

Letters to the Editor

Ancient Species Flocks and Recent Speciation Events: What Can Rockfish Teach Us About Cichlids (and Vice Versa)?

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The large assemblage of northeastern Pacific species of rockfish (genus *Sebastes*) has been proposed to be the modern result of an ancient explosive speciation event (Johns and Avise 1998). This radiation has been compared to the more recent radiation of cichlids in the Great African lakes (Greenwood 1991), and by analogy, the *Sebastes* assemblage was termed the “ancient species flock” (Johns and Avise 1998). An increasing number of molecular evolutionary studies focusing on cichlids from the Great African Lakes have uncovered unique modes of speciation. Sexual selection has been shown to play a crucial role in all three Lakes. In the oldest lake, Lake Tanganyika, sexual selection was evoked to explain the comparatively large genetic divergence observed between congeneric *Tropheus* species (Sturmbauer and Meyer 1992). Indeed, these species did not show any morphological differences besides coloration, although there was significant genetic divergence at the mitochondrial control region level [14.5% sequence divergence (Sturmbauer and Meyer 1992)]. On the other hand, haplochromine species from the most recent lake, Lake Victoria, and the species flock from the evolutionary intermediate lake, Lake Malawi, were found to exhibit a vast array of color patterns but little genetic divergence (Crapon de Caprona and Fritzsche 1984; Meyer et al. 1990; Seehausen et al. 1997; McElroy and Kornfield

1990; McKaye et al. 1993; Deutsch 1997). Furthermore, lineage sorting was shown to be incomplete in the Malawi species flock, locally called mbuna, using mitochondrial control region sequences (Bowers et al. 1994; Kornfield and Parker 1997; Parker and Kornfield 1997). Recent microsatellite studies, however, show that mbuna species could be distinguished using fast-evolving molecular markers (Kornfield and Parker 1997; van Oppen et al. 1997; Markert 1998; Arnegard et al. 1999).

Thus, the relationship between speciation and divergence at the morphological and genetic level in cichlids (as in most organisms) is not simple (Meyer 1993). While few morphological differences besides coloration patterns were found in Victoria haplochromines, Malawi mbunas (within genera) and Tanganyika *Tropheus* species, levels of genetic divergence at the mitochondrial control region level were either large—14.5% sequence divergence for the genus *Tropheus*—or very limited—2.3 and 8.2% sequence divergence for the whole Victoria and Malawi species flocks, respectively. A similar situation appear to exist in the *Sebastes* species flock, where examples of species groups that exhibit little or no morphological difference also exist [i.e., *Sebastes rubrivinctus* and *S. babcocki*; *S. rosenblatti*, *S. eos*, and *S. chloresticus*; *S. carnatus* and *S. chrysomelas*; *S. helvomaculatus* and *S. simulator* (Rocha-Olivares et al. 1999a,b)]. What levels of genetic divergence should we expect in these species? If these species groups have been maintained for an extensive period of time, as the idea of an ancient species flock would suggest, we

should expect significant genetic divergence levels. Conversely, if the evolutionary origin of these groups is recent, we should expect these species to exhibit little genetic divergence.

In order to investigate speciating events in rockfishes, we have selected a pair of closely related species, *Sebastes carnatus*, the gopher rockfish, and *Sebastes chrysomelas*, the black-and-yellow rockfish. Gopher rockfish and black-and-yellow rockfish are morphologically and meristically identical (Phillips 1957; Miller and Lea 1972). Their color patterns, however, are different. Over an identical dark background, gopher rockfish have pink blotches, while black-and-yellow rockfish have yellow blotches. More importantly, ecological characteristics differ in these two species (Larson 1980a, b). Black-and-yellow rockfish are restricted mostly to water shallower than 10–16 m, while gopher rockfish are generally found in waters deeper than 16 m (Hallacher 1984; Larson 1980a, b). This difference in depth preference, however, does not result in different growth rates or significant differences in diet (Larson 1980a–c). After comparing depth segregation, competition, and habitat usage among these two species of rockfish, Larson (1980a–c) concluded that there was no evidence of social dominance between these fish and suggested that light intensity has a direct or indirect effect on the depth distributions of these species. Thus, gopher rockfish and black-and-yellow rockfish show similarities to cichlid species in several ways. Their morphological and meristic similarity is reminiscent of several Malawi mbuna species (which are, ironically, also called rockfish) (Ribbink et al. 1983; Bowers et al. 1994; Kornfield and Parker 1997). Furthermore, their segregation by depth is also similar to the ecological segregation that was observed in haplochromine Victoria cichlids (Goldsmith et al. 1990).

Considering the comparison between gopher rockfish and black-and-yellow rockfish sequence divergences with similar divergences in cichlids, we sequenced a 251-bp portion of the mitochondrial control region for 47 individuals (35 gophers and 12 black-and-yellows) which were sampled over their distribution range (Fig. 1). Control regions in these species were found to be fairly variable, as the average sequence divergence between gopher rockfish and black-and-yellow rockfish was 3.8%, and the haplotype diversity was 0.95 (40 haplotypes of 47 individuals). However, phylogenetic reconstructions using the neighbor-joining and the maximum-parsimony methods did not result in the separation of gopher rockfish and black-and-yellow rockfish into distinct clades (Fig. 2). Furthermore, the alternative constrained topology that would place gopher rockfish and black-and-yellow rockfish individuals into two separate monophyletic assemblages, which would require an additional 17 steps, was rejected by a Kishino and Ha-

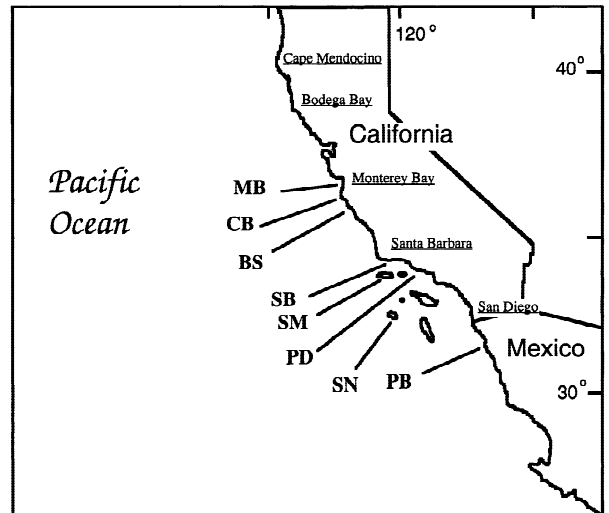


Fig. 1. Sample collections. Samples were collected by scuba divers with pole spears at the following sites: Monterey Bay (MB), Carmel Bay (CB), Big Sur (BS), Santa Barbara (SB), Point Dume (PD), Punta Banda (PB), San Miguel Island (SM), and San Nicolas Island (SN).

segawa test ($p < 0.02$) and a T-PTP test ($p < 0.01$) (Kishino and Hasegawa 1989; Faith 1991). Therefore, our data indicate that *Sebastes carnatus* and *S. chrysomelas* lineages cannot be sorted using mitochondrial control region sequences.

Previous attempts to separate these two species using slower molecular markers such as allozymes (Seeb 1986) and nuclear ITS sequences (Hunter 1994) failed to find fixed differences between black-and-yellow rockfish and gopher rockfish. Taken together, all the evidence points toward two possible hypotheses. One possibility is that these two species are simply color morphs of a single species. Since their description in 1880 (Jordan and Gilbert 1880a, b), scientists have questioned the taxonomic status of these two species (Hubbs and Schultz 1933; Phillips 1957; Chen 1986). However, some elements negate this possibility. The color morph separation by depth is difficult to explain (Alesandrini 1997). Although phenotypic plasticity may be evoked, no intermediate color morphs have been described. An alternative explanation is that gopher rockfish and black-and-yellow rockfish are genuine species.

Since mitochondrial control regions are among the fastest molecular markers available, why can't we separate black-and-yellow rockfish and gopher rockfish? One possibility is that there is currently active gene flow between species. While gene flow between black-and-yellow and gopher rockfish is possible, our data suggest that this is unlikely. Indeed, no haplotypes are shared between species, while several haplotypes are shared among individuals within a species. This is the case for black-and-yellow rockfish individuals CB2 and CB3 and for gopher rockfish individuals CB1 and BS1, individuals CB12 and BS10, individuals BS9 and CB8, and in-

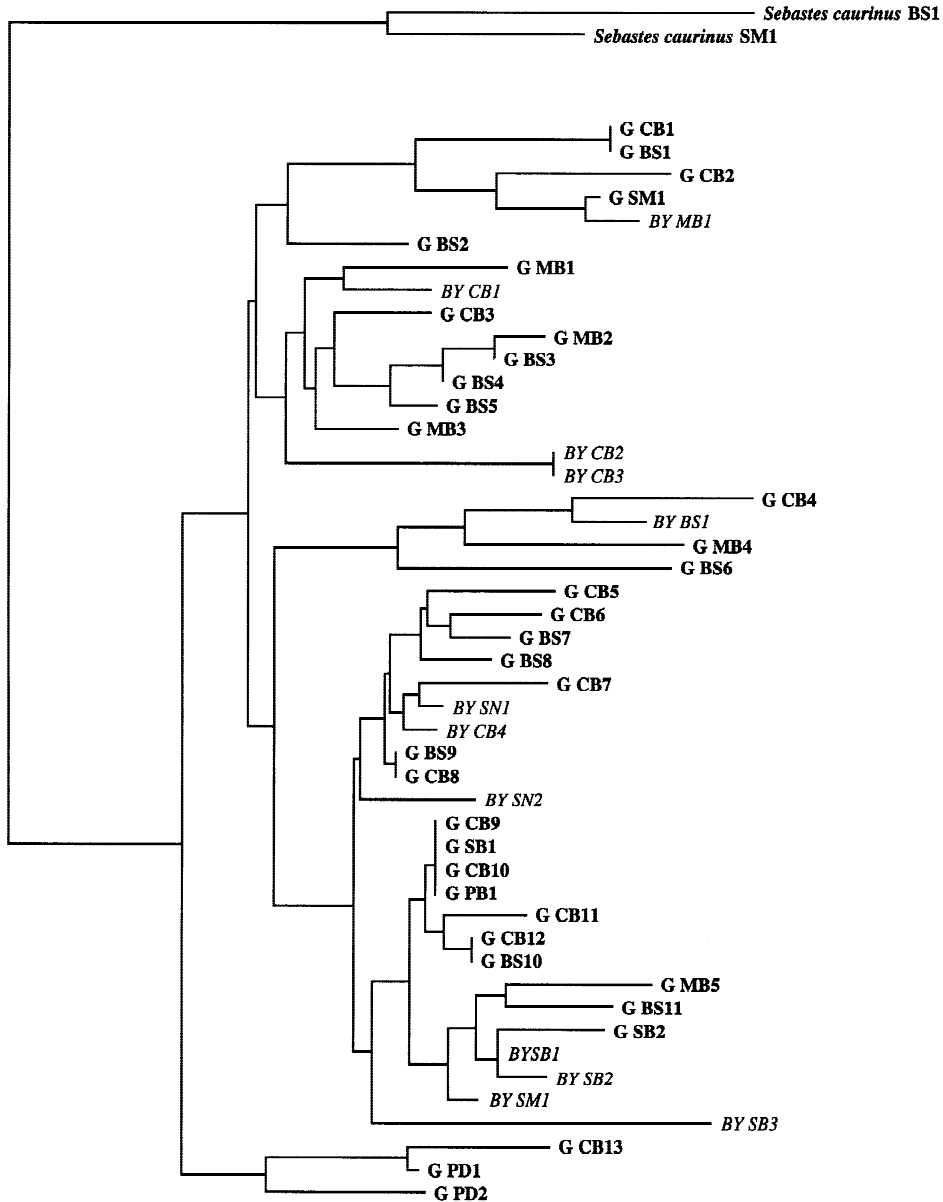


Fig. 2. Phylogenetic relationships of *Sebastes carinatus* (labeled G) and *Sebastes chrysomelas* (labeled BY) individuals based on mitochondrial control regions [neighbor-joining method using PAUP 4.0 (Swofford 1998)]. Over 3000 trees resulted from a maximum-parsimony analysis (tree length, 136 steps), but none of them separated gopher rockfish and black-and-yellow rockfish into distinct clades (not shown). A list of locality abbreviations is given in Fig. 1. PCR products were amplified using the following primers: Roc-F, 5' TAT CAA CAT TAA TTT ATA TTA AC 3', and Roc-R, 5' CAA TAA CCG TTG GCA TTA A 3' (denaturation, 35 cycles at 94°C for 45 s; annealing, 48°C, for 45 s; extension, 72°C for 60 s). The closely related copper rockfish, *Sebastes caurinus*, was used as an outgroup (Rocha-Olivares et al. 1999a, b).

dividuals CB9, SB1, CB10, and PB1. An alternative hypothesis is that speciation occurred recently, from a large and genetically diverse ancestral population, in the absence of recent bottleneck at each speciation event (Avice 1994). Our data are compatible with this hypothesis, as the genetic diversity of each species (0.98 for G and 0.98 for BY) is similar to the genetic diversity of the combined species (0.95). Furthermore, if this hypothesis is correct, it is possible to estimate the minimum time of species divergence by determining the sequence divergence between the most closely related individuals of the

two species. Sequence divergence between our two species ranges from 0.4 to 7.8%. Using currently accepted ranges for D-loop substitution rates [2 to 10%/Myr (Grant and Bowen 1998)] and assuming an absence of gene flow between species, the minimum divergence time between black-and-yellow rockfish and gopher rockfish is approximately 40,000 years.

The concept of the "ancient species flock" coined by Johns and Avice (1998) may suggest that a main burst of *Sebastes* speciation happened in the distant past and that we are now witnessing the results of this original "big

bang.” Our data show that speciation mechanisms may still be active in *Sebastes*. As speciation in marine organisms is still poorly understood (Palumbi 1992, 1994), it is difficult to pinpoint the mechanisms of speciation in rockfish. However, as in several cichlid assemblages, depth segregation and coloration differences suggest that ecological and sexual selections may play important roles in this system. Extensive analysis of closely related *Sebastes* species using fast-evolving markers, such as microsatellites, should determine if recent speciation events are a common feature of the *Sebastes* species flock.

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References

- Alessandrini S (1997) Genetic differentiation and population structure in the gopher rockfish (*Sebastes carnatus*) and the black-and-yellow rockfish (*S. chrysomelas*) along the California coast, MSc dissertation. University of California, Santa Cruz, Santa Cruz
- Arnegard ME, Markert JE, Danley PD, Stauffer JR Jr, Ambali AJ, Kocher TD (1999) Population structure and colour variation of the cichlid fish *Labeotropheus fuelleborni* Ahl along a recently formed archipelago of rocky habitat patches in southern Lake Malawi. *Proc R Soc Lond B* 266:119–130
- Awise JC (1994) Molecular markers, natural history and evolution. Chapman & Hall, New York
- Bowers N, Stauffer JR, Kocher TD (1994) Intra- and interspecific mitochondrial DNA sequence variation within two species of rock dwelling cichlids from Lake Malawi Africa. *Mol Phylogenet Evol* 3:75–82
- Chen LC (1986) Meristic variation in *Sebastes* (Scorpaenidae), with an analysis of character association and bilateral pattern and their significance in species separation. NOAA Technical Report National Marine Fisheries Service 45
- Crapon de Caprona M-D, Fritsch B (1984) Interspecific fertile hybrids of haplochromine cichlidae (Teleostei) and their possible importance for speciation. *Neth J Zool* 34:503–538
- Deutsch JC (1997) Colour diversification in Malawi cichlids: Evidence for adaptation, reinforcement or sexual selection? *Biol J Linn Soc* 62:1–14
- Faith DP (1991) Cladistic permutation tests for monophyly and non-monophyly. *Syst Zool* 40:366–375
- Goldschmidt T, Witte F, de Visser J (1990) Ecological segregation in zooplanktivorous haplochromine species (Pisces: Cichlidae) from Lake Victoria. *Oikos* 58:343–355
- Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *J Hered* 89:415–426
- Greenwood PH (1991) Speciation. In: Keenleyside MHA (ed). *Cichlid fishes: Behaviour, ecology, and evolution*. London, Chapman and Hall, p 378
- Halacher LE (1984) Relocation of original territories by displaced black-and-yellow rockfish, *Sebastes chrysomelas*, from Carmel Bay, California. *Calif Fish Game* 70:158–162
- Hubbs CL, Schultz LP (1933) Descriptions of two new American species referable to the genus *Sebastes*, with notes on related species. *Univ Wash Publ Biol* 2:15–44
- Hunter KA (1994) Incipient speciation in rockfish (*Sebastes carnatus* and *Sebastes chrysomelas*), MSc dissertation. California State University Northridge, Northridge
- Johns GC, Awise JC (1998) Tests for ancient species flocks based on molecular phylogenetic appraisals of *Sebastes* rockfishes and other marine fishes. *Evolution* 52:1135–1146
- Jordan DS, Gilbert CH (1880a) Description of a new species of “rockfish” (*Sebastichthys carnatus*) from the coast of California. *US Nat Mus Proc* 3:73–75
- Jordan DS, Gilbert CH (1880h) Description of a new species of “rockfish” (*Sebastichthys chrysomelas*) from the coast of California. *US Nat Mus Proc* 3:365–366
- Kishino H, Hasegawa M (1989) Evaluation of the Maximum Likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J Mol Evol* 29:170–179
- Kornfield I, Parker A (1997) Molecular systematics of a rapidly evolving species flock: The mbuna of Lake Malawi and the search for phylogenetic signal. In: Kocher TD, Stepien CA (eds) *Molecular systematics of fishes*. Academic Press, New York
- Larson RJ (1980a) Territorial behavior of the black and yellow rockfish and gopher rockfish (Scorpaenidae, *Sebastes*). *Mar Biol* 58:111–122
- Larson RJ (1980b) Competition, habitat selection and the bathymetric segregation of two species of rockfish (*Sebastes*). *Ecol Monogr* 50:221–239
- Larson RJ (1980c) Influence of territoriality on adult density in two rockfishes of the genus *Sebastes*. *Mar Biol* 58:123–132
- Markert JA (1998) Population structure and phylogenetic history of the Lake malawi species flock, PhD dissertation. University of New Hampshire, Durham
- McElroy DM, Kornfield I (1990) Sexual selection, reproductive behavior, and speciation in the mbuna species flock of Lake Malawi (Pisces, Cichlidae). *Environ Biol fish* 28:273–284
- McKaye KR, Howard JH, Stauffer JR Jr, Morgan RP II, Shonhiwa F (1993) Sexual selection and genetic relationships of a sibling species complex of bower building cichlids in Lake Malawi Africa. *Jap J Ichthyol* 40:15–21
- Meyer A (1993) Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trends Ecol Evol* 8:279–284
- Meyer A, Kocher TD, Basasibwaki P, Wilson AC (1990) Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* 347:550–553
- Miller DJ, Lea RN (1972) Guide to coastal marine fishes of California, Fish Bulletin 157. State of California, Department of Fish and Game
- Nei M (1987) *Molecular evolutionary genetics*. Columbia University Press, New York
- Palumbi SR (1992) Marine speciation on a small planet. *Trends Ecol Evol* 7:114–118
- Palumbi SR (1994) Genetic divergence, reproductive isolation and marine speciation. *Annu Rev Ecol Syst* 25:547–572
- Parker A, Kornfield I (1997) Evolution of the mitochondrial DNA control region of the mbuna (Cichlidae) species flock of Lake Malawi, East Africa. *J Mol Evol* 45:70–83
- Phillips JB (1957) A review of the rockfishes of California (Family Scorpaenidae), Fish Bulletin 104. State of California, Department of Fish and Game, Marine Fishes Branch
- Ribbink AJ, Marsh BA, Marsh AC, Ribbink AC, Sharp BJ (1983) A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. *So Afr J Zool* 18:149–310
- Rocha-Olivares A, Kimbrell CA, Eitner BJ, Vetter RD (1999a) Evolution of a mitochondrial cytochrome b gene sequence in the species-rich genus *Sebastes* (Teleostei, Scorpaenidae) and its utility in testing the monophyly of the subgenus *Sebastomus*. *Mol Phylogenet Evol* 11:426–440
- Rocha-Olivares A, Rosenblatt RH, Vetter RD (1999b) Molecular evolution, systematics, and zoogeography of the rockfish subgenus

- Sebastomus* (*Sebastes*, Scorpaenidae) based on mitochondrial cytochrome b and control region sequences. *Mol Phylogenet Evol* 11:441–458
- Seeb LW (1986) Biochemical systematics and evolution of the scopaeid genus *Sebastes*, PhD dissertation. University of Washington, Seattle
- Seehausen O, van Alphen JJM, Witte F (1997) Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 277:1808–1810
- Sturmbauer C, Meyer A (1992) Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature* 358:578–581
- Swofford DL (1998) PAUP: Phylogenetic analysis using parsimony, Version 4.0.d64. University of Washington, Seattle
- van Oppen MJH, Turner GF, Rico C, Deutsch JC, Ibrahim KM, Robinson RL, Hewitt GM (1997) Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fishes. *Proc R Soc Lond B* 264:1358–1370