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MOLECULAR SYSTEMATICS OF THE GRACKLES AND ALLIES, AND THE EFFECT OF ADDITIONAL SEQUENCE (CYT *B* AND ND2)

KEVIN P. JOHNSON[1] AND SCOTT M. LANYON

Bell Museum of Natural History and Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, Minnesota 55108, USA

ABSTRACT.—Within the New World blackbirds, the lineage of grackles and their allies contains several species that have been extremely well studied by avian biologists. Using mitochondrial DNA (mtDNA) sequences, we present a phylogeny for the grackles and allies that serves as a basis for comparative studies in this group. We compare two gene regions and determine that ND2 is evolving more rapidly than cytochrome *b*. However, this difference in evolutionary rate does not result in significant incongruence between phylogenies derived from the two gene regions independently. A combined weighted analysis provides a completely resolved phylogeny for this group. In general, this phylogeny has higher support than the phylogenies derived from the two genes independently. Two major clades are identified in this combined phylogeny (1) a completely South American clade, and (2) a largely North American/Caribbean/Central American clade. This phylogeny has important implications for the study of behaviors and morphology. *Received 16 April 1998, accepted 8 January 1999.*

The Red-winged Blackbird (*Agelaius phoeniceus*) and other North American blackbird relatives (e.g. *Quiscalus quiscula* and *Molothrus ater*) are among the most intensively studied avian species in North America (e.g. Friedmann and Kiff 1985, Orians 1985, Searcy and Yasukawa 1995, Beletsky and Beadle 1996). However, the lack of explicit knowledge of the phylogenetic relationships of these species and their close relatives hampers attempts to place the behavior, morphology, and physiology of these species in an evolutionary context. Several comparative studies of blackbirds have been conducted using poorly resolved and/or supported phylogenies. These studies include size dimorphism and mating systems (Björklund 1991, Webster 1992), brightness (Irwin 1994) and song repertoires (Gray and Hagelin 1996). Given the varied evolutionary questions that are of interest in this group, it is important to examine trait evolution in a historical context based on a well-resolved and well-supported phylogeny.

Lanyon and Omland (1999) present evidence from cytochrome-*b* (cyt *b*) DNA sequences for five main lineages within the traditionally recognized New World blackbirds (Icteridae): (1) grackles and allies, (2) caciques and oropendolas, (3) orioles, (4) meadowlarks and allies, and (5) the single cup-nesting cacique. Here, we present a phylogeny based on mtDNA sequences for the lineage to which the Red-winged Blackbird belongs: the grackles and allies.

The grackles and allies are distributed throughout much of the New World. As currently described (Sibley and Monroe 1990), this lineage consists of 40 species in 15 genera; 9 of the genera are monotypic, suggesting a general lack of understanding of relationships within the group. The behaviors exhibited by this group encompass much of the variation in breeding biology found in birds (including obligate brood parasitism and polygyny). In addition, detailed studies of several species (e.g. Red-winged Blackbird; Searcy and Yasukawa 1995, Beletsky and Beadle 1996) have greatly advanced our understanding of several biological phenomena including polygyny, territoriality, breeding physiology, and sexual selection. Systematic studies of this group have been limited, but an initial analysis of partial cyt-*b* gene sequences (Lanyon 1994) suggested that some genera, as currently defined for this group, are not monophyletic (e.g. *Agelaius* and *Molothrus*). In addition, Lanyon's (1992, 1994) analyses provided an initial working hypothesis of systematic relationships that can be used as a guide for further study.

In addition to clarifying phylogenetic rela-

[1] Present address: Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA. E-mail: johnson@biology.utah.edu

tionships within the clade of grackles and their allies, we use this study to explore issues that have troubled molecular systematists. One such issue concerns the strategies that may be employed to increase support for weakly supported nodes. Two possibilities involve gathering more data (1) sample additional taxa, or (2) sequence additional gene regions. Although sampling additional taxa may greatly improve phylogenetic estimation by breaking up long branches (Felsenstein 1978, Hillis 1996, Sanderson 1996), in many systematic studies this is not feasible (either because all extant taxa have been sampled, or additional taxa are not available). Because we have sampled nearly all representative species within the grackles-and-allies clade, we explore the effects of the addition of another gene (ND2) on the resolution and support for the phylogeny of the grackles and allies.

A second major issue is how to weight DNA sequence characters in phylogenetic analyses. We explore the effects of several different weighting options. In addition, we compare the two protein-coding gene regions (cyt *b* and ND2) in terms of their rate and mode of sequence evolution. Finally, we interpret the evolutionary history of this group in light of phylogenetic hypothesis derived from these genes and examine the implications of this phylogeny for taxonomy, biogeography, and character evolution.

METHODS

Samples.—We sampled 34 of 40 ingroup species of grackles and their allies, including representatives of all but one (*Hypopyrrhus*) of the 15 genera recognized by Sibley and Monroe (1990). We used samples of the species within this clade used by Lanyon and Omland (1999), which is eight more species than Lanyon (1994) used in an earlier analysis of *Agelaius*. In addition, we included six outgroup taxa, with at least one representative from each of the four other major clades within Icteridae (Lanyon and Omland 1999). Each species was represented in our study by a single individual.

DNA sequencing.—We sequenced all samples for an 879 base-pair (bp) portion of the cyt-*b* gene (see Lanyon [1994] for primers). We also sequenced ND2 for these samples using the primers L5215 (Hackett 1996), H6313 and L5758 (Johnson and Sorenson 1998), and H5776 (constructed for this study; 5′-TGG GAR ATG GAG GAR AAG GC-3′). We amplified both cyt *b* and ND2 from total genomic DNA extracts. We performed PCR in 50-μL reaction volumes using 0.5 μL of Thermo flavus polymerase (Epicentre Technologies), 3 μL of 10 micromolar solution for each primer, 3.9 μL of 25 micromolar $MgCl_2$, 2.5 μL of 20× reaction buffer, 35 μL of distilled water, and between 1 and 5 μL of total genomic DNA extracts.

We used a Perkin Elmer DNA Thermal Cycler 480 to perform the reactions, with reaction conditions of one cycle of 3 min at 93°C, 1 min at 50°C, and 2 min at 72°C followed by 35 cycles of 1 min at 93°C, 1 min at 52°C, and 1 min, 20 s at 72°C. A 10-min extension at 72°C and a hold at 4°C followed these cycles. We purified PCR products using a Qiagen PCR purification kit with the manufacturer's protocols. We used an ABI Prism Dye Terminator Reaction kit FS with manufacturer's protocols for sequencing reactions. We sequenced each PCR product for ND2 using all four of the primers given above and for cyt *b* using the primers L14841, H15299, B3, B4, and B5 (see Lanyon 1994). We purified sequencing reactions using Centrisep columns packed with 0.05 g Sephadex (Sigma) in 0.8 mL water and followed manufacturer's protocols. We dried products in a Centrivap vacuum concentrator and ran them on an acrylamide gel with an ABI 377 automated sequencing machine.

We aligned resulting chromatograms of complementary strands and reconciled them using Sequencher 3.1 (GeneCodes). We also aligned sequences between species using Sequencher 3.1. This produced sequences that included the entire ND2 gene (1,035 bp) and a large portion (879 bp) of the cyt-*b* gene for each taxon (Genbank accession numbers AF089004 to AF089014, AF089016, AF089018, AF089020 to AF089027, AF089037, AF089039 to AF089046, AF089051 to AF089052, AF089054 to AF089058, AF089060, AF089064, AF089066, AF099278, AF099314, AF099354 to AF099360, and AF109931 to AF109962).

Comparison of gene regions.—To explore the nature of base substitutions in the two gene regions, we examined the proportion of base positions that were variable and potentially phylogenetically informative for both gene regions analyzed separately. These proportions were compared using a *z*-approximation statistic (Milton and Arnold 1990). We computed the number of transitions and transversions over each codon position for all pairwise comparisons for both genes using MEGA (Kumar et al. 1993). To determine the potential of various base positions for experiencing multiple substitutions, we constructed plots of the number of transitions and transversions at each base position against total percent sequence divergence. In addition, we constructed a plot of total percent divergence in ND2 against total percent divergence in cyt *b* to further compare rates of substitutions in the two genes. We do not present statistical analyses of these plots because of the non-independence of the many pairwise comparisons. We estimated the "native" transition-to-transversion ra-

tio for both genes by plotting third-position transitions against transversions from pairwise comparisons (Sturmbauer and Meyer 1992). We used this ratio as a guide in constructing transversion weighting schemes. We performed these analyses to determine the initial suitability, in terms of sequence variation, of these two genes for phylogenetic analysis.

Phylogeny and sensitivity to weighting scheme.—To determine the sensitivity of the reconstructed phylogeny to weighting scheme, we conducted parsimony searches using PAUP* (v. d60 to d62; Swofford 1997) on the 34 ingroup and 6 outgroup species using several different weighting schemes of transversions over transitions. We performed searches using 1:1, 2:1, and 5:1 weighting of transversions over transitions with both gene regions independently and combined. We conducted a partition homogeneity test (Farris et al. 1994, Swofford 1997) using the two genes as partitions under equal weighting to determine if combining sequences from the two gene regions in a combined analysis was justified (Bull et al. 1993).

To assess the degree to which results of the parsimony analyses are robust to selection of characters and taxa, we employed bootstrap (Felsenstein 1985) and jackknife (Lanyon 1985) manipulations, respectively. For bootstrapping, we independently analyzed the cyt-*b* data set, the ND2 data set, and then the combined data with both 1:1 and 2:1 weighting of transversions over transitions. In the jackknife analysis (Lanyon 1985), we examined only the combined data set with both 1:1 and 2:1 weighting of transversions over transitions. We constructed 34 jackknife pseudoreplicate data sets with a single ingroup taxon deleted from each set.

Results

Comparison of gene regions.—For the regions sequenced, ND2 was more variable than cyt *b*. Of 1,035 bases for ND2, 473 (45.7%) were variable and 380 (36.5%) were potentially phylogenetically informative; for cyt *b*, 331 of 878 (37.7%) base pairs were variable and 258 (29.4%) were potentially phylogenetically informative. These differences were statistically significant with the *z*-approximation statistic (both $P < 0.001$). These differences also translated into a difference in the proportion of variable amino-acid residues (30.1% for ND2 and 14.0% for cyt *b*; $P < 0.001$).

Overall percent sequence divergence between groups of interest ranged from around 4% within ingroup genera to 12% between the ingroup and outgroup (Table 1). Plots of the number of transitions and transversions at third positions in pairwise comparisons against percent sequence divergence (Fig. 1) showed clear differences between cyt *b* and ND2. Transversions appear to accumulate more rapidly in cyt *b* than in ND2, whereas pairwise transitions appear to level off after an initial increase in cyt *b* but show no leveling off in ND2. Although the accumulation of transversions at first and second positions are very similar for cyt *b* and ND2, transitions at first and second positions appear to accumulate more rapidly in ND2 than in cyt *b* (not shown).

TABLE 1. Pairwise divergence between selected taxa of icterids.

Comparison	Average percent divergence
Agelaius cyanopus vs. *Molothrus badius*	7.9
Agelaius cyanopus vs. *Agelaius tricolor*	9.0
Other *Molothrus* vs. *Molothrus badius*	9.1
Other *Molothrus* vs. *Scaphidura*	4.9
Euphagus vs. *Quiscalus*	7.4
Dives vs. rest of ingroup	8.6
Nesopsar vs. rest of ingroup	8.8
Outgroup vs. ingroup	12.2

Transitions at first and second positions could account for the increased number of amino-acid differences in ND2. A plot of percent sequence divergence in ND2 against that for cyt *b* (Fig. 2) also suggests that ND2 is evolving at a faster rate (or is less prone to multiple substitutions). Although at low divergences (<5%), cyt *b* and ND2 are accumulating substitutions at similar rates, above this level of divergence, ND2 accumulates substitutions more rapidly. The "native" transition/transversion ratio (Sturmbauer and Meyer 1992) is approximately 5:1 for both genes.

The unweighted (1:1) ND2 tree shows similar resolution (28 of 32 potential nodes) to the unweighted (1:1) cyt-*b* tree (29 of 32 potential nodes), and the ingroup topologies from cyt *b* and the ND2 unweighted (1:1) analyses are very similar, with 18 of 28 resolved nodes in the ND2 topology also present in the cyt-*b* topology. The 2:1 weighted topologies are also similar, with 20 of 29 resolved ingroup nodes in the 2:1 ND2 topology present in the 2:1 cyt *b* topology. Between the cyt-*b* and ND2 50% bootstrap topologies, no nodes disagree above the 50% bootstrap level; however, the 50% ND2 bootstrap topology shows more resolution. The

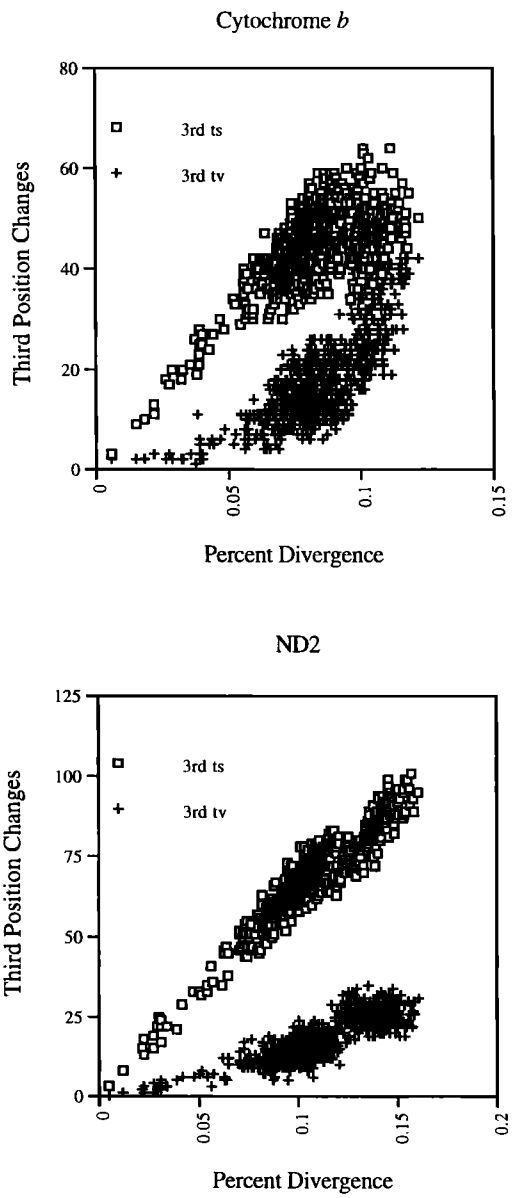

FIG. 1. Plots of transitions (ts) and transversions (tv) at third-codon positions against total percent sequence divergence in pairwise comparisons based on cyt *b* (upper) and ND2 (lower).

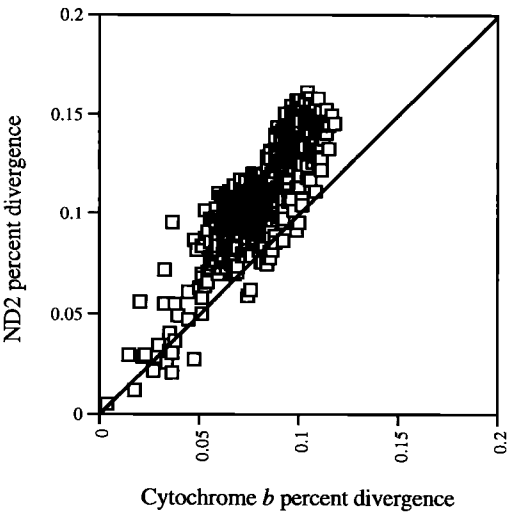

FIG. 2. Plot of percent sequence divergence in ND2 against that of cyt *b* in pairwise comparisons of all sequences. The line shows the expectation under the assumption that the two genes evolve at equal rates.

similar phylogenetic signal in both genes is also confirmed by the partition homogeneity test. Using cyt *b* and ND2 as the two data partitions, the signal between these two partitions does not differ significantly ($P = 0.56$).

Phylogeny and sensitivity to weighting scheme.—The trees resulting from the different weighting schemes (1:1, Fig. 3; 2:1 and 5:1, Fig. 4) on the combined data set are completely compatible; however, the weighted topology (Fig. 4) is more fully resolved. This suggests that tree topology is relatively insensitive to weighting scheme. Weighting of 2:1 or 5:1 of transversions over transitions resulted in the same single fully dichotomous most-parsimonious tree. The analysis of the combined data set produced topologies containing nodes that were not present in the separate analyses of gene regions, suggesting that support in both genes exists for nodes that do not appear in the individual analyses.

Another way to assess the success of combining data sets (two different genes) is to examine the change in bootstrap support from the separate analyses to the combined analysis. A total of 12 nodes showed an increase in bootstrap support from the independent analyses to the combined analysis. Four nodes remained at 100% bootstrap support from the separate to the combined analyses. Only four nodes showed a slight decreases in bootstrap support (none greater than 5% and all from relatively high values greater than 90%). Two additional nodes (not present in the separate analyses) attained bootstrap support at the 50% level. These results indicated that combining the data

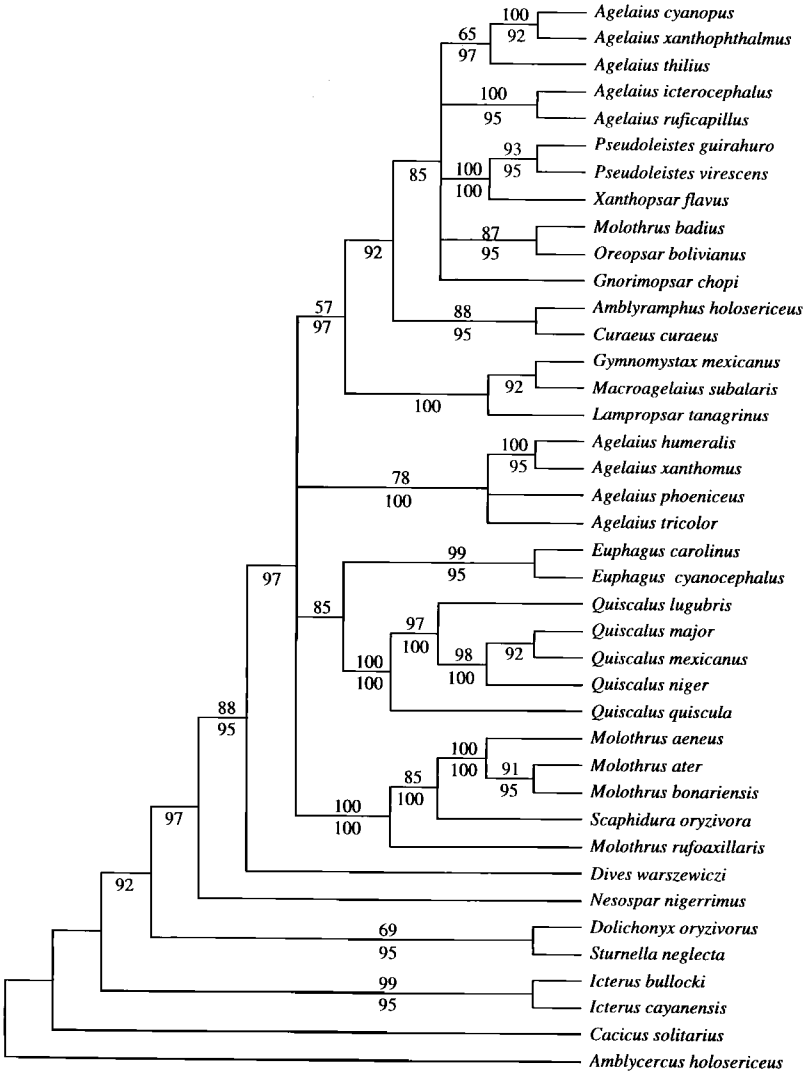

FIG. 3. Consensus of seven most-parsimonious trees (l = 3,044, RC = 0.171) constructed from cyt-*b* and ND2 sequences with no weighting of transversions over transitions. Numbers above branches are bootstrap values, and numbers below branches are jackknife proportions.

sets increased resolution and support for the topology.

We assessed the jackknife support for nodes using the 1:1 and 2:1 weighting schemes. Jackknife topologies were well resolved (Figs. 3 and 4). Of 33 ingroup nodes with 2:1 weighting of transversions, 27 received jackknife support at the 90% or higher level. Because transitions at third positions appear to have a strong potential for multiple substitutions in cyt *b* (Fig. 1), and because transitions accumulate at a rate five times higher than transversions, we prefer weighting schemes that give higher weight to transversions. In addition, most of the topology was insensitive to weighting scheme and was well supported by bootstrap and jackknife replicates. Thus, we use the weighted (2:1 and 5:1) topology (Fig. 4) as the best estimate of the phylogenetic relationships among the grackles and allies.

This tree shows two major clades in the grackles and allies (groups 1 and 2 in Fig. 4). One of these clades (group 1) is distributed exclusively in South America. The other, group 2,

is distributed mainly in North America, Central America, and the Caribbean with the exception of *Molothrus rufoaxillaris* and *Scaphidura oryzivora*. These two large clades are sister to each other, and in turn are sister to *Dives warszewiczi* and *Nesopsar nigerrimus* (two taxa from the Caribbean and Central America). Within the North American clade, *Agelaius* is sister to *Molothrus*, and *Euphagus* is sister to *Quiscalus*. Within this topology, *Agelaius* and *Molothrus* are not monophyletic, a conclusion supported by bootstrap replicates (see below).

DISCUSSION

Comparison of gene regions.—The differences in the rates and patterns of sequence substitution between cyt *b* and ND2 in the grackles and their allies are striking. However, these differences are not dramatic enough to cause significant differences in phylogenetic signal (Bull et al. 1993). The differences in substitution rates between these genes are similar to those found in tanagers (Hackett 1996) but are in stark contrast to results obtained for these two genes under similar levels of divergence in dabbling ducks (Johnson and Sorenson 1998). This suggests that these two genes evolve differently in different lineages. This could have important implications for phylogeny reconstruction using these two genes across lineages. Specifically, cyt *b* appears to show a dramatic difference in pattern of evolution between blackbirds and waterfowl. Because few other studies have included ND2, it is difficult to extrapolate these patterns to other studies that have included only cyt *b*. The reasons for differences in patterns of sequence evolution in different taxa require additional investigation.

Phylogeny and sensitivity to weighting scheme.—The phylogenetic relationships indicated by the combination of these two gene regions are relatively insensitive to weighting of transversions over transitions. In general, increased resolution is attained with weighting, and this resolution is completely consistent with a consensus of topologies derived from equal weighting. In addition, much of this topology is well supported by both bootstrap and jackknife replicates. In comparing the 50% bootstrap topology derived from transversion weighting and the cyt *b* and ND2 combined analysis with topologies derived from mtDNA restriction fragments (Freeman and Zink 1995), we note several differences. We use Freeman and Zink's (1995: figure 1b) well-supported topology for comparison. First, the monophyly of the grackles and allies is not demonstrated in their study, but it is strongly supported in our analysis and the analysis of Lanyon and Omland (1999). Second, the monophyly of a brood-parasitic cowbird clade is present in our analysis but not supported by theirs. Finally, the sister relationship between *Euphagus* and *Quiscalus* is well supported in the sequence data but is not evident in the restriction-site data. At present, it is unclear why the dramatic differences between phylogenies derived from the same linkage group (mtDNA) should occur. Rate differences between data sets (Bull et al. 1993), errors in character scoring, or the inclusion of poorly supported nodes could account for these differences. It seems more likely, however, that differences in these data sets simply are a consequence of the inability of nine six-base enzymes to adequately sample the genome.

We also compared our weighted topology (Fig. 4) with a phylogeny derived from 23 skeletal characters for the genus *Quiscalus* and outgroups (Björklund 1991). *Quiscalus* is not monophyletic in Björklund's (1991) study, whereas the monophyly of *Quiscalus* receives strong support in the molecular data from our study (Figs. 3 and 4). One node present in both analyses (morphological and molecular) is a clade including *Q. major*, *Q. mexicanus*, and *Q. niger*. Other than this, little similarity exists between the trees from the two data sets. We bootstrapped the skeletal data set to determine the level of support for Björklund's (1991) topology and determined that only one node was found in more than 50% of bootstrap replicates (*Q. major* + *Q. niger*). This analysis suggests that the topological incongruence between the morphological and molecular data sets is due to a small number of morphological characters relative to the number of molecular characters (i.e. "sampling error" sensu Bull et al. 1993).

Because the topology generated from our study is generally well resolved and well supported, we feel justified in suggesting the following taxonomic revisions. *Molothrus badius* (Bay-winged Cowbird) is the sole member of *Molothrus* that is not an obligate brood parasite. Our current study indicates that its affinities lie

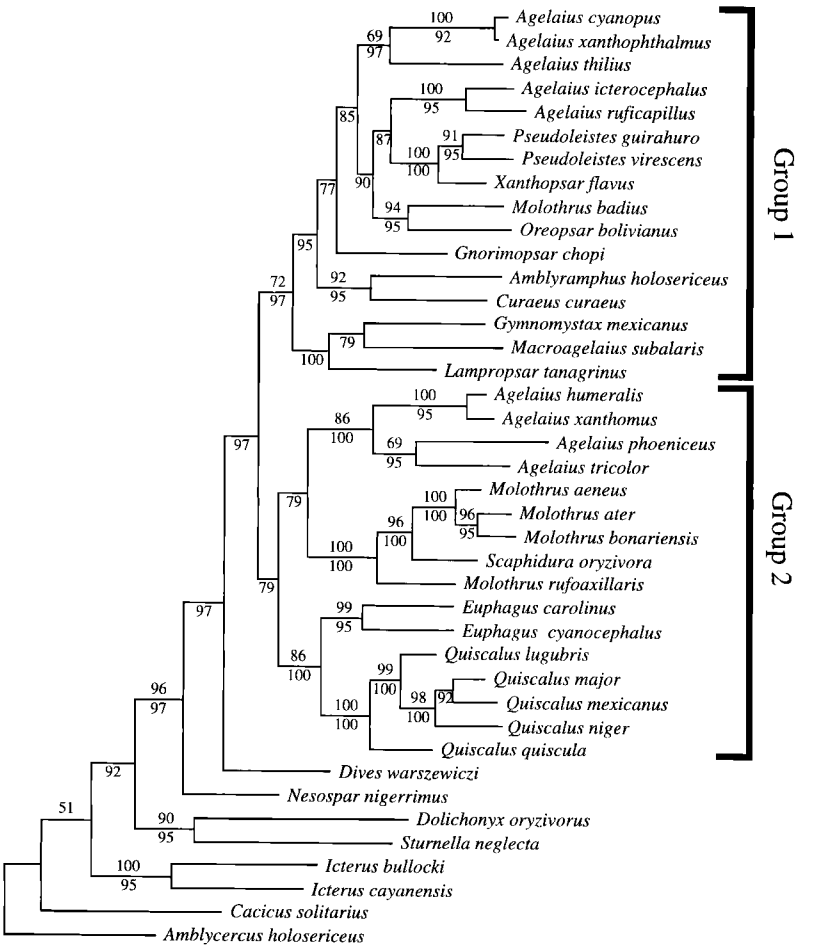

FIG. 4. Single most-parsimonious tree (l = 3,594, RC = 0.194) constructed from cyt-*b* and ND2 sequences with 2:1 weighting of transversions over transitions. Other weighting schemes higher than 2:1 produced an identical topology for the ingroup. Numbers above branches are bootstrap values, and numbers below branches are jackknife proportions. Two major clades discussed in the text are labeled group 1 and group 2.

with the monotypic *Oreopsar bolivianus* (Bolivian Blackbird). Although this sister-group relationship has not been suggested previously, it is supported by both gene regions and by the combined analysis. Furthermore, this conclusion is not sensitive to changes in character weighting or to data-set resampling manipulations. The unusual nesting characteristics of these two species make their close affinity especially interesting. *Oreopsar bolivianus* is the only hole-nesting icterid, and *Molothrus badius* is the only nest parasite within the subfamily. To make the genus *Molothrus* monophyletic, and to recognize the phylogenetic affinity of these two species, we transfer the Bay-winged Cowbird to the genus *Oreopsar,* and it becomes *Oreopsar badius* (Vieillot) 1819.

The monotypic genus *Schaphidura* clearly falls within *Molothrus* in our analysis. Paraphyly of *Molothrus* with respect to *Schaphidura oryzivora* (Giant Cowbird) is strongly supported by our study. Neither changes in weighting scheme nor the two resampling manipulations alter this conclusion. Therefore, we recommend merging these two genera to produce a single genus that contains all five of the brood-parasitic icterids. Because *Molothrus* is the older of these two names, the Giant Cowbird becomes *Molothrus oryzivorus* (Gmelin) 1788.

Our study provides strong evidence that the

South American *Agelaius* (i.e. *cyanopus, xanthophthalmus, thilius, icterocephalus,* and *ruficapillus*) are not closely related to North American and Caribbean *Agelaius.* However, we have no strong evidence to suggest which of the other South American taxa are these species' closest allies. Indeed, it is not clear that the South American *Agelaius* are even their own closest relatives (the weighted tree shows them as paraphyletic). Although it is disconcerting to allow this group of species to remain as a paraphyletic taxon, we believe this to be the prudent decision at this time. More character sets are needed to identify and confirm phylogenetic relationships of these five South American species.

The relatively distant relationship between *Agelaius* and *Nesopsar* deserves comment. Bond (1950) suggested that *Nesopsar* should be considered a subgenus of *Agelaius,* and Wiley and Wiley (1980) designed a study of evolutionary adaptation based on the explicit assumption that the "closest relatives [of *Neospar*] are blackbirds in the genus *Agelaius.*" The intent of Wiley and Wiley was to study "two species that share a relatively recent phylogenetic origin but differ in their current environments." Our data suggest that these taxa are not closely related. The forest dwelling *Nesopsar* is identified as the sister taxon of all other typical blackbirds, an assemblage that includes grassland, marsh, and forest species. *Nesopsar* is the basal member of the lineage of grackles and their allies and therefore is not sister to any species of *Agelaius.*

Implications.—It is often the case that phylogenetic relationships reflect biogeographic distributions (Wiley 1988). That is, species that inhabit adjacent biogeographic zones often are close relatives. This is clearly the case in the lineage of grackles and allies. One major clade is distributed exclusively in South America and the other is distributed mainly in Central and North America and the Caribbean. This pattern suggests that certain components of biogeographic distribution are phylogenetically conserved. It is interesting to note that the brood-parasitic cowbirds are the exception to this pattern. This clade (within the Central/North American clade) has representatives throughout the New World. This also suggests that a pattern links brood parasitism with a lack of phylogenetic conservation of biogeographic distribution. Recent range expansions by *Molothrus ater* (Mayfield 1965) and *Molothrus bonariensis* (Post et al. 1993) also suggest that cowbird species are prone to changes in biogeographic distribution. An examination of biogeographic patterns in other obligate brood parasites would reveal whether repeatable patterns exist in these characteristics.

The phylogenetic relationships of the brood-parasitic cowbirds are identical to those presented by Lanyon (1992). In addition, our work provides further evidence that obligate brood parasitism within the blackbirds evolved only once. Although *Molothrus* (*Oreopsar*) *badius* parasitizes nests, it incubates its eggs and provides parental care. Our analysis also indicates that the evolution of nest parasitism is not linked to the evolution of brood parasitism in the other cowbirds because these two groups are in separate parts of the tree. The reasons for the origins of brood parasitism are still heavily contested (Rothstein 1990, Lanyon 1992), but this phylogeny provides a framework for considering hypotheses concerning the origin of brood parasitism.

The fact that several species of marsh-nesting blackbirds were previously placed in *Agelaius* suggests that several characters are convergent with marsh nesting. Presumably, *Agelaius* species were previously grouped because of these similarities and their propensity to breed in marshes. A phylogenetic reconstruction of marsh nesting (Fig. 5) indicates that this behavior has evolved independently four to six times within the grackles and their allies. Repeated, evolutionarily independent invasions of similar habitats can pose problems for phylogenetic studies if suites of other characters evolve as an adaptive complex in response to the invasion of a novel habitat. Caution is required when selecting characters for use in phylogenetic analyses such that these characters are independent.

Although convergence can obscure phylogenetic relationships, it provides a tremendous opportunity for the evolutionary biologist to study adaptation. Because presumably similar habits and morphology have converged between the North American and South American *Agelaius* groups, this presents the opportunity to study the factors that have been responsible for this apparent convergence. Although the Red-winged Blackbird has been

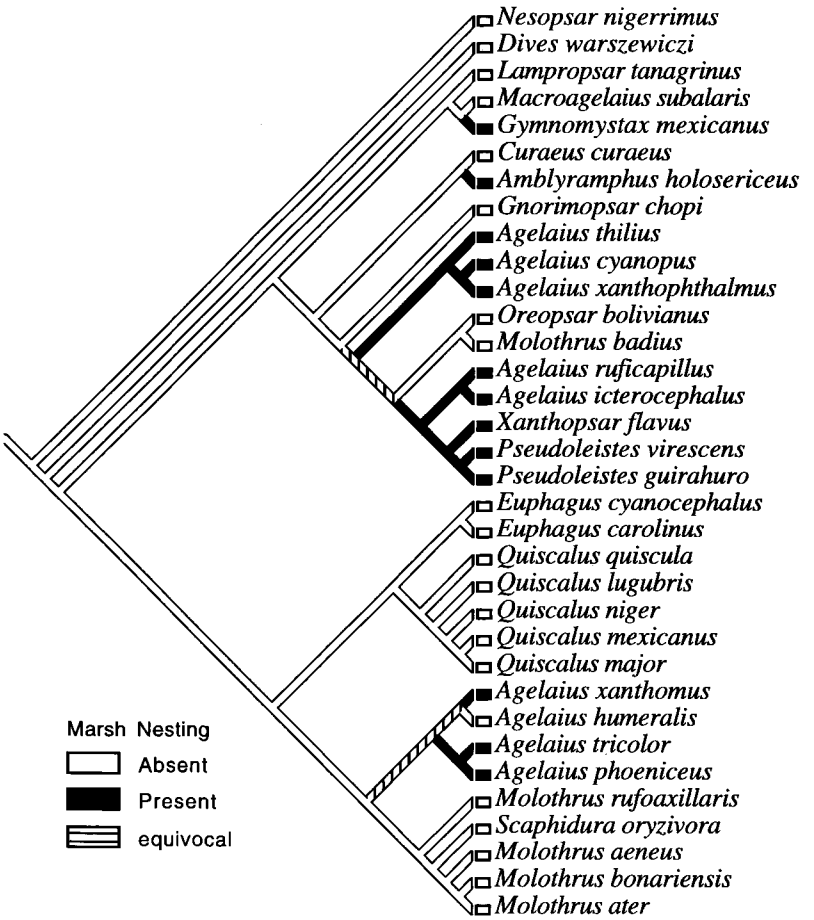

FIG. 5. Phylogenetic reconstruction of marsh nesting over the weighted phylogeny using unordered parsimony. Black branches represent marsh nesting and white branches the absence of marsh nesting.

well studied, the behaviors of most of South American species of *Agelaius* remain poorly known. We suggest that the phylogeny for grackles and allies presented here, coupled with the rich body of literature concerning the group 2 clade and the range of behaviors exhibited by the group, make the study of members of the South American clade (group 1) a priority for improving our understanding of avian behavioral evolution.

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