelagoes on other plates: across a tectonic boundary, archipelagoes sometimes have very different faunas. Springer (131) suggested that the fish species tend to remain on archipelagoes of a particular plate, despite the potential for dispersal across plate boundaries (123), and that "plate effects" have built up over a long time (see also 66). The generality of this pattern is not clear, however, and further research is warranted.

Isolation by Distance

Oceanic currents are sometimes able to carry larvae far from their parents (121–123). For example, populations of spiny lobsters in Bermuda seem to be dependent on long distance larval transport along the Gulf Stream (52). However, there may be a limit to gene flow even in species with larvae that can disperse long distances (144, 145). Although long-lived larvae may drift for many months (114, 121), *successful* transport over long distances may be rare (62). Larvae that disperse over long distances may have a greater chance of wafting into unfavorable environments than do larvae that disperse short distances. This is coupled with a diffusion effect: The density of larvae thins with increasing distance from the center of larval production so that settlement events per available area decline with distance from the source of propagules. Lecithotrophic larvae can also be constrained by energy supply; long periods in the plankton consume energy stores, leaving little metabolic reserve for metamorphosis (114; planktotrophic larvae may not always have these limits—95).

Geographic patterns of genetic variation of marine fish and invertebrates suggest that isolation by distance occurs, but only over the largest geographic scales. Isolated islands in the Pacific Ocean, like the Hawaiian and Society Islands, appear to harbor populations with reduced genetic variation (98, 103, 150). These reductions are probably due to two physical factors. First, these islands are a long distance from neighboring archipelagoes. Second, equatorial currents flow westward toward the center of the Indo-West Pacific, and so both Hawaii and the Society Islands are "upstream" from the rest of the Indo-West Pacific. When the equatorial current breaks down, or when large water masses move from west to east across the Pacific during El Niño years (153), this dispersal barrier may disappear (115).

Isolation by distance effects may be weakest in species that inhabit continental margins, where extreme populations are connected through intermediate, stepping-stone populations. We have found that Atlantic and Pacific populations of the sea urchins *Strongylocentrotus droebacheinsis* and *S. pallidus* can be very similar genetically (102, 104). This pattern can change for populations on different sides of an ocean basin where no intermediate populations exist. For example, littorinid snails with planktotrophic larvae have little genetic divergence along the east coast of North America but are very divergent on opposite sides of the Atlantic (9, 10).

Behavioral Limits to Dispersal

The physical barriers discussed above can play an important role in limiting gene flow and creating genetic structure within oceanic populations even if larvae are passive planktonic particles. However, additional aspects of marine

life histories can lead to limited genetic dispersal. Burton & Feldman (19) showed that genetic differences in marine organisms can occur on a geographic scale that is much less than that predicted by their dispersal potential. For some species, dispersal occurs at a stage during which the individual can control its movements. For example, fresh water eels spawn in marine habitats, and their larvae migrate from spawning grounds to continental river mouths (2). American and European populations of eels both breed in the Sargasso Sea, but adult populations are genetically distinct (2). This suggests that these larval fish can control the direction of their migration from the joint breeding ground to the rivers inhabited by adults. Larger marine animals, like turtles and whales, have long been known to be capable of this type of migration, and genetic structure in these species is on a geographic scale far smaller than their potential range of movement (4, 14).

However, small larvae and adults may also have some control over their dispersal. Burton & Feldman (19) showed that the intertidal copepod *Tigriopus californicus* showed strong genetic differences over just a few kilometers of coastline. One explanation for this pattern is that juveniles and adults may have behavioral adaptations that prevent their being swept off the rocky outcrops that they inhabit. Such behavioral nuances are known for the amphipod *Gammarus zaddachi,* which migrates in and out of estuaries by rising into the water only during those seasonal tidal currents that will take individuals seaward in winter and upstream in the spring (57). Crustacean larvae are known to regulate their depth in a complex way that may allow retention in estuaries (27) or return them to coastal habitats after initial transport offshore (107). Few, if any, genetic differences have been attributed to these larval behavioral abilities (100, but see 92), but only a small number of species have been examined.

Selection

As shown by several well-known studies in marine systems, gene flow may be curtailed by selection as well as by limited dispersal. In the mussel *Mytilus edulis,* estuarine habitats of Long Island Sound are colonized regularly by migrants from oceanic, coastal zones. However, strong selection at a leucine amino-peptidase locus alters gene frequencies of settlers in the Sound, creating a strong genetic cline (53, 75). In the salt marsh killifish, *Fundulus heteroclitus,* selection at one of the lactate dehydrogenase (LDH) loci appears to create a strong cline in gene frequencies along the steep temperature gradient of the east coast of North America (reviewed in 108). Temperature and allozyme properties combine in these fish to create differences in development rate, swimming endurance, oxygen transport, and patterns of gene expression (108). A cline in mitochondrial haplotypes also parallels the LDH cline, and these concordant patterns suggest a dual role for phylogenetic history and natural

selection in the divergence of southern and northern populations of this fish (11).

Recent History

One of the most surprising marine genetic patterns was discovered in the widespread oyster *Crassostrea virginica*. Despite a larval dispersal stage in this species that lasts for several weeks, Reeb & Avise (111) demonstrated a strong genetic break midway along the east coast of Florida. Populations north and south of this break differed in mitochondrial DNA sequences by about 3% despite the lack of an obvious barrier to genetic exchange. Populations spanning this break have broadly similar patterns of allozyme variation, a result that had been interpreted as evidence for widespread gene flow (18). Karl & Avise (65) showed that patterns of nuclear DNA differentiation match the mtDNA patterns, not the allozyme patterns, and they suggested that balancing selection is responsible for the allozyme similarities. Reeb & Avise invoked history to explain these varied genetic patterns: populations of estuarine species like *C. virginica* may have been isolated during periods of low sea level in the Pleistocene when large coastal estuaries drained. Thus, the genetic pattern we see today may be far from equilibrium, and it reflects neither contemporary genetic exchange nor the larval dispersal potential of this species.

Unique historical events may have been instrumental in the speciation of stone crabs in the Gulf of Mexico. Western and eastern Gulf populations of *Menippe mercenaria* were probably separated during periods of low sea level during the Pliocene or Pleistocene. Today, two species exist allopatrically in the southeastern United States (12). There is a broad hybrid zone where these species meet in the Gulf of Mexico (13), but there also appears to be a second region where allozyme frequencies are intermediate between species. This second region is on the Atlantic coast of Florida, close to the mouth of the Sewanee Strait, a temporary seaway that connected the Gulf and the Atlantic during periods of high sea level in the Miocene and Pliocene (12). A combination of genetic and geological data suggests that the brief existence of this seaway injected genes from the western Gulf species deep into the range of the eastern Gulf/Atlantic species. Although this injection occurred long ago, the genetic signature of the event persists despite the potential for long distance gene flow in this species (12, 13).

The tropical Pacific ocean has been a backdrop for a great deal of faunistic change in the Pleistocene. Although sea surface temperatures probably did not change much during glacial cycles (24), sea levels changed repeatedly by up to 150 m (105). During sea level regressions, shallow back reefs and lagoons dried out. Higher sea level may have drowned some fringing reefs. Associated with these changes have been many local extinctions and recolonizations by the marine fauna of isolated reefs (48, 76, 105). For example, the cone snail

Conus kahiko is found commonly in the fossil record of Hawaii until about 100,000 years ago, when it disappeared and was replaced by the morphologically similar *Conus chaldaeus* (76).

Recent evidence from two species groups suggests that the Pleistocene may have been a period of rapid speciation. Sibling species of *Echinometra* sea urchins arose and spread throughout the Pacific over the past 0.5–2 million years (103). Likewise, sibling species of butterfly fish in at least two subgenera of *Chaetodon* differentiated from their Indian Ocean counterparts during the past million or so years (91). In the latter case, concordant patterns of species differentiation based on molecular phylogenies strongly suggest that divergence was affected by extrinsic factors such as dispersal barriers during sea level fluctuations (91).

Some taxa have probably been affected more strongly than others by the flush-fill cycle in the Pacific. Soft-sediment (e.g. lagoon-inhabiting) bivalves have low species richness on isolated archipelagoes where such habitats were severely reduced by low sea level. This may explain a previously uncovered but poorly understood pattern of lower bivalve endemicity on isolated islands (66).

Cronin & Ikeya (27a) regard cycles of local extinction followed by recolonization as opportunities for speciation. Their analysis of arctic and temperate ostracods suggests that these opportunities only seldom result in new species. However, there have been a large number of opportunities for speciation during the past 2.5 million years, and as a result, speciation has occurred in 15% to 30% of ostracods during this time period.

MECHANISMS OF GENETIC DIFFERENTIATION

Genetic Differentiation in Large Populations

The types of genetic changes that occur during speciation have fueled debate for many years. A great deal of attention has been focused on small populations derived by colonization of a novel habitat. These founder events (88) can lead to rapid genetic changes that have been described as genetic revolutions (21, 22) or genetic transiliences (138). Such changes are thought to alter substantially the genetic architecture of a population, allowing rapid accumulation of a large number of genetic differences that can then lead to reproductive isolation.

In addition to these genomic reconstructions, normal genetic variants may accumulate more quickly in small than large populations. Under several reasonable models of molecular evolution, most mutations are slightly deleterious. Kimura (69) showed that this type of mutation could drift in a small population as if it were neutral, rising to fixation with about the same probability as a

strictly neutral change. By contrast, in large populations, in which drift is minor, even slightly deleterious mutations will be eliminated by natural selection. Kimura's analysis shows that as population size decreases, the fraction of "nearly neutral" mutations increases. The result is that the overall rate of molecular evolution may increase for small populations as compared to large populations.

It is unlikely that evolutionary models that rely on very small population sizes will explain a large fraction of speciation events among marine organisms with the potential for long-distance dispersal. This is because populations that become allopatrically or parapatrically separated from one another (by some of the mechanisms reviewed above) are likely to be large in extent and in population size. Furthermore, multiple invasions of a new habitat (like an island) are much more likely for marine organisms with long distance dispersal than for gravid female flies, birds, lizards, etc. As a result, the genetic differentiation of allopatric marine populations has been thought to be a slow process, requiring many millions of years to accomplish (89, 117, 131).

Although many efforts have been made to identify and explain major genetic changes during founder events (see 22 for review), other workers have argued that the well-known genetic processes of mutation and selection may be the most powerful forces creating reproductive isolation (5, 6). When selection acts, gene frequencies can shift quickly, even in large populations. Thus, a shifting selective regime can generate large genetic differences very quickly, even between large populations that are not completely isolated. Given the extensive geographic ranges of many marine species, it is not difficult to imagine environmental gradients that impose differential selection in different areas (108). In fact, these types of environmental gradients have produced some of the best-known examples of selection acting on individual allozyme loci (see above). Thus, speciation can result from the shifting adaptive landscape envisioned by Barton & Charlesworth (7), as populations throughout an extensive geographic range adjust to local selective pressures.

Newly Discovered Mechanisms of Genetic Divergence

Our view of the acrobatics of the genome during divergence has changed substantially since the allopatric model was proposed. Molecular tools have revealed a host of evolutionary mechanisms that might contribute to the divergence of genomes in large and small populations. These mechanisms may act along with selection in large populations to promote genetic differentiation of semi-isolated marine populations.

Transposable elements exist in the genomes of virtually all taxa (36, 51), including marine groups like sea urchins (130). Transposons are short stretches of DNA capable of directing their own replication and insertion through either a DNA or an RNA intermediate. They disrupt genome function by inserting

into otherwise functional genes and can greatly increase mutation rate (136). Yet, although they may reduce fitness, transposable elements can spread rapidly through even a large population (42). For instance, natural populations of *Drosophila melanogaster* throughout the world may have been invaded by transposable "P" elements within a period of 20–30 years (118).

Rose & Doolittle (116) suggested that invasion of allopatric populations by different transposable elements may greatly reduce the fitness of hybrids between populations. This is because the mechanisms that limit the copy number of a particular transposable element in a genome may disappear in hybrids (34), allowing rampant transposition and an increase in mutation rate. Rose & Doolittle could not find an obvious case of species formation by invasion of transposons, but the clear demonstration of hybrid dysgenesis in *Drosophila* shows how the basic mechanism can operate (68, 118).

One of the hallmarks of transposable elements is that they exist in multiple copies throughout the genome. Other gene regions, however, also occur as multiple copies. Even though they do not transpose, they often show extraordinary evolutionary dynamics. For example, the nuclear ribosomal genes are typically found in a long tandem array containing hundreds of copies of this gene cluster (reviewed in 54). Although ribosomal genes tend to be variable between species, the multiple gene clusters within the array tend to be identical to one another. If simple mutation and Mendelian inheritance were the only genetic processes occurring in these clusters, we would expect to find a great deal of variation between gene clusters on a chromosome, perhaps even more than we find between species. However, in general, the tandem clusters of ribosomal genes are remarkably similar.

The process that homogenizes multiple copies of a DNA segment within a population has been called concerted evolution and has been documented for a number of multi-gene families (55). Two mechanisms operate during concerted evolution. Unequal crossing-over changes the number of tandem DNA segments on two homologous chromosomes. Through stochastic processes, this gain and loss of segments will result in extinction of some segments and eventual fixation of one type (31). Hillis et al (55) also showed that biased gene conversion operated in tandem arrays of ribosomal gene clusters. In gene conversion, sequences on one chromosome are used to change the sequence of homologous regions of the second chromosome. Biased gene conversion is the preferential replacement of one type of sequence with another. Dover (31, 32) has pointed out that this mechanism could result in the rapid sweep of a particular sequence through a large population. Termed *molecular drive,* this rapid shift in the properties of a genome could play a role in rapid genetic divergence of large populations during speciation (31). Shapiro (126) lists a suite of genetic mechanisms that might contribute to the reorganization of whole genomes during evolution.

None of these mechanisms (gene conversion, concerted evolution, molecular drive, hybrid dysgenesis, etc) has been strongly implicated in particular speciation events (116), and it has been argued that such mechanisms are unnecessary to explain most cases of speciation (6, 7). Yet, modern genetic research continues to uncover mechanisms, like these, that can substantially remold genomes. Furthermore, some of these changes can spread through populations in a nonmendelian way. As a result, the genetic divergence of populations through mutation, selection, and drift can perhaps be augmented by other types of genetic change. For our purposes, it is enough to point out that these mechanisms operate well in large populations, and that there are a plethora of possible mechanisms for generating large genomic differences in relatively short periods of time.

REPRODUCTIVE ISOLATION IN CLOSELY RELATED SPECIES

The formation of species requires the evolution of reproductive isolation (7, 25, 71, 88). If allopatric populations are brought back together, and no barrier to reproduction exists, then whatever genetic differences had accumulated between isolates will be shared throughout the rejoined population. As a result, understanding marine speciation requires an understanding of reproductive isolation between species. The most illuminating examples are likely to be those in which the isolating mechanisms act between two recently derived species. In these cases we are more likely to be examining changes that occurred during speciation (although it is usually impossible to prove this in any given case).

Mechanisms of Reproductive Isolation

In general, reproductive barriers are classified into pre-zygotic and post-zygotic categories (25). For marine species, post-zygotic isolation is seldom studied because of the difficulty of raising offspring through complex life cycles and through long generation times. However, pre-zygotic mechanisms of reproductive isolation are well studied and fall into several broad types.

MATE PREFERENCE   In terrestrial taxa, mate preferences are known to vary between closely related species (e.g. 30), and this form of reproductive isolation is receiving more attention in marine systems. Snell & Hawkinson (128) found mating preferences among sympatric and allopatric populations of the estuarine rotifer *Branchionus plicatilis,* possibly because of species-specific reaction to a diffusable mating signal (41). Male fiddler crabs (genus *Uca*) engage in elaborate courtship displays in which the single large claw is waved and rapped on the substrate. Although morphological differences between species are often slight, the waving and rapping components of courtship often

differ significantly (119). Other crustaceans such as stomatopods (110), am- phipods (132), and isopods (129) also have complex behavioral mechanisms or chemical detection abilities that may isolate sibling species. The behavioral component of assortative mating is the most important in maintaining isolation among several sympatric species of the isopod genus *Jaera* (129). These differences probably arose during the Pleistocene diversification of this genus (129).

The large claw of alpheid shrimp is used in aggression between males or between females of the same species or between males and females of different species. Species separated by the Isthmus of Panama have quickly become reproductively isolated: Male-female pairs from different species are behav- iorally incompatible (73). Although these pairs have been allopatrically sepa- rated by a land-barrier, there are also sympatric shrimp species that appear to be behaviorally isolated in very similar ways. Thus, the mechanism of repro- ductive isolation so clearly seen across the Isthmus of Panama appears to operate within ocean basins as well.

Weinberg et al (147) showed that this type of behavioral change could be detected on a very small geographic scale. In the polychaete genus *Nereis,* males and females react territorially to members of the same sex but form mated pairs after intersexual encounters. Populations of *N. acuminata* from the Atlantic and Pacific coasts of North America showed strong aggression toward each other when a male and female from opposite coasts were paired (147). Surprisingly, east coast populations separated by only 110 km also showed a significant degree of aggression. The common infaunal polychaete *Capitella* is composed of several cryptic species that are reproductively isolated even when they occur sympatrically (46).

Fish can also show strong behavioral control over mate choice. In the tropical genus *Hypoplectrus* (the hamlets), sibling species are defined on the basis of color pattern differences: Few ecological or morphological distinctions can be found among sympatric species (35). Field observations show that spawning is almost exclusively (~ 95%) between individuals of the same color pattern (35). Work within other species has also shown that females can distinguish males on the basis of their color pattern and that they choose mates using species-specific rules (146). This degree of color discrimination is not always observed, however. Among butterfly fish of the genus *Chaetodon,* sibling species are distinguished by discrete color pattern differences. However, mating occurs randomly between species along a narrow hybrid zone in the Indo-West Pacific (91). In this genus, sibling species are largely allopatric as opposed to the largely sympatric distri- bution of behaviorally isolated hamlets (35).

HABITAT SPECIALIZATION   Reproductive isolation can also be associated with habitat specialization. Recently diverged Baltic Sea species of the amphipod

Gammarus have developed marked differences in salinity tolerance that prevent their hybridization (77). A group of hydroid species that inhabits the shells used by hermit crabs shows strict habitat specialization: Different hydroid species use the shells of different hermit crab species (20). Coral species in the genus *Montastrea* appear to segregate on the basis of depth and light levels (72, 74). Knowlton & Jackson (72) discuss other examples from coral reefs of niche use differentiation among sibling species (see also 71). Species of the isopod *Jaera* (129) show slight habitat segregation, but this mechanism of isolation is thought to be less important than the behavioral isolation discussed above.

SPAWNING SYNCHRONY   Many marine species spawn eggs and sperm into the water column or lay demersal eggs that are fertilized externally. For sedentary invertebrates, fertilization success is a strong function of proximity to another spawning individual (84, 106). As a result, selection for spawning synchrony may occur in these species, and closely related species can be isolated by changes in the timing of spawning. Among three sympatric sea cucumber species in the genus *Holothuria* on the Great Barrier Reef, two show strong, seasonal patterns of spawning (50). In the tropical Pacific, the sea urchin *Diadema savignyi* spawns at full moon. A broadly sympatric species, *D. setosum,* spawns at full moon in some localities but at new moon in others. Where spawning overlaps, hybrids between the two species are common (JS Pearse, personal communication). Species in this genus separated by the rise of the Isthmus of Panama have also diverged in spawning time (81, 82). Examples of sympatric species that show differences in the timing of spawning come from hermit crabs (112), bivalves (15, 109), sponges (63a), coral reef fish (39), and gastropods (140). Knowlton (71) lists 26 examples of spawning asynchrony in cryptic or sibling marine species.

However, differences in the timing of spawning are not ubiquitous among sympatric marine species (3, 50, 71). Hundreds of coral species spawn together on the Great Barrier Reef during a few nights in the summer (3). In temperate habitats, numerous species spawn in the spring, sometimes during mass spawning events (106), perhaps because spawning time is constrained by seasonal availability of planktonic food (56). As a result, other mechanisms of reproductive isolation probably exist to limit cross-fertilization among gametes of different species spawned at the same time.

FERTILIZATION   Fertilization is easily studied in many marine species, and a great deal has been discovered about fertilization mechanisms in these taxa. By contrast, there are relatively few studies of fertilization patterns between closely related species. Nevertheless, the data available suggest a number of generalizations.

Some species pairs fertilize readily in the laboratory when their gametes are mixed together. The sea stars *Asterias forbesi* and *A. vulgaris* occur over a narrow sympatric zone along the northeast coast of North America. There is only slight differentiation in spawning season for these species, and sperm and eggs of both species can cross-hybridize (125). Sea urchins in several genera can also cross with one another (but see below) (83, 103, 134, 135). Certain kelp species distributed in the north and south Atlantic can cross-fertilize (although normal offspring are not always produced—139).

Complete fertilization in hybrid crosses is not the most common result, however. Instead, species that can cross-fertilize often do so incompletely or unidirectionally. That is, the eggs of one species will be receptive to the sperm from the second, but the reverse crosses fail (83, 135, 141). Of the three "successful" crosses performed by Buss & Yund (20) between species in the hydroid genus *Hydractinia,* two showed asymmetric success. Rotifer mating preferences show the same pattern (128). These patterns are remarkably similar to the mate choice asymmetries in insects discussed by Coyne & Orr (26). An interesting but unanswered question is why such similar patterns emerge from biological mechanisms as different as marine fertilization and insect mate choice.

In some taxa, certain species' eggs tend to be "choosier" than others. For example, eggs of the sea urchin *Strongylocentrotus droebachiensis* can be cross-fertilized to a greater degree (134) than the eggs of congeners (they are also more easily fertilized at low concentrations of conspecific sperm; see 84). Eggs of the sea urchin *Colobocentrotus atratus,* which occurs only in intertidal areas with high wave action, also show high cross-fertilizability (16). Again there is an analogy to the literature on mate choice in insects. Species differ in the receptivity of females to heterospecific males. Changes in this receptivity have been hypothesized to be important to rapid species formation (64).

In a few known cases, fertilization barriers are reciprocal and strong. Buss & Yund (20) recorded 6 out of 9 crosses between hydroid species that resulted in less than 5% developing eggs, although in this case it has not been conclusively shown that fertilization failure (as opposed to developmental failure) was the cause of these patterns. Sibling species of the serpulid polychaete *Spirobranchus* show strong reciprocal fertilization barriers (86), producing about 5% developing eggs in interspecific crosses. Crosses between four species of abalone showed low fertilization unless sperm concentrations were 100 times normal. Even under these conditions, only 10–30% of the eggs were fertilized (on average), except in one cross (and in only one direction) which produced 96% fertilization (80). Among Hawaiian sea urchins in the genus *Echinometra,* we have shown that there are strong reciprocal barriers to fertilization (93, 103). This result has been observed for the two species in this

genus on Guam (93), although the species complexes on Okinawa and in the Caribbean show some cases of asymmetric gamete compatibility (83, 141).

Selection for Reproductive Isolation?

A classic problem in speciation research is distinguishing those changes that occur during speciation from those that occur afterwards as a result of reproductive isolation. For example, do mate choice differences arise by random drift between partially or completely isolated populations? Or is there selection for reduced mating between species that are already developmentally incompatible because of evolutionary changes at other loci (88)? Work on species separated by the Isthmus of Panama has shown that reproductive isolation at the fertilization (83) and mate recognition levels (73) can arise without contact between newly formed species. These changes, especially incomplete, asymmetric barriers to fertilization, are probably due to random drift of these characters in isolated populations (83). They cannot be due to selection for reproductive isolation because these species have been geographically isolated since their initial separation in the Pliocene (82). Interestingly, the patterns of mate recognition and fertilization failure seen across Panama are also seen between sympatric species in the Caribbean, the eastern Pacific, and the tropical Pacific (73, 83, 103, 141). This suggests that reproductive divergence without reinforcing selection has occurred in at least some of these cases, or that the signatures of selection and random divergence are remarkably similar.

Some of the strongest evidence for the operation of selection in reducing hybridization comes from comparison of allopatric and sympatric species in the same species group (26), but this type of evidence is rare in marine taxa. Snell & Hawkinson (128) showed that sympatric populations of *Brachionis* rotifers had stronger mating discrimination than did allopatric populations; this is consistent with the idea of selective reinforcement of reproductive barriers. Isopod species exhibit a slight pattern in this direction—the average hybridization rate for females from sympatric populations is roughly half that of allopatric species—but males show no geographic effects (Ref. 129, Table 1). Sympatric species of alphaeid shrimp are more different morphologically than are allopatric species with the same degree of genetic divergence (N Knowlton, personal communication), suggesting that selection is acting differently in sympatric vs allopatric comparisons.

Examples in which an allopatric/sympatric difference is not seen strongly include urchins (83) and butterfly fish (91). In the latter case, species with overlapping distributions show less discrimination in mate choice experiments than do species that are allopatric. These divergent results suggest that selection for increased or decreased reproductive isolation may occur in marine systems,

but it is not ubiquitous, and significant isolation can evolve over reasonably short time periods without it.

GENETICS OF REPRODUCTIVE ISOLATION—A MISSING LINK

The preceding pages highlight the large amount of information on mechanisms of genetic divergence of marine populations. Likewise, there have been many studies of the ways in which recently diverged marine species have become reproductively isolated. But a large gap remains between these two types of information. We know why genetic change might take place, but not how these changes affect reproductive isolation. We know what types of physiological, ecological, or sensory changes give rise to reproductive isolation, but not which genetic changes have produced them. The link between genetics and reproductive isolation is largely missing.

Recently, interest has increased in genetic divergence of particular loci that are strongly involved in reproductive isolation and species recognition (25). For some systems, it has been possible to examine the evolution of proteins that are involved in creating barriers to gene flow. For example, two genes are involved in the control of reproductive season in some insects (137). Coyne (25) lists other examples in which only a few loci affect reproductive isolation; but even for terrestrial taxa, the list is very short.

Mechanisms of Reproductive Isolation and the Evolution of Recognition

Part of the reason for this lack of understanding is that studies of reproductive isolation are phenomenological: They describe the interactions of individuals within and between species in order to detect the phenomenon of reproductive isolation. This approach is sufficient to understand the nature of species differentiation, but it does not explain the mechanisms of reproductive isolation and leaves open the question of how those mechanisms evolved. Reproductive isolation can involve many different molecular, physiological, or sensory systems (see above for just a few examples), and so it is difficult to generalize from one isolation mechanism to another.

A few aspects of reproductive isolation, however, can be investigated at all levels (molecular, physiological, sensory) and in practically all taxa. One such aspect is recognition, the means by which individuals recognize members of the same species and distinguish them from other individuals in the environment. Most modes of reproductive isolation involve some component of recognition, except for strict allopatry (e.g. the Isthmus of Panama) or strong habitat selection (77).

Surprisingly, very little is known about the recognition process in marine

species. Some fish are thought to use color patterns as a mating cue (35, 91). Limb vibrations appear to be part of the courtship process in some crustaceans (110, 119, 129). A diffusable pheromone can induce mating behavior in rotifers (128). Bioluminescent ostracods may broadcast their identity with patterns of bioluminescence (J Morin, personal communication). Sperm attraction to eggs has been documented in a large number of invertebrate taxa (see for example 94a). In none of these systems has the genetics of recognition been determined, in part because of the potentially complex nature of genetic control over some of these recognition processes.

Evolutionary studies of recognition that have been performed to date have focused on simple interactions that are amenable to genetic analysis. Examples include the *per* locus effects on mate signaling in *Drosophila* (148), the S-allele system that mediates self-incompatibility in plants (23), the mating type loci in protozoa (94), and loci governing fertilization in marine invertebrates (101, 142).

Although these studies involve only a few simple examples, they are derived from a range of taxa. Thus, it is interesting that there are so many similarities at the genetic level. Comparisons of amino acid sequences for proteins involved in gamete or mating type discrimination reveal a general pattern of large differences among species (23, 79, 94, 148) or among alleles within species (23, 93). These differences often exceed those predicted on the basis of silent changes in these proteins, and so they appear to reflect the action of some type of selection for variation sensu Hughes & Nei (59, 23, 79). The nature of these selective forces is well understood for self-incompatibility in plants, because in these systems there is strong selection for heterozygosity (151). By contrast, the existence of similar selective forces is unclear for marine invertebrates.

An additional problem is that reproductive isolation is unlikely to be caused by large differences at a single genetic locus. Dobzhansky (29), Mayr (88), and many other evolutionary biologists have pointed out that if reproductive isolation is caused by a single, large, dominant mutation at a single locus, then the individuals possessing that mutation cannot breed and so have zero fitness. A recessive mutation might drift in a population until it is expressed in many individuals at the same time, but this mechanism is likely to operate well only in small populations. Orr (99) discussed the impact of maternal inheritance on this result.

However, recognition loci seldom act alone. In most cases there are both signals and signal receptors, and these are likely to be produced through the action of different loci (self-incompatibility systems of plants and protists are major exceptions in that they involve only a single locus—23, 94). Where recognition occurs because of the interaction of at least two loci, mathematical models have shown how polymorphisms can be maintained within populations (70) and lead to reproductive divergence (78, 152). In general, polymorphisms

are maintained if individuals with a particular allele at one locus prefer mates with a particular allele at the other locus. If there are multiple combinations of these matched alleles, they can be stable within populations (152; SR Palumbi, submitted). This type of model, developed to understand sexual selection, has seldom been applied to marine systems. As a result, the application of these results to concrete examples of speciation of marine organisms is lacking.

However, marine species have provided some of the best mechanistic views of the recognition process. This is because many marine invertebrates spawn eggs and sperm into the water. In these taxa, complex pre-mating behavioral differences are limited to the interactions of short-lived gametes, avoiding the complex behavioral genetics that might dominate reproductive isolation in many vertebrates or arthropods. For species that spawn at the same time (e.g. the hundreds of coral species that mass spawn in the Pacific—3), or for temperate invertebrates that are slaved to strong seasonal reproduction (106), such gamete interactions may determine levels of hybridization between species, or determine fertilization success within species.

Two gamete recognition systems have received the most attention. In abalone, sperm penetrate the outer chorion layer through the action of a protein called lysin (142). Although the mechanisms of lysin action are obscure, the protein has been purified and its secondary structure determined (127). Lysins act efficiently only on the chorion coatings of their own species (142), and fertilizations between abalone species are low except at high sperm concentration (80). Amino acid sequences of lysins from several species show high ratios of replacement to silent site changes (79) as discussed above. Furthermore, the areas of high amino acid replacement occur along a part of the protein that appears, from the crystalline structure, to play an important functional role (127).

The other well-studied system is the fertilization mechanism of sea urchins. In this class, a sperm protein called bindin attaches sperm to the vitelline coat of eggs and promotes egg-sperm fusion (37, 44). Bindin is expressed only in sperm where it occurs in a tightly packed vesicle. After the acrosome reaction, bindin coats the outside of the acrosomal process. The mature bindin shows a central area of high amino acid conservation: 95% of the amino acids are conserved between urchins separated by 150–200 million years. By contrast, the flanking regions both show large sequence differences between species (40, 43, 96)

A series of detailed experiments has failed to show a simple relationship between bindin sequence and attachment of bindin to the egg receptors. Although small pieces of the bindin protein, synthesized as peptides, can show species specificity (97), there is no single substitution that alters overall bindin-egg interactions (85). Between very closely related sea urchins that show

gamete incompatibility, we have shown multiple amino acid substitutions, many clustered in a short region of the protein (101), as well as a suite of insertion/deletion events. Interestingly, many bindin alleles occur in the species we have studied, and these alleles differ from one another in ways that are qualitatively similar to the differences we have seen between species (high amino acid substitution, plus rampant insertion/deletion events) (E Metz, SR Palumbi, in preparation). These results suggest that there is a continuum of bindin function and that differences in amino acid sequence accumulate to give rise to more and more differentiated sperm-egg binding properties.

Of course, the egg receptor plays an equally critical role in this process. Recently, the gene for the sea urchin egg receptor has been isolated and sequenced (38). Although comparative sequence data are not yet available for a large number of species, preliminary results suggest that the extracellular component of the egg receptor is highly variable between species (38). The ability to analyze both sperm attachment and egg receptor proteins in sea urchins makes this system particularly interesting in the analysis of gamete interactions.

Directions for Future Research

One of the largest gaps in our knowledge about speciation remains the link between genetic divergence and mechanisms of reproductive isolation (25). Even in systems amenable to formal genetics, like *Drosophila,* an understanding of the genetics of speciation is only slowly emerging (25).

Unfortunately, for many species it is not possible to perform the genetic miracles that are commonplace in a *Drosophila* laboratory. Marine species are especially difficult to raise because of long generation times, complex life cycles, or obscure mating requirements. In terrestrial systems, these limitations are sometimes overcome by using the natural laboratories of hybrid zones to illuminate the genetic nature of species boundaries. Such studies are rare in marine systems (see 12, 91) but may be profitably used in the future.

A complementary approach is suggested by recent successes in understanding the evolutionary genetics of gamete recognition proteins. Here, a physiological process (gamete binding and fusion) was explored with the full power of the modern molecular toolbox, and the genes responsible for the phenomenon were isolated. Without formal genetics, these studies have shown the importance of particular modes of molecular evolution to the evolution of species recognition.

For some marine taxa, this approach is especially appealing because the simple mating dynamics of free-spawning invertebrates eliminates many of the complexities of mate choice in behaviorally complex vertebrates or arthropods. These simple mating cues (e.g. the mating pheromone of rotifers—128

or simple gamete recognition processes—104) allow the possibility of unraveling reproductive isolation at the genetic level.

Perhaps osmoregulatory differences in amphipods, or chemosensory systems in rotifers, or visual pigment differences in fish could be understood in terms of the gene products that create the physiological, ecological, or sensory differences responsible for currently recognized patterns of reproductive isolation. Although such research is technically difficult and may not uncover *all* the genes responsible for reproductive isolation, this approach can serve as a strong alternative to the study of the genetics of reproductive isolation.

CONCLUSIONS

Although examples of genetic homogeneity over large distances are common in marine systems, there are also many examples of population structure in marine species with high dispersal potential. Such species probably do not "see" the ocean as a single, undifferentiated habitat, either because of environmental differences among localities or because of a large number of physical mechanisms known to produce at least partial isolation between populations. Genetic divergence within these partially isolated gene pools is probably not as slow as thought originally. Various mechanisms exist to generate genetic differences between large isolated populations. Some of these mechanisms include evolutionary processes that have only recently been recognized, whereas others include fairly standard applications of selection theory. Finally, history has played a strong role in the development of marine biogeographic patterns. Cycles of sea level rise-and-fall during the Pleistocene have affected near-shore marine communities, and these cycles were probably exacerbated by the steepening of latitudinal thermal gradients. As a result, even populations that are well connected today by gene flow may have been isolated in the very recent past.

The link between genetic divergence of populations and reproductive isolation is poorly known for marine (or terrestrial) species. How do genetic changes lead to the physiological, ecological, or sensory differences that define sibling species? How do they create reproductive isolation? What are the mechanisms by which species recognition evolves? Studies of gamete recognition show how a focus on the mechanisms of reproductive isolation can lead to the discovery of the genes responsible for species recognition.

This suggests a general approach to speciation research that is based on investigations of the physiological, ecological, and sensory differences that give rise to species recognition and perhaps to reproductive isolation. Such investigations would lead to increased understanding of the underlying genetic mechanisms by which recognition evolves within and between species, and

they provide important evidence to help fill major gaps in our understanding of speciation.

Literature Cited

1. Avise JC. 1992. Molecular population structure and biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos* 63:62–76

2. Avise JC, Helfman GS, Saunders NC, Hales LS. 1986. Mitochondrial DNA differentiation in North Atlantic eels: Population genetic consequences of an unusual life history pattern. *Proc. Natl. Acad. Sci. USA* 83:4350–54

3. Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, et al. 1986. Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. *Mar. Biol.* 90:379–94

4. Baker CS, Perry A, Abernethy B, Alling A, Bannister J, et al. 1993. Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proc. Natl. Acad. Sci. USA* 90:8239–43

5. Banse K. 1986. Vertical distribution and horizontal transport of planktonic larvae of echinoderms and benthic polychaetes in an open coastal sea. *Bull. Mar. Sci.* 39:162–75

6. Barton NH. 1989. Founder effect speciation. In *Speciation and Its Consequences*, ed. D Otte, JA Endler, pp. 229–56. Sunderland, Mass: Sinauer. 679 pp.

7. Barton NH, Charlesworth B. 1984. Genetic revolutions, founder effects and speciation. *Annu. Rev. Ecol. Syst.* 15:133–64

8. Benzie JA, Stoddart JA. 1992. Genetic structure of crown-of-thorns starfish (*Acanthaster planci*) in Australia. *Mar. Biol.* 112:631–39

9. Berger EM. 1973. Gene-enzyme varia-
tion in three sympatric species of *Littorina. Biol. Bull.* 145:83–90

10. Berger EM. 1977. Gene-enzyme variation in three sympatric species of *Littorina*. II. The Roscoff population with a note on the origin of North American *Littorina. Biol. Bull.* 153:255–64

11. Bernardi G, Sordino P, Powers DA. 1993. Concordant mitochondrial and nuclear DNA phylogenies for populations of the teleost fish *Fundulus heteroclitus. Proc. Natl. Acad. Sci. USA* 90:9271–74

12. Bert TM. 1986. Speciation in western Atlantic stone crabs (genus *Menippe*): the role of geological processes and climatic events in the formation and distribution of species. *Mar. Biol.* 93: 157–70

13. Bert TM, Harrison RG. 1988. Hybridization in western Atlantic stone crabs (genus *Menippe*): evolutionary history and ecological context influence species interactions. *Evolution* 42:528–44

14. Bowen BW, Meylan AB, Avise JC. 1989. An odyssey of the green sea turtle: Ascension Island revisited. *Proc. Natl. Acad. Sci USA* 86:573–76

15. Boyden CR. 1971. A comparative study of the reproductive cycles of the cockles *Cerastoderma edule* and *C. glaucum. J. Mar. Biol. Assoc. UK* 51:605–22

16. Branham JM. 1972. Comparative fertility of gametes from six species of sea urchins. *Biol. Bull.* 142:385–96

17. Brothers EB, Williams DM, Sale PF. 1983. Length of larval life in twelve families of fishes at "One Tree Lagoon", Great Barrier Reef, Australia. *Mar. Biol.* 76:319–24

18. Buroker NE. 1983. Population genetics of the American oyster *Crassostrea*

virginica along the Atlantic coast and the Gulf of Mexico. *Mar. Biol.* 75:99–112

19. Burton RS, Feldman MW. 1982. Population genetics of coastal and estuarine invertebrates: does larval behavior influence population structure? In *Estuarine Comparisons*, ed. VS Kennedy, pp. 537–51. New York: Academic. 709 pp.

20. Buss LW, Yund PO. 1989. A sibling species group of *Hydractinia* in the northeastern United States. *J. Mar. Biol. Assoc. UK* 69:857–74

21. Carson HL. 1982. Speciation as a major reorganization of polygenic balances. In *Mechanisms of Speciation*, ed. C Barigozzi, pp. 411–33. New York: Liss. 546 pp.

22. Carson HL, Templeton AR. 1984. Genetic revolutions in relation to species phenomena: The founding of new populations. *Annu. Rev. Ecol. Syst.* 15:97–131

23. Clark AG, Kao T-H. 1991. Excess nonsynonymous substitution at shared polymorphic sites among self-incompatibility alleles of Solanaceae. *Proc. Natl. Acad. Sci. USA* 88:9823–27

24. CLIMAP Project. 1976. The surface of the ice age earth. *Science* 191:1131–37

25. Coyne JA. 1992. Genetics and speciation. *Nature* 355:511–15

26. Coyne JA, Orr HA. 1989. Patterns of speciation in *Drosophila*. *Evolution* 43:362–81

27. Cronin TW, Forward RB. 1986. Vertical migration cycles of crab larvae and their role in larval dispersal. *Bull. Mar. Sci.* 39:192–201

27a. Cronin TW, Ikeya N. 1990. Tectonic events and climatic change: Opportunities for speciation in cenozoic marine ostracoda. In *Causes of Evolution: A Paleontological Perspective*, ed. RM Ross, WD Allmon. Chicago: Univ. Chicago Press. 479 pp.

28. Darwin CR. 1872. *On the Origin of Species by Natural Selection*. New York: Appleton. 5th ed. 447 pp.

29. Dobzhansky TH. 1937. *Genetics and the Origin of Species*. Reprinted 1982. New York: Columbia Univ. Press. 364 pp.

30. Dobzhansky TH. 1970. *Genetics of the Evolutionary Process*. New York: Columbia Univ. Press. 505 pp.

31. Dover G. 1982. Molecular drive, a cohesive mode of species evolution. *Nature* 299:111–17

32. Dover G. 1989. Linkage disequilibrium and molecular drive in the rDNA gene family. *Genetics* 122:249–52

33. Emlet RB, McEdward LR, Strathmann RR. 1987. Echinoderm larval ecology

viewed from the egg. *Echinoderm Stud.* 2:55–136

34. Engels WR. 1981. Hybrid dysgenesis in *Drosophila* and the stochastic loss hypothesis. *Cold Spring Harbor Symp. Quant. Biol.* 45:561–65

35. Fischer EA. 1980. Speciation in the Hamlets (*Hypoplectrus:* Serranidae)—a continuing enigma. *Copeia* 1980:649–59

36. Flavell AJ. 1992. *Ty1-copia* group retrotransposons and the evolution of retroelements in the eukaryotes. *Genetics* 86:203–14

37. Foltz KR, Lennarz WJ. 1993. The molecular basis of sea urchin gamete interactions at the egg plasma membrane. *Dev. Biol.* 158:46–61

38. Foltz KR, Partin JS, Lennarz WJ. 1993. Sea urchin egg receptor for sperm: sequence similarity of binding domain and hsp70. *Science* 259:1421–25

39. Foster SA. 1987. Diel and lunar patterns of reproduction in the Caribbean and Pacific segeant major damselfishes *Abudefduf saxatilis* and *A. troschelii.* *Mar. Biol.* 95:333–43

40. Gao B, Klein LE, Britten RJ, Davidson EH. 1986. Sequence of mRNA coding for bindin, a species-specific sea urchin sperm protein required for fertilization. *Proc. Natl. Acad. Sci. USA* 83:8634–38

41. Gilbert JJ. 1963. Contact chemoreceptors, mating behavior and sexual isolation in the rotifer genus *Brachionus*. *J. Exp. Biol.* 40:625–41

42. Ginzburg LR, Bingham PM, Voo S. 1984. On the theory of speciation induced by transposable elements. *Genetics* 107:331–41

43. Glabe CG, Clark D. 1991. The sequence of the *Arbacia punctulata* bindin cDNA and implications for the structural basis of species-specific sperm adhesion and fertilization. *Dev. Biol.* 143:282–88

44. Glabe CG, Vacquier VD. 1977. Species-specific agglutination of eggs by bindin isolated from sea urchin sperm. *Nature* 267:836–38

45. Gould SJ. 1982. Introduction. See Ref. 19

46. Grassle JF, Maciolek NJ. 1992. Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples. *Am. Nat.* 139:313–41

47. Grassle JP, Grassle JF. 1976. Sibling species in the marine pollution indicator *Capitella* (Polychaeta). *Science* 192:567–69

48. Grigg RW. 1988. Paleooceanography of coral reefs in the Hawaii-Emporer chain. *Science* 240:1737–43

49. Hansen TA. 1983. Modes of larval de-

velopment and rates of speciation in early tertiary neogastropods. *Science* 220:501–2

50. Harriot VJ. 1985. Reproductive biology of three congeneric sea cucumber species, *Holothuria atra, H. impatiens,* and *H. edulis* at Heron Island, Great Barrier Reef. *Aust. J. Mar. Freshwater Res.* 36:51–57

51. Hartl DL, Lozovskaya ER, Lawrence JG. 1992. Nonautonomous transposable elements in prokaryotes and eukaryotes. *Genetica* 86:47–53

52. Hateley JG, Sleeter TD. 1993. A biochemical genetic investigation of spiny lobster (*Panulirus argus*) stock repleneshment in Bermuda. *Bull. Mar. Sci.* 52:993–1006

53. Hilbish TJ, Koehn RK. 1985. The physiological basis of natural selection at the LAP locus. *Evolution* 39:1302–17

54. Hillis DM, Dixon MT. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. *Q. Rev. Biol.* 66:411–53

55. Hillis DM, Moritz C, Porter CA, Baker RJ. 1991. Evidence for biased gene conversion in concerted evolution of ribosomal DNA. *Science* 251:308–10

56. Himmelman JH. 1979. Factors regulating the reproductive cycles of two Northeast Pacific chitons *Tonicella lineata* and *T. insignis. Mar. Biol.* 50: 215–25

57. Hough AR, Naylor E. 1992. Biological and physical aspects of migration in the estuarine amphipod *Gammarus zaddachi. Mar. Biol.* 112:437–43

58. Hourighan TF, Reese ES. 1987. Mid-ocean isolation and the evolution of Hawaiian reef fishes. *Trends Ecol. Evol.* 2:187–91

59. Hughes AL, Nei M. 1988. Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals over dominant selection. *Nature* 335:167–70

60. Incze LS, Ortner PB, Schumacher JD. 1990. Microzooplankton, vertical mixing and advection in a larval fish patch. *J. Plankton Res.* 12:365–79

61. Jablonski D, Flessa K, Valentine JW. 1985. Biogeography and paleobiology. *Paleobiology* 11:75–90

62. Jackson JBC. 1986. Modes of dispersal of clonal benthic invertebrates: Consequences for species' distributions and genetic structure of local populations. *Bull. Mar. Sci.* 39:588–606

63. Johannesson K. 1988. The paradox of Rockall: Why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dis-persal stage (*L. littorea*)? *Mar. Biol.* 99:507–13

63a. Johnson MF. 1978. Studies of the reproductive cycles of the calcareous sponges *Clathrina cortiacea* and *C. blanca. Mar. Biol.* 50:73–79

64. Kaneshiro KY. 1980. Sexual solation, speciation and the direction of evolution. *Evolution* 34:437–44

65. Karl SA, Avise JC. 1992. Balancing selection at allozyme loci in oysters: Implications from Nuclear RFLPs. *Science* 256:100–2

66. Kay EA. 1983. Patterns of speciation in the Indo-West Pacific. In *Biogeography of the Tropical Pacific,* ed. FJ Radovsky, PH Raven, SH Sohmer, 72:15–31. Honolulu: Bishop Mus. Press. 221 pp.

67. Kay EA, Palumbi SR. 1987. Endemism and evolution in Hawaiian marine invertebrates. *Trends Ecol. Evol.* 2:183–87

68. Kidwell M. 1982. Hybrid dygenesis in *Drososphila melanogaster.* A syndrome of aberrant traits inducing mutation, sterility and male recombination. *Genetics* 86:813–33

69. Kimura M. 1979. Model of effectively neutral mutations in which selective constraint is incorporated. *Proc. Natl. Acad. Sci. USA* 76:3440–44

70. Kirkpatrick M. 1982. Sexual selection and the evolution of female choice. *Evolution* 36:1–12

71. Knowlton N. 1993. Sibling species in the sea. *Annu. Rev. Ecol. Syst.* 24:189–216

72. Knowlton N, Jackson JBC. 1994. New taxonomy and niche partitioning on coral reefs: jack of all trades or master of some? *Trends Ecol. Evol.* 9:7–9

73. Knowlton N, Weigt LA, Solorzano LA, Mills DK, Bermingham E. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* 260: 1629–32

74. Knowlton N, Weil E, Weigt LA, Guzman HM. 1992. Sibling species in *Montastraea annularis,* coral bleaching, and the coral climate record. *Science* 255:330–33

75. Koehn RK, Newell RI, Immerman F. 1980. Maintenance of an aminopeptidase allele frequency cline by natural selection. *Proc. Natl. Acad. Sci. USA* 77:5385–89

76. Kohn AJ. 1981. *Conus kahiko,* a new Pleistocene gastropod from Oahu, Hawaii. *J. Paleontol.* 54:534–41

77. Kolding S. 1985. Genetic adaptation to local habitats and speciation process within the genus *Gammarus* (Amphipoda: Crustacea). *Mar. Biol.* 89: 249–55

78. Lande R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. USA* 78:3721-25

79. Lee YH, Vacquier VD. 1992. The divergence of species-specific abalone sperm lysins is promoted by positive Darwinian selection. *Biol. Bull.* 182:97-104

80. Leighton DL, Lewis CA. 1982. Experimental hybridization in abalone. *Int. J. Inverbr. Reprod. Dev.* 5:273-82

81. Lessios HA. 1981. Reproductive periodicity of the echinoids *Diadema* and *Echinometra* on the two coasts of Panama. *J. Exp. Mar. Biol. Ecol.* 50:47-61

82. Lessios HA. 1984. Possible prezygotic reproductive isolation in sea urchins separated by the Isthmus of Panama. *Evolution* 38:1122-48

83. Lessios HA, Cunningham CW. 1990. Gametic incompatibility between species of the sea urchin *Echinometra* on the two sides of the Isthmus of Panama. *Evolution* 44:933-41

84. Levitan DR. 1993. The importance of sperm limitation to the evolution of egg size in marine invertebrates. *Am. Nat.* 141:517-36

85. Lopez A, Miraglia SJ, Glabe CG. 1993. Structure/function analysis of the sea urchin sperm adhesive protein bindin. *Dev. Biol.* 156:24-33

86. Marsden J. 1992. Reproductive isolation in two forms of the serpulid polychaete, *Spirobranchus polycerus* (Schmarda) in Barbados. *Bull. Mar. Sci.* 51:14-18

87. Maynard NG, 1976. The relationship between diatoms in the surface sediments of the Atlantic Ocean and the biological and physical oceanography of overlying waters. *Paleobiology* 2:91-121

88. Mayr E. 1942. *Systematics and the Origin of Species*. Reprinted 1982. New York: Columbia Univ. Press. 334 pp.

89. Mayr E. 1954. Geographic speciation in tropical echinoids. *Evolution* 8:1-18

90. McGowan JA, Walker PW. 1985. Dominance and diversity maintenance in an oceanic ecosystem. *Ecol. Monogr.* 55:113-18

91. McMillan WO. 1994. *Speciation, species boundaries, and the population biology of Indo-West Pacific butterflyfishes (Cheatodontidae)*. PhD thesis. Dep. Zool., Univ. Hawaii, Honolulu, HI

92. McMillen-Jackson AL, Bert TM, Steele P. 1994. Population genetics of the blue crab (*Callinectes sapidus* Rathbun): modest populational structuring in a background of high gene flow. *Mar. Biol.* 118:53-65

93. Metz EC, Kane RE, Yanagimachi H, Palumbi SR. 1994. Fertilization between closely related sea urchins is blocked by incompatibilities during sperm-egg attachment and early stages of fusion. *Biol. Bull.* In press

94. Miceli C, La Terza A, Bradshaw RA, Luporini P. 1992. Identification and structural characterization of cDNA clone encoding a membrane-bound form of the polypeptide pheromone Er-1 in the ciliate protozoan *Euplotes raikovi*. *Proc. Natl. Acad. Sci. USA* 89:1988-92

94a. Miller RL. 1985. Demonstration of sperm chemotaxis in echinodermata: Asteroidea, Holothuroidea, Ophiuroidea. *J. Exp. Zool.* 234:383-414

95. Miller SE, Hadfield MG. 1990. Developmental arrest during larval life extends life span in a marine mollusc. *Science* 248:356-58

96. Minor J, Gao B, Davidson E. 1989. The molecular biology of bindin. In *The Molecular Biology of Fertilization*, ed. H Schatten, G Schatten, pp. 73-88. San Diego: Academic. 384 pp.

97. Minor JE, Britten RJ, Davidson EH. 1993. Species-specific inhibition of fertilization by a peptide derived from the sperm protein bindin. *Mol. Biol. Cell* 4:375-87

98. Nishida M. Lucas JS. 1988. Genetic differences between geographic populations of the crown-of-thorns starfish throughout the Pacific region. *Mar. Biol.* 98:359-68

99. Orr HA. 1991. Is single-gene speciation possible? *Evolution* 45:764-69

100. Ovenden JR, Brasher DJ, White RW. 1992. Mitochondrial DNA analyses of the red rock lobster *Jasus edwardsii* supports an apparent absence of population subdivision throughout Australasia. *Mar. Biol.* 112:319-26

101. Palumbi SR. 1992. Marine speciation on a small planet. *Trends Ecol. Evol.* 7:114-18

102. Palumbi SR, Kessing B. 1991. Population biology of the trans-arctic exchange: mtDNA sequence similarity between Pacific and Atlantic sea urchins. *Evolution* 45:1790-805

103. Palumbi SR, Metz E. 1991. Strong reproductive isolation between closely related tropical sea urchins (genus *Echinometra*). *Mol. Biol. Evol.* 8:227-39

104. Palumbi SR, Wilson AC. 1989. Mitochondrial DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. *Evolution* 44:403-15

105. Paulay G. 1990. Effects of late Cenozoic sea-level fluctuations on the bivalve fau-

nas of tropical oceanic islands. *Paleobiology* 16:415–34

106. Pennington JT. 1985. The ecology of fertilization of echinoid eggs: The consequences of sperm dilution, adult aggregation, and synchronous spawning. *Biol. Bull.* 169:417–30

107. Phillips BF, McWIlliam PS. 1986. The pelagic phase of spiny lobster development. *Can. J. Fish. Aquat. Sci.* 43:2153–63

108. Powers DA. 1987. A multidisciplinary approach to the study of genetic variation within species. In *New Directions in Physiological Ecology*, ed. ME Feder, A Bennet, W Burggren, RB Huey, pp. 38–70. Cambridge, UK: Univ. Cambridge Press. 364 pp.

109. Rae JG. 1978. Reproduction in two sympatric species of *Macoma* (Bivalvia). *Biol. Bull.* 155:207–29

110. Reaka ML, Manning RB. 1981. The behavior of stomatopod crustacea and its relationship to rates of evolution. *J. Crustacean Biol.* 1:309–27

111. Reeb CA, Avise JC. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. *Genetics* 124:397–406

112. Reese ES. 1968. Annual breeding seasons of three sympatric species of hermit crabs, with a discussion of the factors controlling breeding. *J. Exp. Mar. Biol. Ecol.* 2:308–18

113. Rex MA, Stuart CT, Hesler RR, Allen JA, Sanders HL, Wilson GDF. 1993. Global scale latitudinal patterns of species diversity in the deep-sea benthos. *Nature* 365:636–39

114. Richmond RH. 1987. Energetics, competency, and long distance dispersal of planula larvae of the coral *Pocillipora damicornis*. *Mar. Biol.* 93:527–33

115. Richmond RH. 1990. The effects of the El Niño/Southern oscillation on the dispersal of corals and other marine organisms. In *Global Ecological Consequences of the 1982–83 El Niño-Southern Oscillation*, ed. PW Glynn, pp. 127–40. Amsterdam: Elsevier. 563 pp.

116. Rose MR, Doolittle F. 1983. Molecular biological mechanisms of speciation. *Science* 220:157–62

117. Rosenblatt RH. 1963. Some aspects of speciation in marine shore fishes. In *Speciation in the Sea*, pp. 171–80. *Syst. Assoc. Special Publ. 5*

118. Rubin GM, Kidwell MG, Bingham PM. 1982. The molecular basis of P-M hybrid dysgenesis: The nature of induced mutations. *Cell* 29:987–94

119. Salmon M, Ferris SD, Johnston D, Hyatt

G, Whitt GS. 1979. Behavioral and biochemical evidence for species distinctiveness in the fiddler crabs, *Uca speciosa* and *U. spinicarpa. Evolution* 33:182–91

120. Saunders NC, Kessler LG, Avise JC. 1985. Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphemus. Genetics* 112:613–27

121. Scheltema RS. 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shoal-water benthic marine gastropods. *Biol. Bull.* 140:284–322

122. Scheltema RS. 1986. On dispersal and planktonic larvae of benthic invertebrates: An eclectic overview and summary of problems. *Bull. Mar. Sci.* 39: 290–322

123. Scheltema RS, Williams IP. 1983. Long distance dispersal of planktonic larvae and the biogeography and evolution of some Polynesian and Western Pacific molluscs. *Bull. Mar. Sci.* 33:545–65

124. Schopf TJM. 1979. The role of biogeographic provinces in regulating marine faunal diversity through geologic time. In *Historical Biogeography, Plate Tectonics, and the Changing Environment*, ed. J Gray, AJ Boucot. pp. 449–57. Corvallis: Oregon State Univ. Press. 500 pp.

125. Schopf TJM, Murphey LS. 1973. Protein polymorphism of the hybridizing seastars *Asterias forbesi* and *Asterias vulgaris* and implications for their evolution. *Biol. Bull.* 145:589–97

126. Shapiro JA. 1992. Natural genetic engineering in evolution. *Genetica* 86:99–111

127. Shaw A, McRee DE, Vacquier VD, Stout CD. 1993. The crystal structure of lysin, a fertilization protein. *Science* 262:1864–67

128. Snell TW, Hawkinson CA. 1983. Behavioral reproductive isolation among populations of the rotifer *Branchionus plicatilis. Evolution* 37:1294–305

129. Solignac M. 1981. Isolating mechanisms and modalities of speciation in the *Jaera albifrons* species complex (Crustacea, Isopoda). *Syst. Zool.* 30:387–405

130. Springer M, Davidson EH, Britten RJ. 1991. Retroviral-like element in a marine invertebrate. *Proc. Natl. Acad. Sci. USA* 88:8401–4

131. Springer V. 1982. Pacific plate biogeography with special reference to shorefishes. *Smithson. Contrib. Zool.* 367:1–182

132. Stanhope MJ, Connelly MM, Hatwick B. 1992. Evolution of a crustacean com-

munication channel: behavioral and ecological genetic evidence for a habitat-modified, race specific pheromone. *J. Chem. Ecol.* 18:1871–87

133. Stanley SM. 1986. Population size, extinction, and speciation: the fission effect in Neogene Bivalvia. *Paleobiology* 12:89–110

134. Strathmann M. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast.* Seattle: Univ. Wash. Press. 670 pp.

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

136. Syvanen M. 1984. The evolutionary implications of mobile genetic elements. *Annu. Rev. Genet.* 18:271–93

137. Tauber CA, Tauber MJ, Necholo JR. 1977. Two genes control seasonal isolation in sibling species. *Science* 197:592–93

138. Templeton AR. 1982. Mechanisms of speciation—a population genetic approach. *Annu. Rev. Ecol. Syst.* 12:23–48

138a. Todd CD, Lambert WJ. 1993. *Population genetics of intertidal nudibranch molluscs: Does a planktonic larva confer dispersal?* Presented at Larval Ecol. Meet., Port Jefferson, NY

139. tom Dieck (Bartsch) I, de Oliveira EC. 1993. The section Digitatae of the genus *Laminaria* (Phaeophyta) in the northern and southern Atlantic: crossing experiments and temperature responses. *Mar. Biol.* 115:151–60

140. Tutschulte T, Connell JH. 1981. Reproductive biology of three species of abalones (Haliotis) in southern California. *Veliger* 23:195–206

141. Uehara T, Shingaki M. 1984. Studies on the fertilization and development in the two types of *Echinometra mathaei* from Okinawa. *Zool. Sci.* 1:1008

142. Vacquier VD, Carner KR, Stout CD. 1990. Species specific sequences of ab-alone lysin, the sperm protein that creates a hole in the egg envelope. *Proc. Natl. Acad. Sci. USA* 87:5792–96

143. Valentine JW. 1973. *Evolutionary Paleoecology of the Marine Biosphere.* Englewood Cliffs, NJ: Prentice-Hall

144. Vermeij GJ. 1987. *Evolution and Escalation: An Ecological History of Life.* Princeton, NJ: Princeton Univ. Press. 527 pp.

145. Vermeij GJ. 1987. The dispersal barrier in the tropical Pacific: implications for molluscan speciation and extinction. *Evolution* 41:1046–58

146. Warner RR, Schultz ET. 1992. Sexual selection and male characteristics in the bluehead wrasse, *Thalassoma bifasciatum*: Mating site acquisition, mating site defense, and female choice. *Evolution* 46:1421–42

147. Weinberg JR, Starczak VR, Mueller C, Pesch GC, Lindsay SM. 1990. Divergence between populations of a monogamous polychaete with male parental care: premating isolation and chromosome variation. *Mar. Biol.* 107:205–13

148. Wheeler DA, Kyriacou CP, Greenacre ML, Yu Q, Rutila JE, et al. 1991. Molecular transfer of a species-specific behavior from *Drosophila simulans* to *Drosophila melanogaster.* *Science* 251:1082–85

149. Wilson GD, Hesler RR. 1987. Speciation in the deep sea. *Annu. Rev. Ecol. Syst.* 18:185–207

150. Winans GA. 1980. Geographic variation in the milkfish *Chanos chanos.* I. Biochemical evidence. *Evolution* 34:558–74

151. Wright S. 1939. The distribution of self-fertility alleles in populations. *Genetics* 24:538–52

152. Wu C-I. 1986. A stochastic simulation study on speciation by sexual selection. *Evolution* 39:66–82

153. Wyrtki K. 1985. Sea level fluctuations in the Pacific during the 1982–83 El Niño. *Geophys. Res. Lett.* 12:125–28

CONTENTS