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SHORT COMMUNICATIONS

The genus *Caryothraustes* (Cardinalinae) is not monophyletic.—The importance of a well-corroborated phylogeny for assessing the evolution of morphological and behavioral traits of birds has been emphasized recently by the analyses of Hackett and Rosenberg (1990), Prum (1990, 1993), Lanyon (1992), Peterson and Burt (1992), and others. Unfortunately, phylogenetic hypotheses at the within-family level are lacking for the vast majority of bird genera and species, and generic allocation of many species is not based on explicit hypotheses, much less data.

The genetic analyses of Tamplin et al. (1993) indicated that the Yellow-shouldered Grosbeak, currently known as *Caryothraustes humeralis,* was not closely related to other cardinaline grosbeaks and perhaps not to cardinalines as a whole. Unfortunately, they did not have access to genetic material of the other two species currently in the genus *Caryothraustes,* both of which are superficially similar to *C. humeralis* in having large, thick bills, black face patterns, and plumage predominantly greenish-yellow and gray. Recent availability of genetic samples of the Yellow-green Grosbeak (*C. canadensis*) permits us to assess the monophyly of the genus *Caryothraustes.* The other species (Black-faced Grosbeak *C. poliogaster*) is extremely similar to *C. canadensis* and differs from it primarily in having gray rather than yellow belly and undertail coverts. *Caryothraustes canadensis* and *C. poliogaster* are allospecies whose close relationship has never been questioned; in fact, Paynter (1970) considered them conspecific. To make our analyses comparable to those of Tamplin et al. (1993), we used protein electrophoresis.

Materials and methods.—Specimens were chosen from representatives of genera within the Cardinalinae as follows: *Caryothraustes humeralis* (Louisiana State Univ. Museum of Natural Science frozen tissue number B-9328), *C. canadensis* (B-1413, B-1414), Northern Cardinal (*Cardinalis cardinalis*) (B-2339), Blue-black Grosbeak (*Cyanocompsa cyanoides*) (B-4871), Rose-breasted Grosbeak (*Pheucticus ludovicianus*) (B-3345), Slate-colored Grosbeak (*Pitylus grossus*) (B-9662), Streaked Saltator (*Saltator albicollis immaculatus*) (B-5254), and Dickcissel (*Spiza americana*) (B-16822). These taxa were chosen to represent evenly the clades depicted in the parsimony tree for Cardinalinae reported by Tamplin et al. (1993). The monotypic genera *Periporphyrus* and *Rhodothraupis* were not included because tissue samples were not available. The non-cardinaline emberizid Plush-capped Finch *Catamblyrhynchus diadema* was used as an outgroup in all phylogenetic analyses. Voucher specimens and frozen tissues are housed in the Louisiana State Univ. Museum of Natural Science.

Homogenates of pectoral muscle were prepared following the methods of Selander et al. (1971). Procedures for starch-gel electrophoresis followed Selander et al. (1971) and Harris and Hopkinson (1976).

Twenty presumptive gene loci were surveyed: adenosine deaminase (ADA, Enzyme Commission number 3.5.4.4); adenylate kinase (AK, 2.7.4.3); aldolase (ALD, 4.1.2.13); alpha-glycerophosphate dehydrogenase (αGPD, 1.1.1.8); creatine kinase (CK, 2.7.3.2); glucose phosphate isomerase (PGI, 5.3.1.9); glutamate-oxaloacetate transaminase (GOT-1, GOT-2, 2.6.1.1); isocitrate dehydrogenase (IDH, 1.1.1.42); lactate dehydrogenase (LDH, 1.1.1.27); malate dehydrogenase (MDH-1, MDH-2, 1.1.1.37); malic enzyme (ME, 1.1.1.40); mannose phosphate isomerase (MPI, 5.3.1.8); peptidase (PEP-B, leucyl-glycyl-glycine; PEP-C, leucyl-alanine; 3.4.11); phosphoglucomutase (PGM, 2.7.5.1); 6-phosphogluconate dehydrogenase (6-PGD, 1.1.1.44); sorbitol dehydrogenase (SODH, 1.1.1.14); and hemoglobin (Hb).

Allozyme data were analyzed using phenetic and phylogenetic approaches. Nei's (1978)

genetic distances (*D*) were generated using the BIOSYS-1 program of Swofford and Selander (1981). To determine the most suitable method of phenetic analysis, the distance matrix was tested for evolutionary rate heterogeneity using both the Fitch and Kitsch programs of PHYLIP (Felsenstein 1993). The Fitch program constructs a phenogram using the Fitch-Margoliash method (Fitch and Margoliash 1967) and does not assume an equivalent molecular clock acting across all lineages. The Kitsch program uses a similar algorithm but assumes equal branch lengths; thus, any incongruence between results of these two methods may indicate the presence of some degree of rate heterogeneity (Felsenstein 1990) and precludes UPGMA clustering which was used by Tamplin et al. (1993). A suitable alternative is the neighbor-joining method of Saitou and Nei (1987), which makes no assumptions concerning evolutionary rates. Archie et al. (1989) demonstrated the necessity of large sample sizes when performing phenetic analyses, contrary to findings of Gorman and Renzi (1979). However, the priority may shift from large sample sizes to increased numbers of characters in phylogenetic analyses (Kesner 1994). Furthermore, scarcity of suitable tissues for members of *Caryothraustes* precludes large samples.

Phylogenetic analysis was conducted using the programs PAUP (Swofford 1993), FREQPARS (Swofford and Berlocher 1987), and MacClade (Maddison and Maddison 1992). An exhaustive search was performed using PAUP to determine minimum-length trees with the loci coded as characters and the alleles coded as character states. MacClade was used to determine the length of alternative tree topologies including that of the distance phenogram. MacClade was also used to determine the length of trees with forced monophyly of *Caryothraustes*.

FREQPARS is a useful program for investigating allozyme data because it assigns each internal node a realistic allele frequency (Swofford and Berlocher 1987). However, it cannot perform branch-and-bound searches (Hendy and Penny 1982) and thus cannot guarantee that all minimum-length trees are found. Therefore, following the method of Page (1990), we generated minimum-length trees using PAUP and entered them as user trees into FREQPARS for comparison. We compared all minimum-length parsimony trees and the distance phenograms using this method.

Results.—Eleven of the 20 loci surveyed here were polymorphic (Table 1). The two individuals of *Caryothraustes canadensis* were identical allozymically; therefore, only one was included in all other analyses. *Caryothraustes humeralis* differed from *C. canadensis* at seven of the 20 loci examined (Nei's $D = 0.431$). The genetic distances separating all other genera within Cardinalinae ranged from 0.180 to 0.531 (mean = 0.377).

The topologies of the Fitch and Kitsch trees were dissimilar, thereby suggesting heterogeneity of evolutionary rates among lineages. Therefore, cluster analysis of the distance data was performed using the neighbor-joining program of PHYLIP. The resulting phenogram (Fig. 1A) indicates that the two species of *Caryothraustes* are not more similar to each other than they are to other genera within Cardinalinae. Also, neither Fitch nor Kitsch trees depict *Caryothraustes* as sister taxa.

Parsimony analysis yielded 10 minimum-length trees with 34 steps and a consistency index of 0.783 (excluding uninformative characters). These 10 trees differed from each other concerning the arrangement of *Cardinalis, Pheucticus,* and *Caryothraustes canadensis,* and a 50% majority-rule consensus tree clearly shows that *Caryothraustes* is not monophyletic (Fig. 1B). There are 2334 trees that are one step longer than the minimum length trees, and the shortest tree depicting *Caryothraustes* as monophyletic is two steps longer (6%) than the shortest trees. The topology of the neighbor-joining phenogram is included among the 2334 near-minimum-length trees with 35 steps.

When analyzed during FREQPARS, all parsimony trees had a length of 66 steps and were shorter than the neighbor-joining phenogram (67 steps). The tree constructed by FREQ-

TABLE 1

ALLELIC VARIATION AT 11 POLYMORPHIC LOCI IN EIGHT REPRESENTATIVES OF CARDINALINAE AND THE OUTGROUP

Taxon	Locus										
	ADA	ALD	GPD	GOT-1	IDH	LDH	PEP-B	PEP-C	PGD	PGM	Hb
Caryothraustes humeralis	c	b	a	b	b	a	d	d	c	b	a
C. canadensis	a	c	a	a	d	a	b	f	c	a	a
Cardinalis cardinalis	c	a	a	a	e	a	e	c	c	a	a
Cyanocompsa cyanoides	c	b	a	a	c	a	a	h	c	ab	a
Pheucticus ludovicianus	c	b	c	a	c	b	b	i	c	a	a
Pitylus grossus	e	a	a	a	f	a	b	a	b	b	a
Saltator albicollis	e	b	b	a	f	a	a	e	b	b	a
Spiza americana	c	b	b	a	f	a	c	g	d	ab	b
Catamblyrhynchus diadema	c	d	a	a	a	a	d	a	b	a	a

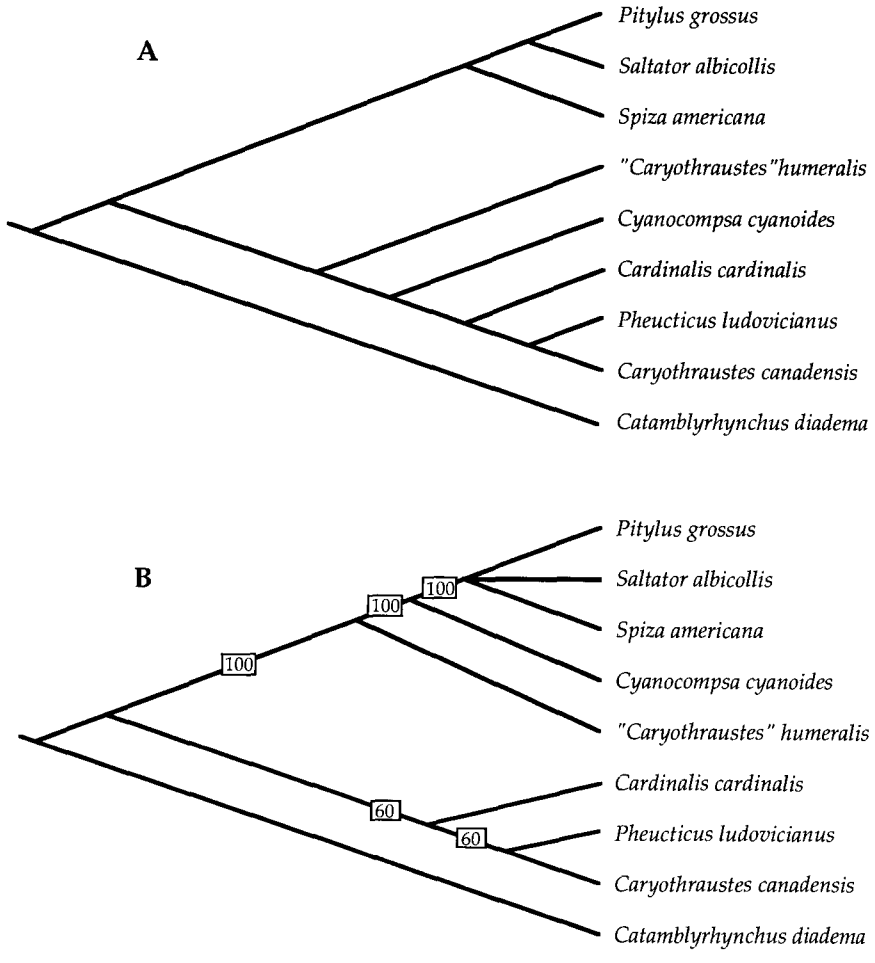

FIG. 1. Neighbor-joining phenogram (A) and fifty-percent majority-rule consensus tree of the ten most parsimonious trees found by PAUP (B) for eight genera of the Cardinalinae and the outgroup. Boxed values on branches leading to clades on the parsimony tree indicate percentage of the ten trees in which the clades were found.

PARS was one step longer than the PAUP trees (67 steps) and showed similarities to both the PAUP trees and the neighbor-joining phenogram. Therefore, all phylogenetic analyses support the 10 minimum-length trees found by PAUP, summarized in Fig. 1B. Importantly, in no analysis (phenetic or maximum-parsimony) was *Caryothraustes* shown to be a monophyletic group.

Discussion.—Based on his examination of external morphology and plumage, Hellmayr (1938:50) long ago noted that *Caryothraustes humeralis* "probably deserves generic separation" from the other two *Caryothraustes* species. Tamplin et al. (1993) summarized an-

ecdotal natural history information that also suggested that *humeralis* was not a member of the genus *Caryothraustes*. The size-corrected morphometric analysis conducted by Tamplin et al. (1993) also failed to support a close relationship between *humeralis* and other *Caryothraustes* species, and, like Hellack and Schnell (1977), Tamplin et al. found that *humeralis* was more similar in morphology and allele frequencies to *Cyanocompsa* than to other cardinalines. Our phylogenetic analysis strongly suggests that *Caryothraustes* is not monophyletic, and that *humeralis* is more closely related to a group of cardinalines consisting of saltators, *Pitylus* grosbeaks, and buntings than to other grosbeaks (including *C. canadensis*) and cardinals.

The species *humeralis* has always been placed in either *Caryothraustes, Pitylus,* or *Saltator* (Hellmayr 1938). The type species for the genus *Caryothraustes* Linnaeus is *C. canadensis*. Our data indicate that *Spiza* and *Cyanocompsa* are more closely related to *Saltator* and *Pitylus* than is *humeralis*. Thus, to allocate *humeralis* to *Saltator* or *Pitylus* would create a paraphyletic genus. Therefore, we are in the process of naming a new genus for *humeralis* (Remsen and Demastes, unpubl. data).

Our analysis of genetic relationships within the Cardinalinae (Fig. 1) supports a previous analysis of allele frequency data (Tamplin et al. 1993) that indicates that the cardinalines may consist of two major clades: (1) the saltators (*Saltator* and *Pitylus*) and Dickcissel (*Spiza*), and (2) the grosbeaks (*Pheucticus*) and cardinals (*Cardinalis*). Placement of the buntings (*Passerina*), grosbeaks of the genera *Cyanocompsa* and *Guiraca,* and other taxa is problematic, and may require the application of higher-resolution techniques (e.g., analysis at the nucleic acid level) to resolve more clearly the phylogeny of the Cardinalinae.

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Genetic structure in a wintering population of American Coots.—American Coots (*Fulica americana*) wintering on the Savannah River Site (SRS), near Aiken, South Carolina, arrive in stages and exhibit temporally stable patterns of site fidelity. Site fidelity in color-marked coots was observed both throughout the winter and across years (Potter 1987) on various portions of Par Pond reservoir on the SRS. In addition, Brisbin et al. (1973) and Potter (1987) found that cesium-137 body burdens of coots differed significantly between