

Genetic connectivity among color morphs and Pacific archipelagos for the flame angelfish, *Centropyge loriculus*

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Abstract Color variation is used in taxonomic classification of reef fishes, but it may not reliably indicate evolutionary divergence. In the central Pacific, there are three color morphs of the flame angelfish, *Centropyge loriculus*: a red morph that occurs primarily in the Hawaiian archipelago, the endemic Marquesan color morph with reduced black markings, and an orange morph that occurs throughout the rest of Oceania. The red and orange morphs co-occur at Johnston Atoll (1,300 km south of Hawai'i), but intermediate forms have not been reported. To determine whether the three color morphs represent distinct evolutionary lineages, we compared 641 base pairs of mitochondrial cytochrome *b*. Forty-one closely related haplotypes were observed in 116 individuals. Analysis of molecular variance (AMOVA) indicated no significant genetic structure among color morphs ($\Phi_{ST} = 0.011$, $P = 0.147$). Likewise, there was no significant pairwise structure between sampling locations, separated by up to

5,700 km, after a Bonferroni correction ($\Phi_{ST} = 0.000$ – 0.080 , $P = 0.0130$ – 0.999). Genetic studies in conjunction with larval distribution data indicate that *Centropyge* species are highly dispersive. While there is a strong geographic component to the distribution of color morphs in *C. loriculus*, we find no evidence for corresponding genetic partitioning. We do not rule out an adaptive role for color differentiation, but our data do not support emerging species.

Introduction

Coloration plays an important role in the taxonomic classification of reef fishes, yet the evolutionary significance of these vivid displays is uncertain (McMillan et al. 1999; Bernardi et al. 2002). Color pattern is frequently the sole character used to distinguish closely related species; this is justified if it serves as a primary cue in the recognition of conspecifics (Randall 1998; McMillan et al. 1999). However, employing coloration in taxonomy may be problematic if variation is a result of phenotypic plasticity, rather than reproductive isolation (Grady and Quattro 1999). Another concern is that coloration may evolve rapidly (Endler et al. 2005) outpacing conventional morphological and genetic characters.

The family Pomacanthidae (marine angelfishes) is characterized by morphological similarity among congeners, and color patterns are often used as diagnostic characters (Pyle and Randall 1994). Pygmy angelfishes of the genus *Centropyge* (Kaup 1860) include 32 species divided into three subgenera and six species complexes (Pyle 2003). Previous research efforts have used the number of lateral-line scales or vertical scale

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rows as diagnostic characters, but Pyle (2003) found that these may be unreliable, varying with the size of the specimen. He found no meristic or morphological characters that unambiguously distinguish species, except when accompanied by color differences.

The “*bispinosa*” complex of the genus *Centropyge* includes five species (*C. bispinosa*, *C. ferrugata*, *C. loriculus*, *C. potteri*, and *C. shepardi*). The flame angelfish, *C. loriculus* (Günther 1874), is characterized by an overall orange to red color with up to seven broad black bars on the side of the body. The edges of the anal and dorsal fins are black with a blue margin, and the posterior margin of each has alternating blue and black markings (Fig. 1). Most populations have small black spots scattered across their dorsal and anal fins and adjacent portions of their bodies, although there is some variation throughout the range of *C. loriculus*, which spans most of Oceania (Pyle 2003).

Centropyge loriculus in the Marquesas Islands have almost no black coloration (Fig. 1). Aquarists have long recognized a “Hawaiian” color morph (hereinafter referred to as the “red morph”), which exhibits a bright red coloration and a reduced number of black markings on the body. It possesses fewer and generally smaller black spots scattered across the dorsal and anal fins, compared to conspecifics from other locations (the latter hereinafter referred to as the “orange morph”). The red morph also does not exhibit the black margin on the posterior edge of the operculum or on the dorsal edge of the pectoral-fin base as consistently observed in the orange morph. Notably, the two color morphs coexist at Johnston Atoll, the closest reef habitat about 1,300 km south of the Hawaiian Archipelago (Fig. 1).

In cursory field surveys at Johnston Atoll, no individuals were observed with an intermediate phenotype (Pyle 2003).

Although color variation can be attributed to differences in diet or habitat, the red and orange morphs appear to retain their differences where they co-occur and when placed in a “common garden” aquarium setting (R. L. Pyle, personal observation). It is thus possible that positive assortative mating, linked with differences in coloration and markings, inhibits hybridization between the two morphs, and geographic isolation may maintain the third Marquesan color morph. Such barriers to gene flow might warrant the elevation of these color morphs to species status. However, in a previous survey of Atlantic *Centropyge* spp., sister taxa defined exclusively by coloration were found to be genetically indistinguishable, with high connectivity across the western Atlantic (Bowen et al. 2006b).

Here we investigate whether color differences in the Pacific *C. loriculus* are associated with molecular evolutionary divergence. We also assess the genetic connectivity of this reef species occurring from Hawai’i to Vanuatu, a range of over 5,700 km. To accomplish these goals, we sequenced 641 base pairs (bp) of the mitochondrial cytochrome *b* gene in 116 individuals from throughout the Central and West Pacific.

Materials and methods

C. loriculus specimens were collected from ten localities (Table 1; Fig. 1), including the Hawaiian Islands ($n = 6$), Johnston Atoll (28), Palmyra (9), Christmas

Fig. 1 Map of ten collection sites and three color morphs (red at top right, orange in middle, Marquesan at bottom right) photos by J.E. Randall

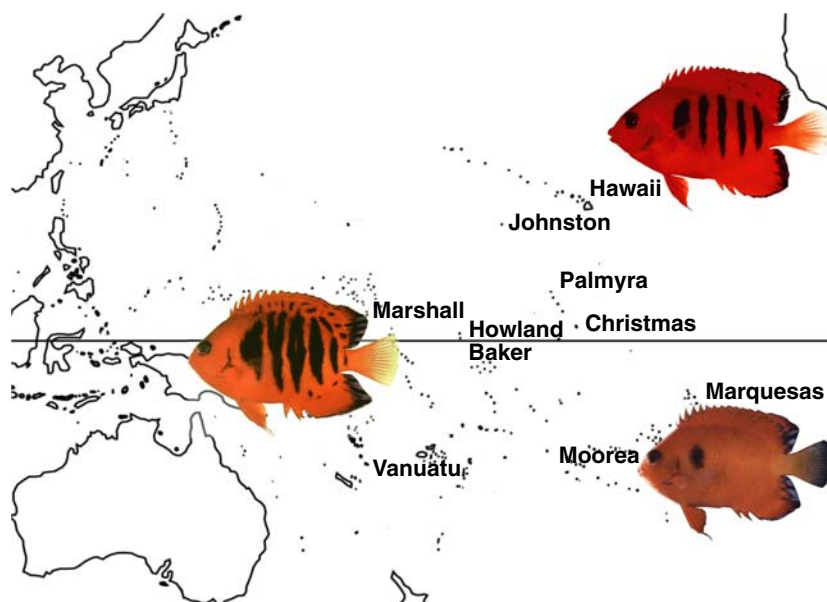


Table 1 Sample size and descriptive statistics for cytochrome *b* data of 116 *Centropyge loriculus*

Location	<i>N</i>	No of haplotypes	No of unique haplotypes	Haplotype diversity (<i>h</i>)	Nucleotide diversity (π)	Tajima's <i>D</i>	Fu's <i>F_S</i>
Marquesas							
Marq. morph	10	8	4	0.93	0.0034	-1.7295*	-4.7782**
Moorea	6	4	2	0.80	0.0026	-1.3369	-0.8335
Vanuatu	8	7	1	0.96	0.0026	-1.2801	-5.1619**
Marshall	13	8	5	0.80	0.0034	-1.3247	-3.3655**
Baker	10	9	5	0.98	0.0050	-2.0100*	-5.2597**
Howland	9	7	2	0.94	0.0053	-1.3907	-2.2092
Christmas	17	11	6	0.88	0.0026	-2.1809*	-8.4184**
Palmyra	9	6	1	0.83	0.0024	-1.7666*	-2.8718**
Johnston							
Orange morph	10	6	1	0.84	0.0026	-0.8427	-2.2900
Red morph	18	8	2	0.80	0.0022	-1.8362*	-3.7975
Hawai'i							
Red morph	6	5	0	0.93	0.0026	-1.3369	-2.51803

*Indicates significance at 0.05 level, **indicates significance at 0.02 level

Island, Kiribati (17), Howland Island (9), Baker Island (10), Marshall Islands (13), Marquesas Islands (10), Vanuatu (8), and Moorea (6). Low sample size in the Hawaiian Islands was due to extreme scarcity and the corresponding difficulty in locating specimens. The six Hawaiian and 18 of the 28 Johnston Atoll specimens exhibited the red color morph ($n = 24$). The specimens from the Marquesas had reduced black markings ($n = 10$), and the remainder ($n = 82$) exhibited the orange color morph. To assess the monophyly of the species, we sampled a single specimen from every other species within the “*bispinosa*” complex, including *C. bispinosa*, *C. ferrugata*, *C. shepardi* and *C. potteri*. Specimens were captured with nets and spears. Tissue samples were stored in a salt-saturated-DMSO buffer (Amos and Hoelzel 1991) or 95% ethanol.

Total genomic DNA was extracted using a standard salting out procedure (Sunnucks and Hales 1996). An approximately 700 bp fragment of the mtDNA cytochrome *b* gene was amplified using the following primer sequences designed by Song et al. (1998) and Taberlet et al. (1992), respectively: heavy strand 5'G T GACTTGAAAACCACCGTTG and light strand 5' AATAGGAAGTATCATTCGGGTTTGATG. Each 50 μ L reaction consisted of: 0.26 μ M each primer, 2 mM dNTPs, 3 mM MgCl₂, 0.5 units of polymerase, and 30–80 ng extracted DNA. All reagents were manufactured by Bionline Inc., London. Thermal cycling parameters for polymerase chain reactions (PCR) consisted of an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 94°C, 60 s; 50°C, 30 s; 72°C, 45 s and a final extension at 72°C for 10 min. The PCR product was prepared for automated sequencing using the DNace clean up kit (Bionline). PCR products were sent to Macrogen Inc. (Seoul, South Korea) for

sequencing in the forward and reverse directions on an ABI 3730XL Automated Sequencer. DNA sequences were edited using Sequencher (v4.52b; Gene Codes Corporation, Ann Arbor, MI, USA) and are available in Genbank (accession nos. DQ489742–DQ489786).

Sequences were aligned using ProAlign (v0.5 alpha 1; Loytynoja and Milinkovitch 2003). Phylogenetic trees were constructed in PAUP (v4.0b10; Swofford 2002). The Akaike Information Criteria (AIC) within the program Modeltest (v3.7; Posada and Crandall 1998) was used to determine the best model for constructing neighbor joining (NJ, Saitou and Nei 1987) and maximum likelihood trees (using the heuristic method). A pruned tree was constructed using ten representative *C. loriculus* haplotypes and single specimens from all other members of the “*bispinosa*” species complex. Confidence in each node was assessed by bootstrapping with 1,000 replicates. A statistical parsimony network was constructed using the computer program TCS (v1.21; Clement et al. 2000), which estimates gene genealogies from DNA sequence data. Clades within the TCS diagram were nested by hand. Genetic variation across geographic space was interpreted using GeoDis (v2.4; Posada et al. 2000) and biological significance was assessed with the inference key by Templeton (2004).

Molecular diversity and structure indices were calculated overall, among color morphs and geographic sites using Arlequin (v3.0; Excoffier et al. 2005). Estimates of haplotype diversity (*h*) and nucleotide diversity (π) were calculated using the algorithms in Nei (1987) as implemented in Arlequin. Tajima's *D* (Tajima 1989) and Fu's *F_S* (Fu 1997) were calculated for each site to assess neutrality and population expansion using 1,000 permutations. Analysis of molecular variance (AMOVA) among color morphs was performed using 10,000 permutations.

Population pairwise Φ_{ST} values (a value similar to F_{ST} which incorporates sequence divergence among haplotypes) were calculated using 10,000 permutations for significance. An exact test for population differentiation based upon haplotype frequencies was calculated using 100,000 steps in the Markov chain and 1,000 dememorization steps. The significance level after Bonferroni Correction was 0.0011.

Time depths for phylogenetic separations in *Centro-pyge* are based on a sequence divergence estimate of 2% per MY between lineages, or 10^{-8} within lineages (Bowen et al. 2001). We used the shape of the nucleotide mismatch distribution to test for exponential population growth (Rogers and Harpending 1992). Resampled distributions of Harpending's (1994) raggedness statistic were used to assess the fit of the mismatch distribution to the growth model. The equation, $\theta = 2N_f\mu$, was used to estimate female effective population size at the time of population expansion (θ_0) and for present-day populations (θ_1) (Rogers and Harpending 1992). N_f is the effective female population size, and μ is the per haplotype mutation rate (6.41×10^{-6} based on the rate of 10^{-8} per site multiplied by 641 bp and the generation time). Tau ($\tau = 2\mu t$) was estimated from the crest of the mismatch distribution and provides an estimate in generations (t) of the interval to population coalescence. Generation time for *Centro-pyge* was estimated to be one year, based on captive observation of maturity at 220 days and a lifespan of at least 3 years (F. Baensch and M. M. F. Lutnesky, personal communication).

Results

Among the 116 specimens of *C. loriculus* from ten geographic regions, we found 47 polymorphic sites in 641 bp of cytochrome *b*. Forty-one haplotypes were observed, of which 26 were unique to a single individual. The most common haplotype was shared by 38 individuals from all geographic locations and color morphs (Fig. 3).

The data was best fit by the HKY + I model which allows for different transition and transversion substitution rates and unequal nucleotide frequencies (Hasegawa et al. 1985). Maximum likelihood and neighbor joining analyses including all specimens produced a comb-like tree (not shown). The pruned neighbor-joining tree indicates monophyly and shallow coalescence in *C. loriculus* (Fig. 2) and is identical to a maximum likelihood tree in describing the relationships among species. The divergences between *C. loriculus* and the other species of the “*bispinosa*” complex

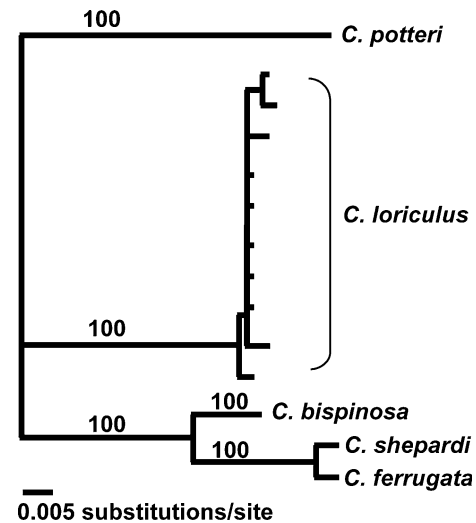
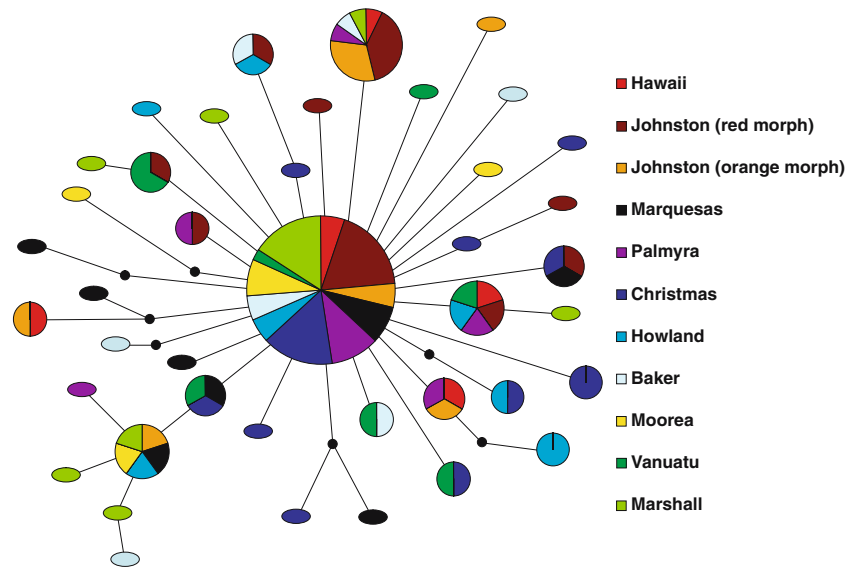


Fig. 2 Neighbor joining tree (with 1,000 bootstrap replicates) showing relationships within the “*bispinosa*” species complex of the genus *Centro-pyge*. Only ten *C. loriculus* haplotypes are shown with one specimen from *C. potteri*, *C. shepardii*, *C. ferrugata*, and *C. bispinosa*

are high ($d = 0.107$ – 0.121), whereas the deepest divergence within *C. loriculus* ($d_{max} = 0.005$) is over an order of magnitude lower. The parsimony network demonstrates a lack of divergence between geographic regions as well as color morphs (Fig. 3). Seventeen primary level clades nest into two secondary clades (not shown). A permutation analysis (1,000 resamples) of all clades reveals no geographic association of haplotypes ($P = 0.460$ – 1.000). Nested clade analyses of geographic distances within and among lineages are inconclusive based on the inference key by Templeton (2004). We conclude that nested clade analysis may not be informative for the star-like networks (see Fig. 3) which often characterize marine organisms. Tajima's D and Fu's F_S are designed to detect selection; however, these tests are also sensitive to population expansion. Significant values with both tests indicate expansion at the following populations: Marquesas, Baker, Christmas and Palmyra (Table 1).

Haplotype diversity is high overall ($h = 0.88$) and within sample locations ($h = 0.80$ – 0.98 ; Table 1). Nucleotide diversity is low overall ($\pi = 0.0031$) and within sample locations ($\pi = 0.0022$ – 0.0053), indicating shallow divergences in this mtDNA genealogy. AMOVA results reveal no significant difference among the three color morphs ($\Phi_{ST} = 0.011$, $P = 0.147$). The Φ_{ST} comparisons between geographical locations also did not indicate genetic structure. While comparisons between Johnston Atoll and four locations (the Marshall Islands, Christmas Island, Vanuatu and Howland Island) were significant at $\alpha = 0.05$, none of the pairwise comparisons ($\Phi_{ST} = 0.00$ – 0.082) indicate significant population differentiation

Fig. 3 Statistical parsimony network for *Centropyge loriculus* based upon 41 haplotypes of the mtDNA cytochrome *b* gene. The size of circles is proportional to the number of individuals displaying the haplotypes, unique haplotypes are represented by ovals, circles represent shared haplotypes. Each uninterupted line represents a single mutation; black dots represent hypothetical haplotypes



($P = 0.0140-0.999$) after a Bonferroni correction (significance level = 0.00110, Table 2). An exact test of population differentiation based upon haplotype frequencies is not significant among color morphs (global P value 0.150 ± 0.04) or sample locations (global P value 0.380). Comparisons between Johnston Atoll and three locations (Christmas Island, Howland and Marquesas Islands) showed pairwise P values of less than 0.05, but none of the exact tests of pairwise differentiation were significant after a Bonferroni correction (significance level = 0.00110, Table 2). There is an overall pattern of genetic homogeneity with the possible exception of Johnston Atoll, which has low but significant levels of differentiation prior to Bonferroni correction.

The mismatch distribution of *C. loriculus* fits the exponential growth model of Harpending et al. (1993) and hence could be used to estimate historical and current demographic parameters. The mismatch $\tau = 2.09$ was used to estimate the most recent population expansion at approximately 160,000 years BP. The estimate of

initial female population size is $N_f = 0$, indicating a founder event or severe population crash. The contemporary female effective population size is estimated at $N_f = 2.6 \times 10^8$.

Discussion

The three color morphs of *C. loriculus* are sufficiently distinct to raise the possibility of taxonomic assignments. The red and orange types co-occur at Johnston Atoll without apparent intermediates, yet we found no differences in haplotype frequencies ($\Phi_{ST} = 0.003$, $P = 0.329$). Although there is ample sequence diversity within cytochrome *b*, with 41 haplotypes in 116 individuals, there is no evidence of genetic partitioning among color morphs (Fig. 3) or geographic locations (Table 2).

These results corroborate conclusions based on phylogenetic analyses, indicating a shallow population

Table 2 Pairwise population Φ_{ST} values (below diagonal)

	Marquesas	Moorea	Vanuatu	Marshall	Baker	Howland	Christmas	Palmyra	Johnston	Hawai'i
Marquesas	–	1.0000	0.5589	0.7439	1.0000	0.7840	0.7890	1.0000	0.0370	1.0000
Moorea	0.0000	–	0.3329	0.9402	1.0000	0.8393	0.9150	1.0000	0.3691	1.0000
Vanuatu	0.0008	0.0000	–	0.0549	0.7963	0.5242	0.4478	0.3615	0.1265	0.7743
Marshall	0.0000	0.0000	0.0397	–	0.4297	0.2933	0.5509	0.8753	0.1617	0.6871
Baker	0.0000	0.0000	0.0000	0.0049	–	0.7441	0.2676	0.7893	0.0832	1.0000
Howland	0.0126	0.0000	0.0188	0.0326	0.0000	–	0.3256	0.5571	0.0351	0.7744
Christmas	0.0000	0.0000	0.0000	0.0658	0.0023	0.0316	–	0.5714	0.0069	0.4685
Palmyra	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	–	0.6051	0.9680
Johnston	0.0496	0.0377	0.0685	0.0780	0.0230	0.0819	0.0495	0.0000	–	0.6600
Hawai'i	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	–

All values non-significant after a Bonferroni correction ($\alpha = 0.0011$). Exact test of sample differentiation P values (above diagonal)

history embedded in a deep organismal lineage (Fig. 2; see Grant and Bowen 1998). While we regard time estimates as provisional, it is apparent that *C. loriculus* diverged from its nearest extant relative in the late Miocene or Pliocene, approximately 5–6 million years BP ($d = 11\text{--}12\%$), while contemporary individuals coalesce to a common ancestor on the order of 160,000 years BP ($d = 0.2\text{--}0.5\%$).

Before dissecting these results, two caveats merit consideration. First, the red color morph occurs in the Hawaiian Islands, but our small sample size ($n = 6$) reflects its low density. Conclusions are based primarily upon the 18 red individuals collected at Johnston Atoll. However, given that all six Hawaiian and 16 red individuals from Johnston share haplotypes with orange specimens from other locations, we believe that the conclusion of genetic homogeneity among color morphs is robust. Second, using a maternally inherited mtDNA marker, we cannot reject the possibility of either population structure or evolutionary divergence in the nuclear genome. Biparentally-inherited (nuclear) markers subject to faster mutation rates, such as microsatellite loci, may reveal population partitioning. There is little reason to suspect that nuclear markers would show a fundamentally different pattern, though, because *Centropyge* spp. are pelagic spawners (Thresher 1984) and unlikely candidates for sex-biased dispersal.

Incongruence between color-based taxonomy and genetic structure in the genus *Centropyge* has been previously documented. West Atlantic *Centropyge argi* and *C. aurantonotus* are defined by coloration yet their mtDNA control region sequences are indistinguishable (Bowen et al. 2006b). Several other coral-reef fishes exhibit color differences in the absence of molecular divergence. Hamlet morphospecies (genus *Hypoplectrus*, family Serranidae) are differentiated by color but do not uniformly sort into distinct genetic lineages (McCartney et al. 2003; Ramon et al. 2003). Three closely related species of butterflyfishes (genus *Chaetodon*, family Chaetodontidae) show conspicuous color differences, yet only one exhibits assortative mating and genetic divergence (McMillan et al. 1999). Four species of Pacific damselfishes (genus *Dascyllus*, family Pomacentridae) vary in geographical range and color pattern, but genetic analyses reveal that a single color morph is shared by three mtDNA lineages, while one lineage contains two color morphs (Bernardi et al. 2002). Color morphs of the arceye hawkfish (*Paracirrhites arcatus*, family Cirrhitidae) are indistinguishable on the basis of mtDNA haplotypes for specimens collected throughout a broad Indo-Pacific range (G. Bernardi and E. DeMartini personal communication). Despite the

above examples, most reef fishes demonstrate concordance between coloration and mtDNA divergence, especially in wrasses (genus *Halichoeres*, family Labridae: Rocha 2004) and damselfishes (family Pomacentridae Doherty et al. 1994; Bay et al. 2006).

These examples show that coloration may be an uncertain basis for taxonomy and may not be concordant with genetic partitioning in coral reef fishes. Several theories have been proposed to explain why geographic segregation of color morphs is not accompanied by phylogenetic divergence. The incongruence observed in *C. loriculus* and other coral reef fishes may be explained by recent isolation, incomplete species boundaries, phenotypic plasticity, or selection. In the next four sections, we explore each of these hypotheses.

Recent isolation

Striking color differences in the absence of phylogenetic partitioning can result from recent reproductive isolation via allopatry or assortative mating, with shared mtDNA haplotypes reflecting historical (previous) connectivity (McCartney et al. 2003). Divergence in color morphs or other morphological characters may outpace genetic divergence due to the time lag required for mtDNA mutation and lineage sorting. On average this process requires $4N_e$ generations where N_e is the effective population size (Avice 2004). Therefore, a recent isolation event may be reflected in color morphs but not detected in mtDNA sequences. For example, the Atlantic yellowhead wrasse (*Halichoeres garnoti*, family Labridae) exhibits striking color differences between Caribbean and Bermudan populations in the absence of mtDNA divergence (Rocha 2004). However, the population in Bermuda is likely less than 15,000-years-old, post-dating the water temperature increase after the last glacial maximum. This may be an insufficient amount of time for mtDNA lineage sorting between the two color morphs. Sympatric Caribbean hamlet morphospecies (genus *Hypoplectrus*) show assortative mating with respect to their strikingly distinct color patterns but only modest frequency differences in microsatellite alleles and mtDNA (McCartney et al. 2003).

Sympatric red and orange color morphs of *C. loriculus* at Johnston atoll are genetically indistinguishable. The lack of intermediate forms could indicate assortative mating. However, the haplotype frequency differences used to support the hypothesis of recent isolation in other fish species (Campton et al. 2000; McCartney et al. 2003; Craig et al. 2006) are not exhibited by the

three *C. loriculus* color morphs. For this reason, we provisionally discount the explanation of recent isolation but cannot rule out extremely recent separations, as observed in the Bermudan wrasse.

Incomplete species boundaries

Genetic differentiation may be prevented by occasional hybridization between established morphospecies that come into secondary contact. This hypothesis has been proposed for the Caribbean hamlet multispecies complex, based on the presence of two ancient mtDNA lineages, coupled with the observation of rare mixed-morph matings (Ramon et al. 2003). Although positive assortative mating may predominate, a low level of introgression between color morphs is sufficient to prevent monophyly in mtDNA (Takahata and Slatkin 1984). Hybridization has been previously documented in pygmy angelfish. *C. loriculus* × *C. potteri* hybrids occur in Hawai'i and are identified by their intermediate coloration (Pyle and Randall 1994), but no mtDNA introgression has been detected between these phylogenetically distinct species (JKS personal observation). Our *C. loriculus* survey did not identify deep lineages that may have been transplanted between historically isolated color morphs (Fig. 3). Hence, hybridization or incomplete boundaries between evolutionary lineages is an unlikely explanation for the incongruence between coloration and mtDNA variation in *C. loriculus*.

Phenotypic plasticity

Phenotypic plasticity may account for color variation in fishes, especially when exposed to different environmental regimes. Aquaculture experiments demonstrate that pigments such as carotenoids can induce a wide range of coloration in fish (e.g., Ako et al. 1999). However, color morphs of *C. loriculus* retain their distinction when maintained under similar aquarium conditions (RLP personal observation). Furthermore, the red and orange color morphs co-occur at Johnston Atoll, reducing the likelihood that diet or habitat is responsible.

Selection

In Pacific butterflyfishes (genus *Chaetodon*), selection may maintain color pattern differences in cases where gene flow is sufficient to homogenize mtDNA haplo-

types (McMillan et al. 1999). The different color patterns of *Chaetodon punctatofasciatus* and *C. pelewensis* are not accompanied by population level genetic divergence, except at the extremes of their respective ranges. In principle, modest gene flow where the species overlap should be sufficient to homogenize color morphs. Yet, color differences may be maintained by sexual selection. Based upon field observations and captive breeding, McMillan et al. (1999) hypothesized that an intermediate color morph may be at a selective disadvantage in establishing and defending a territory, if it is unable to signal conspecifics. Coloration may be relevant to mate recognition in *Centropyge* species which are known to form harems with 2–7 females (Moyer and Nakazono 1978; Lutnesky 1992). Overt differences in coloration may reduce the likelihood of successful reproduction; Pyle and Randall (1994) suggested that individuals with an intermediate color morph may be excluded from *Centropyge* harems or may have the lowest rank within harems.

Conclusion

The evolutionary significance of coloration is poorly understood and likely to be multi-faceted (Andersson 1994). Our data do not support nomenclatural distinction of the red, orange and Marquesan color morphs of *C. loriculus*, as the variation does not appear to reflect reproductive isolation or genetic divergence. However, we cannot deny that color is an important diagnostic character for the taxonomy of this genus. We agree with Wiens (2004) and Lee (2004) that molecular analyses are valuable but cannot replace the morphological and ethological characters used to define species boundaries.

Reproductive behavior and coloration may co-evolve rapidly, so that genetically similar individuals are effectively isolated (e.g., cichlids; Dominey 1984). In contrast, some fishes have highly divergent color patterns, and yet they hybridize (e.g., hamlets; McCartney et al. 2003; Ramon et al. 2003). In resolving taxonomy and evolutionary lineages, each case must be considered independently, using morphological and molecular data where possible, with the recognition that taxonomic distinctions based on either coloration or genetics alone can be hazardous.

The flame angelfish exhibits high connectivity across 5,700 km, remarkable for a diminutive reef fish. Sharing of haplotypes across such broad geographic range may be partially explained by the 38-day pelagic larval duration (PLD) of *C. loriculus* (Thresher and Brothers 1985). However, PLD alone is a poor predictor of population

structure in reef fishes (Kinlan et al. 2005; Bay et al. 2006; Bowen et al. 2006a), and connectivity may depend on larval behavior more than larval duration (Leis and Carson-Ewart 1998; Taylor and Hellberg 2003; Jones et al. 2005). *Centropyge* larvae have been caught in oceanic trawls (MCZ 73476, 73518, 73521, 73531-2, 73546, 735554-6, 81683, 82468, 158311, 163525; <http://www.mcz.harvard.edu/Departments/Fish/>) in the mid-Atlantic (e.g. 40°20'N, 50°42'W) to the exclusion of many common and abundant reef fishes with longer PLDs (D. Smith and K. Hartel personal communication). Hence, *Centropyge* spp. appear to have extensive oceanic dispersal. These observations provide an obvious explanation for the genetic homogeneity among locations, but leave open the question of how color morphs remain geographically segregated. In a genus where coloration is intimately connected to reproductive behavior, sexual selection is the most likely mechanism for geographic partitioning of color morphs in a highly dispersive species.

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