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TESTS FOR ANCIENT SPECIES FLOCKS BASED ON MOLECULAR PHYLOGENETIC APPRAISALS OF *SEBASTES* ROCKFISHES AND OTHER MARINE FISHES

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Abstract.—The concept of species flocks has been central to previous interpretations of patterns and processes of explosive species radiations within several groups of freshwater fishes. Here, molecular phylogenies of species-rich *Sebastes* rockfishes from the northeastern Pacific Ocean were used to test predictions of null theoretical models that assume random temporal placements of phylogenetic nodes. Similar appraisals were conducted using molecular data previously published for particular cichlid fishes in Africa that epitomize, by virtue of a rapid and recent radiation of species, the traditional concept of an intralacustrine “species flock.” As gauged by the magnitudes of genetic divergence in cytochrome *b* sequences from mitochondrial DNA, as well as in allozymes, most speciation events in the *Sebastes* complex were far more ancient than those in the cichlids. However, statistical tests of the nodal placements in the *Sebastes* phylogeny suggest that speciation events in the rockfishes were temporally nonrandom, with significant clustering of cladogenetic events in time. Similar conclusions also apply to an ancient complex of icefishes (within the Notothenioidei) analyzed in the same fashion. Thus, the rockfishes (and icefishes) may be interpreted as ancient species flocks in the marine realm. The analyses exemplified in this report introduce a conceptual and operational approach for extending the concept of species flocks to additional environmental settings and evolutionary timescales.

Key words.—Cytochrome *b*, mitochondrial DNA, phylogeny, speciation, species flock.

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Biologists have long been intrigued by the existence of extraordinary assemblages of related species that are sometimes referred to as “species flocks” (Greenwood 1984). From Darwin’s fascination with the species-rich geospizine finches of the Galapagos Islands to Meyer’s and colleagues (1990) recent molecular appraisal of the radiation of cichlid fishes in Lake Victoria and in certain African crater lakes (Schliewen et al. 1994), studies of monophyletic groups characterized by explosive speciation and adaptive radiation have had considerable impacts on ecological, evolutionary, and speciation theory (Echelle and Kornfield 1984).

Greenwood (1984) and Ribbink (1984) have advanced various criteria for a species flock. Typically, these include geographic circumscription, endemism, monophyly, and explosive speciation (Woltereck 1931) relative to sister groups. In the ichthyological literature (where the term has been used most widely; Echelle and Kornfield 1984), species flocks typically have been discussed with respect to speciose groups in closed lacustrine environments (Martens 1997). Classic examples include the diverse (but now nearly extinct) cyprinid minnows of Lake Lanao in the Philippines (Herre 1924), the cottoid sculpins of Lake Baikal in Russia (Berg 1965), the pupfishes in Laguna Chichancanab in Mexico (Humphries and Miller 1981), and the cichlid species flocks of the African Rift Valley lakes (Moore 1903; Greenwood 1981). Greenwood’s definition of a species flock does not include a requirement that the speciations be recent, but in practice the term usually is applied to groups thought to have arisen from recent adaptive radiations. The most striking example is the cichlid species flock of more than 200 species in Lake Victoria, where remarkable morphological and trophic diversity (Fryer and Iles 1972; Meyer 1993) appears to have arisen within the last few thousand years (Johnson et al. 1996).

Sebastes rockfishes of the California Current region of the northeast Pacific Ocean also have a large number of species that, like the African cichlids, are similar in general body

plan but nonetheless display great diversity in color, ecology, and behavior. Different species are specialized for habitats ranging from rocky intertidal to the deep shelf, and from mesopelagic to epibenthic. In the heart of the rockfish distribution in central California, about 65 species occur in general sympatry. The larval life-history period, part of which typically involves a pelagic phase, lasts from one to three months on average (Moser 1996). The great majority of rockfish species are endemic and restricted to the northeast Pacific continental shelf and shoreline in waters less than 500 m deep (Phillips 1957; Miller and Lea 1972; Rosenthal et al. 1988; Love 1991). Smaller numbers of rockfish species also occur in other ocean basins (Barsukov 1981; Seeb 1986) such as the North Atlantic where four species occur that probably stem from Pacific forms via trans-Arctic dispersal event(s) (for trans-Arctic dispersal documented in other taxonomic groups, see Dunton 1992 and Ortí et al. 1994).

Previous work based on allozymes yielded much larger genetic distances (mean Nei’s $D = 0.34$ between species; Seeb 1986) than might be expected for a species flock of recent origin. Also, rockfish fossils are known from the Lompoc deposits on the central California coast that date to the Upper Miocene (Jordan 1921; Barsukov 1991). A quandary arising from these observations motivated the current study. What would a recent species flock (such as the African cichlids) look like, phylogenetically, several million years from now? Although we cannot predict the future, an analogous question can be posed by looking backward in time: What phylogenetic footprints might characterize an ancient species flock viewed today?

A rich tradition in paleontology involves attempts to distinguish bursts of speciation and extinction from stochastic “background” rates for these phenomena. In the 1970s, members of the MBL group at Woods Hole were the first to critically examine changes of species diversity in the fossil record against patterns predicted under stochastic models of spe-

ciation and extinction (Raup et al. 1973). These studies raised cautionary notes about interpreting evolutionary patterns in the absence of appropriate statistical null models. Later developments in the statistical analysis of nonrandom cladogenesis have included relative rate tests of diversification (Sanderson and Donoghue 1994), skewness tests of tree topologies (Slowinski and Guyer 1989), and effects of varying rates of cladogenesis on phylogeny reconstruction (Nee et al. 1992, 1994, 1995; Harvey et al. 1994).

Recently, Wollenberg et al. (1996) introduced a procedure for testing phylogenies estimated from data on extant species for departures from "null" trees generated under a Markovian process of stochastic, lineage-independent branching and extinction events. The method should be ideally suited for identifying ancient bursts of speciation in molecular (or other) phylogenies estimated from living species, even when the exact branching order of particular lineages is uncertain (as may often be the case in phylogenetic appraisals that involve many taxa, close nodes, and limited amounts of genetic or other information; see Hoelzer and Melnick 1994). Here we apply this statistical procedure to a large group of marine fishes (genus *Sebastes*) that might prove to represent an ancient species flock.

Specifically, in this study we have generated and phylogenetically analyzed (in the context of the Markovian speciation/extinction models) sequence data from an 855-bp segment of the mitochondrial cytochrome *b* gene (*cyt b*) in 28 species of rockfishes plus one outgroup. Similar statistical analyses also are applied here to an allozyme-based appraisal of rockfish phylogeny previously reported (Seeb 1986). For comparison and contrast, we also apply these methods to molecular phylogenies for a cichlid species flock in Lake Victoria (Meyer et al. 1990), and for a group of icefishes (within Notothenioidei). The latter is the only assemblage of marine fishes, to our knowledge, for which the term species flock previously has been invoked (Bargelloni et al. 1994; Ritchie et al. 1996).

MATERIALS AND METHODS

Laboratory Procedures.—Tissue samples representing 27 species of rockfishes and one species of thornyhead (*Sebastes alascanus*) were collected near Monterey Bay, California. One rockfish species from the Atlantic Coast was also obtained. For 19 of these species, two individuals were sequenced, such that the overall study involved acquisition of 48 sequences total.

Total DNA was extracted from liver tissues following standard phenol/chloroform protocols (Taggart et al. 1992). Initially, "universal" *cyt b* primers (Kocher et al. 1989; Palumbi et al. 1991) with modifications made by Meyer et al. (1990) were used to PCR-amplify rockfish DNA under low stringency conditions. The best success involved primers located in the glutamine tRNA and threonine tRNA genes that flank the *cyt b* gene. Preliminary sequences were obtained from these amplifications and used to design the following rockfish-targeted primers (where RK refers to rockfish, and "L" and "H" refer to light and heavy strands): RK-Glu-L 5'-TCGTTGTTATTCAACTACAAG-3'; RK-CB2L 5'-CCA-AATGTCCTTTTGAGGTGCCACCG-3'; and RK-CB2H 5'-

GGTGATAACGGTGGCACCTCAAAAGG-3'. The fourth primer, CB3H (in Palumbi et al. 1991), worked satisfactorily and, thus, was not redesigned.

These primers amplified the entire *cyt b* gene in each of the samples tested. To obtain high-quality single-stranded template for sequencing, pairs of the universal primers were used with one member of each pair biotinylated, and then repeated with the alternate primer biotinylated. PCR products were purified using Wizard Preps (Promega), and heavy and light strands were separated using magnetic beads coated with streptavidin (Dynal) according to the manufacturer's guidelines. Single-stranded template was used in standard chain termination sequencing using the rockfish optimized primers and Sequenase (US Biochemical). In total, an 855-bp fragment of the 5' end of the *cyt b* gene, including 24 bp of the glutamine tRNA gene, was sequenced from each specimen. Both heavy and light strands were sequenced from all individuals.

Phylogenetic Analyses.—Sequences were aligned using the pileup program in the GCG package (Genetics Computer Group Inc.), and checked by eye. The data matrix first was examined for statistically significant phylogenetic signal using RASA (Lyons-Weiler et al. 1996). Phylogenies then were generated using parsimony, distance, and maximum-likelihood (ML) criteria as implemented in PAUP* (Swofford 1996). Significance was assessed by bootstrap resampling (Felsenstein 1985) using 500 pseudoreplicates in PAUP*.

Statistical Tests for Nonrandom Cladogenesis.—To test for ancient bursts of speciation using the statistical approach of Wollenberg et al. (1996), the relative temporal placements of speciational nodes in a phylogeny must be estimated. This, in turn, normally requires that the external nodes in a tree be "right justified" (contemporaneous) such that all extant species coexist at the same (present) time on the associated temporal axis of the tree. To achieve this end, two additional tree-building approaches were employed. For sequence data (i.e., for the rockfishes, cichlids, and icefishes), ML trees with contemporaneous tips were generated. This approach constrains the trees to a uniform rate of evolution across lineages. For data in the form of distance matrices (i.e., for distance-based summaries of both sequence data and allozyme data), KITSCH trees (least-square trees with contemporaneous tips) were generated using PHYLIP (Felsenstein 1993).

The ML approach began by generating an ML tree under a 2n transition/transversion model with HKY variant. Evolutionary rates for first, second, and third position sites as well as for noncoding sites were estimated separately. For each ML phylogeny, most-parsimonious trees were found and analyzed for ML score. The tree with the greatest log-likelihood score then was used to calculate the initial likelihood parameter values. After obtaining the best ML tree assuming the initial values, this ML tree was used to calculate new likelihood parameter values to generate a final ML tree. This ML tree was then converted to one with contemporaneous tips.

Onto each contemporaneous-tips ML or KITSCH tree, a standardized temporal scale ranging from time zero (the earliest node in the tree) to time one (the present) was superimposed to identify the relative temporal placements of all internal nodes in the tree. Following Wollenberg et al. (1996),

these nodal times in each tree were used to generate a cumulative distribution function (cdf; Sokal and Rohlf 1995) of normalized branching times for statistical comparison against the appropriate null expectations under Markovian models in which speciations/extinctions are stochastic with respect to time. These tests involve use of a Kolmogorov-Smirnov (K-S) *D*-statistic calculated from the observed and expected cdfs (details in Wollenberg et al. 1996).

In addition to the rockfishes, several other datasets were analyzed similarly to illustrate this conceptual approach to the identification of putative ancient species flocks. In each case, to examine the effect of scale (choice of outgroup) on the statistical analyses (see Wollenberg et al. 1996), the outermost taxon as identified by a midpoint rooting criterion was removed from each original tree, and the entire statistical procedure was conducted anew on the truncated phylogeny.

RESULTS

Patterns of Sequence Variation in Rockfishes.—The *cyt b* nucleotide sequences of rockfishes displayed characteristic evolutionary features similar to those reported elsewhere for this gene (Esposti et al. 1993). These included a high transition/transversion ratio (ca. 8 : 1, largely reflective of synonymous transitions) in pairwise species comparisons and a consistent paucity of guanine residues at the third position of codons (but no significant difference in base composition across the taxa; χ^2 tests of homogeneity of base frequencies across taxa for all sites and each codon position individually).

In the 855-bp sequence, 239 sites were polymorphic overall in this study, 130 of these (54%) were informative phylogenetically, and 114 (88%) of the phylogenetically informative sites occurred in the third position. Several lines of evidence suggest that the interspecific genetic distances observed in rockfishes are in a range likely to be phylogenetically instructive. First, all genetic distances among the *Sebastes* species (range 0.009–0.0801, uncorrected *p*) were far below the mean genetic distance between *Sebastes* and *Sebastolobus* (0.160), indicating that substitutions at the variable positions are far from saturated. Second, significant linear correlations between uncorrected genetic distances and the numbers of transitions and transversions per site ($r^2 = 0.97$ and 0.53 , respectively) suggest that few multiple substitutions have occurred at most nucleotide positions. Third, using the RASA approach, the a priori appraisal of phylogenetic signal in the rockfish data was highly significant for the intrageneric pairwise comparisons ($P < 0.001$) as well as when rooted with the *Sebastolobus* outgroup ($P < 0.001$).

Pairwise *cyt b* gene comparisons among the rockfishes also were analyzed with respect to synonymous versus nonsynonymous changes adjusted for transition/transversion bias (Li 1993). For comparisons among the *Sebastes* species, the overwhelming majority of variation was at synonymous sites ($K_S = 0.154$, $K_A = 0.003$), whereas for comparisons between *Sebastes* and *Sebastolobus*, sequences were virtually saturated for synonymous changes and at nonsynonymous sites showed more than 10-fold higher values than in the intrageneric comparisons ($K_S = 0.786$, $K_A = 0.039$). This trend is reiterated in pairwise comparisons between the inferred amino acid sequences. Within the *Sebastes*, the average number of amino

acid substitutions was 1.6, whereas 16–19 substitutions distinguished *Sebastes* and *Sebastolobus*.

Temporal Speciation Patterns in Rockfishes.—As judged by bootstrap criteria, phylogenetic trees generated under either parsimony or minimum evolution (distance-based) criteria gave poor resolution of most of the internal nodes (Fig. 1A,B). The parsimony and distance-based trees (as well as the ML tree; Fig. 1C) tended to agree well with each other only for some of the relatively recent nodes (those that also displayed bootstrap support greater than 50%). Nonetheless, in the current study we are interested primarily in the relative temporal distribution of nodes rather than the precise branching topology of the phylogeny. Indeed, if many speciations truly were close to one another in time, poor resolution of branching order of numerous internal nodes would be an expected signature of an explosive radiation.

To statistically assess possible clustering in the temporal spacing of speciation events, the ML phylogeny with contemporaneous tips (Fig. 2A) was used to generate an empirical cdf (Fig. 3A) for comparison against the null cdf for stochastically generated trees composed of 29 extant species (Wollenberg et al. 1996). The outcome was not significant (Table 1; but see Discussion). The analysis was repeated after removal of the distant *Sebastolobus* species (Figs. 4A, 5A), and this time a highly significant outcome was achieved (Table 1) in a direction signifying an ancient temporal clustering of speciations. This signature of a close temporal grouping of ancient nodes is registered by an empirical cdf that falls mostly above and to the left of the null cdf (Fig. 5A).

We similarly analyzed a multilocus allozyme dataset (Seeb 1986) containing 50 species of *Sebastes* and one *Scorpaena* outgroup (Figs. 2C, 3C). We also reanalyzed the allozyme dataset with only the *Sebastes* species included (Figs. 4C, 5C). In both cases, statistically significant outcomes were obtained in which the empirical cdfs again were mostly above and to the left of the relevant null cdfs (Figs. 3C, 5C; Table 1).

Temporal Speciation Patterns in Cichlids and Icefishes.—One striking contrast between the rockfishes (current study) and the Lake Victoria cichlids (Meyer et al. 1990) is evident from the frequency distributions of pairwise genetic distances at *cyt b* (Fig. 6). In both studies, the same size and region of the *cyt b* gene was monitored, but the rockfish species on average proved much more divergent from one another than the cichlids, as would be expected from the fossil evidence (Jordan 1921).

To examine statistically the phylogenetic signature of the recent cichlid species flock in a temporal context, we used the published mtDNA sequences from Meyer et al. (1990) to generate a contemporaneous-tips ML phylogeny (Fig. 2B) for subsequent analysis by the Wollenberg et al. (1996) approach. The empirical cdf (Fig. 3B) of normalized branching times in the cichlid tree departed highly significantly from the appropriate null expectation in a direction indicative of a recent temporal clustering of nodes (Table 1). In other words, the observed cdf curve falls significantly below and to the right of the null cdf expectation. We then repeated the analysis after removal of the outgroup taxon (Figs. 4B, 5B). The outcome again showed a highly significant recent temporal clustering of nodes relative to the null expectations for

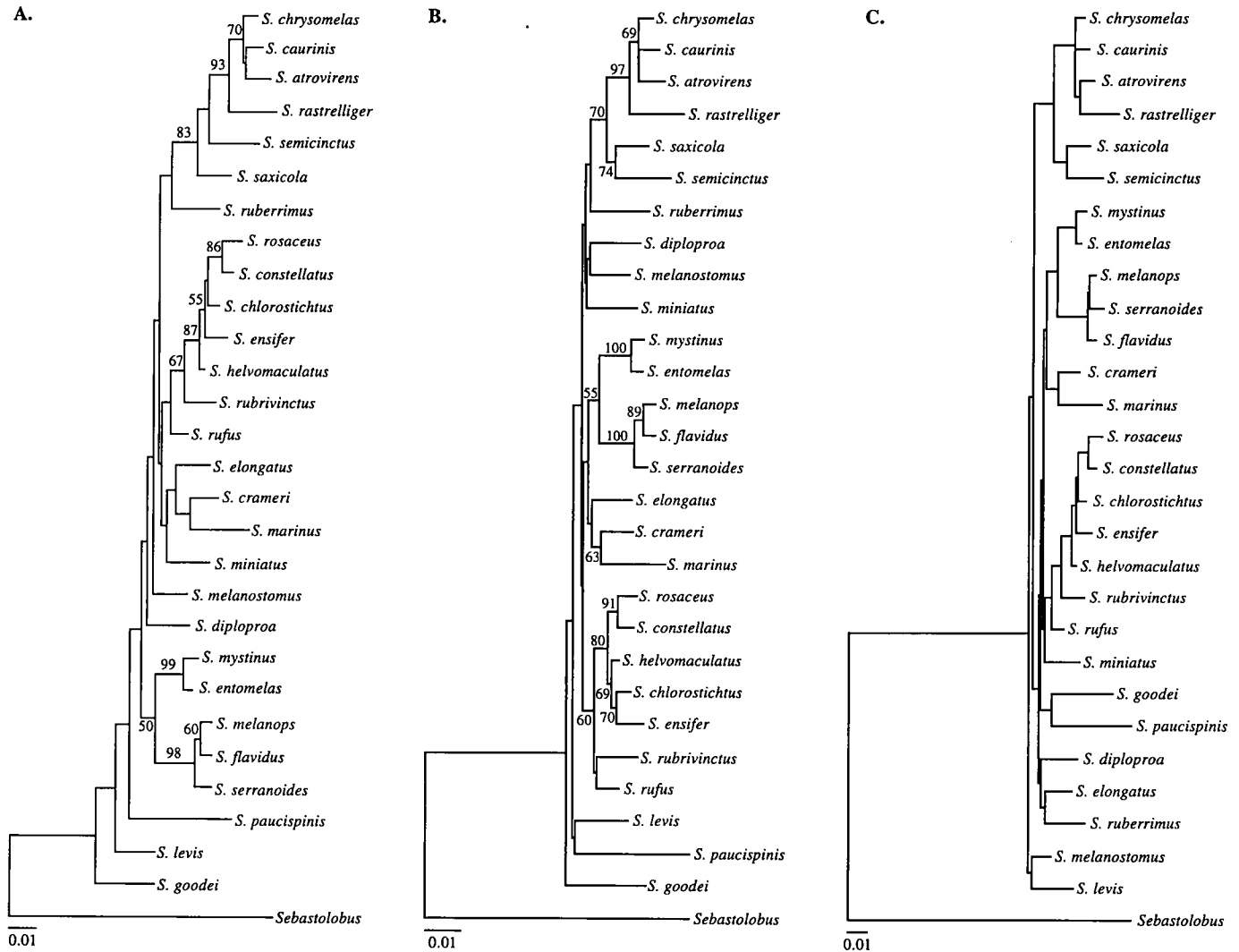


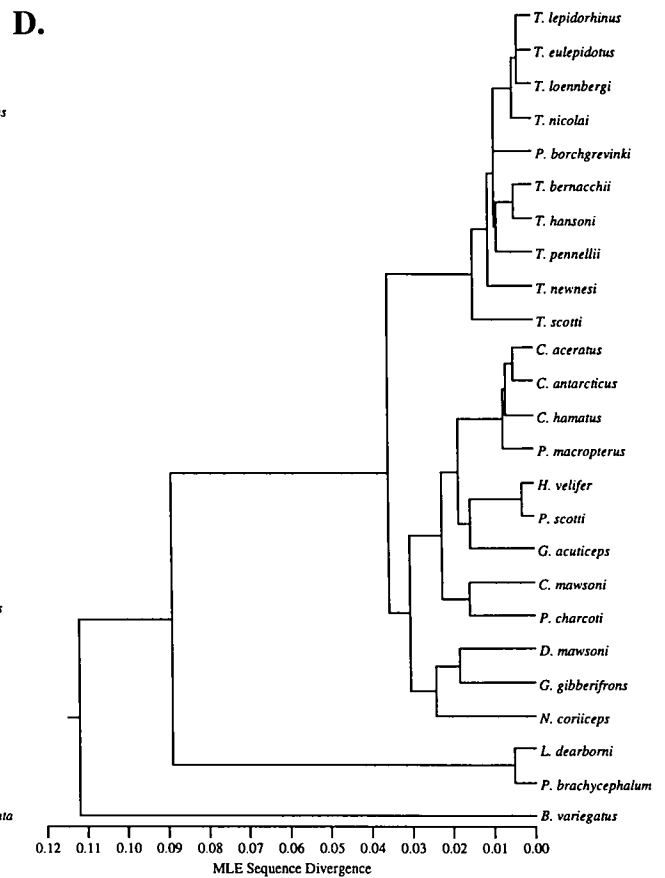
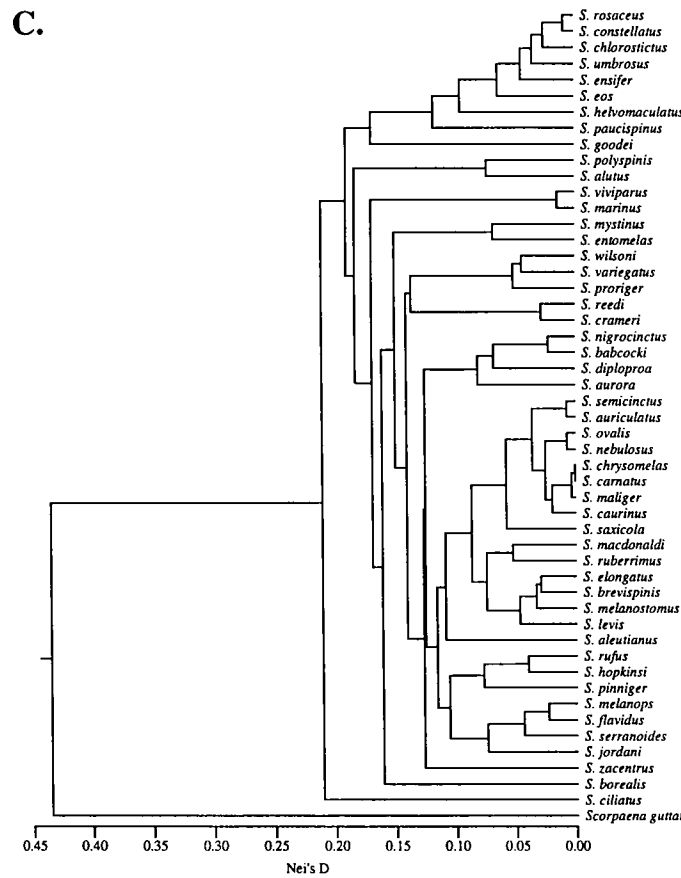
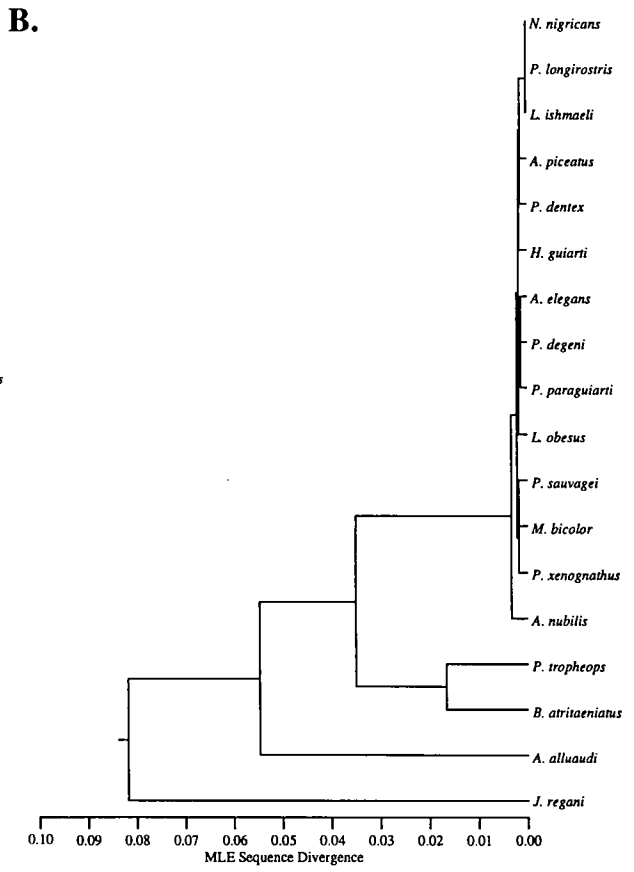
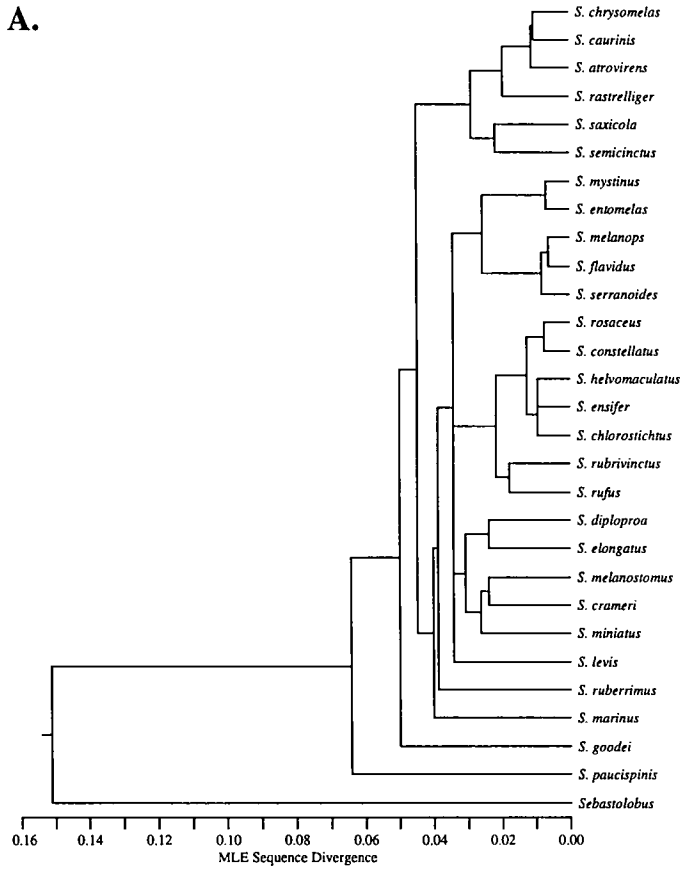
FIG. 1. Phylogenetic reconstructions for rockfishes based on *cyt b* gene sequences. (A) One of six equally most-parsimonious trees. Nodes with bootstrap support greater than 50% for 500 pseudoreplicates are indicated. The topology of the bootstrap consensus tree is entirely consistent with the most-parsimonious tree presented except for a single node uniting *Sebastes saxicola* and *S. semicinctus* at 58% (not shown). (B) Minimum evolution (distance) phenogram based on genetic distance estimates corrected for multiple substitutions using Kimura's two-parameter model. The topology of this phenogram is entirely consistent, for nodes with greater than 50 percent bootstrap support, with that of a consensus tree of 500 pseudoreplicates. (C) Maximum-likelihood tree allowing rate heterogeneity. The scale bar represents 1% corrected sequence divergence.

stochastically generated trees of the same size (Fig. 5B; Table 1).

For additional comparison, the combined mitochondrial 12s and 16s rRNA gene sequences for the Notothenioid icefish (Bargelloni et al. 1994; Ritchie et al. 1996) were reanalyzed for possible temporal groupings of internal nodes. For consistency, the aligned dataset was used to generate a ML phenogram (Fig. 2D) using the same approach as employed

for *Sebastes*. The cdf of normalized branching times in this tree did not differ significantly from the null expectation generated under a Markovian process of stochastic speciation and extinction for a clade of this size (Fig. 3D; Table 1). However, removal of the basal Bovichtidae from the analysis yielded a significant result for an ancient radiation of the remaining notothenioid families (Figs. 4D, 5D; Table 1). This corresponds to a key innovation (as suggested by Bargelloni

FIG. 2. Putative species flock phylogenies reconstructed with the constraint for a uniform rate of evolution across lineages. (A) Maximum-likelihood phylogeny for the rockfish *cyt b* data rooted with a *Sebastolobus* outgroup. (B) Maximum-likelihood phylogeny for the Lake Victoria cichlid species flock using *cyt b* and control region data rooted with *Julidochromis* from Lake Tanganyika. (C) Distance-based phylogeny of rockfishes from allozyme data of Seeb (1986), generated by the KITSCH algorithm and rooted with outgroup *Scorpaena*. (D) Maximum-likelihood phylogeny for the Notothenioid icefishes based on 12s and 16s rRNA gene sequences and rooted with *Bovichtus*. The sequence divergence scales were maximum-likelihood estimates for sequence data or Nei's *D* for allozyme data.



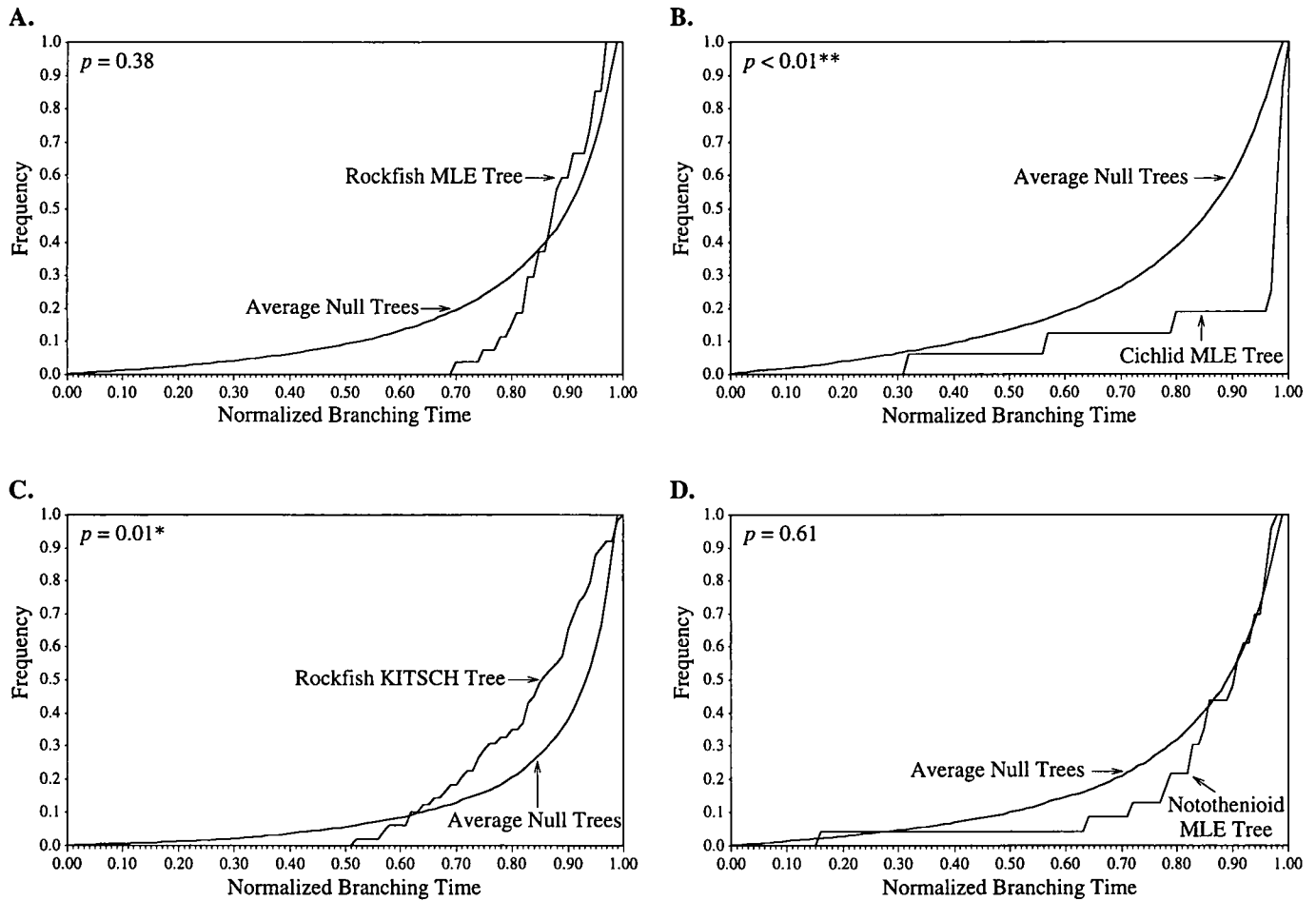


FIG. 3. Cumulative distribution functions (cdfs) for the putative species flock phylogenies of Figure 2, and the appropriate null cdfs. (A) Rockfish *cyt b* analyses. (B) Cichlids of Lake Victoria. (C) Rockfish allozyme analyses. (D) Notothenioid icefishes.

et al. 1994) of antifreeze glycopeptides (AFGPs) after the divergence of the Bovichtidae. Ritchie et al. (1996) proposed a second radiation of the trematomids within the Notothenioidae. To test this suggestion, the most basal trematomid represented was employed as the outgroup and the statistical analyses were repeated. Results indicate a significant ancient clustering of internal nodes for the trematomids (Table 1).

DISCUSSION

The primary intent of this study has been to consider whether the concept of "species flocks" might be extended to particular taxonomic assemblages of fishes inhabiting the marine realm, and into situations in which the species' radiations may have been comparatively ancient. The approach involves estimating the relative temporal placements of internal nodes in phylogenetic trees estimated from molecular data on extant species and statistically evaluating these nodal placements against appropriate null models that assume temporally stochastic speciation and extinction events. Our goal is not to contradict efforts to define species flocks in the strict sense, but rather to augment the usual criteria with the realization that the most essential characteristic is a statistically significant burst of explosive speciation among closely related species at some point in time.

Rockfishes.—Available evidence supports the idea that *Sebastes* rockfishes generally satisfy the criteria of a species flock as defined by Greenwood (1984). They constitute a species-rich and probably monophyletic assemblage of fishes rather distantly related to other taxa such as the thornyheads (*Sebastolobus*). Most rockfish species are endemic and geographically confined to the continental shelf of the northern Pacific region, where they are restricted in range by ocean temperatures and other ecological limits. Many of the species are broadly sympatric within this general area.

The analyses of molecular data presented here support an additional notion implicit in most discussions of species flocks: that they involve numerous speciation events spaced relatively close together in time. The statistical tests employed in this study, based on phylogenetic appraisals of living taxa, were designed to distinguish temporal clustering of nodes from null patterns under temporally stochastic speciation and extinction scenarios. Phylogenies for rockfishes generated from both *cyt b* gene sequences and allozyme data often revealed significant evidence for temporal groupings of relatively old nodes. In particular, the empirical cdfs of normalized branching times in the rockfish trees usually departed significantly from appropriate null expectations in a direction indicative of past temporal clustering of speciation events.

TABLE 1. Outcomes of statistical tests for nonrandom temporal patterning of internal nodes in the estimated phylogenies for three putative species flocks.

Phylogeny	Number of taxa	Data source	Reference	K-S <i>D</i> statistic	Probability
Rockfishes	29 ¹	mtDNA <i>cyt b</i>	current study	0.19	0.38
	28			0.50	< 0.01**
Rockfishes	51 ²	allozymes	Seeb 1986	0.29	0.01*
	50			0.48	< 0.01**
Cichlids	18	mtDNA <i>cyt b</i> and control region	Meyer et al. 1990	0.64	< 0.01**
	16			0.65	< 0.01**
Icefishes	25 ³	mtDNA 12s and 16s rDNA	Bargelloni et al. 1994	0.15	0.61
	24 ⁴			0.39	0.01*
	10 ⁵	rDNA	Ritchie et al. 1996	0.47	0.04*

¹ Includes one *Sebastolobus* taxon.

² Includes one *Scorpaena* taxon.

³ Species of Notothenioidei (includes two zoarcoid taxa).

⁴ Notothenioids with antifreeze proteins (includes two zoarcoid taxa).

⁵ Species of Trematominae.

* $P < 0.05$

** $P < 0.01$

Several important caveats apply to the current analyses. First, as with any phylogeny-based inference, the accuracy of conclusions is limited by the accuracy of the phylogenetic methods. The rockfish phylogenies presented are subject to the same limitations that apply to any *cyt b* (Meyer 1994) or allozyme-based phylogenetic treatments. In addition, they are limited by a particular requirement of the statistical tests employed: that trees have contemporaneous tips. The algorithms employed to produce ML or KITSCH trees with contemporaneous tips contain implicit assumptions regarding molecular clocks.

With the ML approach employed here, the validity of the clock constraint was examined using a likelihood ratio test (LRT) that asks whether the null hypothesis of equal rates of evolution is significantly worse than the alternative of rate heterogeneity (Felsenstein 1981; Huelsenbeck and Rannala 1997). In the current context, a LRT test is likely to suggest a better fit under rate heterogeneity because the many long, terminal branches inherent in the tree for a true ancient species flock would permit considerable latitude for rate adjustments along these lineages. Indeed, in preliminary LRT analyses we found that a no-clock ML tree for rockfishes was statistically better than its counterpart tree under a ML clock. However, this test as conducted was not ideal because it failed to evaluate the best from the entire universe of uniform-rate ML trees (the appropriate null hypothesis) against its counterpart no-clock ML tree. Such approaches were too intensive computationally for implementation here.

Another more traditional perspective pertaining to molecular clocks is germane to the current study. Lineage separations for any relatively ancient taxonomic group (whether a species flock or not) might appear to be compressed in time due solely to saturation effects in the nucleotide substitution curves. However, the rockfish separations appear to be well within a range where saturation effects at protein-coding genes should not confound distance estimates seriously (Moritz et al. 1987).

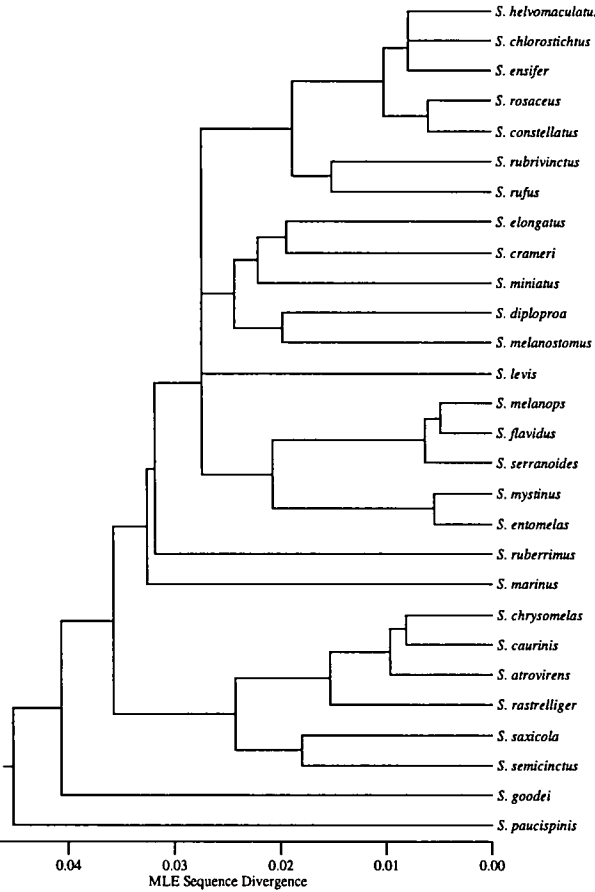
A second caveat is that only subsamples of rockfish species were included in the current surveys, and that only limited genetic information (*cyt b* sequences and multilocus allozyme

data) was tapped. However, the *cyt b* sequences were gathered from species collected without known bias with respect to genetic relationships. Thus, no conscious effort was made to sample genetically distant rockfishes that would bias statistical outcomes in favor of inferring ancient bursts of speciations, nor to preferentially sample genetically close species that would bias in favor of recent radiations.

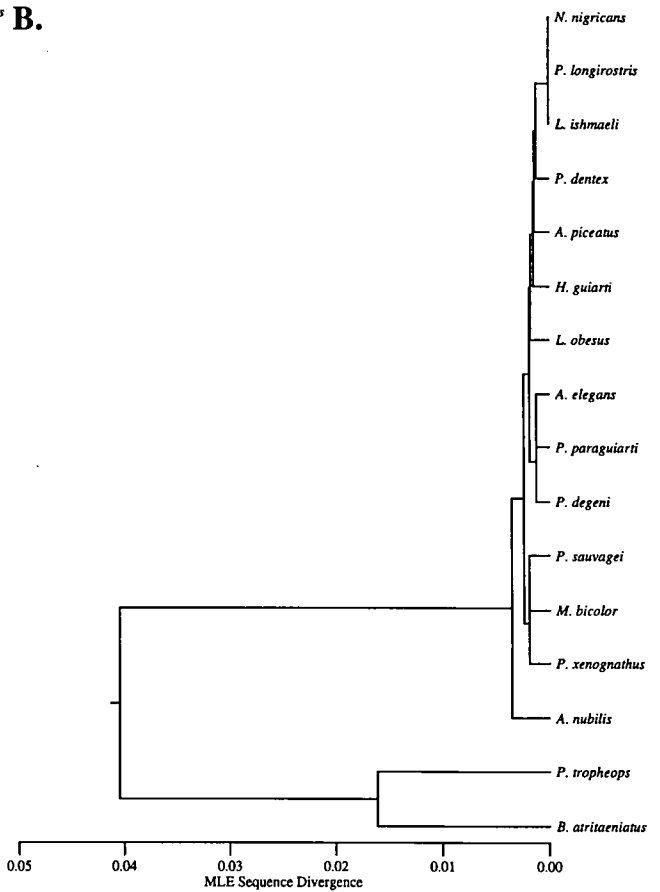
Nee et al. (1994) have presented theoretical models that show how small samples from the total number of extant taxa can lead to a spurious impression of a decrease in effective cladogenesis through time. To maintain the simplicity of the null model employed here, species birth and extinction rates are assumed to be equal such that effective cladogenesis is stochastic with respect to time. Thus, equations (33) and (34) in Nee et al. (1994) are not directly applicable. Nonetheless, our fraction of species sampled within *Sebastes* ($f = 0.43$) falls well above the range where a spurious impression of decreased cladogenesis occurs. Furthermore, Seeb's (1986) allozyme study included 48 of the 65 rockfish species ($f = 0.74$) in the northeastern Pacific, yet yielded conclusions about ancient speciations consistent with those from the *cyt b* analyses. Nonetheless, the overall conclusions might change as more extant rockfish species and/or additional genes are included in subsequent studies.

A third caveat concerns choice of outgroups, which as emphasized by Wollenberg et al. (1996) can have important and generally predictable influences on the outcomes of the statistical tests. Specifically, outgroups too distant from a posited radiation will tend to compress nodal placements toward the present in a relative time frame, whereas the mistaken use of an outgroup within a posited radiation will tend to distribute nodal placements across the entire relative time frame (thus potentially resulting in a failure to identify important nonrandom patterns that may exist in the posited phylogeny). For this reason, we first analyzed each dataset in its entirety and then again after removal of outgroup taxa used in the initial appraisal. Outcomes generally were consistent in yielding significant support for temporal clustering of old nodes, with one exception. In the *cyt b* analyses with *Sebastolobus* as outgroup (Fig. 2A), the empirical cdf did not

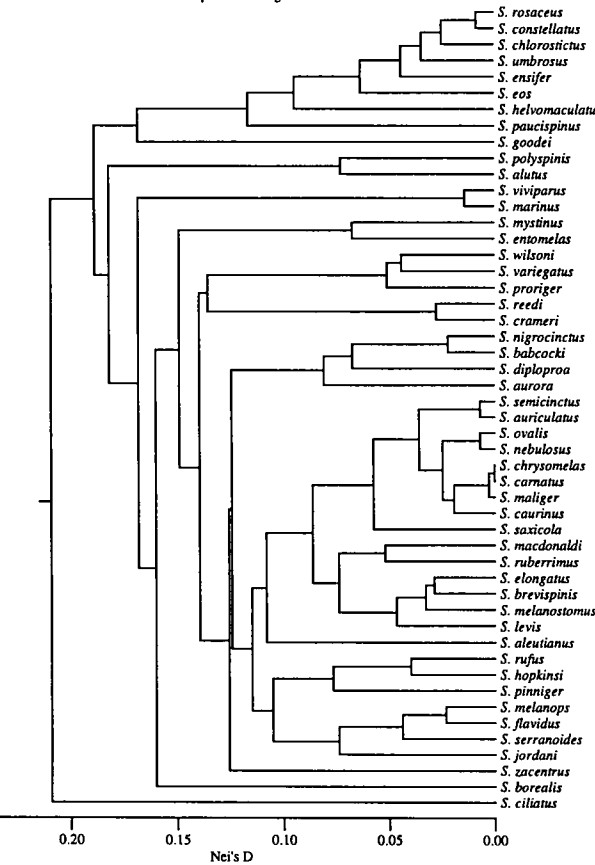
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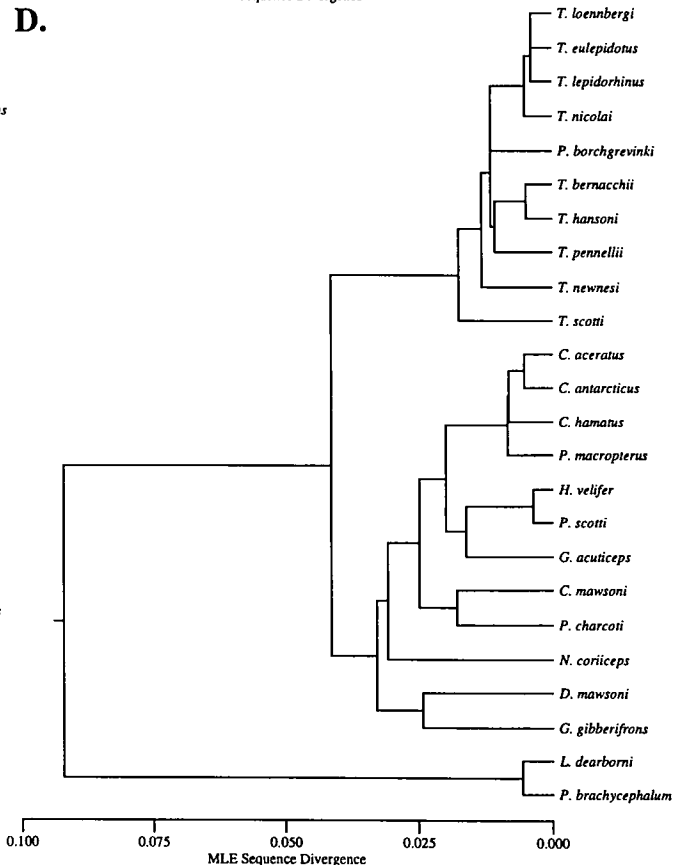
B.



C.



D.



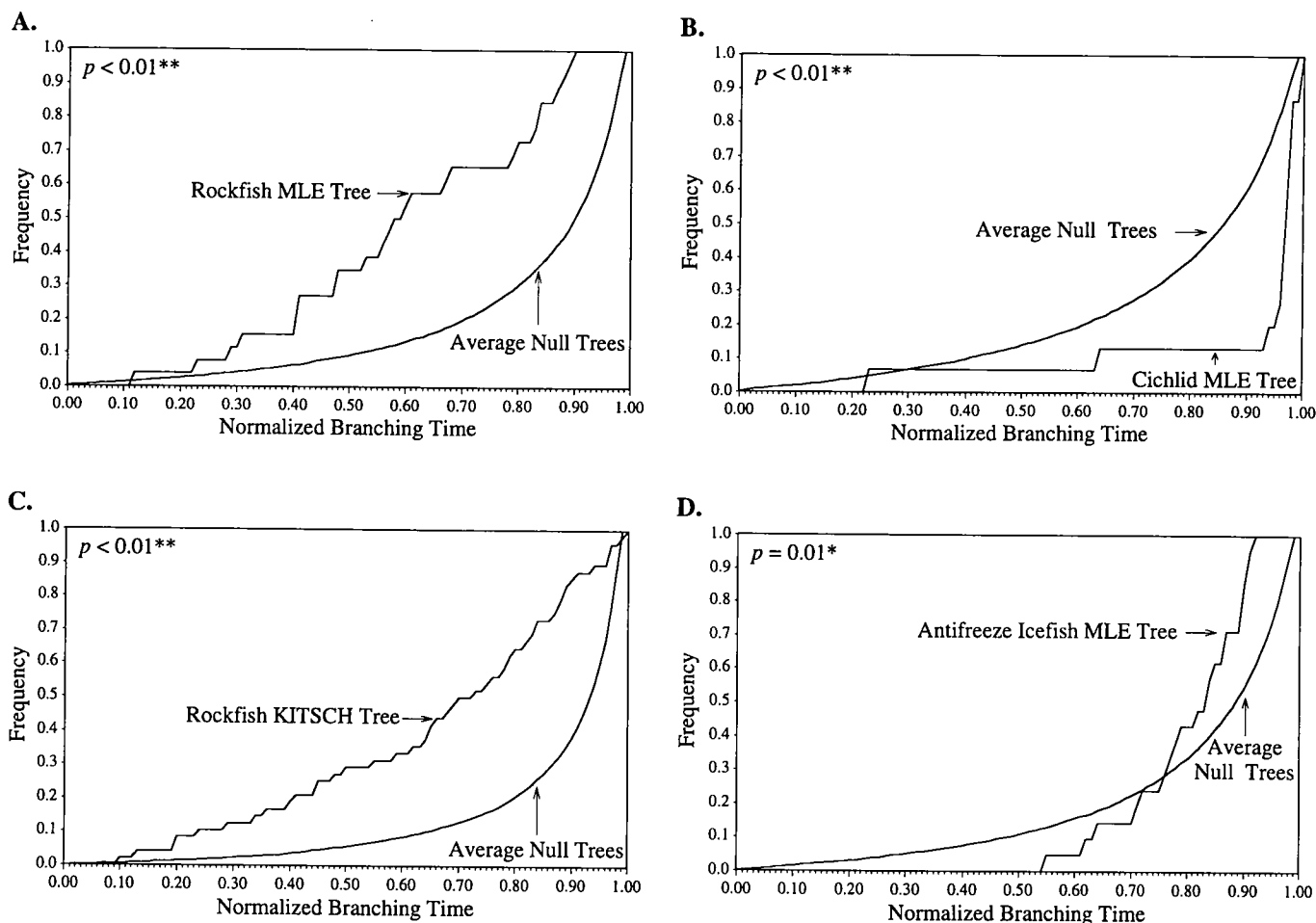


FIG. 5. Effects of outgroup choice on the statistical significance of putative species flocks based on comparisons between the cdfs for the phylogenies in Figure 4 and the appropriate null cdfs. (A) Rockfish *cyt b* analyses. (B) Cichlids of Lake Victoria. (C) Rockfish allozyme analyses. (D) Icefishes with antifreeze proteins.

depart significantly from the null cdf (Fig. 3A; Table 1). Even here, however, a sharp “jump” in the empirical cdf was evident at a normalized branching time near 0.80–0.90 (Fig. 3A). This jump crossed the null cdf curve symmetrically, from below to above, and, thus, failed to register as significant in the Kolmogorov-Smirnov test statistic (which assesses only the maximum departure of an empirical cdf from a null cdf). Although not formally significant in this case, a noticeable discontinuity in the empirical cdf curve suggests that a relatively narrow period in evolutionary time brackets a disproportionate number of internal nodes in the rockfish phylogeny.

Cichlids and Icefishes.—In contrast to the rockfishes, the sharp burst of speciation in the cichlid fishes of Lake Victoria occurred in the very recent past. Not surprisingly, this spe-

ciation pattern was registered in our statistical tests as highly significant departures of the cdf curves below and to the right of the corresponding null cdfs. This outcome also was robust to changes in outgroup and applied even when related cichlids in Lake Malawi were employed.

To our knowledge, the only other marine fish assemblage to which the term species flock has been applied involves icefishes in the Notothenioidei (Bargelloni et al. 1994), which includes the Trematominae (Ritchie et al. 1996). In our analyses, a significant temporal clustering of nodes was registered for the notothenioids that have acquired antifreeze proteins (AFGPs) and for the Trematominae within this group, but not for the Notothenioidei as a whole. Of course, these conclusions are subject to the same caveats as mentioned above for rockfishes. Although the icefishes and the rockfishes cur-

FIG. 4. Effects of outgroup choice on temporal pattern of speciation for the putative species flocks. Phylogenies were reconstructed as in Figure 2, except that the most-basal outgroup for each phylogeny was removed from the analysis. (A) Maximum-likelihood phylogeny for the *Sebastes* based on *cyt b* data and rooted with *S. paucispinus*. (B) Maximum-likelihood phylogeny for the Lake Victoria cichlids rooted with *Astatoreochromis*. (C) KITSCH phylogeny for the *Sebastes* based on allozyme data and rooted with *S. ciliatus*. (D) Maximum-likelihood phylogeny for the icefishes possessing antifreeze proteins, rooted with the zoarcoids. The sequence divergence scales were maximum-likelihood estimates for sequence data or Nei's *D* for allozyme data.

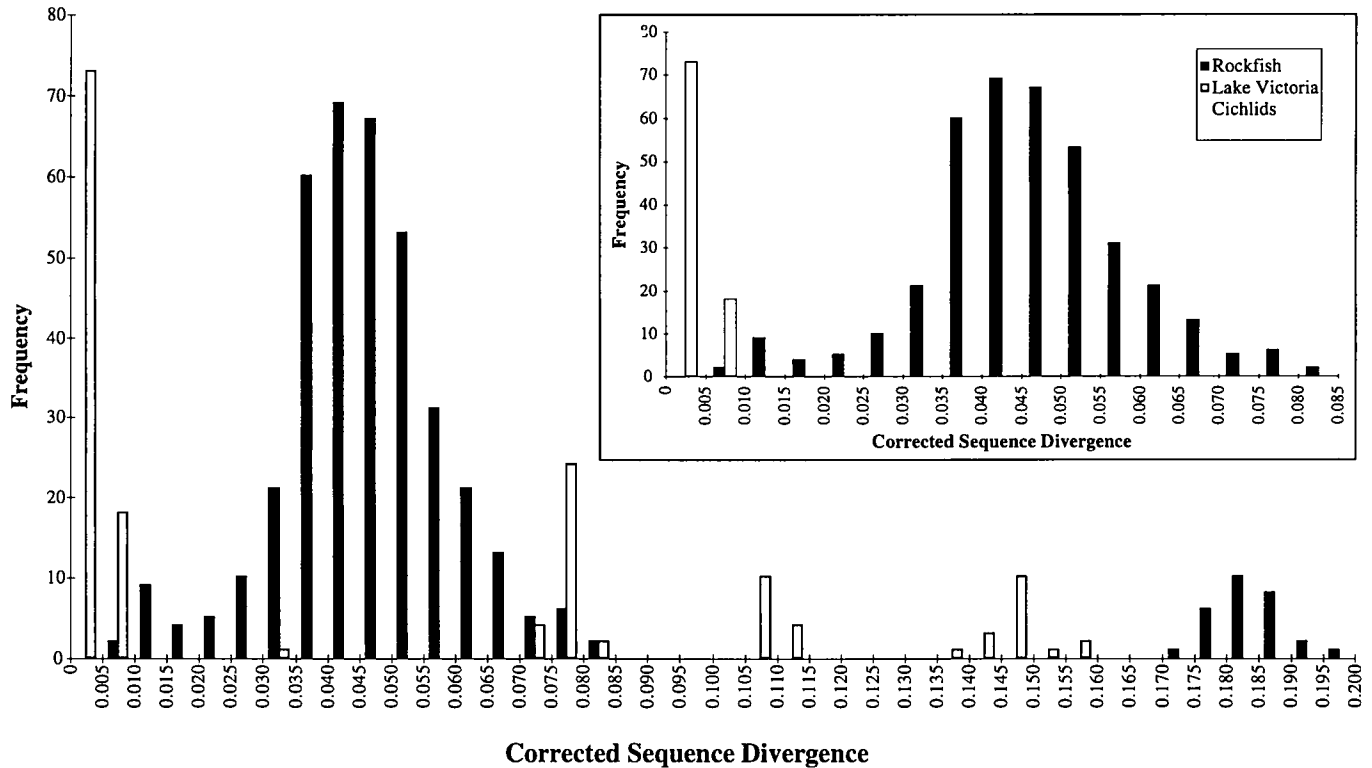


FIG. 6. Frequency distribution of interspecific pairwise genetic distances in the *cyt b* gene for the rockfish (black bars) and cichlid (white bars) datasets. Distance estimates were corrected for multiple substitutions using the Kimura two-parameter model. The inset shows the same data, but with outgroup taxa (non-*Sebastes* rockfishes and cichlids outside of Lake Victoria) removed.

rently provide the only available instances of what justifiably might be considered ancient species flocks in the marine realm, many more such groups probably remain to be discovered.

Absolute Time.—All the analyses presented here involve assessments of relative rather than absolute time. Nonetheless, it may be instructive to consider briefly the possible absolute ages of the various species flocks. Clearly, by all available evidence the cichlid species flock in Lake Victoria has arisen recently, within the last few thousand years. By contrast, the rockfish and icefish radiations appear to be much more ancient. From transversions in rRNA gene sequences, Bargelloni et al. (1994) estimated the origin of the notothenioid radiation to be 12 million years ago. Using the same calibration, Ritchie et al. (1996) estimated an average age of the trematomid radiation at about 3.4 million years ago.

If we employ for the rockfishes the molecular clock calibration used by Meyer et al. (1990) for *cyt b* in the Lake Victoria cichlids (1.25% sequence evolution per lineage per million years), then the maximum age of the rockfish species flock is possibly greater than 3.6 million years. Alternatively, it has been suggested that poikilotherm mtDNA evolves up to five times slower than mtDNA in endotherms (review in Rand 1994). Under this assumption, the oldest rockfish separations may have occurred about 18 million years ago, and the rapid radiation of species (as registered by the first significant departure of the observed from the null cdf curves; Fig. 5A) may have occurred about 8.5 million years ago. Another estimate comes from a consideration of fossil evi-

dence. As noted previously, the earliest fossil rockfishes known are from the Upper Miocene (10–12 million years ago). Using this date as a provisional estimate of the origin of the group, a proportionately scaled time estimate for the first significant radiation within the group then becomes about 5 million years ago. Although all of these estimates are highly provisional, they do show unequivocally that these marine assemblages are vastly older than the species flock in Lake Victoria.

Conclusions.—Like the paleontological assessments initiated by the MBL group in the 1970s (Raup et al. 1973), the current approach merely tests for significant temporal pattern in evolution and cannot directly address questions of evolutionary process: how and why the species flocks evolve. Nonetheless, the approach exemplified here is an important first step in deciding when a suspected evolutionary pattern (in this case, a rapid proliferation of species in a relatively short time span) departs significantly from null expectations and hence is worthy of further efforts toward causal interpretation.

For the Lake Victoria cichlid species flock, traditional explanations that invoke microallopatric speciation include: fluctuations in lake level that created separate lake basins during periods of low water, followed by reintegration during periods of high water; or behavioral preferences for different microhabitats within the lake. For these, and more forcefully for some crater lake cichlid species flocks (Schliewen et al. 1994), mechanisms of true sympatric speciation have been suggested as well (reviewed in Martens 1997).

For rockfishes and other relatively old species flocks in the marine realm, causal processes underlying speciation patterns will be even more difficult to decipher because particular events will have been blurred by the large spatial scales and obscured by evolutionary time. Nonetheless, we hope to have suggested a general conceptual orientation and empirical approach that may prove useful in promoting further thought about the nature of speciation in the marine realm (Palumbi 1994). Perhaps this approach will help point the way to interesting new lines of inquiry into both pattern and process in evolution.

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