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EVOLUTIONARY GENETICS OF BIRDS II. CONSERVATIVE PROTEIN EVOLUTION IN NORTH AMERICAN SPARROWS AND RELATIVES

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Abstract

Avise, J. C., J. C. Patton, and C. F. Aquadro (Departments of Zoology and Genetics, University of Georgia, Athens, Georgia 30602) 1980. Evolutionary genetics of birds II. Conservative protein evolution in North American sparrows and relatives. Syst. Zool., 29:323–334.—Differentiation at 20–21 protein-coding genes was examined by conventional techniques of starch-gel electrophoresis among twelve species and seven genera of North American sparrows and relatives, Emberizidae, subfamily Emberizinae. One species representing Fringillidae was also included. Data were summarized in a distance matrix which was subsequently used to infer phylogenetic trees by a variety of methods. Results were generally consistent with current classification. Two salient results were unanticipated: 1) the relatively close genetic similarity of *Pipilo* to group I Emberizinae; 2) the relatively large genetic distance of *Calcarius* from other Emberizinae. A search of the literature revealed that the distribution of a behavioral characteristic, "bilateral scratching," had led to a prediction of phylogenetic relationships for these genera fully consistent with the protein information. This result is significant because it lends support to proposals that some behavioral traits are extremely valuable phylogenetic markers. Levels of protein divergence in birds are compared to previous estimates for other vertebrate taxa. At corresponding levels of the taxonomic hierarchy, birds consistently exhibit far smaller genetic distances than do many fishes and other vertebrates. [North American sparrows; Emberizidae; Fringillidae; protein evolution; electrophoresis; evolutionary relationships.]

An important question in evolutionary biology concerns whether taxonomic categories in different classes or phyla are equivalent (Van Valen, 1973). This question is difficult to answer because many criteria for attempting such comparisons may be employed, different criteria may yield different conclusions, and there may not be a unique way of weighting relevant criteria even if they could be recognized. The problem is important beyond the desirability of consistency in classification. Comparisons of rates and patterns of evolutionary divergence among very different kinds of organisms can provide insights into processes causally responsible for evolutionary change. In a significant example of this approach, Allan Wilson and colleagues argue that from a "frog's perspective," the morphological differences among certain primates are profound while the differences in allelic composition of structural genes are small (King and Wilson, 1975; Cherry, Case, and Wilson, 1978). This led to the suggestion that structural gene changes and morphological divergence proceed independently (a conclusion not readily apparent from comparisons among closely related species—Avise, 1974), and has stimulated hypotheses that regulatory gene changes are responsible for morphological evolution (Wilson, 1976; Wilson et al., 1977).

Many analogous and homologous genes and the proteins they encode are found in virtually all organisms. They provide a common yardstick for comparisons of relative levels of genetic divergence among taxa belonging to disparate groups. In the first paper of this series (Avise et al., 1980), we observed among congeneric species of thrushes, genetic distances typical of values reported between conspecific populations in other classes of vertebrates. Furthermore, intergeneric distances in thrushes were less than or

equal to intrageneric distances in most fishes and tetrapods. Similar observations have been reported for other bird taxa (Smith and Zimmerman, 1976; Barrowclough and Corbin, 1978). Before explanatory hypotheses relating conservative protein evolution to unique aspects of avian biology are erected and tested, it is imperative to establish whether the phenomenon is general to birds.

In this paper, we continue our biochemical survey of Aves by examining frequencies of electromorphs encoded by 20–22 loci in 12 species and 7 genera of Emberizidae, subfamily Emberizinae. Also included as an "outgroup" comparison is the purple finch (*Carpodacus purpureus*), a member of the closely allied family Fringillidae. Most previous multilocus genetic studies of North American sparrows and relatives have compared electromorph and vocal dialect divergence among conspecific populations (Baker, 1974, 1975; Baker and Fox, 1978; Handford and Nottebohm, 1976; Nottebohm and Selander, 1972). We are concerned with the mean levels of genetic divergence between emberizid taxa relative to previous estimates for birds and other vertebrates. The data are also of relevance to evolutionary relationships of species within Emberizidae.

MATERIALS AND METHODS

Taxa examined and laboratory techniques.—Species examined in this study are listed in Table 1. Most specimens were collected and frozen within a few hours after they had died by collision with a television tower adjacent to Tall Timbers Research Station near Tallahassee, Florida. Since the birds were in migration, we do not know their breeding locales. *Amphispiza bilineata, Calcarius ornatus,* and *Pipilo fuscus* were collected near Alpine, Texas and *Carpodacus purpureus* near Athens, Georgia, all in the winter of 1978.

A traditional classification of primarily seed-eating birds lumps cardinal-grosbeaks, buntings, and similar "sparrows" and "finches" into a very large family Fringillidae (A.O.U. checklist, 1957). More recently a so-called "Basel sequence" has been introduced (Mayr and Greenway, 1956; Paynter, 1970). This brings together in a family Emberizidae the presumably related groups of emberizine buntings and sparrows, cardinal-grosbeaks, and tanagers, and distinguishes these from the revised family Fringillidae, a current member of which is *Carpodacus* examined in this study. All other species examined here are currently placed in Emberizidae, subfamily Emberizinae (buntings and American sparrows).

Within Emberizinae, seven groups of genera are recognized. The first group includes *Calcarius, Zonotrichia, Junco, Ammodramus, Spizella,* and *Amphispiza,* and the sixth group includes *Pipilo.* In this paper, we will employ for purposes of discussion the classification of the "Basel sequence" as reflected in the recent reference list of Morony et al. (1975).

We used standard techniques of horizontal starch-gel electrophoresis to assay electromorph products of 20–22 genetic loci. These procedures are described in detail in Avise et al. (1980) and references therein. Electrophoretic conditions providing good clarity of protein banding in sparrows were very similar to those previously employed for thrushes, with the following modifications: 1) creatine kinase (muscle form, *CK*-3), α-glycerophosphate dehydrogenase (*GPD-2*), and tetrazolium oxidase (*TO*) were scored off the Poulik buffer system; 2) adenylate kinase (*ADK*) was scored off tris-citrate I; 3) peptidase (*PEP*) was scored with leucylglycylglycine as substrate.

Electromorphs were assigned numerical values according to mobility relative to the common electromorph at each locus in *Ammodramus sandwichensis,* the species arbitrarily chosen as standard. Electromorph designations were determined by comigration in gels with previously assayed electromorphs of known

TABLE 1. SPECIES EXAMINED IN THIS STUDY AS CLASSIFIED BY MORONY ET AL. (1975). HETEROZYGOSITIES WERE DETERMINED BY DIRECT COUNTS OF PROPORTIONS OF INDIVIDUALS HETEROZYGOUS PER LOCUS, AVERAGED ACROSS 20–22 ASSAYED LOCI.

Species	English name	Sample size	H ± SE
Emberizidae			
Emberizinae			
1) *Zonotrichia melodia*	Song sparrow	7	0.019 ± 0.014
2) *Zonotrichia georgiana*	Swamp sparrow	10	0.051 ± 0.021
3) *Zonotrichia albicollis*	White-throated sparrow	10	0.079 ± 0.029
4) *Junco hyemalis*	Dark-eyed junco	10	0.058 ± 0.037
5) *Ammodramus sandwichensis*	Savannah sparrow	10	0.049 ± 0.022
6) *Ammodramus henslowii*	Henslows sparrow	3	0.076 ± 0.044
7) *Ammodramus savannarum*	Grasshopper sparrow	10	0.045 ± 0.024
8) *Spizella passerina*	Chipping sparrow	11	0.065 ± 0.030
9) *Spizella pusilla*	Field sparrow	6	0.083 ± 0.039
10) *Amphispiza bilineata*	Black-throated sparrow	7	0.072 ± 0.038
11) *Calcarius ornatus*	Chestnut-collared longspur	3	0.061 ± 0.036
12) *Pipilo fuscus*	Brown towhee	5	0.010 ± 0.010
Fringillidae			
Carduelinae			
13) *Carpodacus purpureus*	Purple finch	4	0.036 ± 0.019
	Totals	96	0.054

mobility in other sparrows, and do not directly correspond to designations earlier reported for thrushes.

Methods of phylogenetic tree formation.—We have argued elsewhere for use where possible of a qualitative, cladistic method of analysis which utilizes presence or absence of electromorphs as data base in formation of cladograms (Patton et al., 1980; Avise et al., 1980). This approach, which links species into clades when they share derived electromorphs (synapomorphs), and which utilizes "outgroup" taxa for determination of ancestral electromorphs (plesiomorphs), basically follows the conceptual outline of Hennig (1966). The resulting phylogenetic trees have defined character states along all branches and hence are very testable. In our experience, a serious drawback of this approach can be the empirically low proportion of electromorphs which contribute to clade identification. For reasons discussed later, this approach was not readily applicable to the current emberizid data. Consequently, we have formed various dendrograms and trees by conventional methods of analysis which manipulate values in similarity or distance matrices.

Genetic divergence between species was measured using the statistics, genetic similarity ($\bar{I}$) and genetic distance ($\bar{D}$) (Nei, 1972). The matrix of genetic distances was employed to generate evolutionary trees according to three procedures: 1) UPGMA (unweighted pair-group method of analysis with arithmetic means—Sneath and Sokal, 1973); 2) F-M (the method of Fitch and Margoliash, 1967); 3) Wagner (the method described and developed by Farris, 1972).

There is no general concensus on the "best" method of tree formation. UPGMA offers advantages of conceptual and methodological simplicity. It involves an iterative averaging procedure, assumes homogeneity of rate of evolution across lineages, and generates a single tree. F-M is a related procedure involving iterative averaging but in which the assumption of homogeneous evolutionary rates is relaxed. Negative branch lengths (which are difficult to interpret biologi-

TABLE 2. ELECTROMORPHS (AND THEIR FREQUENCIES) OBSERVED IN NORTH AMERICAN SPARROWS AND RELATIVES. SPECIES ARE NUMBERED AS IN TABLE 1. ALL SAMPLES APPEARED MONOMORPHIC FOR THE SAME ELECTROMORPH (OR EXHIBITED ONLY RARE VARIANTS) AT *LDH-2*, *MDH-2*, *GOT-2*, *ADK*, *PGI*, AND *IDH-2*.

Protein character	Species												
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
CK-1	100	100	100	100	100 (0.95) 90 (0.05)	100	100	100	100	100	100 (0.17) 80 (0.83)	100	95
CK-3	100	100	100	100	100	100	200	100	100	100	98	100	100
GOT-1	100 (0.93) 10 (0.07)	100 (0.95) 10 (0.05)	100 (0.95) 115 (0.05)	100	100	100	100	100	100	100	100	100	20
GPD-1	100	100 (0.85) 80 (0.15)	100	125	100 (0.88) 110 (0.12)	—	—	150	150	140	50	130	90 (0.12) 75 (0.88)
GPD-2	100	100 (0.89) 150 (0.11)	100 (0.95) 150 (0.05)	100 (0.95) 150 (0.05)	100 (0.94) 150 (0.06)	100	100 (0.44) 150 (0.06) 95 (0.50)	100	100	100	70	100	100 (0.88) 145 (0.12)
Hb	−100	−100	−100	−100	−100	−100	−100	−100	−100	−100	−80	−100	−100
IDH-1	100	100	100 (0.95) 300 (0.05)	100	100	100	100	100 (0.95) 200 (0.05)	100 (0.92) 125 (0.08)	100	100 (0.67) 200 (0.33)	100	205
LDH-1	150	150	100	105	100	150	200	350	350	340	300	150(?)	300
MDH-1	100	100	100	100	100	100	90	100	100	100	100	100	100
PEP	100	100	100 (0.88) 110 (0.12)	100 (0.50) 110 (0.06) 90 (0.39) 80 (0.05)	100 (0.80) 110 (0.05) 90 (0.15)	100 (0.34) 110 (0.66)	100 (0.90) 90 (0.05) 120 (0.05)	100 (0.80) 110 (0.20)	100 (0.42) 105 (0.58)	90 (0.66) 80 (0.34)	88	100	110
PGD	100	100 (0.95) 90 (0.05)	100 (0.79) 85 (0.11) 130 (0.05) 75 (0.05)	100 (0.85) 80 (0.15)	100	100 (0.66) 115 (0.17) 105 (0.17)	100 (0.90) 85 (0.05) 78 (0.05)	100 (0.88) 85 (0.12)	100 (0.50) 80 (0.50)	100	80 (0.50) 120 (0.50)	100	140 (0.88) 120 (0.12)
PGM	100	100	100 (0.80) 80 (0.20)	100	100 (0.95) 50 (0.05)	100	100 (0.95) 125 (0.05)	100	100	100 (0.93) 130 (0.07)	100	100 (0.90) 50 (0.10)	105
PT-1	—	100	100	100	—	100	100	100	100	100	100	95	100
PT-2	100	100	100 (0.93) 105 (0.07)	100	100	—	100	100	100	100	120	100	100
TO	100	100	100	100	100	100	100	75	75	75	100	100	2 band

cally) are possible. Furthermore, for any given data set a large number of alternative F-M trees can be generated and evaluated. The Farris algorithms for the Wagner approach provide approximations to most-parsimonious trees, and hence assume that observed distances among species are minimum estimates of true distances. Wagner trees do not assume homogeneous evolutionary rates. The Wagner approach usually results in formation of a single tree, and when based on certain distance metrics (such as Nei's $\bar{D}$), may occasionally exhibit negative branch lengths.

We have employed a goodness-of-fit criterion proposed by Prager and Wilson (1976; see also Farris, 1972) for evaluating the precision with which a phylogenetic tree reflects the distance matrix from which it was formed. Their statistic is

$$F = 100 \sum_{i=1}^{n} |I_i - O_i| / \sum_{i=1}^{n} I_i,$$

where for n pairwise comparisons of species, I and O are the input values of the original matrix and the output values of the tree, respectively. Smaller values of F indicate better fit. Apparently, for many but not all data sets, F-M trees yield the lowest F values, UPGMA intermediate, and Wagner trees the highest values of F (Prager and Wilson, 1978).

RESULTS

Electromorph frequencies are presented in Table 2. Not included in the table is information for a heart-predominant malic enzyme (*ME-1*) which could not be reliably compared across species, but which nonetheless was included in heterozygosity estimates because homozygotes and heterozygotes within species could be distinguished. Estimates of genic variation within samples from each species were determined by direct counts of mean proportions of individuals heterozygous per locus. The resulting heterozygosity (H) values per species range from 0.010 ± 0.010 in *Pipilo fuscus* to 0.083 ± 0.039 in *Spizella pusilla* (Table 1). The overall mean heterozygosity in assayed species of Emberizidae and Fringillidae is $\bar{H} = 0.054$. This estimate is nearly identical to mean values reported in other birds ($\bar{H} = 0.052$ in Muscicapidae and Mimidae, Avise et al., 1980; $\bar{H} = 0.046$ in Parulidae, Barrowclough and Corbin, 1978), and in many other groups of vertebrate species (Nevo, 1978). Passeriform birds certainly do not appear depauperate in within-species genic variability.

Genetic similarities and distances calculated from the data in Table 2 are presented in Table 3. Nei's $\bar{D}$ values ($\bar{D} = -\ln \bar{I}$) may be interpreted as estimated mean numbers of electrophoretically detectable codon substitutions per locus accumulated since the evolutionary separation of populations or species. Observed $\bar{D}$ values range from 0.024 in the comparison of *Spizella passerina* and *Spizella pusilla* to 0.795 in the comparison of *Carpodacus purpureus* and *Calcarius ornatus*. Members of each of three pairs of species, *Spizella passerina–S. pusilla*, *Zonotrichia melodia–Z. georgiana*, and *Ammodramus sandwichensis–Zonotrichia albicollis*, showed no significant electromorph frequency differences at 21 assayed loci. In each of 95 other pairwise comparisons of species, electromorph frequency distributions at one or more loci appeared totally distinct or nearly so, although with our sample sizes uncommon shared electromorphs could have gone undetected.

In this study, we were unable to satisfactorily apply the qualitative cladistic approach to tree formation for several reasons. First, we had originally chosen *Carpodacus purpureus* and *Pipilo fuscus* as outgroup taxa, based on the classification of Morony et al. (1975). This proved to be an inappropriate choice, since *Pipilo* now appears genetically very closely allied to most other Emberizinae examined (see below). Second, *Calcarius ornatus* was not originally cho-

TABLE 3. GENETIC DISTANCES (ABOVE DIAGONAL) AND SIMILARITIES (BELOW DIAGONAL) BETWEEN SPECIES OF SPARROWS AND RELATIVES, BASED UPON ELECTROMORPH FREQUENCIES AT 20–21 LOCI AND CALCULATED ACCORDING TO NEI'S (1972) FORMULAS. SPECIES ARE NUMBERED AS IN TABLE 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	—	0.028	0.086	0.149	0.057	0.114	0.253	0.198	0.235	0.243	0.617	0.078	0.621
2	0.973	—	0.057	0.110	0.082	0.074	0.203	0.157	0.190	0.201	0.534	0.097	0.549
3	0.918	0.944	—	0.119	0.032	0.130	0.220	0.167	0.197	0.209	0.535	0.165	0.522
4	0.862	0.896	0.888	—	0.136	0.129	0.223	0.171	0.187	0.176	0.514	0.172	0.530
5	0.945	0.921	0.969	0.873	—	0.162	0.247	0.192	0.225	0.223	0.591	0.129	0.596
6	0.892	0.929	0.878	0.879	0.850	—	0.259	0.187	0.209	0.211	0.467	0.137	0.508
7	0.776	0.816	0.803	0.800	0.781	0.772	—	0.282	0.319	0.327	0.585	0.275	0.739
8	0.821	0.854	0.846	0.843	0.825	0.830	0.754	—	0.024	0.141	0.622	0.221	0.526
9	0.791	0.827	0.821	0.830	0.798	0.812	0.727	0.976	—	0.152	0.589	0.258	0.530
10	0.784	0.818	0.811	0.838	0.800	0.810	0.721	0.868	0.859	—	0.631	0.267	0.546
11	0.540	0.586	0.586	0.598	0.554	0.627	0.557	0.537	0.555	0.532	—	0.636	0.795
12	0.925	0.907	0.848	0.842	0.879	0.872	0.759	0.801	0.773	0.766	0.529	—	0.638
13	0.537	0.578	0.593	0.589	0.551	0.602	0.477	0.591	0.589	0.579	0.451	0.528	—

sen as an outgroup taxon but proved to be biochemically very distinct from other Emberizinae. Third, no matter which taxa are finally chosen as outgroups, the particular allelic distributions observed (Table 2) permit designation of very few synapomorphic states, and hence provide poor delineation of clades.

In lieu of this qualitative approach, we employed several means of tree generation which begin with a distance matrix (Table 3) and hence reference the original character states only indirectly. Figure 1 shows a dendrogram for sparrows and relatives constructed by UPGMA. Values along the horizontal scale indicate mean genetic distances at which species members of various clusters are joined. One large cluster involving 11 species of Emberizinae appears very distinct ($\bar{D} \approx 0.58$) from *Carpodacus purpureus* and *Calcarius ornatus*, which are also very distinct from one another as already mentioned. Within the large cluster, *Ammodramus savannarum* appears phentically most divergent. This is largely due to its possession of autapomorphic (unique) electromorphs at *MDH-1* and *LDH-1* (Table 2). Other clusters of species appear as well, but all join at levels of $D \leq 0.22$.

Also shown in Figure 1 is a tree with identical branching structure constructed according to the F-M procedure. The calculated amounts of evolution in various lineages are shown by numerical values along branches of the tree. We constructed a total of eight alternative F-M trees. Although some of the alternatives exhibited fewer negative branch lengths, none provided a better fit to the distance matrix as evaluated by *F*. Figure 2 shows the Wagner tree constructed according to Farris' (1972) procedure. The branching structure appears considerably different from F-M and UPGMA trees, particularly for species which are phenetically similar. The more distant branches (to *Carpodacus* and *Calcarius*) remain distinct. This Wagner tree was rooted according to a procedure suggested by Farris (1972).

For our data, the F-M tree provides the best fit to the distance matrix, followed by UPGMA and the Wagner tree. Values of *F* are 9.2, 11.0, and 12.6, respectively. These estimates of fit and their order are very similar to those reported by Prager and Wilson (1978) for other data sets of comparable size.

Of the 13 species examined, 11 appear very similar phenetically to one another. With about 20 assayed loci, each locus for which a pair of species exhibits a fixed allelic difference would contribute to an increase in $\bar{D}$ of roughly 0.05, and large differences in allele frequencies at a given polymorphic locus would contribute

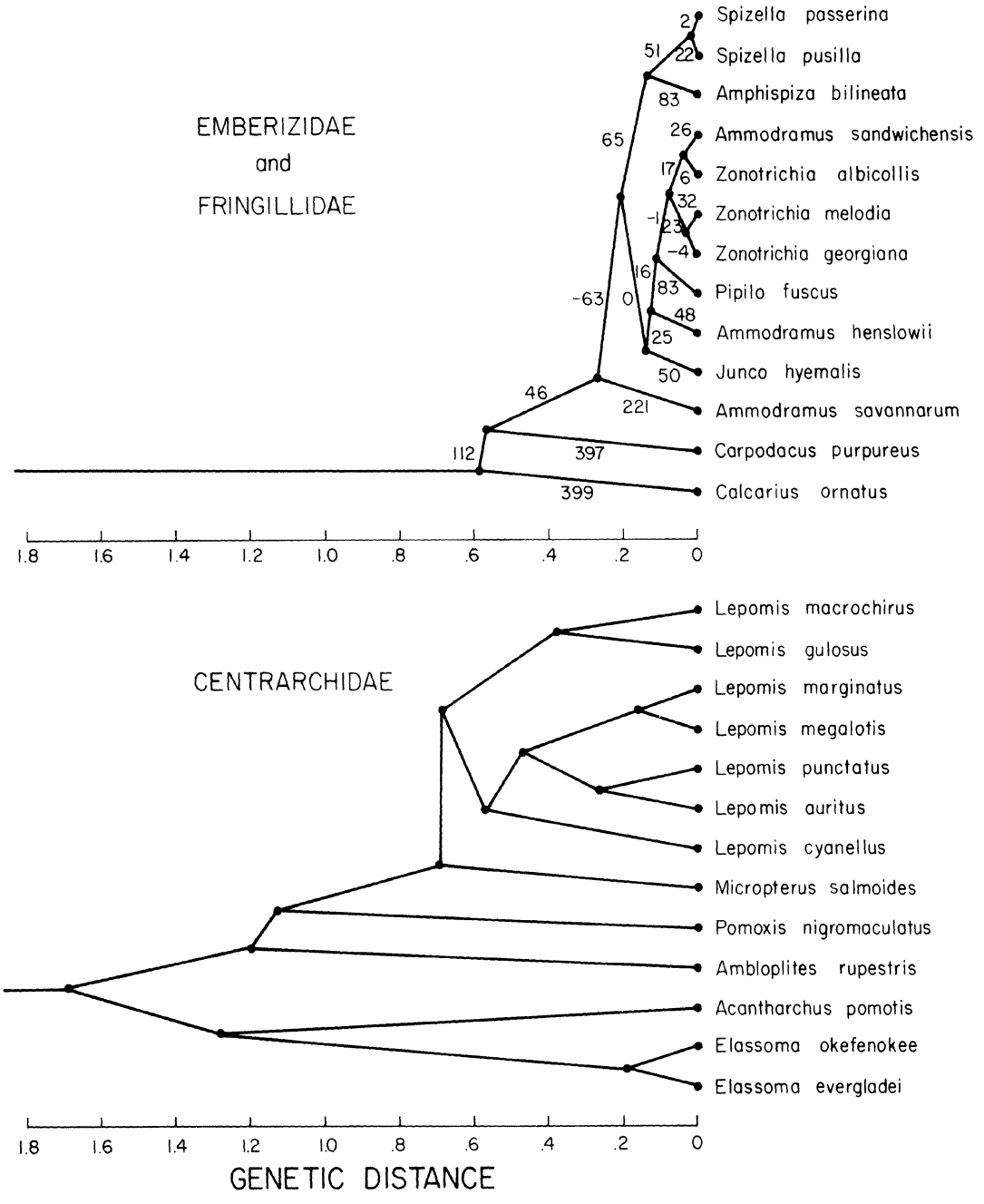

FIG. 1.—*Above:* Dendrogram of North American sparrows and allies derived from UPGMA of distance coefficients based on electromorph frequencies at 20–21 loci. Numbers refer to branch lengths (Nei's $\bar{D}$ ×1000) in a tree with identical branching structure constructed using the Fitch-Margoliash procedure. *Below:* Dendrogram of representative North American sunfishes, Centrarchidae, derived from UPGMA of distance coefficients based on electromorph frequencies at 11–14 loci.

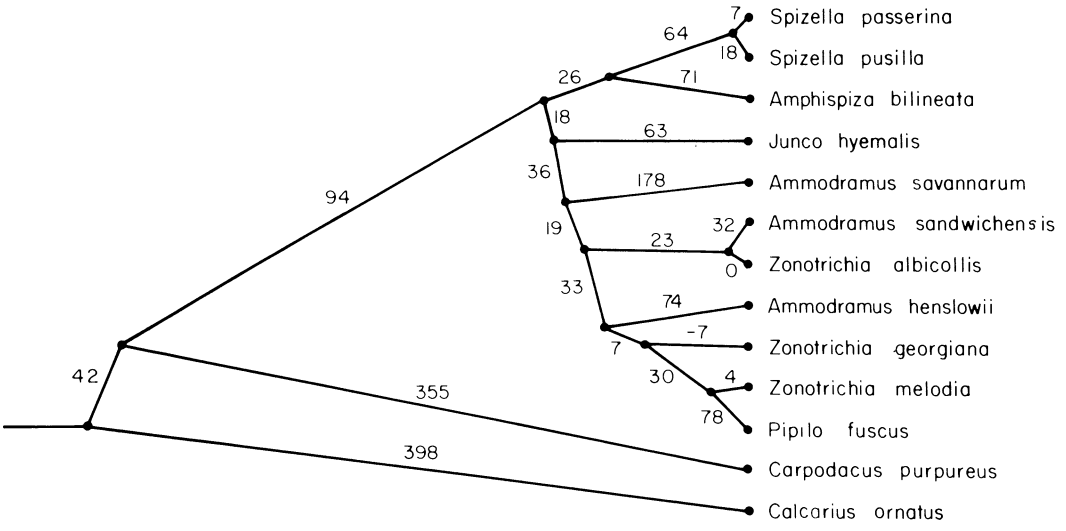

FIG. 2.—Wagner phylogenetic tree of North American sparrows and relatives constructed by Farris' (1972) algorithms. Numbers are branch lengths in units of Nei's $\bar{D}$ (×1000).

almost as much to $\bar{D}$. Thus, levels of divergence among these 11 species are due to the equivalent of major electromorph frequency shifts at only 0–4 of 21 assayed loci. Considering the rather small sample sizes of several species available to us, and the modest number of loci examined, it would be unwise to attach too much significance to the *relative* branching order among these 11 species, although we can be quite certain of their very close evolutionary relationships. Nei (1978) suggests that sampling biases (even from a sample size of one individual where $\bar{H} = 0.05$) are not too important when $\bar{D}$ is large, greater than about 0.15, but can become serious when $\bar{D}$ is smaller than this. Our reservations concerning the significance of the relative branching orders of these 11 species are further supported by the observation that different methods of data analysis, beginning with a common data base, can yield rather different outcomes (compare Figs. 1 and 2).

By contrast, the observed distances of *Carpodacus* and *Calcarius* from the other species are due to major electromorph frequency differences at 9–10 of 21 loci. Their genetic distinctness is readily apparent in all methods of data summary.

DISCUSSION

Evolutionary relationships.—Species in group I of Emberizinae are Palearctic or Nearctic grassland forms, generally with plain or streaked brown plumage. American genera represented in this study are *Spizella, Amphispiza, Ammodramus, Zonotrichia,* and *Junco.* The genus *Calcarius* is Holarctic. *Pipilo,* placed in group VI of Emberizinae, is also limited to the New World. Conventional thought regards Emberizinae as having originated and diversified in the New World, while Old World genera (including buntings of the important genus *Emberiza*) represent descendents of an invasion by New World forms (Harrison, 1967; Mayr, 1946).

With respect to this subfamily, our data demonstrate very close genetic relationships among all strictly American genera in group I. Surprisingly, however, *Calcarius* appears genetically very distant from these genera, while *Pipilo,* which is North American but placed in group VI,

is genetically very similar to the North American sparrows. These latter results appear inconsistent with predictions of current classification.

Greenlaw (1977) and Harrison (1967) have recently utilized the distribution of a behavioral characteristic, "double-scratching," to infer possible evolutionary relationships in Emberizinae (see also Hailman, 1973). The double-scratch is a rapid, bilateral backward kick used to scrape the surface of the ground or leaf litter during feeding. According to Harrison (1967), the Emberizinae "morphologically ... show little differentiation and it is therefore of interest that they appear to be divisible into two groups by a behavioral character (bilateral scratching), the presence or absence of which does not appear to be determined by, or correlated with, their immediate needs or surroundings." This scratching motion appears confined to American species of Emberizinae, and has been observed in all strictly New World genera assayed in this study, including *Pipilo* (Greenlaw, 1977). It appears to be absent in Old World genera such as *Emberiza*. It is also absent in *Calcarius*.

On the basis of this information, Harrison (1967) suggested that modern longspurs (*Calcarius*) are descendants of ancestral bunting stock which had lost the double-scratch behavior. This stock is represented by *Emberiza* in the Old World. Greenlaw (1977) gave a different interpretation: "I find no *a priori* reason to think that bilateral scratching is as old as the subfamily .... It is easier to believe that the habit arose after the earliest emberizine stock had already diversified." In Greenlaw's view, *Emberiza* represents an early divergence of emberizine stock prior to the acquisition of double-scratch behavior, and that *Calcarius* represents a secondary diversification after colonization of North America by Old World *Emberiza*-like forms.

According to either scenario, *Calcarius* should be more closely related (cladistically) to *Emberiza* than to North American sparrows. In Greenlaw's view, the towhee (*Pipilo*) should be cladistically allied with North American sparrows, distinct from *Calcarius*. Harrison's scenario does not yield unequivocal predictions about the evolutionary relationships of *Pipilo* to group I Emberizinae versus *Calcarius*. Predictions would depend upon time and pattern of splitting of *Pipilo* from ancestral emberizine forms.

Although we have not examined *Emberiza*, our data are wholly consistent with the remainder of Greenlaw's predictions. If genetic divergence as assayed by electromorph frequencies is even crudely proportional to time, the evolutionary line leading to modern *Calcarius* must have separated from the lineage leading to group I Emberizinae earlier than did the line leading to *Pipilo*.

Many authors have suggested that relative to other vertebrates, Aves as a whole and many groups of Passeriformes in particular, are comparatively uniform in anatomy and physiology (Bock, 1969; Prager and Wilson, 1975; Romer, 1966). This structural conservatism has resulted in considerable taxonomic controversy and confusion, and has stimulated searches for other kinds of evolutionary information. Perhaps more prevalent in the avian literature than elsewhere are attempts to utilize various behavioral characteristics as phylogenetic markers. It is important to verify the possible phylogenetic significance of these behavioral markers by independent criteria whenever possible. Results of the present study, in conjunction with Greenlaw's (1977) and Harrison's (1967) inferences based on behavior, demonstrate the utility of this comparative approach, and suggest that some behaviors may be extremely useful in phylogeny.

Based on our limited sample of 21 loci, *Carpodacus* does not appear significantly more (or less) divergent from North American sparrows than does *Calcarius*. Because evolutionary relationships of

TABLE 4. MEAN GENETIC DISTANCES (NEI'S COEFFICIENT) BETWEEN AVIAN POPULATIONS AT VARIOUS LEVELS OF TAXONOMIC DIVERGENCE. ALSO INCLUDED ARE DISTANCES BETWEEN CENTRARCHIDAE (SUNFISH) TAXA; THESE LATTER VALUES ARE FAIRLY CHARACTERISTIC FOR MANY OTHER VERTEBRATE AND INVERTEBRATE GROUPS.

Group	Local populations	Conspecific species	Genera	Families	Reference
Aplonis (starlings)	0.005	0.035	—	—	Corbin et al., 1974
Zonotrichia (sparrows)	0.006	—	—	—	Baker, 1975; Handford and Nottebohm, 1976
Icteridae (blackbirds)	—	0.011	0.248	—	Smith and Zimmerman, 1976
Parulidae (warblers)	—	0.100	0.179	—	Barrowclough and Corbin, 1978
Muscicapidae (thrushes)* and Mimidae (mimic thrushes)	—	0.024	0.344	0.780	Avise et al., 1980
Emerizidae and Fringillidae	—	0.123	0.253	0.592	present study
Centrarchidae (sunfish)	0.024	0.626	1.340	—	Avise and Smith, 1977

* Does not include *Regulus* whose systematic status is in doubt.

genera within and among these and other seed-eating families of birds are poorly understood, future studies involving additional comparisons are needed.

Protein divergence.—Outcomes of a large number of multi-locus comparisons of nonavian vertebrates and invertebrates are now available. Many of these studies have examined sets of loci analogous to those employed in this study. Not surprisingly, there is considerable variance in level of genetic divergence observed, depending in part on the species and groups assayed. Nonetheless, some useful generalizations have emerged (Avise, 1974, 1976; Ayala, 1975). Since we cannot review here all these studies, we have chosen an example (Centrarchidae, North American sunfish) which exhibits patterns of genetic differentiation typical of many nonavian families (Avise and Smith, 1977). What are the mean levels of divergence in protein-coding loci among avian taxa relative to nonavian taxa?

Results are summarized in Table 4. Avian populations show considerably less genetic divergence at all assayed taxonomic levels than do sunfish. The difference is especially clear when dendrograms for representatives of the two groups are compared against a common scale (Fig. 1). The most genetically similar species of the sunfish genus *Lepomis* appear even more distinct than do several genera of Emberizidae. The comparison of *Carpodacus* with North American sparrows yields slightly lower genetic distances than do some comparisons among members of *Lepomis*. Divergent genera of sunfish exhibit genetic distances more than 2.5 times greater than the largest distances observed among any species of birds in this study. These results are probably not an artifact of sampling error in structural loci examined. Even if we make the extreme assumption that all sparrows and relatives examined in this study would be completely distinct in allelic composition at each of the next two loci examined, mean genetic distances would only increase to the following values: between congeners, $\bar{D} = 0.222$; between genera, $\bar{D} = 0.350$; between families, $\bar{D} = 0.687$. Overall conclusions would remain unaltered.

Reasons for the apparent conservative nature of protein evolution in many groups of Passeriforme birds are unclear. An important question is whether the relative conservatism is evident at the DNA level as well. If the pattern is characteristic of most of the bird genome, two possibilities exist: 1) genetic divergence is decelerated in birds, due to a lowered rate of occurrence and/or fixation of mu-

tations; 2) avian taxa are younger than corresponding nonavian taxa. Limited data do exist on thermal stabilities of hybridized single-copy DNA's of *Junco* and several other emberizid genera (Shields and Straus, 1975). Mean differences in thermal stability (ΔTS, a measure which approximates the percent nucleotide divergence) in DNA's extracted from different genera was 3.8 degrees C. This value is roughly similar to that observed between two sibling species of *Drosophila* (Laird and McCarthy, 1968), or between satellite DNA's of two species of the rodent *Mus* (Rice and Strauss, 1973). It is considerably less than the value (ΔTS = 15°C) observed between DNA's of *Mus* and *Rattus* (Shields and Straus, 1975).

Nonetheless, in view of the limited information currently available, it is conceivable that the conservative avian evolution extends only to proteins, or to certain subclasses of proteins. Possible causal factors responsible for the slowdown in protein evolution would include any aspects of biology unique to or prevalent in birds, e.g., high body temperature or ability to withstand high blood pH. We will address these and other possibilities in greater detail elsewhere.

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