

VICARIANCE AND DISPERSAL ACROSS BAJA CALIFORNIA IN DISJUNCT MARINE FISH POPULATIONS

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Abstract.—Population disjunctions, as a first step toward complete allopatry, present an interesting situation to study incipient speciation. The geological formation of the Baja California Peninsula currently divides 19 species of fish into disjunct populations that are found on its Pacific Coast and in the northern part of the Gulf of California (also called the Sea of Cortez), but are absent from the Cape (Cabo San Lucas) region. We studied the genetic makeup of disjunct populations for 12 of these 19 fish species. Phylogeographic patterns for the 12 species can be separated into two major classes: a first group (eight species) showed reciprocal monophyly and high genetic divergence between disjunct populations. A second group (four species) displayed what appeared to be panmictic populations. Population structure between Pacific Coast populations, across the Punta Eugenia biogeographic boundary, was also evaluated. While dispersal potential (inferred by pelagic larval duration) was a poor predictor of population structure between Gulf of California and Pacific populations, we found that population genetic subdivision along the Pacific Coast at Punta Eugenia was always positively correlated with differentiation between Pacific and Gulf of California populations. Vicariant events, ongoing gene flow, and ecological characteristics played essential roles in shaping the population structures observed in this study.

Key words.—Baja California, comparative phylogeography, control region, disjunct populations, Gulf of California, marine fishes.

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The first step toward allopatric speciation is taken when populations become disjunct (Endler 1977). Population disjunctions in marine organisms may derive from several mechanisms, which include vicariant events and limited dispersal capabilities (Palumbi 1992) such as the closing of the Isthmus of Panama, which occurred approximately 3 million years ago (Bermingham and Lessios 1993; Knowlton et al. 1993; Lessios 1998). While the role of population disjunctions is pivotal in understanding the early stages of marine allopatric speciation, relatively few studies have focused on this topic. The paucity of studies in this area is mostly due to the lack of good study systems (Randall 1998; Avise 2000; Lessios et al. 2001; Muss et al. 2001). In addition to the absolute barrier to gene flow presented by the Isthmus of Panama, other geographic locations amenable to study allopatric speciation are the Atlantic north of Cape Canaveral/northern Gulf of Mexico region (Avise 2000) and the Baja California Peninsula region (Riddle et al. 2000a). While the phylogeographic relationships among marine populations in the Atlantic north of Cape Canaveral/northern Gulf of Mexico region have been characterized extensively (e.g., Bert 1986; Bowen and Avise 1990), few studies have focused on the phylogeography of marine species in the Baja California region (Terry et al. 2000; Huang and Bernardi 2001; Stepien et al. 2001).

The Baja California Peninsula is among the most geologically active places on Earth. During the past 5 million years the peninsula has undergone tectonic plate spreading, subduction, and lifting. This geological activity, accompanied by the spreading of the ocean floor along the San Andreas fault between the peninsula and the mainland, created the Gulf of California (also called the Sea of Cortez) over a period

of 3.5 to 12 million years ago (Holt et al. 2000). The northern portion of the Gulf of California is older, possibly as old as the late Miocene, whereas the southern part of the Gulf of California is more recent. Along the peninsula, several seaways were present, possibly allowing marine species to migrate between the Gulf of California and the Pacific Coast of the peninsula (Riddle et al. 2000a,b,c). Overall, three postulated vicariance events may have affected the marine fauna and flora of the region: (1) approximately 4 million years ago, the southern Gulf of California was established and separated from the Pacific Ocean by a small peninsula; (2) approximately 3 million years ago, two seaways were created in the north (northern Gulf) and in the south (Isthmus of La Paz) of a proto-Baja California Peninsula; and (3) approximately 1 to 1.6 million years ago a midpeninsular seaway connected again the Gulf of California with the Pacific Ocean (Riddle et al. 2000a). Phylogeographic studies of desert animals, particularly rodents and lizards, have underscored the importance of these seaways resulting in vicariant events structuring the present-day terrestrial populations (Upton and Murphy 1997; Grismer 1999, 2000; Riddle et al. 2000a,b,c). These geological events, combined with recent glaciations and their associated variations in ocean temperatures and levels have created a biologically unique situation both on land and in the sea (Lindberg 1991; Upton and Murphy 1997; Riddle et al. 2000a). It is likely that colder water temperatures during glaciating periods have also allowed temperate species to migrate along the southern part of Baja California. By contrasting the effects of the closing of older seaways with dispersal events that would have occurred during the more recent glaciation times, disjunct populations of fishes give us a unique opportunity to determine the relative effects of vicariance and dispersal in the marine environment.

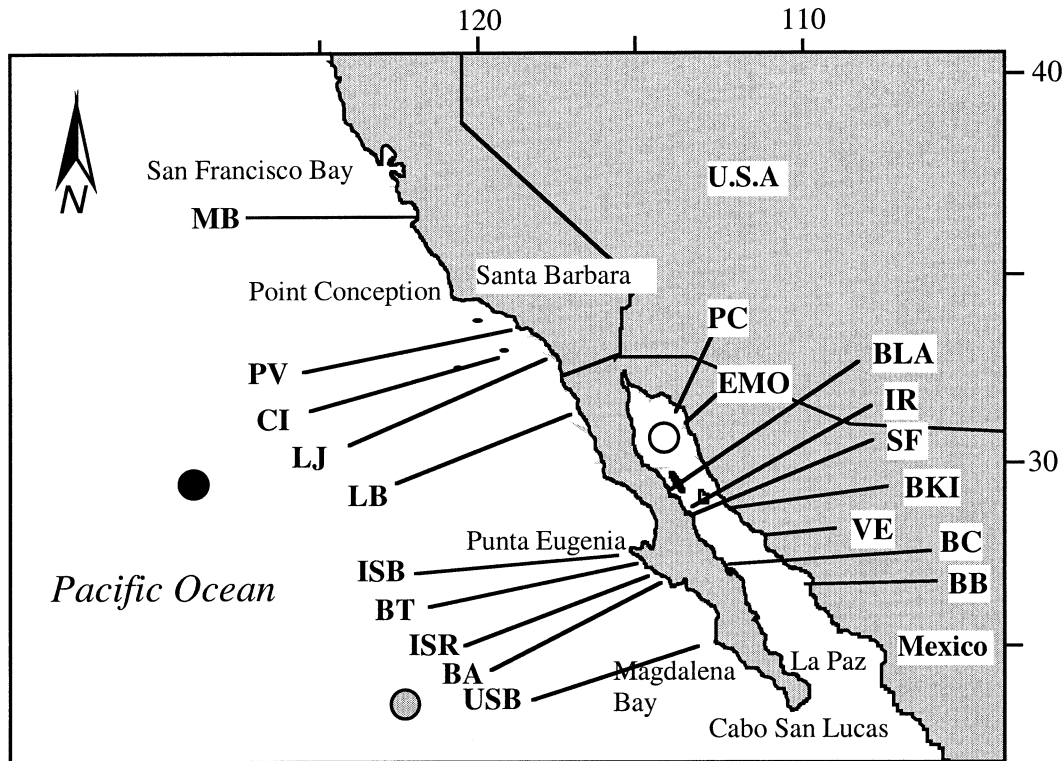


FIG. 1. Sampling localities. Sampling locality codes are given in Table 2.

The Baja California Peninsula region separates the open Pacific Ocean from the Gulf of California. Disjunct populations of marine organisms are present in the northern (upper) region of the Gulf of California and along the northern Pacific Coast of the Baja California Peninsula, but are rare or absent from the warmer southern waters of the Cape region (Cabo San Lucas, Fig. 1). These include marine mammals, invertebrates, and approximately 19 species of fishes (Miller and Lea 1972; Present 1987; Maldonado et al. 1995; Medina and Walsh 2000; Thomson et al. 2000; Table 1). While the concept of disjunction refers to intraspecific geographic separation, in two cases of Pacific Ocean/Gulf of California fishes, species rankings were given to each group. For opaleye, *Girella nigricans/simplicidens*, this ranking was challenged by Orton and Buth (1984) based on an allozyme study. For grunions, *Leuresthes tenuis/sardina*, this ranking was confirmed and supported by an allozyme study and by direct cross-hybridization experiments (Moffat and Thompson 1975; Crabtree 1983). Thus, disjunct assemblages represent a fertile terrain for research on speciation and species concepts (Lessios 1998).

The reef fish species of the Gulf of California form a mixed assemblage, which comprises representatives from the tropical Panamic Province (73% of species) and the temperate Californian Province (10% of species), with the remaining 17% of species being endemic (Walker 1960; Thomson et al. 2000). Similarly, disjunct fish species tend to exhibit both Panamic (70% of species) and Californian (30% of species) province affinities (Table 1). They also represent a highly variable assemblage of taxonomic and ecological groups that encompass 14 families (Table 1). Walker (1960) observed

that the majority of disjunct species exhibit morphological differences between Gulf of California and Pacific Coast populations. Differences, however, were usually small, and often limited to color variations. Thus, the limitations of morphological studies have prompted researchers to use molecular techniques to estimate the levels of genetic isolation of some disjunct fish species (Crabtree 1983; Orton and Buth 1984; Present 1987; Tranah and Allen 1999; Terry et al. 2000; Huang and Bernardi 2001; Stepien et al. 2001).

The presence of populations confined to the northern colder waters of the Gulf of California may result from two different origins. Vicariant events, with the closing of Neogene waterways connecting the northern portion of the Gulf of California to the Pacific (Riddle et al. 2000a) or more recent dispersal around the southern Cape of Baja California (Cabo San Lucas) during periods of oceanic cooling associated with glaciating events (Brusca 1973). The goal of the present study was to use a multispecies approach to define marine phylogeographic patterns for the region. We analyzed data for 12 of the 19 disjunct species. Using different types of molecular data—allozymes, restriction fragment length polymorphisms (RFLPs), and DNA sequences—we were able to use phylogeographic methods to test specific dispersal/vicariance hypotheses (Avice 1992, 1994, 2000; Grismer 2000) and infer their potential evolutionary consequences.

MATERIALS AND METHODS

Species Selection

More than 40 disjunct fish species have been previously described (Present 1987). However, several species listed

TABLE 2. Number of samples collected per locality. Locality positions are shown in Figure 1. LTE, *Leuresthes tenuis/sardina*; HJE, *Hypsoblennius jenkinsi*; CAL, *Chaenopsis alepidota*; ADA, *Anisotremus davidsonii*; LDA, *Lythripnus dalli*; HA, *Hermosilla azurea*; SPU, *Semicossyphus pulcher*; SMA, *Sebastes macdonaldi*. We also summarized information for previously published data: GNI, *Girella nigricans* (Terry et al. 2000); GMI, *Gillichthys mirabilis* (Huang and Bernardi 2001); PMA, *Paralabrax maculatofasciatus* (Stepien et al. 2001).

Sampling locality	Code	LTE	HJE	CAL	ADA	LDA	HA	HSE	SPU	SMA	GNI	GMI	PMA
Pacific Coast California											44	41	17
Monterey Bay	MB	5											
Palos Verdes	PV						7		3				
Catalina Island	CI			7		5							
La Jolla	LJ	1	4		9								
Pacific Coast Baja California											20	15	10
La Bufadora	LB							1					
San Benito Islands	SBI									8			
Bahia Tortugas	BT								5				
Isla San Roque	ISR				2								
Bahia Ascuncion	BA				3			9	2				
Uncle Sam's bank	USB									44			
Total Pacific Coast		6	4	7	14	5	7	10	10	52	64	56	27
Sea of Cortez east coast													
Punta Cholla	PC	5			6			11					
Estero Morua	EMO		3										
Bahia Kino	BKI				5								
Venicia	VE						3						
Bahia de Bacoichibampo	BB						4						
Sea of Cortez west coast													
Bahia de Los Angeles	BLA					5			5	43			
Isla Raza	IR						1						
Bahia San Francisquito	SF								5				
Bahia Conception	BC			4									
Total Sea of Cortez		5	3	4	11	5	8	11	10	43	43	43	36
Total		11	7	11	25	10	15	21	20	95	107	99	63

were likely ranked as disjunct due to either inadequate sampling (e.g., *Hydrologus colliei*), misidentification (e.g., *Merluccius* sp.), or very few records (e.g., *Porichthys notatus*). Some species were also categorized as disjuncts because they are present in the north and absent from the south of the Gulf of California, but have otherwise vast geographic ranges (e.g., *Cetorhinus maximus*, *Urolophus halleri*, *Gymnura marmorata*). Thus, a critical analysis of the original list of disjunct populations left only 19 species that are unequivocally disjunct (Table 1). In this study, we were able to analyze 12 of these 19 species. We obtained data from the literature for six species and original data for nine species. In cases for which new and literature data were available, both sets of data are presented. For the remaining seven species, *Hypsopsetta guttulata* and *Xenetremus ritteri* live in deeper habitats and are uncommon. The giant sea bass, *Stereolepis gigas*, is rare and protected. Samples for *Pleuronichthys verticalis*, *Zalembeus rosaceus*, *Scorpaena guttata*, and *Hypsoblennius gentilis* were obtained but only from one location (results not shown). The closest available relative was used as an out-group, which in most cases is a congeneric species, for the Mexican rockfish, midpoint rooting was used (Table 1).

Collections and DNA Samples

Sequence and RFLP data for *Paralabrax maculatofasciatus*, *G. nigricans*, and *Gillichthys mirabilis* were from the literature (Tranah and Allen 1999; Terry et al. 2000; Huang and Bernardi 2001; Stepien et al. 2001). Allozyme data for *G. nigricans*, *Leuresthes* spp., *Lythripnus dalli*, and *Hypsob-*

lennius jenkinsi were from the literature (Crabtree 1983; Orton and Buth 1984; Present 1987; Waples 1987). Original samples used in this study were collected with hand nets (*H. jenkinsi*, *L. dalli*, *L. gilberti*), beach seines (*L. sardina*, *L. tenuis*, *Colpichthys regis*), hook-and-line (*Sebastes macdonaldi*), and pole spears while scuba or free diving (all other species). Sampling locations and sizes are listed in Table 2. After collection, samples were immediately placed in 95% ethanol and stored at ambient temperature in the field, and then at 4°C in the laboratory. Muscle or liver tissue was later dissected from these samples. Total genomic DNA was prepared from 75–150 mg of muscle or liver tissue by proteinase K digestion in lysis buffer (10 mM Tris, 400 mM NaCl, 2 mM EDTA, 1% SDS) overnight at 55°C. This was followed by purification using chloroform extractions and alcohol precipitation (Sambrook et al. 1989).

Polymerase Chain Reaction Amplification and Sequencing

Amplification of the mitochondrial control region (also called D-loop) was accomplished with universal primers or custom primers (CR-A and CR-E, Lee et al. 1995; Rocha-Olivares and Vetter 1999). The control region was not consistently amplified for the bluebanded goby (*L. dalli*) and the sargo (*A. davidsonii*). We therefore used the cytochrome *b* region as a molecular marker. Amplification protocols and primers for the gobies followed Huang and Bernardi (2001), for the sargo followed Bernardi and Crane (1999), and for the Mexican rockfish followed Rocha-Olivares and Vetter (1999). For all other amplifications, each 100- μ l reaction

contained 10 to 100 ng of DNA, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 2.5 units of *Taq* DNA polymerase (Perkin-Elmer, Norwalk, CT), 150 mM of each dNTP, and 0.3 mM of each primer and was amplified with a cycling profile of 45 sec at 94°C, 45 sec at 48°C, and 1 min at 72°C, for 35 cycles. After purification following the manufacturer's protocol (Perkin-Elmer), sequencing was performed in both directions with the primers used in the PCR amplification on an ABI 373 automated sequencer (Applied Biosystems, Foster City, CA).

Phylogenetic Analyses and Population Structure

We used the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems) to align the DNA sequences. Phylogenetic relationships were assessed using the neighbor-joining and the maximum-parsimony methods implemented by the software package PAUP (ver. 4.0, Swofford 1998). Topological confidence was evaluated with 100 bootstrap replicates (Felsenstein 1985) for neighbor joining and also with 100 replicates using the fast-step method for maximum parsimony (only one tree kept at each replicate). In both neighbor joining and maximum parsimony, bootstrapping analysis was performed with equal weighting of transitions and transversions due to the low probability of multiple hits among recently diverged haplotypes. Gene flow (inferred from fixation indices F_{ST}) and haplotype diversity were calculated using the software package DNAsp (ver. 3, Rozas and Rozas 1997) following Hudson et al. (1992).

RESULTS

DNA Sequences

Portions of the 5' end of the mitochondrial control region (D-loop) were obtained for seven species. Once aligned, these regions had a length of 414 bp for *L. tenuis/sardina*, 363 bp for *H. jenkinsi*, 347 bp for *Chaenopsis alepidota*, 382 bp for *Hermosilla azurea*, 365 bp for *Halichoeres semicinctus*, 429 bp for *Semicossyphus pulcher*, and 515 bp for *S. macdonaldi*. We also obtained a portion of the 5' end of the cytochrome *b* for two species. Once aligned, these sequences had a length of 703 bp for *L. dalli* and 692 bp for *A. davidsonii*. Very few indels were found in the D-loop regions, none were found in the cytochrome *b* region. When found, indels were removed from the data analysis. *Chaenopsis alepidota* displayed a sequence on the 5' end of its control region (5'TAAAAAATT3') that was repeated up to 10 times. Repeats on the 5' end of the control region are not uncommon in fishes (Lee et al. 1995). This region was removed from the analysis. For all the analyzed species, transitions were more common than transversions (ratios Tr/Tv = 14–2.9), as is expected due to the close genetic relationship of disjunct populations, thus we never observed saturation effects (not shown) and characters were not weighed.

Phylogenetic Relationships and Gene Flow Levels between Disjunct Populations

Phylogenetic relationships for each species are shown in Figure 2. For species with large numbers of individuals (*G. mirabilis*, *G. nigricans*, *P. maculatofasciatus*, *S. macdonaldi*)

trees were simplified for clarity. Complete trees for the first three species can be found in the literature (Terry et al. 2000; Huang and Bernardi 2001; Stepien et al. 2001). Species displayed two distinct patterns of phylogenetic relationships.

The first group, which corresponds to the majority of the studied species (eight species, 66%) resulted in phylogenetic relationships that grouped Gulf of California and Pacific individuals into two distinct clades, with fixed differences and low levels of gene flow between them (Fig. 2a). In this group, one exception was found in the case of *G. mirabilis*, where two individuals from the Gulf of California grouped with Pacific Coast individuals (Fig. 2a). Since *G. mirabilis* is often used as bait fish and specimens were collected close to fishing villages, these two individuals were assumed to have been recently transferred by fishermen (Huang and Bernardi 2001).

In this group of disjunct species, sequence divergence between Gulf of California and Pacific populations varied between 11.6% to 1.06% for D-loop and 2.21% to 1.34% for cytochrome *b* (Table 1). Gene flow varied between F_{ST} values of 0.97 to 0.51 (Nm between 0.01 and 0.48 migrants per generation; Table 1).

The second group included four species. For these, Pacific and Gulf of California populations did not separate into distinct clades and showed high levels of gene flow. Gene flow varied between F_{ST} values of 0.00 to 0.02 ($Nm > 27$ migrants per generation; Table 1). Phylogenetic relationships for these species are shown in Figure 2b.

A second level of phylogenetic pattern was also observed. For six species of the 12 disjunct species studied here, we were able to obtain Pacific Coast samples from the southern Baja California coast (gray circles, Fig. 2) and from California (black circles, Fig. 2). A phylogeographic break in the Punta Eugenia region, which coincides with a biogeographic break (Briggs 1974), has been suggested before (Bernardi 2000; Bernardi and Talley 2000; Terry et al. 2000; Huang and Bernardi 2001). In the present study, gene flow between these two regions (across Punta Eugenia) was found to be either very high or very low. In the four species showing limited gene flow between Gulf of California and Pacific regions, gene flow was also limited between northern and southern populations along the Pacific Coast. Indeed, *G. mirabilis*, *G. nigricans*, *P. maculatofasciatus*, and *A. davidsonii* showed average values of $F_{ST} = 0.385$ across Punta Eugenia. *Girella nigricans* individuals collected north of Punta Eugenia that grouped with individuals collected south of Punta Eugenia (Fig. 2a) were all from a single population (Naples Reef) that may exhibit unique recruitment patterns (Terry et al. 2000). Furthermore, in the case of *G. nigricans*, Gulf of California individuals seemed more closely related to the individuals collected south of Punta Eugenia than these latter individuals are to the other Pacific Coast individuals (Figure 2a). However, statistical tests could not reject the alternative topology that would group both Pacific clades together (Terry et al. 2000).

In contrast with the above pattern, when gene flow was high between Gulf of California and Pacific populations, it was also high within Pacific populations for *H. semicinctus* and *S. pulcher*. These species showed average values of $F_{ST} = 0.01$ across Punta Eugenia. This correlation suggests that gene flow levels may be more influenced by species-specific

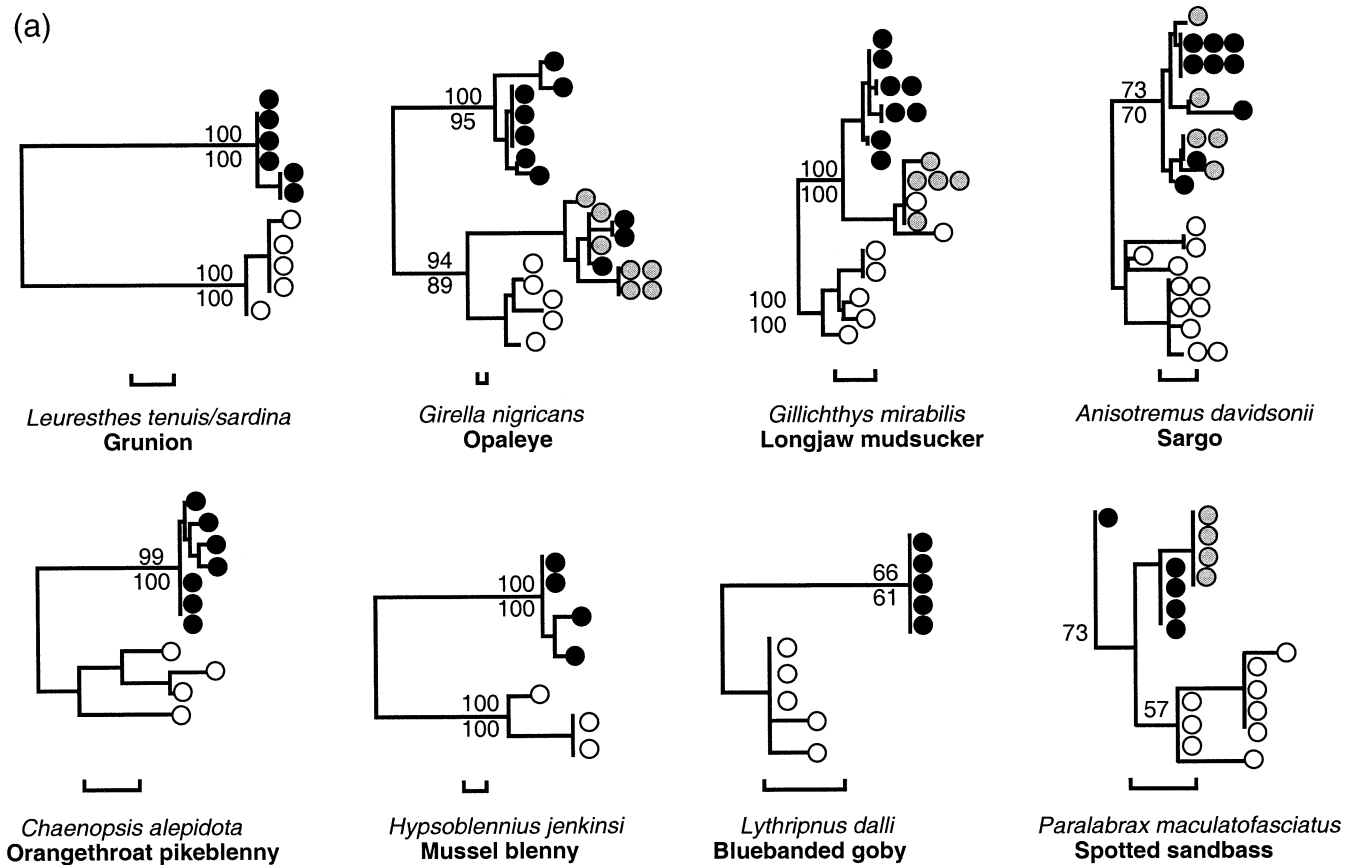


FIG. 2. Phylogenetic relationships of disjunct populations of fishes based on mitochondrial DNA. Phylogenetic relationships were based on a portion of the cytochrome *b* for *Lythripnus dalli*, *Gillichthys mirabilis*, and *Anisotremus davidsonii* and on a portion of the control region for all the remaining species. Each individual is represented by a white circle if collected in the Sea of Cortez, a black circle if collected on the Pacific Coast north of Punta Eugenia, and a gray circle if collected on the Pacific coast south of Punta Eugenia. Bootstrap values are given when above 50%. Neighbor-joining bootstrap values are given above the nodes, maximum-parsimony ones are given below the nodes. Scale bar represents a sequence divergence of 0.5% (Kimura two-parameter). Trees with large numbers of individuals (*G. mirabilis*, *Girella nigricans*, *Paralabrax maculatofasciatus*, *Sebastes macdonaldi*) were simplified; all other trees include all individuals.

characteristics rather than the shared influence of extrinsic factors.

DISCUSSION

The two major phylogeographic patterns between Gulf of California and Pacific populations presented above may result from older vicariant events (pattern 1, Fig. 2a) and recent or ongoing dispersal, with high levels of gene flow (pattern 2, Fig. 2b).

Dispersal

The presence or absence of genetic separation between Gulf of California and Pacific populations may be due to differences in dispersal potential between species. The majority of reef fish species exhibit a bipartite life history with a pelagic larval phase (where dispersal is likely to be high) and a benthic adult phase (where dispersal is usually reduced). Population structure in marine fishes has been proposed to be directly linked to the pelagic larval duration (PLD), the expected structure in fish populations being inversely correlated to the PLD. High population structure is predicted when PLD is short, and low population structure

is associated with long PLD. However, genetic studies that have addressed this question by studying several species with variable PLDs did not find a general relationship between PLD and gene flow (Waples 1987; Doherty et al. 1995; Shulman and Bermingham 1995; Riginos and Victor 2001).

Unfortunately, few PLD data are available for our species. *Girella nigricans*, which display a strong phylogeographic break, have a pelagic larval stage of 2 to 4 months (Waples 1987). *Paralabrax maculatofasciatus* and *L. dalli*, which display a shallow phylogeographic break have a pelagic larval stage of approximately 2 months and 4 months, respectively (Waples 1987). *Semicossyphus pulcher* and *H. semicinctus*, which show no phylogeographic break, have a pelagic larval stage of 37 days and 30 days, respectively (Victor 1986). These partial data indicate that there does not seem to be a simple correlation between PLD and the establishment of a particular phylogeographic pattern in Gulf of California and Pacific disjunct populations. It has been recently shown that larval retention in marine fish may be important even in the presence of long PLD (Jones et al. 1999; Swearer et al. 1999). In the Gulf of California, larval retention has been observed (Brogan 1994) and may have played a role in shaping the population structure of some endemic species (Riginos and

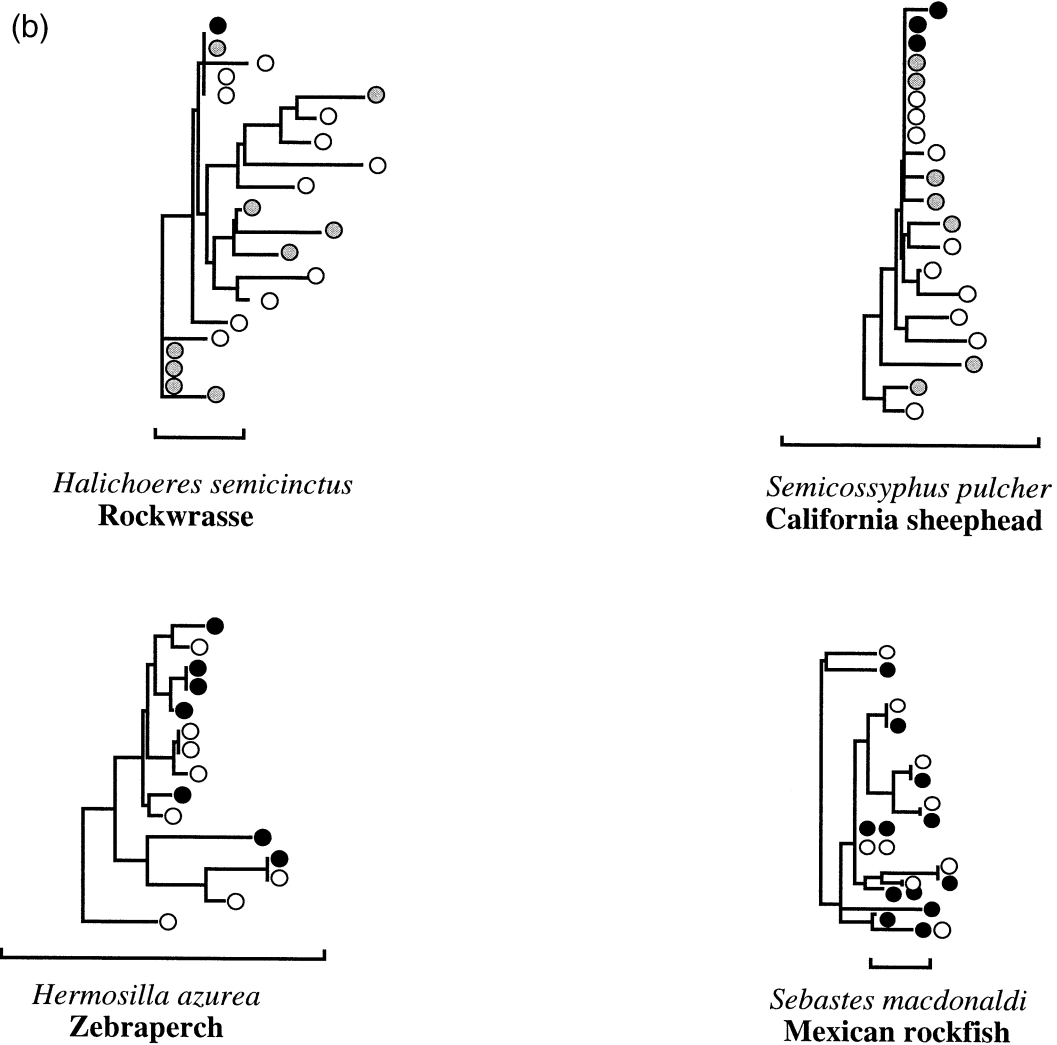


FIG. 2. Continued.

Nachman 2001; Riginos and Victor 2001). However, no data on larval retention are available for our disjunct species. We therefore conclude that PLD alone is a poor predictor of actual genetic disjunction between Gulf of California and Pacific populations.

Ongoing contact between populations could result from two possible mechanisms. First, larvae could be entrained around Cabo San Lucas from or into the Gulf of California without being detected. Evidence of this possibility is given by the occasional collection of vagrant larvae of disjunct species south of Cabo San Lucas (Present 1987). The occurrence of larvae in that region is also probably underestimated due to the paucity of systematic surveys. Another possibility for ongoing contact is by migration of adult fishes via deeper water. This is likely to be the case for at least one species, the California sheephead, *S. pulcher*. Indeed, *S. pulcher* is capable of feeding in deeper colder waters (e.g., on urchins). *Semicossyphus pulcher* is occasionally caught in deep water in the cape region (Cabo San Lucas, depth of 60 m, Scripps collection 59–219). Furthermore, *S. pulcher* is only found in the northern Gulf of California in shallow water

during the winter months, when the surface water is cool. We have not been able to collect or see any specimens during the warm summer months, suggesting that individuals may be migrating to deeper water during parts of the year.

If allopatric populations are indeed presently unconnected by migration and gene flow, our lack of resolution may be the result of very recent disjunction with insufficient time for lineage sorting (Avice 2000). The use of other, more rapidly evolving molecular markers, such as microsatellites, could help resolve this issue.

Vicariance

Vicariant events responsible for Gulf of California and Pacific fish population disjunctions may be fairly recent (Pleistocene) or relatively older (Neogene). The establishment of disjunct populations in the northern Gulf of California was originally proposed to be the result of Pleistocene migrations around the Cape region by temperate species, when the water temperatures in the southern Gulf of California were cooler and did not represent such a strong phys-

iological barrier to cold-adapted fishes (Brusca 1973). Dispersal would later have been prevented by the physiological barrier of warm water present in the southern Gulf of California, thus keeping populations in a disjunct state. But are the warm waters of the lower Gulf of California truly effective physiological barriers to dispersal? The northern Gulf of California shows a wide range of temperatures. During the winter months, water temperatures are indeed much lower than in the southern Gulf of California region (12°C vs. 23°C), however, during the summer months, temperatures in the upper Gulf of California are higher than in the lower Gulf of California (30°C vs. 27°C). At that time, *A. davidsonii* and *G. nigricans*, two disjunct species that show strong genetic divergence between Gulf of California and Pacific populations, are among the most abundant subtidal fishes in the northern Gulf of California (Thomson et al. 2000). Southern warm temperatures therefore cannot be regarded as effective physiological barriers to these species, and temperature cannot be considered solely responsible for keeping disjunct populations separated. Thus, if separation between populations occurred during the Pleistocene, cooler temperatures in the southern portion of Baja California may have facilitated migration of individuals or larvae across the cape region. However, the maintained separation of these populations is unlikely to be due to present-day water temperatures. An alternative hypothesis that also results in the establishment of disjunct populations during the Pleistocene is that temperate fishes crossed the Baja California Peninsula north of the Cape, using a waterway that was present in the La Paz region (Walker 1960). This hypothesis is different from the previous one, as it does not require ecological interactions and competition between tropical fishes from the Cape region and the migrant temperate species, yet it still needs southern warm water as a mechanism to maintain populations separated.

The majority of species showed fixed differences between Gulf of California and Pacific populations with different levels of sequence divergence. Three species showed few fixed differences, *L. dalli* (blue-banded goby), *P. maculatofasciatus* (spotted bass), and *C. alepidota* (orangethroat pikeblenny). Using molecular clocks, genetic divergence between Gulf of California and Pacific populations of spotted bass were estimated to have occurred between 120,000 and 600,000 years ago (Stepien et al. 2001). According to these authors, such estimates are compatible with a Pleistocene separation of disjunct populations, which could have been created by migration through the waterway in the La Paz region. The similarity of pattern between *L. dalli*, *P. maculatofasciatus*, and *C. alepidota* together with similar estimated times of divergence (200,000 to 900,000 years ago for *C. alepidota* and 200,000 to 400,000 years ago for *L. dalli*) suggests that common mechanisms may have been at play for these species as well.

Five species show high levels of sequence divergence: *G. nigricans* (opaleye), *L. sardina/tenuis* (grunion), *A. davidsonii* (sargo), *H. jenkinsi* (mussel blenny), and *G. mirabilis* (longjaw mudsucker). Such high levels of sequence divergence and the presence of fixed differences indicate long separation times between populations. Using molecular clocks, time of divergence between Gulf of California and Pacific popula-

tions have been estimated at 0.76–2.3 million years ago (*G. mirabilis*), and 0.3–2.2 million years ago (*G. nigricans*; Terry et al. 2000; Huang and Bernardi 2001). These times were considered to be compatible with a separation of individuals by the Baja California Peninsula with the closure of more ancient waterways. During the Neogene, the northern part of the Baja California Peninsula was divided by waterways that eventually were closed, approximately 1 million years ago, by the uplifting of the peninsula (Upton and Murphy 1997; Riddle et al. 2000a). The similarity of phylogeographic patterns between opaleye, longjaw mudsucker, sargo, mussel blenny, and grunion, together with similar time of divergence between disjunct populations (1.3 to 2.6 million years ago for sargo, 0.3 to 2.0 million years ago for Mussel blenny, 0.4 to 3 million years ago for grunion) again suggest that common historical events may have shaped the population structure of these species.

Our results are therefore compatible with a vicariant scenario in which Gulf of California and Pacific populations of *G. nigricans*, *L. sardina/tenuis*, *A. davidsonii*, *H. jenkinsi*, and *G. mirabilis* would have been connected via extinct Neogene waterways. Subsequently, this connection was severed by the closing of the waterways and isolation was maintained by a lack of dispersal around the Cape region. In contrast, species that exhibit population separations, but low levels of genetic divergence may be the result of more recent physical separation, possibly during the Pleistocene. These species are *L. dalli*, *P. maculatofasciatus*, and *C. alepidota*. No genetic evidence of present-day dispersal between the Gulf of California and the Pacific could be found (fixed differences were always present).

Ecological Factors

Thus far, mostly historical factors have been considered to explain our results. Other abiotic factors with direct influences on the ecology of the species, such as tides, salinity, and temperature, are also likely to play an important role. Tidal amplitudes along the Gulf of California are extreme. The northern Gulf of California experiences huge tidal amplitudes (over 10 m), while tidal fluxes are almost nonexistent in the southern Gulf of California. Four of the species that exhibit strong population structure have an important part of their life history linked to the intertidal zone. *Gillichthys mirabilis* and *H. jenkinsi* live exclusively in the intertidal, and *Leuresthes* and *Girella* spawn or nurse (respectively) in the intertidal. Assuming that the portion of life history spent in the intertidal region is important for the overall dispersal of these species, the lack of suitable intertidal habitat in the southern region of the Gulf of California is likely to have played an important role in creating an effective ecological barrier to gene flow for these species. In general, the ecology of Gulf of California fishes is poorly known. It is likely that further ecological studies on these species will provide some other clues as to the reasons for the observed differences in phylogeographic patterns between disjunct species.

Evolutionary Consequences: Speciation

While sympatric speciation has been modeled as a possible component of speciation (Dieckmann and Doebeli 1999;

Kondrashov and Kondrashov 1999) and is currently the subject of much theoretical and empirical work, it is likely that allopatric speciation plays an essential part and is probably the most common mechanism for incipient speciation in disjunct species (Endler 1977). Galapagos finches are probably the best-known example of terrestrial disjunctions that resulted in speciation events (e.g., references in Grant 1999). In contrast, relatively few studies have evaluated the role of population disjunctions on speciation of marine organisms (Stepien and Rosenblatt 1991; Palumbi 1994; Burton 1998; Hellberg 1998). Recently, studies that have focused on the relationship between genetic divergence and species formation have used cytochrome *b* sequences for a wide range of vertebrate species (Johns and Avise 1998) or both cytochrome *b* and D-loop sequences for fish species (McCune and Lovejoy 1998). By studying cases of recognized sister species, as well as sister clades within species, McCune and Lovejoy were able to provide a range of genetic divergences expected for intra- and interspecific groups. Comparing our data with McCune and Lovejoy's, we found that *L. tenuis/sardina*, *G. nigricans*, and *H. jenkinsi* were well within the interspecific domain. The sargo, *A. davidsonii*, was close to the overlap zone between inter- and intraspecific genetic divergences, and all other species were within the intraspecific region.

Molecular clocks and genetic divergence estimates need to be considered carefully. The results presented here are based on a single locus, and better estimates of divergence time would be obtained if multiple loci were used (Edwards and Beerli 2000). Our results show that either population divergence differences between species correspond to different histories, or that, if species have been separated for similar times, then their genetic divergences did not follow uniform rates. This phenomenon is well known and is best exemplified by the studies of genetic divergences between populations across the Isthmus of Panama, where actual time of divergence is better known (Knowlton et al. 1993; Bermingham et al. 1997; Lessios 1998).

Conclusion

We studied 12 of the 19 known disjunct species of marine fishes that are separated by the Baja California Peninsula. Therefore, the results presented here are likely to give a fair representation of the situation of disjunct Gulf of California and Pacific fish populations. Eight species showed strong genetic divergences (and fixed differences) between Gulf of California and Pacific populations, whereas four showed high levels of gene flow between populations. Among those eight species, three or four seemed to correspond to incipient speciation events, indicating that population disjunctions, which have traditionally been considered as the first step toward speciation, are probably responsible for the creation of new geminate species. Vicariance, ecology, and dispersal limitation are all factors that appear to have played important roles in these speciation events.

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