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STRONG GENETIC DIVERGENCE AMONG POPULATIONS OF A MARINE FISH WITH LIMITED DISPERSAL, *ACANTHOCHROMIS POLYACANTHUS*, WITHIN THE GREAT BARRIER REEF AND THE CORAL SEA

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Abstract.—*Acanthochromis polyacanthus* is an unusual tropical marine damselfish that uniquely lacks pelagic larvae and has lost the capacity for broad-scale dispersal among coral reefs. On the modern Great Barrier Reef (GBR), three color morphs meet and hydridize at two zones of secondary contact. Allozyme electrophoreses revealed strong differences between morphs from the southern zone but few differences between morphs from the northern counterpart, thus suggesting different contact histories. We explore the phylogeography of *Acanthochromis polyacanthus* with mitochondrial cytochrome *b* region sequences (alignment of 565 positions) obtained from 126 individuals representing seven to 12 fish from 13 sites distributed over 12 reefs of the GBR and the Coral Sea. The samples revealed three major clades: (1) black fish collected from the southern GBR; (2) bicolored fish collected from the GBR and one reef (Osprey) from the northern Coral Sea; (3) black and white monomorphs collected from six reefs in the Coral Sea. All three clades were well supported (72–100%) by bootstrap analyses. Sequence divergences were very high between the major clades (mean = 7.6%) as well as within them (2.0–3.6%). Within clades, most reefs segregated as monophyletic assemblages. This was revealed both by phylogenetic analyses and AMOVAs that showed that 72–90% of the variance originated from differences among groups, whereas only 5–13% originated within populations. These patterns are discussed in relation to the known geological history of coral reefs of the GBR and the Coral Sea. Finally, we ask whether the monospecific status of *Acanthochromis* should be revisited because the sequence divergences found among our samples is substantially greater than those recorded among well-recognized species in other reef fishes.

Key words.—Coral reef fish, Coral Sea, cytochrome *b*, founder effect, Great Barrier Reef, phylogeography, population structure.

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There is a general presumption in marine ecology that the high fecundity of marine organisms and the production of planktotrophic (self-feeding) larvae are associated with broad propagule dispersal and high gene flow over vast distances (Ward et al. 1994; Shaklee and Bentzen 1998). However, recent molecular surveys have begun to show the full extent of cryptic speciation resulting from limited gene flow (Knowlton 1993; Knowlton and Jackson 1994). Partly for reasons of access, information about speciation in the sea is still in a formative state (Palumbi 1992, 1994) and most attention so far has been given to invertebrate taxa and often to species with local dispersal and small population sizes. In contrast, marine fish generally are considered to have high dispersal and large populations, which makes them poor candidates for differentiation at scales smaller than the major oceanographic recirculations that determine their stock structures (Sinclair 1988). One of the exceptions is an unusual tropical damselfish, *Acanthochromis polyacanthus*, which lacks pelagic larvae.

Acanthochromis polyacanthus is a monotypic genus established for an unusual damselfish found in the Indo-West Pacific from the Philippines to the Great Barrier Reef (GBR). This species is the only damselfish known to lack pelagic larvae (Robertson 1973). Instead it lays small clutches of unusually large eggs that are defended by both parents for an extended period of time (Thresher 1983). Larvae hatching from these eggs are large and advanced relative to confamilials. After hatching, the fry school together near the nest site, rather than dispersing into the water column, and continue to be defended by the parents until they are fledged into the surrounding habitat at relatively large sizes (Thresher 1985; Kavanagh 2000). The absence of a pelagic larval stage has great significance for the population dynamics and colonizing ability of *A. polyacanthus*. Unlike species with pelagic larvae, there is no obvious mechanism for dispersal between isolated habitats. This extreme philopatry is associated with strong spatial patterns in color and genetic characters.

In the first systematic survey of these variations, Doherty et al. (1994) described three color morphs from the central and southern GBR. Allozyme analysis found strong differences at three loci between northern and southern morphs, suggestive of secondary contact between stocks that had been isolated during Pleistocene sea-level regressions. Planes and Doherty (1997a) showed that an old river channel delineates the contact zone under modern sea levels. Reefs on the northern side of this channel are populated exclusively by bicolored fish, whereas reefs on the southern edge are populated exclusively by dark fish. At the mouth of this channel, both phenotypes were found to coexist in a classic pattern of habitat partitioning on Hyde Reef with narrow hybrid zones on each side of the reef. Despite this evidence of outcrossing, there were steep and concordant clines of color and gene frequencies around the perimeter of this reef that appeared to be maintained over ecological time scales by assortative mating. Although signs of introgression were detected at both

FIG. 1. Sampling localities and color morphs of *Acanthochromis polyacanthus* in the GBR and the Coral Sea. At Hyde Reef two sites were sampled, one for each color morph.

local and regional scales, secondary contacts in the central GBR were still characterized by strong genetic heterogeneity and limited gene flow between color morphs.

On the northern GBR (Planes and Doherty 1997b), the bicolored morph meets and breeds with a monochromatic white morph, which was presumed to represent a third stock. Unlike the southern contact zone between bicolored and black fish, these hybrid zones were not characterized by concordant patterns of color and genetic markers. Either their genomes were homogenized through previous contacts or the color polymorphism has evolved very recently (Allen 1975).

To date, only allozymes have been used to describe the genetic structure within populations of *A. polyacanthus*. Although capable of detecting bottlenecks on gene flow, these markers are limited in their ability to reconstruct phylogeographic history; unlike modern techniques based upon DNA sequencing (e.g. Avise 1992, 1994; McMillan and Palumbi 1995; Bowen and Grant 1997; Bernardi 2000; Terry et al. 2000). In addition, some mitochondrial regions, such as cytochrome *b*, have been used extensively in fish studies, where divergence rates have been independently estimated on several occasions (Meyer 1993). In this study, we analyzed the genetic variability of the cytochrome *b* region of *A. polyacanthus* from several sites on the GBR and from isolated reefs in the adjacent Coral Sea. Our objectives were to determine the phylogenetic relatedness of different color morphs within this species and to test the hypothesis that reefs in the Coral Sea may provide a refugium for coral reef species during periods of low sea level (Davies 1989). On the basis of our discoveries, we were obliged also to ask whether *Acanthochromis* is truly a monotypic genus.

MATERIALS AND METHODS

Samples and DNA Extraction

In 1997 and 1998, samples of 40 to 50 *A. polyacanthus* were collected from 13 sites distributed over 12 reefs of the GBR and the Coral Sea (Fig. 1). Two sites were sampled on Hyde Reef, the only reef to have two sympatric morphs.

Three main color morphs were distinguished in this study (Fig. 1). A bicolored morph was sampled from locations in the northern and central GBR. At Hyde Reef, the morph

coexists with a black monochromatic morph that dominates reefs in the southern GBR. Variants on both of these morphs were also observed in the Coral Sea. In addition, reefs from the central section of the Queensland Plateau had monochromatic white morphs similar to those found in the far north of the GBR (Planes and Doherty 1997b).

Fish were speared and placed immediately in ice slurry. Soon afterward, they were stored at −20°C until dissection, which was done on the same day. Tissue samples from pectoral fins were stored at 4°C in DMSO solution saturated with NaCl. Tissues were digested overnight at 55°C in 500 μl of extraction buffer (400 mM NaCl, 10 mM Tris, 2 mM EDTA, 1% SDS, and 20 μg/μl proteinase K). We purified the DNA by standard chloroform extraction and isopropanol precipitation (Sambrook et al. 1989). Only a random sample of 12 individuals per sites was used for subsequent DNA analysis with considering two groups (i.e., two color morphs) for Hyde Reef.

Because *A. polyacanthus* is a monotypic genus, sequences from species of two closely related genera, *Chromis chromis* and *Dascyllus trimaculatus*, which belong to the same subfamily (Chrominae), were used as outgroups (Bernardi and Crane 1999).

Polymerase Chain Reaction and Sequence Analysis

Amplification of the mitochondrial cytochrome *b* region was accomplished using two successive polymerase chain reaction (PCR) amplifications. The first amplification used universal primers CYT1-L and CB3-H (Meyer 1992). Each 100-μl reaction contained 10–100 ng of DNA, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 units of Taq DNA polymerase (Perkin-Elmer, Norwalk, CT), 150 μM of each dNTP, and 0.3 mM of each primer. Each reaction 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. Both DNA strands from 10 individuals were sequenced, and the resulting sequence alignment was used to design an additional specific primer. For the remaining samples, the first PCR amplification was followed by a second amplification (nested PCR) using the same protocol but with CYT1-L and the newly designed primer, CBE-H-APO (5′-GAG GTC TTT GTA TGA AAA GTA-3′) and the product of the first PCR as a template. Ten individuals resulting from this new protocol were sequenced on both DNA strands and, because no differences between strands could be detected, all remaining samples were sequenced on the forward strand. After PCR purification following the manufacturer's protocol (Perkin-Elmer, Applied Biosystems, Foster City, CA), sequencing was performed with the primer used in the PCR amplification on an ABI 373 automated sequencer. Sequence alignment was done with the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems).

Phylogenetic relationships were assessed using the neighbor-joining method (Nei 1987) implemented by the software package PAUP (ver. 4.0; Swofford 1998). Topological confidence was evaluated with 1000 bootstrap replicates for neighbor joining (Felsenstein 1985) and with 1000 replicates using the fast-step method for maximum parsimony (only one tree kept at each replicate). In both neighbor joining and maximum parsimony, bootstrapping analyses were performed with equal weighting of transitions and transversions, as well as with transversions weighted three times as much as transitions. F_{ST} and haplotype diversity were calculated using the software package DNASP (Rozas and Rozas 1997) following Hudson et al. (1992). Population structure was calculated by an analysis of molecular variance (AMOVA; Excoffier et al. 1992), using ARLEQUIN (ver. 1.1; Schneider et al. 1997). We used AMOVA to test the significant partitions of total genetic variance (F_{CT}) among regions defined from the dendrogram and computing two AMOVA with two levels of structuration: one looking at all populations and another only analyzing population of the Coral Sea. The significance of individual *F*-statistics was tested by comparison to null distributions constructed from 1000 random permutations of the original data matrix.

RESULTS

Sequence Analysis and Phylogenetic Reconstruction

Mitochondrial cytochrome *b* region sequences were obtained from 126 individuals. Alignments included 565 positions, 151 of which were variable and 89 were phylogenetically informative. No evidence for size polymorphism due to repeated sequences or heteroplasmy was found. Average pairwise sequence divergences, diversity indexes, and haplotype diversity are shown in Table 1. As expected, transitions were more common than transversions (ratio = 2.7). Yet, when a 3:1 transversions:transitions weighting was used, tree topologies remained unchanged, indicating that saturation had minimal effect on the data.

The phylogenetic reconstruction obtained with the neighbor-joining method is presented in Figure 2. The samples partitioned into three major clades. The first clade (denoted as clade 3 in Fig. 2), which was present in 100% of the bootstrap replicates, included all samples of black fish collected from the southern GBR reefs. This included black fish from Hyde Reef that were in direct contact with bicolored fish. Populations from this region exhibited the lowest diversity (0.64, 0.67, and 0.25, respectively), with a total of 11 haplotypes for 28 individuals and a total of 20 variable sites. The limited variability was particularly evident with the Hyde Reef samples, where only two haplotypes were found. One haplotype was found in seven individuals, whereas the second haplotype, which differed from the first one by only two substitutions, was found in a single individual. Within this clade, each of the reefs formed a separate subclade with individuals from Sykes Reef being the sister clade of a group formed by Hyde (black morphs) and Howards Reefs.

The second and third clades grouped together in 70–97% of the bootstraps. These clades were themselves well supported (Fig. 2). Clade 2 (84–100% of bootstraps) comprised all samples of bicolored fish including the bicolored variant (the white part of the body contained many black speckles) found at Osprey Reef in the northern Coral Sea. This clade showed the highest genetic diversity (0.97) with some extreme values from Yonge (0.97) and Hyde (0.96) Reefs. Such diversity resulted from 30 haplotypes for 44 individuals screened and 38 variable sites. Within this subclade, indi-

TABLE 1. Summary of sequence variation within and among populations of *Acanthochromis polyacanthus*. Number of haplotypes, number of variable sites, and mean sequence of divergence were calculated using DNASP (Rozas and Rozas 1997). Diversity was calculated according to the equation: $h = (1 - \Sigma x_i^2)n/(n - 1)$, where x_i is the frequency of the ith mtDNA type (Nei 1987). B/W, black-and-white bicolored morph; B, black monochromatic morph.

Regions	Sites	*n*	Number of haplotypes	Diversity	Nucleotide diversity index ($\times 10^3$)	Number of variable sites	Mean number of pairwise differences
Coral Sea (clade 1)		54	32	0.97	24.58	65	13.89
South		16	11	0.93	10.32	21	5.83
	Diamond Reef	7	5	0.91	5.73	9	3.24
	Lihou Reef	9	6	0.83	6.29	9	3.56
Central		18	10	0.92	7.32	17	4.14
	Chilcott Reef	9	5	0.83	2.85	4	1.61
	Flinders Reef	9	5	0.83	4.92	10	2.78
North		20	11	0.87	3.89	15	2.20
	Willis Reef	9	5	0.86	3.05	4	1.72
	Herald Cay	11	8	0.89	4.18	13	2.36
Northern Great Barrier Reef (clade 2)		44	30	0.97	7.40	38	4.18
	Osprey Reef	12	6	0.85	4.21	9	2.38
	Yonge Reef	9	8	0.97	8.73	11	4.93
	Pith Reef	12	7	0.83	2.87	9	1.62
	Hyde Reef (B/W)	11	9	0.96	6.92	17	3.91
Southern Great Barrier Reef (clade 3)		28	11	0.86	6.04	20	3.41
	Hyde Reef (B)	8	2	0.25	2.75	2	1.56
	Sykes Reef	10	4	0.64	1.69	4	0.96
	Howards Reef	10	5	0.67	5.62	12	3.18
Total		126	73	0.98	45.11	146	25.49

viduals from Hyde Reef formed a distinct clade, whereas the other reefs showed a low level of mixing (discussed below).

Clade 1 (54–75% of bootstraps) included all Coral Sea samples except for Osprey Reef. This clade was divided into three subclades (Diamond and Lihou Reefs, Chilcott and Flinders Reefs, Willis Reef and Herald Cay). These pairings correspond to latitudinal separation within the Coral Sea and all were well supported (95%, 99%, and 100% of bootstraps, respectively). Within the southern and central Coral Sea subclades, individuals from each reef also formed distinct clades, which in turn were well supported (Fig. 2). Overall the Coral Sea clade showed similar diversity levels (0.97) to the northern GBR samples with 32 haplotypes from 54 individuals, albeit with a higher number of variable sites (65 vs. 38).

Thus, in most cases, individuals from the same reef grouped together as a monophyletic group. Excluding Hyde Reef, which is the only reef to have two morphs, there were only two exceptions to this general pattern: (1) Willis Reef and Herald Cay (both from the Coral Sea); (2) Yonge, Osprey, and Pith Reefs.

Population Structure

Population structure analysis, using an AMOVA, closely matched the phylogenetic results presented above (Table 2). The overall separation of the samples into three distinct clades accounted for 71.5% of the variance of the data. When the Coral Sea populations were analyzed separately, the partitioning of northern, central, and southern reefs also accounted for over 70% of the variance in the data (Table 3). Alternatively, when Coral Sea samples were excluded, the partitioning of reefs from the northern and southern GBR explained 89.7% of the variance. Clearly, *A. polyacanthus* populations are highly structured by geography.

F_{ST}-values were also found to be extremely high between reefs, both between and within the different clades (Table 4). For example, within the southern GBR (clade 3), which was discriminated strongly from the two other clades, F_{ST}-values among individual reefs within this tight group ranged between 0.51 and 0.79. With one exception (Willis and Herald Reefs), F_{ST}-values among reefs within the Coral Sea (clade 3) were even more extreme, ranging between 0.63 and 0.97. Once corrected for multiple tests (using the sequential Bonferroni correction), only a single F_{ST}-value was not significant ($F_{ST} = 0.116$ between Willis Reef and Herald Cay). All other values were significantly different from zero, with most values higher than 0.9.

Sequence Divergence

Sequence divergences were very high among the major clades (mean = 7.6% sequence divergence) as well as within the clades (2.0–3.6%). Such high divergence levels are even more remarkable for the two color morphs in contact on Hyde

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FIG. 2. Phylogenetic relationships of *Acanthochromis polyacanthus* individuals, using the neighbor-joining method. Numbers above the major nodes indicate bootstrap support using the neighbor-joining method with equal weights for transitions and transversions and when transversions were weighted three times as much as transitions. Numbers below the major nodes correspond to bootstrap support using the maximum-parsimony method with equal weights and 3:1 weights for transversions and transitions. Only values above 50% are shown. In all cases, 1000 bootstrap replicates were used. *Chromis chromis* and *Dascyllus trimaculatus* were used as outgroups.

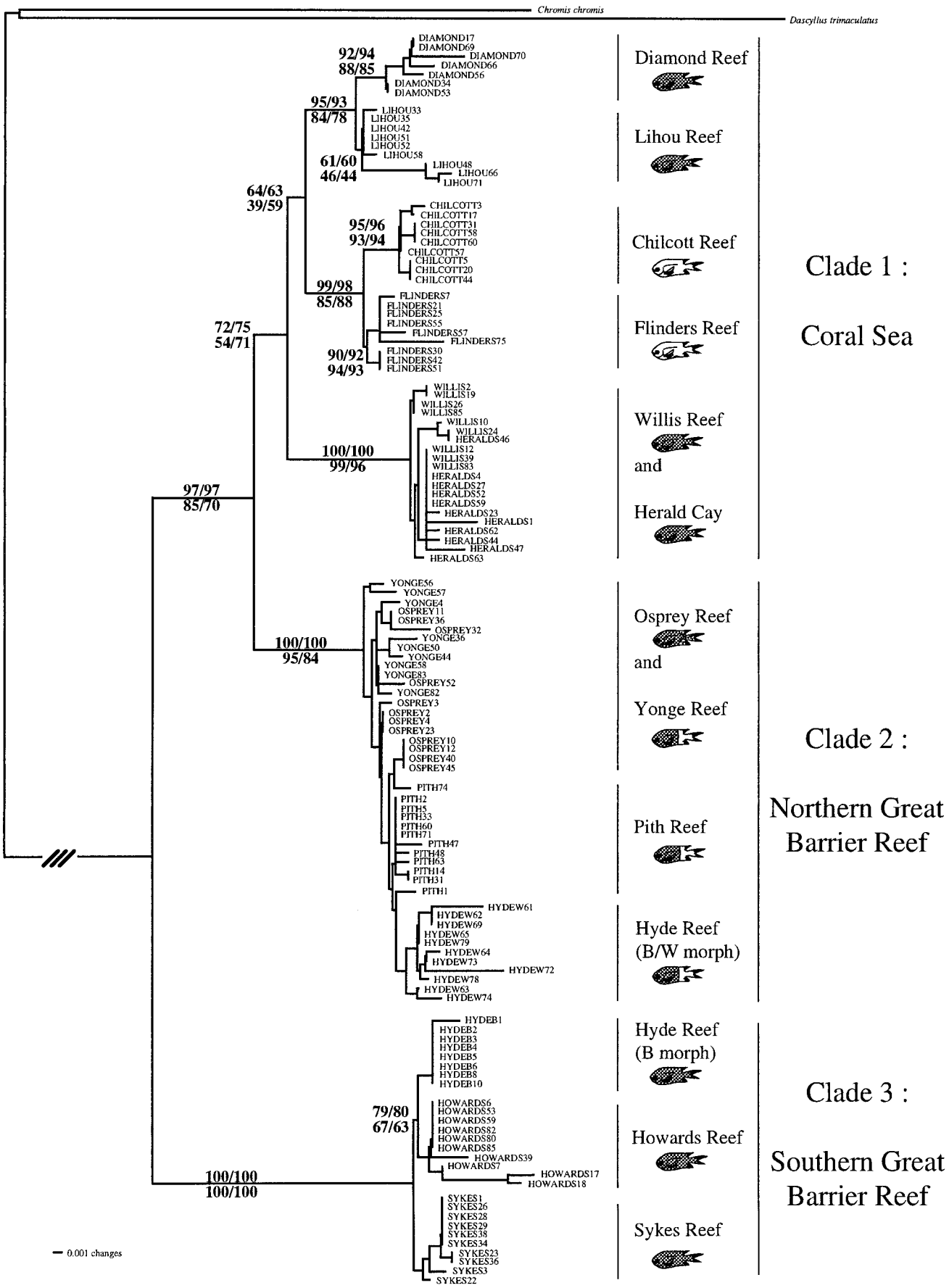
Chromis chromis
Dascyllus trimaculatus
DIAMOND17
DIAMOND69
DIAMOND70
DIAMOND66
DIAMOND56
DIAMOND34
DIAMOND53
92/94
88/85
95/93
84/78
LIHOU33
LIHOU35
LIHOU42
LIHOU51
LIHOU52
LIHOU58
61/60
46/44
LIHOU48
LIHOU66
LIHOU71
64/63
39/59
CHILCOTT3
CHILCOTT17
CHILCOTT31
CHILCOTT58
CHILCOTT60
CHILCOTT57
CHILCOTT5
CHILCOTT20
CHILCOTT44
95/96
93/94
99/98
85/88
FLINDERS7
FLINDERS21
FLINDERS25
FLINDERS55
FLINDERS57
FLINDERS75
FLINDERS30
FLINDERS42
FLINDERS51
72/75
54/71
90/92
94/93
WILLIS2
WILLIS19
WILLIS26
WILLIS85
WILLIS10
WILLIS24
HERALDS46
WILLIS12
WILLIS39
WILLIS83
HERALDS4
HERALDS27
HERALDS52
HERALDS59
HERALDS23
HERALDS1
HERALDS62
HERALDS44
HERALDS47
HERALDS63
100/100
99/96
97/97
85/70
YONGE56
YONGE57
YONGE4
OSPREY11
OSPREY36
OSPREY32
YONGE36
YONGE50
YONGE44
YONGE58
YONGE83
OSPREY52
YONGE82
OSPREY3
OSPREY2
OSPREY4
OSPREY23
OSPREY10
OSPREY12
OSPREY40
OSPREY45
100/100
95/84
PITH74
PITH2
PITH5
PITH33
PITH60
PITH71
PITH47
PITH48
PITH63
PITH14
PITH31
PITH1
HYDEW61
HYDEW62
HYDEW69
HYDEW65
HYDEW79
HYDEW64
HYDEW73
HYDEW72
HYDEW78
HYDEW63
HYDEW74
HYDEB1
HYDEB2
HYDEB3
HYDEB4
HYDEB5
HYDEB6
HYDEB8
HYDEB10
79/80
67/63
HOWARDS6
HOWARDS53
HOWARDS59
HOWARDS82
HOWARDS80
HOWARDS85
HOWARDS39
HOWARDS7
HOWARDS17
HOWARDS18
100/100
100/100
SYKES1
SYKES26
SYKES28
SYKES29
SYKES38
SYKES34
SYKES23
SYKES36
SYKES3
SYKES22
0.001 changes
Diamond Reef
Lihou Reef
Chilcott Reef
Flinders Reef
Willis Reef
and
Herald Cay
Osprey Reef
and
Yonge Reef
Pith Reef
Hyde Reef
(B/W morph)
Hyde Reef
(B morph)
Howards Reef
Sykes Reef
Clade 1 :
Coral Sea
Clade 2 :
Northern Great
Barrier Reef
Clade 3 :
Southern Great
Barrier Reef

TABLE 2. Results of hierarchical analysis of molecular variance for the three levels of geographic structure derived from the neighbor-joining dendrogram computed from phylogenetic relationships among 565 pairs of base DNA sequences of cytochrome *b*. *P*-values, calculated from a random permutation test (10,000 replicates), represent the probability of obtaining by chance alone a more extreme variance component and Φ-statistic than the observed values (Excoffier et al. 1992).

Source of variation	df	Variance components	Percentage of variance	Φ-statistics	*P*
Among clades	2	12.43	71.47	$\Phi_{SC} = 0.739$	<0.0001
Among population within clades	10	3.67	21.09	$\Phi_{ST} = 0.926$	<0.0001
Within populations	115	1.29	7.44	$\Phi_{CT} = 0.715$	<0.0001

Reef, where they hybridize (Planes and Doherty 1997a). The sequence divergence between these two color morphs was found to be 7.98%. Overall, population pairwise sequence divergences varied from 0.41% (Willis vs. Herald) to 8.14% (bicolored fish from Hyde reef vs. black fish from Howards Reef; Table 4). Such divergences are mainly due to fixed mutations restricted to each reef, but the number of haplotypes within each reef also enhanced the diversity found within a population. This effect was somewhat limited in populations from the southern GBR where genetic diversity was low.

DISCUSSION

Acanthochromis polyacanthus is atypical among marine fish for its lack of pelagic larvae. As a result, it displays an unusual degree of spatial patterning in color and genetic characters at geographic and regional scales (Doherty et al. 1994; Planes and Doherty 1997a,b). This study, the first to apply modern molecular markers to this species, suggested that populations on most of the reefs sampled were monophyletic groups. Although this might be expected for the isolated reefs of the Coral Sea, it was equally true for continental shelf reefs forming the archipelagic GBR, which is of relatively modern origin in its present configuration (see below). This conclusion was supported both by phylogenetic analyses and by AMOVAs that showed 72–90% of the variance among samples came from differences among groups compared with 5–13% from within populations. On Hyde Reef, the only one to have more than one color morph, the differences between samples collected from the same reef were among the greatest in the whole study.

Overall, the population structure of *A. polyacanthus* described from cytochrome *b* sequences was characterized by extreme differentiation between color morphs, significant differentiation among reefs inhabited by individuals of the same color morph, and monophyletic origins suggested for the majority of samples. The extreme divergence between color morphs and the large differentiation among reefs suggest long periods of isolation, whereas the limited diversity within most reefs suggests some recent modification in the population size. The last feature might be explained through recent founder events and/or recent bottlenecks in population size leading to lineage extinction. Given the different impacts from changing sea levels predicted for shelf and oceanic reef habitats (Davies 1989), it is likely that modern populations of *A. polyacanthus* on reefs from the GBR and Coral Sea do not share the same history.

Acanthochromis polyacanthus on the Great Barrier Reef

The molecular clock for divergence in cytochrome *b* sequences has been calibrated at 1.0–2.5% per million years (Irwin et al. 1991; Martin et al. 1992). At this rate, the differences measured between clades of *A. polyacanthus* from the northern and southern GBR (7.7%) correspond to isolation since the early Pliocene (5 million years ago).

The foundations of the GBR have been traced back to the appearance of reef facies left by fringing reefs growing in the Eocene (38–54 million years ago), although the modern structure was built by high sea levels associated with Pleistocene aggradational phases starting 1.5 million years ago (Symonds et al. 1983). Data on sea-level change during the Pliocene and early Pleistocene show variations over a range of −100 to +20 m relative to the present level (Chappell 1983; Davies 1989; Lambeck and Nakada 1990). During each regression, most of the continental shelf was exposed to the atmosphere, with huge losses of habitat space and biodiversity. While some reef growth may have continued on the steep continental margins during some of these retreats, it is likely that discharges from major terrestrial catchments would have introduced significant breaks into the fringing coralline habitat. Modern distributions suggest that habitat discontinuities at subkilometer scales provide effective barriers to the dispersal of *A. polyacanthus* within reefs (P. J. Doherty, unpubl. data), which suggests high potential for the

TABLE 3. Results of hierarchical analysis of molecular variance for the three levels of geographic structure observed within clade 1 (Coral Sea). *P*-values, calculated from a random permutation test (10,000 replicates), represent the probability of obtaining by chance alone a more extreme variance component and Φ-statistic than the observed values (Excoffier et al. 1992).

Source of variation	df	Variance components	Percentage of variance	Φ-statistics	*P*
Among groups[1]	2	3.72	72.08	$\Phi_{SC} = 0.518$	<0.0001
Among population within group	3	1.35	14.47	$\Phi_{ST} = 0.865$	<0.0001
Within populations	48	1.25	13.45	$\Phi_{CT} = 0.721$	>0.05

[1] Groups were defined as north, central, and south populations of the Coral Sea, distinguished from phylogenetic relationships among 565 pairs of base DNA sequences of cytochrome *b*.

TABLE 4. Pairwise comparisons of populations are represented by the F_{ST}-values above the diagonal. Values were calculated using DNASP (Rozas and Rozas 1997). Below the diagonal is the pairwise mean sequence divergence.

	Coral Sea						Northern Great Barrier Reef					Southern Great Barrier Reef			
	South		Central		North										
Color	Diamond Black	Lihou Black	Chilcott White	Flinders White	Willis Black	Herald Black	Osprey B/W[1]	Yonge B/W	Pith B/W	Hyde B/W B/W	Hyde B Black	Sykes Black	Howards Black
Diamond	—	0.573	0.967	0.805	0.880	0.873	0.885	0.875	0.909	0.865	0.960	0.958	0.930
Lihou	1.41	—	0.823	0.781	0.855	0.849	0.866	0.854	0.889	0.850	0.952	0.952	0.926
Chilcott	3.08	2.58	—	0.627	0.921	0.909	0.912	0.906	0.931	0.890	0.974	0.970	0.942
Flinders	2.71	2.55	1.04	—	0.882	0.973	0.879	0.870	0.903	0.862	0.956	0.954	0.926
Willis	3.50	3.22	3.74	3.36	—	0.116*	0.914	0.908	0.933	0.895	0.972	0.969	0.941
Herald Cay	3.77	3.40	3.93	3.56	0.41	—	0.907	0.900	0.923	0.891	0.962	0.961	0.936
Opsrey	4.16	3.81	4.11	3.73	4.34	4.53	—	0.180	0.367	0.479	0.960	0.958	0.935
Yonge	4.28	3.91	4.23	3.86	4.46	4.64	0.56	—	0.496	0.527	0.958	0.956	0.929
Pith	4.28	3.91	4.16	3.84	4.41	4.60	0.56	0.77	—	0.397	0.972	0.969	0.946
Hyde B/W	4.78	4.42	4.62	4.37	4.94	5.13	0.99	1.28	0.80	—	0.945	0.945	0.923
Hyde B	7.91	7.81	7.31	6.92	7.33	7.52	7.35	7.45	7.43	7.98	—	0.788	0.508
Sykes	7.89	7.96	7.43	7.03	7.45	7.63	7.40	7.58	7.54	8.02	0.63	—	0.571
Howards	8.07	7.98	7.49	7.12	7.50	7.61	7.52	7.63	7.61	8.14	0.70	0.85	—

[1] Morph with substantial black speckles on the white posterior.
* $P < 0.05$.

distribution of this species to be disrupted during glacial-driven falls in sea level.

Doherty et al. (1994) assumed that the GBR was colonized from the north by a single stock of *A. polyacanthus* and hypothesized that black fish evolved into a regional stock because of persistent retreat into a glacial refugium south of the influence from the Fitzroy catchment of central Queensland. They attributed the low heterozygosity levels of the southern stock to bottlenecks in population size during these retreats (see also Vrijenhoek et al. 1985; Leberg 1992). The data from the cytochrome *b* sequences challenge this hypothesis by suggesting that the separation between northern and southern color morphs was of more ancient origin (early Pliocene) and therefore unlikely to have occurred in north east Australia. The corollary is that the GBR has been colonized more than once by at least two different stocks of *A. polyacanthus*. Under this scenario, the black fish in the peripheral southern populations would represent the first invasion. In this scenario we must consider that black southern individuals appear phylogenetically older than black-and-white ones from the northern GBR and older than black individuals from the Coral Sea (Fig. 2). Limited diversity of the black individuals would still have to result from bottleneck events, whereas the low level of diversity in black and white individuals is the result of a recent founder effect.

Although the extreme difference in sequence divergence (7–8%) between bicolored and black fish on the GBR can be attributed to an ancient split in lineages reinforced by periodic bottlenecks in the peripheral southern stock, modern populations of the same morph displayed differences among reefs within regions that are too great to have evolved in situ. For example, the three samples of black fish from the southern GBR (Sykes, Howards, Hyde) varied by an average of 0.73%, which is equivalent to the divergence expected over 2.9–7.3 $\times 10^5$ years between identical demes. The three samples of bicolored fish from the central and northern GBR (Hyde, Pith, Yonge) were slightly more variable at an average of 0.95, corresponding to 3.8–9.5 $\times 10^5$ years of separation if all of the difference were due to standard molecular clock evolution of cytochrome *b*. Given that these populations were established less than 10,000 years ago during the last marine transgression (Chappell 1983; Davies 1989), most of these differences could reflect founder effects and the slightly different outcomes between stocks may reflect chance sampling from regional demes of different diversity. In another perspective, the diversity observed within a reef (in the southern GBR) associated with the time of the last transgression give a mean divergence rate of 8.5% to 28% per million years. Because we cannot know for sure that all haplotypes within a single reef are novel (generated by mutation after a single colonization event), these estimates provide only an upper limit on the rate of evolution resulting in divergence. This divergence rate is certainly higher than any previous estimates for tropical marine fauna (Bermingham and Lessios 1993; Knowlton et al. 1993; Bermingham et al. 1997; Knowlton and Weigt 1998). Therefore, it is difficult to draw any conclusion regarding this scenario that identified founder effects as the main process driving the population structure of *A. polyacanthus*. Analysis of other genes would help in establishing the rate of divergence and therefore drawing con-

clusion on whether bottleneck or founder effects have driven the genetic structure.

Acanthochromis polyacanthus in the Coral Sea

Based on geological evidence, Davies (1989) proposed that offshore reefs on the Queensland and Marion Plateaus in the Coral Sea could have provided refugia for reef biota displaced from the continental shelf during regressions. Benzie and coworkers (Benzie 1991; Benzie and Williams 1992; Benzie et al. 1994) proposed the same model for several species of invertebrates (foraminiferan, mollusc, and sponge) that show high levels of gene flow (i.e., no significant differentiation) between populations from the GBR and Coral Sea. In contrast, the very high divergence of populations of *A. polyacanthus* between these locations is consistent with negligible gene flow over ecological time scales yet there is evidence that the reefs in the Coral Sea must have been colonized over large water gaps.

The Queensland Plateau, which supports most of the Coral Sea reefs, separated from the Australian mainland in the late Cretaceous (~65 million years ago) and reached its current position during the Eocene (~55 million years ago). This epoch marked the development of significant reef growth in the Coral Sea and along the continental margins of northern Australia. Starting around 40 million years ago, the Queensland Plateau subsided and reached its current level about 25 million years ago. During this subsidence, the reefs of the Coral Sea were formed in their current position (Mutter and Kraner 1980).

The age of the Coral Sea reefs provide strong evidence that *A. polyacanthus* did not colonize them before they reached their present position (i.e., not when the Queensland Plateau was still part of the Australian landmass). Our data show the black fish on the southern GBR represents the oldest lineage of *A. polyacanthus*. Discounting 1% of the divergence for founder effects (see above), the difference between the two stocks on the GBR represents a phylogenetic split starting 2.5–7.0 million years ago, long after the Coral Sea reefs had stabilized in their current position. The populations in the Coral Sea show the same level of difference to the black fish of the southern GBR as the bicolored fish of the central and northern GBR. This means three things: (1) that the Coral Sea populations are younger than the black fish of the southern GBR; (2) that the Coral Sea populations are of similar age to the bicolored fish on the GBR and may both be derived from common stock; and (3) that the Coral Sea populations must have been founded by pelagic migrants of *A. polyacanthus*.

Although this last conclusion revises conventional wisdom about this species, it is consistent with the presence of *A. polyacanthus* in other places accessible only over large open blue water (e.g., Vanuatu; Allen 1975). Within our data, it is supported also by the unexpected similarity of populations (0.6% divergence) on Osprey Reef, which is the most northern reef in the Coral Sea, and Yonge Reef, which is one of the nearest shelf reefs to the west. Despite proximity, these two reefs are separated by about 100 km of open water, previously thought to be an impassable barrier to the dispersal of a species without pelagic larvae. Regardless of improbability, the two populations share bicolored body markings (albeit with additional black flecking on the white posteriors of fish from Osprey) and mutations. Although little detail is known about ocean currents in the Coral Sea, satellite altimetry shows a dominant northerly flow in the general circulation at this latitude and the presence of seasonal gyres (D. Burrage, pers. comm.). Although dispersal must be possible, it is clearly rare. We found no haplotype common to the two populations and there is other evidence that they have diverged at the nuclear level. During our previous work on allozymes (Doherty et al. 1994), we analyzed fish from Osprey Reef and found that they had lost all variation at seven loci coding for allozymes that are polymorphic in bicolored fish from the northern GBR (S. Planes, unpubl. data). For this reason, we can conclude that the population on Osprey Reef was founded from the bicolored fish of the northern GBR rather than vice versa.

Following the same logic, we suggest that the bicolored stock from the GBR also provided the extreme migrants of *A. polyacanthus* that colonized other oceanic reefs on the Queensland Plateau even though modern populations do not share the same coloration. Sequence data do not support alternative scenarios such as common ancestry with the black stock on the southern GBR nor migration through Osprey Reef. Based on sequence divergence, there is weak support for a hypothesis that reefs on Queensland Plateau were colonized through the Flinders Reefs complex with subsequent radial radiation outward across the plateau. Curiously, under this scenario, migration along the east-west axis seems to have been stronger than migration along the north-south axis (the inverse of the sequence divergences), but there is no information about the oceanography of this basin during periods of low sea level. Not surprisingly, these isolated oceanic reefs generally showed high levels of divergence among themselves indicative of strong founder effects, long periods of isolation, or both. Equally unsurprising, we found that *A. polyacanthus* was absent from several reefs in the Coral Sea that have apparently suitable habitat for this species. Two of these reefs were isolated singletons on the Marion Plateau in the southern Coral Sea and therefore difficult targets. However, we cannot explain the absence of *A. polyacanthus* on Holmes Reef, when surrounded by potential sources, other than through the vagaries of chance colonization or extinction.

Is Acanthochromis a Monotypic Genus?

The biological species concept has been discussed and revised during the last 50 years as taxonomists, ecologists, evolutionary biologists, and palaeontologists have brought morphological, distributional, reproductive, and phylogenetic perspectives to their definitions. One thing that is clear is that speciation is a dynamic process that has not followed the same pathway for all organisms nor attained a single state for those that share common characters (Coyne et al. 1988; Palumbi 1992; Gosling 1994). The growing literature on hybrids (e.g., Barton 2001; Hewitt 2001) provides many examples of populations that have evolved morphologically and/or ecologically into distinct species, yet not acquired complete reproductive isolation (Schwartz 1972; Pyle and

Randall 1994). Typically these situations arise where temporary barriers have subdivided a genome and allowed partial divergence before the barriers are released and the stocks make secondary contact. When the period of isolation is relatively brief (e.g., on the scale of Pleistocene climate variations), secondary contacts between sibling species may result in fertile offspring and the introgression of genes through stable hybrid zones, which may eventually reunite the genome. Although there are numerous examples of stable hybrid zones among terrestrial plants and animals (Barton and Hewitt 1985; Roques et al. 2001), there are few counterparts in the marine environment because of the scale of dispersal offered by pelagic larvae. Thresher et al. (1989) showed that species with pelagic larval duration in excess of a month achieve cosmopolitan distributions and the levels of gene flow across some of the largest water gaps on the planet (Leis 1984) can maintain panmixis between fish populations on opposing continents.

Acanthochromis polyacanthus is not like other fish. Notwithstanding evidence that this species must be able to colonize distant habitats through open water over evolutionary time (see above), the lack of pelagic larvae results in a rare philopatric lifestyle (for a marine fish) in which the dispersal of offspring can be inhibited by habitat discontinuities on the scale of hundreds of meters within reefs. For this reason, it is not surprising that gene sequences in populations of *A. polyacanthus* with a long history of isolation (as in the Coral Sea) have diverged from each other or that different color patterns have been fixed in these isolated populations. Despite different coloration, the species is instantly recognizable in all of these places and has not evolved different meristics (Allen 1975). At issue is the fact that these populations of apparent conspecifics vary from each other by 3–4% in cytochrome *b* sequences, whereas the butterflyfishes show just 2% divergence among well-established species (McMillan and Palumbi 1995) and this level of divergence is often found between species of the same genus (Johns and Avise 1998; Bernardi and Crane 1999).

Even today, the biological species concept relies heavily on reproductive isolation (Mayr 1942). For *A. polyacanthus* on the isolated reefs in the Coral Sea, there is little chance of mixed mating, so their potential fertility is unknown. On the GBR, however, it seems that at least two lineages (black and bicolored) meet in the marine equivalent of secondary contact. Under modern sea levels, *A. polyacanthus* is confined to discrete platform reefs and thus does not mix freely. However, a small number of reefs in the center of the broad contact zone were colonized by both lineages resulting in a wonderful natural experiment. On Hyde Reef, black and bicolored fish partition the reef and produce hybrids at two hybrid zones on opposite sides of the reef perimeter (Planes and Doherty 1997a). If both morphs arrived on Hyde Reef soon after its recent formation, approximately 3000 generations have passed without significant introgression despite the persistent formation of hybrids. Cytochrome *b* differences between black and bicolored fish collected away from the hybrid zone on Hyde Reef were 8%, which was among the highest value detected among any pairwise contrast in this study.

Planes and Doherty (1997a) did not find evidence of hybrid inviability from their electrophoretic study of *A. polyacanthus* on Hyde Reef. Faced with this, they hypothesized that the spatial arrangement of the populations was maintained by a high degree of positive assortative mating with possible reinforcement by differential predation against rare morphs outside the hybrid zone. Sexual selection has been implicated in the explosive radiation of cichlid fishes in the African Great Lakes (Meyer et al. 1990; van Oppen et al. 1997; Seehausen and van Alphen 1999). This diverse fauna contains genuine species flocks, which are morphologically distinct, true breeding lineages with little genetic modification, consistent with their recent evolution (Turner et al. 2001). Despite their recent ancestry, there are few reports of hybrids in the cichlid fishes and we can only assume that habitat specialization (even if some new case explain divergence in African cichlids as a result of reproductive isolation as well; Knight et al. 1998) and philopatry prevent potentially compatible lineages from making contact (similar to the allopatric isolation of *A. polyacanthus* on discrete reefs). Although the lifestyles of cichlids and *Acanthochromis* share many superficial similarities, it may be dangerous to carry this analogy too far.

On Hyde Reef, two lineages that split a long time ago (millions of years if the molecular clock for cytochrome *b* is calibrated appropriately for *Acanthochromis*) can interbreed when given the opportunity but have maintained separate demes on the same small reef for thousands of generations. Under almost any scenario that we can imagine, the hybrid condition on Hyde Reef should have expanded beyond its limited zones if hybrids are fertile and back-crossing is free. The corollary is that the strong tension on Hyde Reef really reflects the reproductive incompatibility of the black and bicolored stocks of this fish. If this can be demonstrated through experimental mating (L. Van Herwerden, unpubl. ms.), there will be an argument for raising these color morphs to species status. At issue then will be the status of the isolated populations in the Coral Sea, which on sequence data can differ among parts of that domain almost as much as they differ from bicolored fish on the mainland. If postmating incompatibility could be demonstrated among these isolated populations, then species status might be warranted for them, also but we have to ask whether uncovering cryptic species at this scale provides a workable taxonomy.

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