ORIGINAL ARTICLE

Phylogeography of the flag cabrilla *Epinephelus labriformis* (Serranidae): implications for the biogeography of the Tropical Eastern Pacific and the early stages of speciation in a marine shore fish

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ABSTRACT

Aim To examine the role of previously described biogeographical boundaries in shaping phylogeographical relationships within and among two putative eastern Pacific sibling species, the flag cabrilla, *Epinephelus labriformis* and the Clipperton grouper, *Epinephelus clippertonensis* (Serranidae).

Location Tropical Eastern Pacific (TEP).

Methods Sequence data from the mitochondrial cytochrome *b* gene were obtained from samples throughout the range of the species. Coalescence analysis, mismatch distributions and an analysis of molecular variance (AMOVA) were used to infer population differentiation.

Results Overall, 49 haplotypes were found among 304 specimens, and there was significant structure corresponding to geographical locality (AMOVA, $\Phi_{ct} = 0.198$, $P < 0.001$; $\Phi_{st} = 0.207$, $P < 0.001$; $F_{st} = 0.169$, $P < 0.001$; $F_{ct} = 0.151$, $P = 0.036$). Coalescence analysis indicates a population expansion at Clipperton Atoll during the mid-Pleistocene.

Main conclusions Our results suggest that previously described barriers to dispersal along the mainland of the TEP may not impinge on the dispersal ability of marine species, such as these groupers, that have long-lived pelagic larvae. In contrast, gene flow between mainland and island populations of the readily distinguishable morphospecies *E. labriformis* and *E. clippertonensis* is restricted. The low level of genetic differentiation between the two species indicates that changes in external colour patterns may evolve more rapidly than genetic markers commonly used to delimit species boundaries. Thus a combination of colour differences and a lack of reciprocal monophyly may act as good indicators of incipient speciation in the marine environment.

Keywords
Clipperton Atoll, cytochrome *b*, *Epinephelus clippertonensis*, *Epinephelus labiformis*, phylogeography, Serranidae, speciation, species barriers, Tropical Eastern Pacific.

INTRODUCTION

Understanding the process and underlying mechanisms of speciation remains a challenging subject in evolutionary biology. Identifying genes that are essential to confer reproductive isolation is difficult and time consuming, and those that have been described as 'speciation genes' vary in terms of demonstrable effect (Swanson & Vacquier, 2002; Tregenza, 2002; Noor, 2003; Pitnick *et al.*, 2003; Ritchie & Noor, 2004; Arnegard *et al.*, 2005). We are therefore faced with the challenge of using putatively neutral markers as a means of developing hypotheses regarding the underlying mechanisms of speciation.

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It has become commonplace in evolutionary biology to evaluate the degree of connectivity among populations of organisms. Such studies are useful in terms of assessing conservation strategies, evaluating ecological processes, and determining the evolutionary history of a group of organisms. Usually these evolutionary processes include restricted gene flow in order for populations to diverge sufficiently for speciation to occur (cf. Avise, 2000 and references therein). Such restrictions in gene flow have long been thought to be most effective when coupled with geographical isolation or disjunction (Endler, 1977; Terry *et al.*, 2000; Bernardi *et al.*, 2003). Hence our evaluation of population divergence is often thought to reflect the early stages of speciation. In the marine environment, the processes governing population divergence are countered by the homogenizing effects of pelagic larval transport. Nonetheless, it is expected that at some early stage of speciation populations will show certain characteristics, such as subtle differentiation, that indicate so-called incipient speciation. These features may emerge prior to complete lineage sorting and reciprocal monophyly at neutral genetic loci (Bowen, 1998).

It has long been a contention in marine biogeography that the presence and duration of the pelagic larval stage plays a critical role in shaping the distributions of species and the degree of connectivity among populations (Ekman, 1953; Hedgecock, 1986; Bonhomme & Planes, 2000). Dispersal theory suggests that organisms possessing long-lived pelagic larvae should, on average, display relatively extensive ranges in comparison with related species with shorter larval periods (Scheltema, 1968). Using the same reasoning, population genetic theory predicts that such organisms should experience high gene flow among populations, and species with large ranges should show little genetic structuring relative to geographical locality (Avise, 2000 and references therein). It is therefore surprising that recent studies have shown that (1) some species with restricted ranges also have long pelagic larval stages relative to congeners with larger geographical ranges, and (2) despite time spent in the plankton, local retention of larvae may play a more important role in settlement processes and population structuring than previously hypothesized (Swearer *et al.*, 1999; Leis & Carson-Ewart, 2000; Taylor & Hellberg, 2003). As restrictions in gene flow are often assumed to be a requisite of speciation, these studies provide mechanisms by which speciation may occur in the absence of physical barriers to dispersal.

The Tropical Eastern Pacific (TEP) has been considered a model system for examining how the distributions of marine shorefishes are affected by habitat discontinuities (Springer, 1959; Rosenblatt, 1967; Waples, 1987; Allen & Robertson, 1994; Hastings, 2000; Mora & Robertson, 2004). In this area, long stretches of rocky coastline are interrupted by two large expanses of sandy shore: the 370-km-wide Sinaloan Gap extending south from Topolobampo, Sinaloa, Mexico to Mazatlán, Mexico; and the 1000-km-wide Central American Gap extending south from the Isthmus of Tehuantepec to the Gulf of Fonseca (Springer, 1959; Walker, 1960; Rosenblatt, 1967; Dawson, 1975; Hastings, 2000; Fig. 1). These barriers may represent an environment that prohibits adult movement in reef species, and/or may limit larval movement due to the distance they must travel before encountering suitable habitat for settlement. These gaps have been hypothesized to play a large role in driving speciation within this region (Hastings, 2000; Pondella *et al.*, 2003). Based on shared overlapping distributions of marine shorefish species, the TEP has been divided into three mainland biogeographical provinces separated by these gaps: Cortez Province, Mexican Province and Panamic Province (Fig. 1). The offshore oceanic islands in this region harbour a unique ichthyofauna but, with the exception

Figure 1 Map of the Tropical Eastern Pacific showing biogeographical provinces and collecting localities for 304 specimens of *Epinephelus labriformis* and *Epinephelus clippertonensis*. Numbers in parentheses are samples sizes for each collecting site.

Figure 2 Photographs of the flag cabrilla, *Epinephelus labriformis* (a) and the Clipperton grouper, *Epinephelus clippertonensis* (b,c). Photographs by D.R.R.

of the Galápagos Islands (Brusca & Wallerstein, 1979), none has been designated as a distinct province. Hence this coastline is a prime setting to test the ability of marine species with pelagic larval stages to traverse barriers of inappropriate habitat, as well as to assess the extent to which habitat gaps play a key role in driving speciation.

The flag cabrilla, *Epinephelus labriformis* (Jenyns, 1840) is a member of an exclusively marine family commonly known as groupers and seabasses (Serranidae). A dominant predator on many reef systems throughout the TEP, the flag cabrilla is commonly taken in artisanal and commercial fisheries throughout its range (Ramírez & Rodríguez, 1990; M.T.C., pers. obs.). The flag cabrilla lives over rocky substrate where it feeds on small fishes and crustaceans (Hobson, 1968; pers. obs.). Typical of other species of grouper, the flag cabrilla exhibits a rapid first year of growth, followed by an asymptotic slowing in upper age classes, and may reach ages in excess of 25 years (Craig *et al.*, 1999). The flag cabrilla possesses a pelagic larval stage that may last as long as 60 days (B. Victor, pers. comm.); recent evidence suggests that, like many other grouper species, it is a protogynous hermaphrodite (B. Erisman and M.T.C., unpubl. data).

Widespread throughout the TEP, the flag cabrilla has been recorded from San Diego, CA, USA to Peru, including the offshore islands of Revillagigedo, Coco and Galápagos (Heemstra & Randall, 1993; Craig *et al.*, 2006). Although previously reported at the Clipperton Atoll (eastern Pacific), *E. labriformis* is replaced there by its recently described sister species, *Epinephelus clippertonensis* Allen & Robertson, 1999. While it was thought that *E. clippertonensis* was restricted to Clipperton Atoll, recent observations indicate that it also occurs at the Alijos Rocks (Baja California, Mexico). Lacking the characteristic white spots and olive green background of the flag cabrilla, the Clipperton grouper has a pinkish-grey colour pattern that is readily identifiable in the field (Fig. 2). However, similarities in their overall morphology make it very difficult to distinguish preserved specimens of *E. labriformis* and *E. clippertonensis*, although they have differences in relative eye size and scalation (Allen & Robertson, 1999).

Population genetic data for grouper species in the TEP are scarce, yet may contribute to an understanding of biogeographical partitions (especially dispersal ability and the strength of hypothesized barriers), and also to the growing body of knowledge on mechanisms of speciation. To address these issues, we sampled flag cabrilla from throughout the TEP and Clipperton grouper from Clipperton Atoll and Alijos Rocks. We used a genetic analysis of these fishes to evaluate three hypotheses regarding these populations: (1) complete speciation despite ongoing gene flow, (2) complete speciation with secondary contact (hybridization), and (3) incomplete or recent speciation with incomplete lineage sorting. Our findings are most consistent with a recent or incomplete speciation event.

MATERIALS AND METHODS

Sequence data were collected for 304 individuals (268 *E. labriformis* and 36 *E. clippertonensis*) at 11 sample sites throughout the TEP (Fig. 1). Tissue samples (muscle, fin clips or gill clips) were taken from individuals collected by spear pole or hook and line, or purchased from artisinal fish markets between 1997 and 2004. Voucher specimens were deposited at the Scripps Institution of Oceanography (SIO) Marine Vertebrates Collection. Tissues were stored in either 95% ethanol or 5× Net solution (Craig *et al.*, 2001) and maintained at ambient temperature while in the field, and at −20 °C in the laboratory. Total genomic DNA was isolated using the DNEasy DNA isolation kit (Qiagen) following the manufacturer's instructions. A 468-bp portion of the mitochondrial cytochrome *b* (cyt *b*) gene was amplified using the polymerase chain reaction (PCR), and unique haplotypes were deposited in Genbank (*E. labriformis*, AY728099–AY728136, DQ007239; *E. clippertonensis*, AY728095–AY728098, DQ007240–DQ007245). The 50-μL PCR reactions were prepared following the instructions included with the Sigma RedTaq Ready Mix, with the addition of 10–100 ng template DNA and 10 pmol forward and reverse primer. Cyt *b* has proven useful in evaluating population-level differentiation in other grouper species (Gilles *et al.*, 2000). PCR and sequencing primers were taken from Gilles *et al.* (2000): 28-for 5′-cgaacgttgatatgaaaaaccatcgttg-3′, 34-rev 5′-aaactgcagcccctcagaatgatatttgtcctca-3′. PCR reactions consisted of 35 cycles of the following step procedure following a 30-s denaturation step at 94 °C: 94 °C for 30 s, 50 °C for 30 s, 72 °C for 45 s. Unincorporated dNTPs and primers were

removed using the Millipore Amicon filter plate. Direct sequencing of PCR products was accomplished using a Megabace 1000 automated DNA sequencer following the manufacturer's instructions included with the ET Dye Terminator chemistry (Amersham Biosciences). Sequences for both forward and reverse directions were used to create a consensus sequence for the final analysis.

Haplotype (gene) diversity (h) and nucleotide diversity (π) were calculated for each site and for the overall sample group using the algorithms of Nei (1987) as implemented in the computer program ARLEQUIN ver. 2.00 (Schneider *et al.*, 2000). Neutrality (equilibrium) was assessed by calculating Tajima's D for each population (Tajima, 1989). Significance was tested using 1000 permutations in ARLEQUIN ver. 2.00. A statistical parsimony network was constructed using the computer program TCS (Clement *et al.*, 2000) using default settings. Φ_{ct} and Φ_{st} values were calculated using ARLEQUIN for samples grouped by the TEP provinces, Clipperton, and Alijos Rocks, and significance was tested using 1000 permutations of the data set. Conventional F statistics based only on haplotype frequency were also calculated according to the same procedure. An exact test of haplotype frequencies among populations was performed using 20,000 replicates of a Markov chain as implemented in ARLEQUIN.

We used mismatch distributions for the Clipperton Atoll and mainland populations to differentiate between past population expansion or stasis models. The limited number of samples from the Alijos Rocks confounded computations and were deemed unreliable, hence they were not considered in further evaluation. When a unimodal distribution was found, we followed Li (1977) and Rogers & Harpending (1992), and fitted estimates of τ, θ_0 and θ_1 to observed mismatch distributions to determine effective population sizes and time to coalescence. Coalescence analysis requires an estimate of generation time and rate of DNA evolution. Rate estimates for cyt b have been obtained for some reef fishes (2% Myr^{-1}; Bowen *et al.*, 2001). We provisionally use this rate (2% Myr^{-1} between lineages) to estimate divergence times between populations of *E. labriformis* and *E. clippertonensis*. However, we recognize that these dates must be interpreted with caution. Generation time in many reef fishes is unknown, including for *E. labriformis* and *E. clippertonensis*. *Epinephelus labriformis* may reach more than 25 years of age (Craig *et al.*, 1999), however, given that the species is probably protogynous, generation time should reflect age at first maturity and age at sex reversal. Thus we chose a generation time of 10 years as an initial estimate.

RESULTS

We analysed 468 bp of the cyt b gene in 304 flag cabrilla (*E. labriformis*) and Clipperton grouper (*E. clippertonensis*) from throughout the TEP. We found 49 unique haplotypes among all individuals. Nucleotide diversity (π) within sampled groups ranged from 0.0012 to 0.004856, and haplotype (gene) diversity (h) from 0.7 to 0.87 (Table 1). AMOVA for all sampled groups indicated significant population structuring ($\Phi_{ct} = 0.198$, $P < 0.001$; $\Phi_{st} = 0.207$, $P < 0.001$; $F_{st} = 0.169$, $P < 0.001$; $F_{ct} = 0.151$, $P = 0.036$). A pairwise comparison of population Φ_{st} values indicated that a majority of the significance was found in comparisons involving the Alijos Rocks and Clipperton Atoll populations (Table 2). Tajima's test of neutrality yielded negative values for all groups (Table 1). Negative values indicate a predominance of low-frequency haplotypes, a possible signature of recent population expansion.

Mismatch distributions for the Clipperton Atoll and mainland populations indicated a pattern consistent with a rapid expansion model (Fig. 3). At Clipperton Atoll haplotypes

Table 1 Sample size and descriptive statistics for cytochrome b data for 304 *Epinephelus labriformis* and *Epinephelus clippertonensis*. *$P < 0.05$

Site	N	Number of haplotypes	Number of unique haplotypes	Gene diversity	Nucleotide diversity	Tajima's D
Cortez Province						
Loreto	38	10	5	0.4609	0.002343	−1.78099*
La Paz	5	3	2	0.7	0.001709	−0.97256
Cabo San Lucas	7	3	0	0.5238	0.001832	−1.35841
Mexican Province						
Mazatlan	30	7	3	0.4644	0.002659	−1.82477*
Puerto Vallarta	49	8	4	0.4464	0.001741	−1.67569*
Huatulco	26	9	3	0.76	0.002774	−1.19676
Panamic Province						
El Salvador	21	5	3	0.5381	0.003663	−1.72296*
Panama	46	9	4	0.5295	0.002147	−1.62131*
East Pacific Islands						
Alijos rocks	12	7	5	0.7727	0.004856	−0.56737
Cocos Island	11	7	3	0.8727	0.004274	−0.66206
Clipperton Atoll	24	6	5	0.4964	0.0012	−1.68244
Galápagos Islands	35	6	2	0.6034	0.002492	−0.54846

Table 2 Pairwise population Φ_{st} values and corresponding significance values (P) for 11 populations of *Epinephelus clippertonensis* and *Epinephelus labriformis*

	Clipperton	Mazatlan	Puerto Vallarta	Panama	Oaxaca	Cocos	Galápagos	Loreto	El Salvador	Eastern Cape	Alijos Rocks
Clipperton	–	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*
Mazatlan	0.46239	–	0.3566	0.2573	0.17068	0.09187	0.03693*	0.4155	0.19305	0.72904	0*
Puerto Vallarta	0.52191	0.00021	–	0.74894	0.23186	0.02663*	0.07504	0.50292	0.34571	0.50124	0*
Panama	0.47746	0.00578	−0.011	–	0.5045	0.04227*	0.20859	0.49698	0.45778	0.35987	0*
Oaxaca	0.44075	0.01509	0.00842	−0.00789	–	0.13613	0.58707	0.15612	0.69805	0.24552	0*
Cocos	0.49537	0.04992	0.09755	0.07337	0.03972	–	0.12573	0.02693*	0.15335	0.0597	0.0003*
Galápagos	0.4925	0.05157	0.03558	0.01203	−0.01358	0.04518	–	0.03693*	0.42521	0.10148	0*
Loreto	0.46893	−0.00078	−0.0057	−0.00516	0.01523	0.07429	0.04761	–	0.20879	0.67904	0*
El Salvador	0.43917	0.014	0.00149	−0.0071	−0.01685	0.0357	−0.00775	0.01015	–	0.35699	0*
Eastern Cape	0.54137	−0.02012	−0.01295	−0.00431	0.01661	0.06436	0.06397	−0.01842	−0.00026	–	0.0003*
Alijos Rocks	0.66178	0.43473	0.55838	0.51581	0.41816	0.35231	0.46131	0.50135	0.4007	0.47503	–

F_{st} values below diagonal; P values above diagonal.

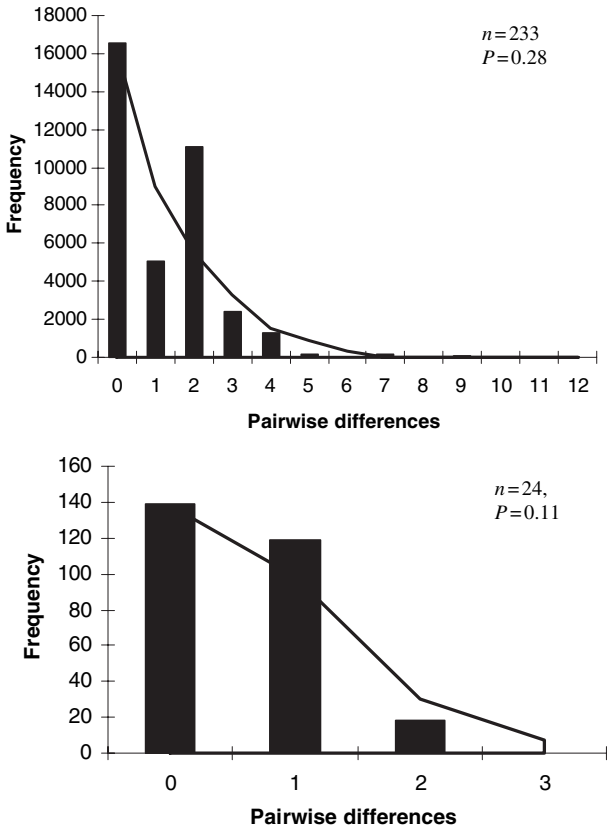

Figure 3 Mismatch distributions for mainland populations of *Epinephelus labriformis* (top) and *Epinephelus clippertonensis* from the Clipperton Atoll (below). *N*, number of individuals sampled. *P* values compare observed (solid bars) with predicted (line) distribution based on a model of rapid population expansion. Values < 0.05 reject the model.

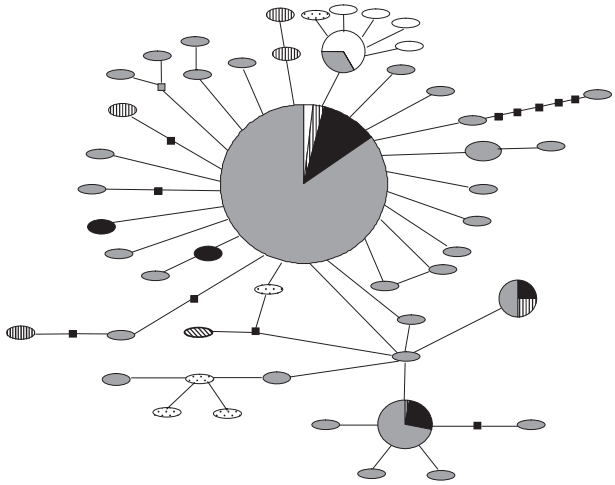

Figure 4 Statistical parsimony network for 304 individuals of *Epinephelus* spp. based on 41 haplotypes of the mitochondrial cyt *b* gene. White, haplotype found at Clipperton Atoll; grey, mainland; black, Galápagos Islands, dotted, Alijos Rocks; straight cross-hatching, haplotype present at Cocos Island. Diagonal hatching, mainland colour morph found at Alijos Rocks. Circles are proportional to the number of individuals displaying the haplotypes; ovals are singleton haplotypes; small squares are single nucleotide changes (missing haplotypes).

coalesce at 392.5 Ka (lower 95% limit 0, upper 95% limit 715 Ka); on the mainland haplotypes coalesce at 1474 Ka (low 95% limit 102 Ka, upper 95% limit 3503 Ka). Effective population size for the initial mainland population was 112. At Clipperton Atoll the initial effective population size was at or near zero.

A statistical parsimony network is presented in Fig. 4. Four of 24 (16.6%) individuals collected at Clipperton Atoll had unique haplotypes; 16 of 24 (66.6%) had haplotypes that were present only in very low frequencies on the mainland (4/222; 1.8%); and four of 24 (16.6%) had the most common mainland haplotype (Fig. 4). For the Alijos Rocks, 10 of 12 individuals (83.3%) had haplotypes that were present only at that locality, while one individual had the most common

haplotype found at Clipperton Atoll. Despite sharing some mitochondrial cyt *b* alleles with the mainland groups, all individuals collected at Clipperton Atoll displayed the *E. clippertonensis* colour pattern, while those on the mainland were exclusively of the *E. labriformis* colour pattern (Fig. 2). At the Alijos Rocks, all individuals showed a colour pattern similar to individuals at Clipperton Atoll, except for one individual which showed the typical *E. labriformis* colour pattern. While some mainland groups possessed private alleles, they did not cluster together in any discernible units. In the Clipperton group the private alleles clustered with the most dominant Clipperton haplotypes.

A tree-based phylogenetic analysis based on the cyt *b* data, including only *E. clippertonensis*, *E. labriformis* and the putative sister taxon *Epinephelus analogus* Gill, 1863, produced a large 'comb' indicating a lack of strong phylogeographical signal (tree not shown).

DISCUSSION

Coalescence analysis

Coalescence analyses indicated a population expansion at Clipperton Atoll during the middle Pleistocene (*c.* 392.5 Ka), with an effective population size near zero. The molecular evolutionary rate and generation times used to estimate these parameters are approximations at best. Thus the estimates of coalescence time and population size should be treated with some caution. However, the range of mutation rates for cyt *b* (1–3% Myr^{-1}) would lead to approximately the same estimate of a population expansion at Clipperton Atoll in the mid-Pleistocene. Given this uncertainty, it seems imprudent to hypothesize the role of any particular geological or climatological event to explain the initial separation of mainland and island populations.

Evolution and species designations

The conservative morphology of the family Serranidae has confounded taxonomists and phylogeneticists alike. In many instances the colour pattern in live individuals is the most useful characteristic for distinguishing closely related species in the field (Heemstra & Randall, 1993). Unfortunately, colours quickly fade in preserved fish and are thus not generally useful for identification of many museum specimens. The description of the Clipperton grouper, *E. clippertonensis* illustrates this dilemma. While the colour pattern of this fish clearly separates it from its sister species (*E. labriformis*), only slight differences in traditional morphological measurements and meristic counts are available to distinguish these species in preserved specimens. In these circumstances, genetic data can provide insight into species boundaries of closely related species (Craig *et al.*, 2001, 2004; Pondella *et al.*, 2003).

Our analysis of genetic data from the mitochondrial cyt *b* gene revealed clustering of the private and shared alleles of the Clipperton and Alijos populations and lack of such grouping of private haplotypes at other island sites, indicating a restriction in gene flow between Clipperton Atoll, Alijos Rocks and the mainland (Fig. 4). The AMOVA analysis provides additional perspective on the evolutionary distinctiveness of the two populations (species) in this study. A comparison of Φ_{st} and F_{st} values indicates a deeper level of divergence when sequence similarity is incorporated ($\Phi_{st} = 0.20671$, $P = 0.00$; $F_{st} = 0.1689$, $P = 0.00$). Quattro *et al.* (2002) suggested that a greater depth in Φ_{st} relative to F_{st} indicates a phylogenetic component with regard to the separation of populations (species). Our data reflect a pattern consistent with this notion, and indicate that the divergence in the populations discussed here reflects a phylogenetic distinctiveness of each population (species).

Several hypotheses may explain the marked differences in life colours, the more subtle differentiation of other morphological features, and the incomplete genetic differentiation between *E. clippertonensis* and *E. labriformis*. One hypothesis is that speciation has occurred despite recent gene flow. While an unlikely scenario, computer simulations indicate that this can occur when additional developmental mechanisms exist (Porter & Johnson, 2002). Felsenstein (1981) described the unlikely possibility of reproductive isolation when gene flow is prominent, concluding that recombination between fitness loci and assortive mating loci causes a breakdown in the favourable allelic combinations, and thus reduces fitness.

Divergence at these island localities with subsequent secondary contact with mainland individuals is also consistent with these data. However, the *E. labriformis* colour morph has not been observed at Clipperton Atoll, nor has the Clipperton colour pattern been documented on the mainland. The mainland colour morph has, however, been reported at the Alijos Rocks. The occurrence of individuals at Clipperton Atoll and Alijos Rocks with certain colour features that resemble those of mainland *E. labriformis* (such as prominent red tip of spinous dorsal fin) may indicate hybridization, but this is impossible to confirm with the available data.

Another, more likely hypothesis is that the evolution of external colours precedes or occurs more rapidly than changes in molecular markers commonly evaluated for evidence of reproductive isolation (species boundaries), and that we are capturing speciation at a relatively early stage in these groupers. For this hypothesis to remain tenable, one would expect a pattern of genetic diversification that reflects recent speciation with incomplete lineage sorting. Our data are consistent with this scenario in that the populations at Clipperton Atoll and Alijos Rocks have not reached reciprocal monophyly at the locus examined, yet show statistically significant differences in gene flow estimates. Furthermore, Clipperton Atoll and Alijos Rocks populations harbour several unique haplotypes that cluster together consistently in a statistical parsimony network, yet share only few 'mainland' haplotypes. The pairwise comparisons of Φ_{st} also support this hypothesis, as both Clipperton Atoll and Alijos Rocks populations show significantly different values in all comparisons (Table 2). This pattern is consistent with the expectations of

incipient species, namely that a current or historical restriction in gene flow has led to incomplete lineage sorting (Bowen, 1998). Coupled with the morphological data presented here, there is a strong case for the hypothesis that we are capturing speciation as it is occurring in nature.

While this phenomenon is relatively uncommon, distinct colour morphs that are thought to represent valid species occur in other fish taxa that lack genetic distinctiveness, such as rockfish of the genus *Sebastes* and hamlets of the genus *Hypoplectrus* (Domeir, 1994; Aguilar-Perrera, 2003; McCartney *et al.*, 2003; Garcia-Machado *et al.*, 2004). Given the importance of coloration and subtle morphological variation in closely related fish species, it is imperative that those performing molecular analyses deposit voucher material, including both whole specimens and photographs taken at time of capture, so that future investigators may confirm identifications.

Despite the lack of reciprocal monophyly at the mitochondrial DNA locus examined and the incomplete lineage sorting, these populations are probably travelling on unique evolutionary trajectories. The cyt *b* data from this analysis, the morphological differences previously documented (Allen & Robertson, 1999), and the distinctive colour patterns support this hypothesis. Given that there exists a specific name in the literature for the Clipperton Atoll population, we therefore suggest that the Alijios Rocks population be treated as *E. clippertonensis* Allen and Robertson as they share the morphological attributes which distinguish it from *E. labriformis* (scale counts, morphology and colour pattern).

The cyt *b* gene has been used with considerable success in elucidating population genetic structure in several fishes (Gilles *et al.*, 2000; Muss *et al.*, 2001; Yamamoto *et al.*, 2004). While other loci may provide finer resolution due to rapid mutation rate (mitochondrial control region; McMillan & Palumbi, 1997), our cyt *b* data indicate that this region provides appropriate resolution. Indeed, comparison of the hypervariable mitochondrial control region, sequenced for a subset of samples from the most distant localities (Loreto and the Galápagos Islands), revealed levels of variation similar to those for cyt *b* (data not shown). The maternal inheritance of mitochondria may confound the ability to detect subtle population differentiation or recent effects of bottlenecks (Avise, 2000). Future studies on this species should aim to assess variation in nuclear markers, including introns and microsatellites.

Phylogeography

The TEP has been divided into biogeographical regions based on the distributions of rocky shore fishes and habitat breaks (see *Discussion* above; Hastings, 2000). These habitat discontinuities have been hypothesized to play a major role in shaping the distribution and evolution of fishes within this coastal region. It would be expected that reef species with long-lived larval stages would be most likely to traverse these boundaries, and should be present in more than one faunal province and/or exhibit little population structure (Riginos & Victor, 2001). Given the distance that larvae must travel to cross the habitat breaks before settlement, some genetic discontinuities would be expected that correlate with the geography, even for widespread species. While the relatively long-lived pelagic larva of *Epinephelus* species is of key importance and is expected to promote gene flow, earlier studies have hypothesized that, in some instances, this attribute may not be a causal factor in predicting species geographical ranges or genetic connectivity. Victor & Wellington (2000) recorded the larval duration of 49 species of TEP shore fishes in the families Pomacentridae (damselfishes) and Labridae (wrasses) from daily otolith increments, and tested for a correlation between geographical range and pelagic larval duration. Surprisingly, they found that many species endemic to small areas possessed some of the longest pelagic larval stages, while species whose ranges were known to extend over large distances had some of the shortest larval durations. Their data suggest a somewhat counterintuitive hypothesis, that beyond a threshold length of time the planktonic duration of pelagic larvae may not drive speciation.

Our data support a panmictic population for mainland *E. labriformis*, as there is little to no genetic divergence correlated with distance along the quasi-linear coastline of the TEP. This suggests that the Sinaloan and Central American gaps are readily crossed by the flag cabrilla, probably during their prolonged larval stage. Despite the long-standing hypothesis and, in some cases, empirical evidence that such biogeographical breaks drive population differentiation (e.g. the Florida Peninsula: Bowen & Avise, 1990; Avise, 1992), it has been shown that, in certain lineages, some of these breaks do not (e.g. Point Conception: Burton, 1998). Thus the role of commonly recognized biogeographical barriers in shaping population differentiation and, ultimately, driving speciation appears to be variable and dependent on complex interactions between organisms and historical aspects of their environment.

The Clipperton Atoll lies some 1100 km from the mainland and 950 km from the nearest offshore islands of the Revillagigedo (Robertson & Allen, 1996). The fish fauna of the Clipperton Atoll has been described in detail (Robertson & Allen, 1996) and is a combination of TEP and western Pacific species. The atoll's isolation and reduced habitat diversity (Robertson & Allen, 1996) probably contribute to its relative paucity of species in comparison with other oceanic islands, in both the TEP and the western Pacific. While these and other islands of the TEP (e.g. Coco, Galápagos) harbour several unique species, it appears as though their degree of isolation is insufficient to drive speciation in the flag cabrilla. Although the Galápagos Islands lie approximately the same distance from the mainland as the Clipperton Atoll, the former is more 'connected' to the mainland by the dominant North Equatorial Current. In contrast, Clipperton Atoll is under the influence of the much weaker North Equatorial Counter Current during the period when flag cabrilla on the mainland probably spawn and are dispersed in the plankton (June–September, B. Erisman, pers. comm.), and would thus have little input of

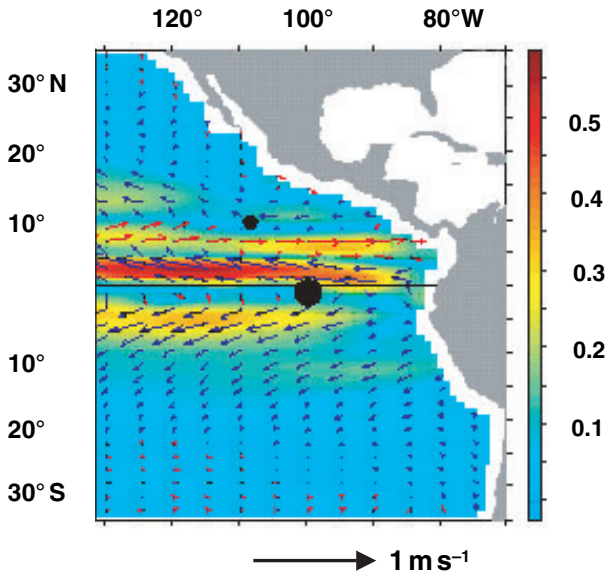

Figure 5 Mean surface current vectors overlying long-term mean, June–September 1994–2004. Coloured scale bar, long-term mean (m s^{-1}), large-scale vector, 1 m s^{-1}. Data from http://www.oscar.noaa.gov (Bonjean & Lagerloef, 2002). Small dot, Clipperton Atoll; large dot, Galápagos Islands.

recruits from mainland populations (Fig. 5). This situation is paralleled by that seen in the blennioid fish genus *Ophioblennius* for mid-Atlantic crossings (Muss *et al.*, 2001). The lack of gene flow to and from the Clipperton Atoll indicates a mechanism for larval retention (Robertson, 2001). The small current vectors surrounding the island during the likely spawning period for the Clipperton grouper (Fig. 5) indicate that perhaps larvae are not dispersed long distances from the island. Alternatively, eddy currents emanating from the islands may return larvae to their natal site.

While a similar evolutionary pattern has occurred at the Alijos Rocks, resulting in the differentiation of the population there, a subtly different mechanism may be responsible. Lying only 300 km from the outer coast of Baja California, the Alijos Rocks appear to be close enough to allow a large degree of larval transport between the island and the mainland. However, this small group of elevated rocks lies at the northernmost range of *E. labriformis* (Craig *et al.*, 2006), thus there are very few individuals on the mainland that may supply the island with propagules throughout most of the year. Instead, it seems probable that, during warm-water periods facilitated by the El Niño Southern Oscillation (ENSO), larvae that would normally be transported in a southerly direction are redirected towards the Alijos Rocks. These individuals could have then formed a founder population that is not under considerable influence by mainland populations, but may be interconnected with other island groups, given the sharing of haplotypes between Clipperton Atoll and the Alijos Rocks (Fig. 3). This hypothesis is supported by the occurrence of one adult *E. labriformis* taken at the Alijos Rocks. The individual was 260 mm standard length and was taken in 2004. Based on previous age and growth analysis for this species (Craig *et al.*, 1999), the individual was probably 6–7 years old. This would indicate that the individual settled during one of the strongest ENSO events on record during 1997–98. Alternatively, if there is no or only little gene flow among island populations, the population at the Alijos Rocks may be a striking example of convergent evolution in that the individuals there share many of the same morphological attributes that distinguish them from mainland *E. labriformis*.

Conservation

Fisheries management strategies are in transition from a species-by-species strategy to an ecosystem model (Sala *et al.*, 2002; Pikitch *et al.*, 2004). However, in order to maintain the biological diversity of any ecosystem effectively, it is important to assess how populations of organisms are interconnected. With a growing desire to implement networks of marine reserves, such data have become critical in understanding population dynamics, identifying source and sink populations, and targeting new areas for reserves (Sala *et al.*, 2002). Additionally, management decisions should take into account the recent evolutionary history of organisms and an accurate assessment of their taxonomic status (Templeton, 2004). Human-induced changes in both population genetic structure and life-history parameters of marine organisms have been documented, suggesting that anthropogenic impacts over relatively short time scales may have effects over evolutionary time scales. Our data provide lessons that may be applicable to management decisions within the TEP by confirming the specific status of a highly endemic island species and the lack of genetic structure in a broadly distributed species. If the observed lack of genetic diversity is a result of a high degree of dispersal, the flag cabrilla may be resilient to local extirpation and able to recolonize an overfished area relatively quickly. While our data suggest panmixia for this broadly distributed species, it is important to consider that a high degree of genetic connectivity may not necessarily indicate contemporary demographic connectedness, given that the number of migrants to maintain genetic diversity is in the order of tens per generation (Mills & Allendorf, 1996). The Clipperton grouper, however, should be treated as an endemic island species that is highly susceptible to extinction, and should be managed accordingly.

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