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PATTERNS OF ALLOZYME, MITOCHONDRIAL DNA, AND MORPHOMETRIC VARIATION IN FOUR SPARROW GENERA

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ABSTRACT.—We sequenced 432 base pairs (bp) of the mitochondrial DNA (mtDNA) cytochrome-*b* gene in all recognized biological species in the genera *Zonotrichia, Passerella,* and *Melospiza,* as well as *Junco hyemalis* and *Pipilo chlorurus.* Our goals were: to estimate the phylogenetic pattern within and among genera; to compare our estimate with previous estimates based on allozymes and mtDNA restriction sites; to map morphometric distances onto the phylogenetic hypothesis; and to determine the extent of geographic variation in two polytypic species, the Fox Sparrow (*Passerella iliaca*) and Song Sparrow (*Melospiza melodia*). There was no geographic pattern to cytochrome-*b* variation within the Song Sparrow, whereas four mtDNA lineages of Fox Sparrows were found; these results corroborate those obtained from mtDNA restriction-site data. Analysis of cytochrome *b* yielded 14 equally-parsimonious trees. Although mtDNA and allozyme trees were statistically congruent, they differed somewhat, and the data were combined to estimate phylogeny; two equally-parsimonious trees resulted. The consensus tree indicated the following relationships: within *Melospiza,* the pattern is {Song Sparrow {Swamp Sparrow [*M. georgiana*], Lincoln's Sparrow [*M. lincolnii*]}}; *Junco* and *Zonotrichia* are sister genera; within *Zonotrichia,* the pattern is {Rufous-collared Sparrow [*Z. capensis*] {White-throated Sparrow [*Z. albicollis*] {Harris' Sparrow [*Z. querula*] {White-crowned Sparrow [*Z. leucophrys*], Golden-crowned Sparrow [*Z. atricapilla*]}}}}; the data could not reliably resolve relationships among the other genera. In general, restriction sites and cytochrome-*b* sequence data yielded congruent phylogenies. Morphometric distances mapped onto the phylogenetic hypothesis revealed instances in which molecular and phenotypic evolution proceeded at different rates, except within *Melospiza,* where the two data sets yielded congruent patterns. Song dialects apparently evolved twice within *Zonotrichia.* *Received 31 August 1994, accepted 6 February 1995.*

KNOWLEDGE OF the evolutionary history of a group of taxa usually increases with the accumulation of diverse character sets. Some types of characters provide evidence for estimating phylogeny, whereas others reveal phenotypic responses to differing environments. Cladistic analysis of molecular characters is commonly used for estimating genealogical relationships (Hillis et al. 1990). Furthermore, comparison of patterns of variation in different molecular character sets can provide a check on the efficacy of each for phylogeny reconstruction. Phenetic analyses of morphometric characters might track phenotypic evolution and not necessarily phylogeny (Zink and Avise 1990). Examination of patterns of morphometric variation in a phylogenetic context can reveal different rates of phenetic change. We undertook an investigation character evolution in a group of emberizid sparrows for which morphometric, allozyme, and mitochondrial-DNA (mtDNA) restriction-site and sequence characters were available.

We sequenced a part of the cytochrome-*b* gene in all species of sparrows in the genera *Zonotrichia, Passerella,* and *Melospiza,* as well as *Junco hyemalis.* Previous allozyme (Zink 1982; Fig. 1) and mtDNA restriction-site analyses of some or all species (Zink et al. 1991; Fig. 2) provide phylogenetic hypotheses against which to test phylogenetic patterns derived from cytochrome-*b* sequences. In addition, these data can be combined to produce a phylogenetic tree based on "total evidence" (Eernisse and Kluge 1993). Questions addressed in our study are: (1) Is intraspecific sequence divergence in two species consistent with mtDNA restriction-site data (Zink and Dittmann 1993, Zink 1994)? (2) What is the interspecific phylogenetic pattern in cytochrome *b*? (3) Is this pattern congruent with that obtained from restriction sites and allozymes? (4) What phylogenetic hypothesis is suggested by combining data sets? (5) Do existing classifications reflect phylogenetic relationships? (6) Does the phylogenetic hypothesis provide insight into whether song dialects have evolved multiple times? (7) Is phenetic varia-

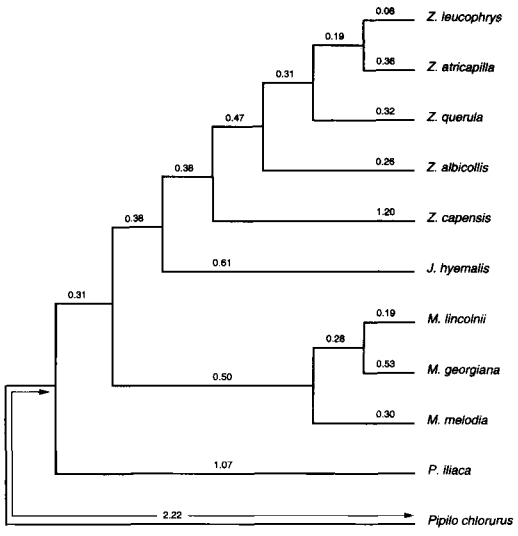

Fig. 1. Pattern of allozymic variation generated by UPGMA phenogram (from Zink 1982). Numbers on tree are branch lengths in units of Rogers' (1972) genetic distance.

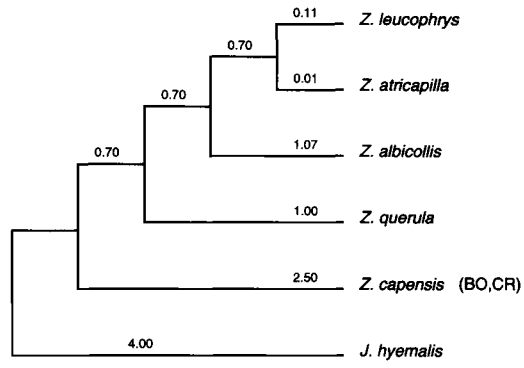

Fig. 2. Phylogenetic pattern of mtDNA restriction-site variation generated by phenetic and phylogenetic analysis (from Zink et al. 1991). Numbers on branches are in units of mtDNA genetic distance estimated from restriction-site variation (Nei and Li 1979).

tion in skeletal measurements (Zink 1982) consistent with the phylogenetic hypothesis, or is morphometric evolution decoupled from phylogeny?

METHODS

The following samples were used for sequencing: Green-tailed Towhee (*Pipilo chlorurus*, $n = 2$); Fox Sparrow (*Passerella iliaca*, 19); Dark-eyed Junco (*Junco hyemalis*, 2); Rufous-collared Sparrow (*Zonotrichia capensis*, 3); Harris' Sparrow (*Z. querula*, 3); White-throated Sparrow (*Z. albicollis*, 2); White-crowned Sparrow (*Z. leucophrys*, 2); Golden-crowned Sparrow (*Z. atricapilla* 2); Song Sparrow (*Melospiza melodia*, 11); Swamp Sparrow (*M. georgiana*, 2); and Lincoln's Sparrow (*M. lincolnii*, 2). *Pipilo chlorurus* was used as an outgroup. Specimens of Fox Sparrows and Song Sparrows represented much of the geographic range of each species, and also were used in restriction-site studies by Zink (1994) and by Zink and Dittmann (1993), respectively. Collecting localities, collection dates, and specimen voucher numbers are associated with the Genebank sequence accession numbers (U40162-U40186).

We purified mtDNA from tissues following procedures in Lansman et al. (1981) and Dowling et al. (1990), as previously reported (Zink and Dittmann 1991). Lincoln's Sparrow mtDNA was extracted from tissue following Carr and Griffith (1987). Using the polymerase chain reaction (Hillis et al. 1990), symmetric amplifications were performed in 30 μl reactions containing 8.33 mM Tris/HCl (pH 8.3), 41.67 mM KCl, primers L14841 and H15299 (made by Operon; numbers refer to primers in Kocher et al. [1989] and Hackett [1992], respectively) at 0.5 μM, each dNTP at 0.2 mM, ca. 2–1,000 ng template DNA, and 0.625 units Thermus Aquaticus polymerase (Perkin Elmer/Cetus, Boehringer Mannheim). A primary cycle of 3 min denaturation at 94°C, 1 min annealing at 50°C, and 1 min extension at 72°C was followed by 33 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C. A final extension at 72°C was carried out for 10 min. An amplification product of 484 base pairs (bp) was produced in all specimens. Electrophoresis of 4 μl of amplification mixture in a 1% agarose gel (Seakem LE, FMC) verified the presence of desired amplified product. The PCR product was filtered (Millipore cat. no. UFC3 TTK OO) to remove excess dNTPs and primers, with volume held constant.

Using the same primers as those used for amplification, double-stranded cycle sequencing was carried out using a Silver Sequence kit (Promega). Light and heavy strands of all samples were sequenced. Sequencing gels were made of 6% acrylamide (GenePage by Amresco) and were run for either 5 h at 1,800 V, or 5.5 h at 60 w, maintaining a temperature of 46° to 49°C, sufficient to read into primer on most samples. Sequences were visualized via silver staining (Silver Sequence, Promega; Caetano-Anolles and Gresshoff 1993), and dried sequence gels were photocopied.

Sequences were aligned visually. Each distinguishable sequence was deemed a unique haplotype; only one representative of each haplotype was used for phylogenetic analysis. We used the computer program MEGA (Kumar et al. 1993) to compute descriptive statistics for nucleotide variation. We used a distance approach to computing trees (neighbor-joining [NJ] method in MEGA; Saitou and Nei 1987) and a maximum-parsimony approach as implemented in the

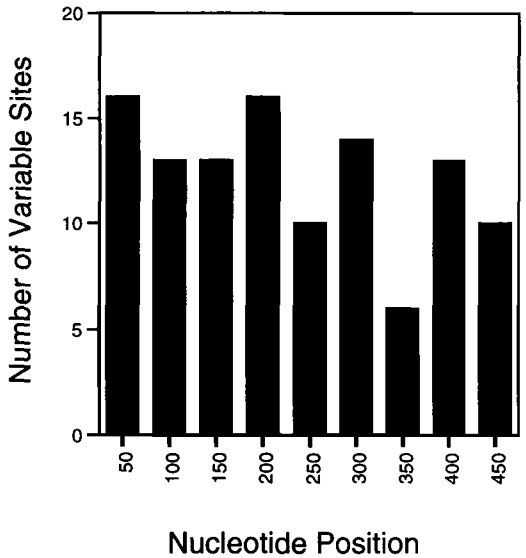

Fig. 3. Distribution of variation across the 432 bp of cytochrome *b* sequenced, beginning at nucleotide position 14965 of the *Gallus gallus* sequence (Genbank accession X52392) and moving from 5′ to 3′.

computer programs PAUP (Swofford 1990), Hennig86 (Farris 1988) and MEGA; variable nucleotide positions were analyzed as unordered. We used the computer program PHYLIP (Felsenstein 1994) to produce a phylogenetic estimate using the maximum-likelihood approach. We used the computer program Random Cladistics (Siddall 1994) to determine if there was phylogenetic signal in our sequence data (PTP test of Faith and Cranston, 1991; g_1 statistic of Hillis 1991). Random Cladistics and PAUP were used to bootstrap data sets (Felsenstein 1985) and establish the degree to which the data supported a particular branching pattern; 1,000 replications were performed, and a majority-rule consensus tree constructed. To compare phylogenetic hypotheses produced from different data sets, we used the computer program COMPONENT (Page 1993). To analyze morphometric distance matrices we used the computer program PHYLIP (Felsenstein 1994), and the routine FITCH.

Results

The distribution of variable nucleotide positions (Fig. 3) shows that variation was distributed throughout this segment of the cytochrome-*b* gene. Overall, the percentages of each nucleotide were: adenine (25.0%), thymine (24.7%), cytosine (33.4%), and guanine (16.9%). These ratios were similar at first- and second-codon positions, but differed considerably at third positions (Table 1), with guanine in extremely low frequency (2.8%; as found in other vertebrates [Kocher et al. 1989]). The average transition:transversion ratio between the outgroup and ingroup (n = 28) was 3.3 ± SD of 1.5. The matrix of pairwise transitions : transversions is available from the authors.

TABLE 1. Percent nucleotide composition for 20 specimens used in phylogenetic analysis.

	Nucleotide			
Position	A	T	C	G
First	24.1	25.0	22.3	28.6
Second	19.0	38.6	23.2	19.2
Third	31.5	11.3	54.4	2.8
Overall	24.9	25.0	33.3	16.9

We identified 27 haplotypes in the 50 specimens examined. Maximum-parsimony and NJ analyses (not shown) of all 27 haplotypes revealed that all conspecific haplotypes grouped together. Because of the difficulty of inferring phylogenetic trees for 27 taxa (Swofford 1991), the data set was analyzed as follows.

To examine the relationships of the six haplotypes within the Song Sparrow, we used its congeners (Swamp Sparrow and Lincoln's Sparrow) as outgroups. Nine positions were variable, and none was phylogenetically informative. Parsimony analysis produced 240 trees (length [*l*] = 18, consistency index [*ci*] = 0.92; retention index [*ri*] = 0.81), the consensus of which was unresolved (not shown).

Among the 11 Fox Sparrow haplotypes, the transition : transversion ratio averaged 6.5:1.0. There were 19 variable nucleotide positions, of which 11 were phylogenetically informative. The numbers of base substitutions at each codon position were: 5 (first), 1 (second), and 13 (third). To examine relationships of haplotypes within the Fox Sparrow, the Dark-eyed Junco and Green-tailed Towhee were chosen as outgroups, because in the phylogenetic analysis of all 27 haplotypes they were the nearest and farthest sister taxa from the Fox Sparrow, respectively. In the NJ tree (using *p*-values [Kumar et al. 1993:16] as the distance coefficient), four groups of samples were resolved, and the analysis suggested that the "iliaca" group was basal (Fig. 4). However, branch lengths among the four groups were not significant statistically (SE test of Rzhetsky and Nei 1992). Maximum-

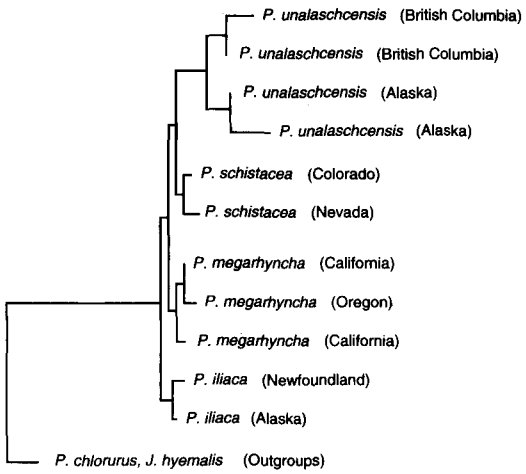

Fig. 4. Neighbor-joining tree depicting phylogenetic relationships among Fox Sparrows based on cytochrome-*b* variation.

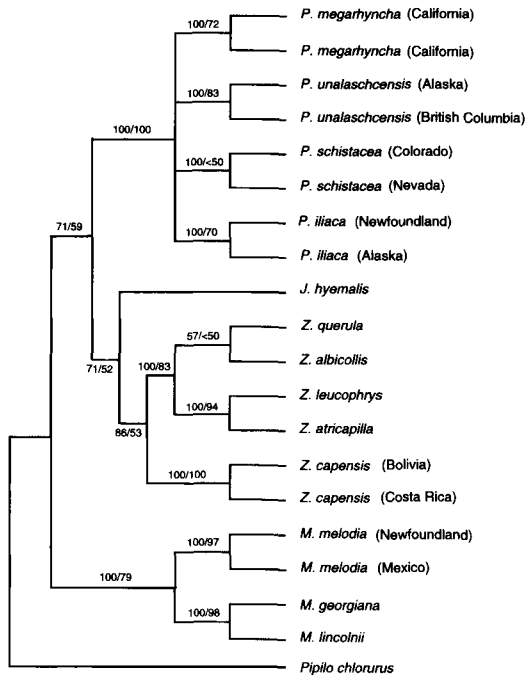

Fig. 5. Majority-rule consensus tree derived from 14 equally-parsimonious trees based on cytochrome-*b* data. First value on branch is percentage of trees out of 14 showing that node and second number is percentage of 1,000 bootstrap replicates showing the node ("< 50" indicates that node was not in bootstrap tree).

parsimony analysis produced two equally-parsimonious trees ($l = 85$, $ci = 0.91$, $ri = 0.87$), each of which resolved the four main groups of Fox Sparrows (Zink 1994). However, relationships among the groups were unresolved in the consensus tree (not shown).

To estimate phylogenetic relationships at the species level, each species was represented by one or two individuals (two if haplotypes differed). For the Fox Sparrow, we used two arbitrary representatives of each of the four major groups and, for the Song Sparrow, we arbitrarily selected an individual from Newfoundland, Canada and Michoacan, Mexico. Reducing the representation of the Fox Sparrow and Song Sparrow resulted in 20 taxa, which permitted more efficient phylogenetic searches, and reduced the effects of unequal sample sizes among species. For this data set, there were 105 variable sites of which 79 were phylogenetically informative. The distribution of variable nucleotide positions across codons was: 18 (first position), 7 (second), and 80 (third). The majority (80%) of nucleotide substitutions were transitions; the transition:transversion ratio was 3.9:1.0.

Randomization tests indicated significant structure in the data matrix (PTP test, $P < 0.001$; $g_1 = -0.51$, $P < 0.001$), which is presumed to indicate phylogenetic signal (Faith and Cranston 1991, Hillis 1991); however, these methods are controversial (Carpenter 1992). All parsimony programs produced identical branching patterns, although there were some differences in tree lengths depending on how missing data were handled. Maximum-parsimony analysis revealed 14 trees ($l = 190$, $ci = 0.63$, $ri = 0.81$), the 50% majority-rule consensus (Fig. 5) of which suggested that the Fox Sparrow was the sister taxon to *Junco* plus *Zonotrichia*, and *Melospiza* was basal to all taxa. Within *Melospiza*, which is monophyletic in our analyses, the Swamp Sparrow and Lincoln's Sparrow are sister taxa, with the Song Sparrow the most basal. The Dark-eyed Junco is the sister taxon to *Zonotrichia*. Within *Zonotrichia*, Rufous-collared Sparrow is basal, Golden-crowned Sparrow and White-crowned Sparrow are sister taxa, and White-throated Sparrow and Harris' Sparrow are intermediately placed (their exact relationships are unclear); the North Temperate species (i.e. excluding *capensis*) are likely a clade. Bootstrap values (Fig. 5) revealed that the groupings of *Z. capensis* to the other *Zonotrichia*, *Junco hyemalis* to *Zonotrichia*, and *Passerella iliaca* to *Junco* plus *Zonotrichia* occurred in less than 50% of the 1,000

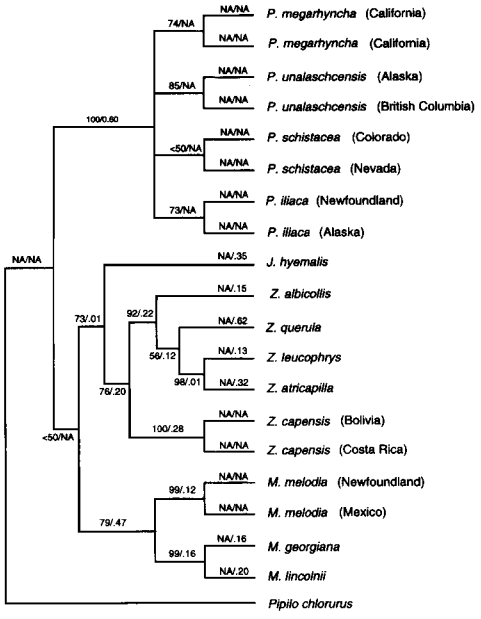

Fig. 6. Consensus of two trees derived from the combined allozyme and cytochrome-*b* sequence data. First number in each series is percentage of 1,000 bootstrap replicates showing that node appeared ("<50" indicates that node was not in bootstrap tree), and second value is morphometric distance (see text). NA = not applicable.

replicate trees. The remaining relationships were more strongly supported by the data. The same pattern of relationships was obtained from the NJ analysis (not shown), with the exception that White-throated Sparrow and Harris' Sparrow were sister taxa. The maximum-likelihood analysis recovered the tree topology in Figure 5.

Relationships inferred from allozyme data (Fig. 1) and cytochrome-*b* sequence data (Fig. 5) differed in the placement of the Fox Sparrow and the White-throated Sparrow. However, the two trees are significantly congruent based on the "quartet test" in COMPONENT. This test examines all possible subsets of four taxa (quartets) and finds the percentage that agree; for 11 taxa there are 330 possible quartets, in which 298 (90%) are identical in our two trees. The NNI comparison (nearest-neighbor interchange; see Page 1993) reveals how many taxa must be moved to transform one tree into another; for our data set, NNI was 2 ($P < 0.001$; Brown and Day 1984). Therefore, we conclude that allozyme and cytochrome-*b* data are significantly congruent, albeit not perfectly so.

To produce a total-evidence tree, we analyzed the allozyme data (Zink 1982) in concert with the sequence data. Allozyme loci were coded as characters and alleles as character states; the most frequent allele was used to represent the species (Buth 1984). Allozyme data did not resolve differences among the four Fox Sparrow groups (Zink unpubl. data), and the groups were treated as undifferentiated. The 19 informative allozyme characters were added to the cytochrome-*b* data set and analyzed as unordered. The combined allozyme and sequence data set yielded two equally-parsimonious trees (Fig. 6). As with the sequence data alone, the two trees differ in the relative relationships of the Fox Sparrow groups (as expected, because the same allozyme characters were added to each of the eight Fox Sparrows). The consensus tree (Fig. 6) depicts the four groups of Fox Sparrows as unresolved, and differs from the sequence data only in that the relationships of *Passerella* and *Melospiza* to the remaining taxa are reversed. However, this pattern is consistent with 4 of the 14 most-parsimonious trees from sequence data. Bootstrap support indicates considerable confidence in most nodes shown.

The matrix of taxonomic distances (Sneath and Sokal 1973) derived from 40 skeletal measurements (Zink 1982) was fitted to the tree depicted in Figure 6. Using a least-squares algorithm (option F, $p = 2$, in PHYLIP), it is apparent that the morphometric distances are partitioned along branches of the tree unevenly. The most extreme example is that for *Zonotrichia querula* and *Z. albicollis*, where the morphometric distances from the common ancestor were 0.74 and 0.15, respectively.

Discussion

Restriction sites vs. sequence data.—Although six of eight specimens exhibited different haplotypes, geographic structure was not evident in the cytochrome-*b* gene sequence of the Song Sparrow, consistent with a restriction-site survey (Zink and Dittmann 1993). Both restriction-site and sequence data reveal four groups of the Fox Sparrow, although only site data provided a robust phylogenetic perspective among groups (Zink 1994); more sequence data are needed for resolution of relationships among the Fox Sparrow groups. Generally, cytochrome *b* is thought not to be useful for intraspecific studies of birds (e.g. Wenink et al. 1994); hence, it would seem

that the Fox Sparrow is more differentiated than most avian species, supporting the suggestion that the four groups are phylogenetic species (Zink 1994). Nonetheless, it is premature to rule out cytochrome-*b* studies of intraspecific variation.

Within *Melospiza*, Kessler and Avise (1985) found that each species was very similar in mtDNA restriction sites, although their distance data weakly support (Zink unpubl. analysis) a grouping of {Swamp Sparrow {Song Sparrow, Lincoln's Sparrow}}, which disagrees with the present analysis. The raw restriction-site data were not published by Kessler and Avise (1985) and could not be added to the cytochrome-*b* data. For *Zonotrichia*, Zink et al. (1991) produced a phylogenetic tree from restriction sites that disagrees with that in Figure 6 because Harris' and White-throated sparrows were reversed; otherwise the two topologies agree. To investigate further sequence and restriction-site variation, we compared mtDNA distances computed from restriction sites (*p*; Nei and Li 1979) and cytochrome-*b* sequences (*p*) for the four groups of Fox Sparrow, *Melospiza* (including *Z. albicollis*), and *Zonotrichia* (all species, including two geographic representatives of *Z. capensis*), and *J. hyemalis*. The correlation coefficient of 0.90 ($n = 33$; $P < 0.001$) indicated significant agreement between restriction-site and cytochrome-*b* data (Fig. 7). Although Wilson et al. (1989) advocated abandoning restriction sites in favor of sequence data, this seems premature because a thorough restriction-site survey might better index genomewide divergence than a sequence data set from a single gene region. Several studies of birds, including this one, indicate agreement between restriction-site data and other evidence (Zink and Avise 1990, Dittmann and Zink 1991, Zink and Dittmann 1991, Zink et al. 1991, Dodge et al. 1995, A. J. Baker pers. comm.).

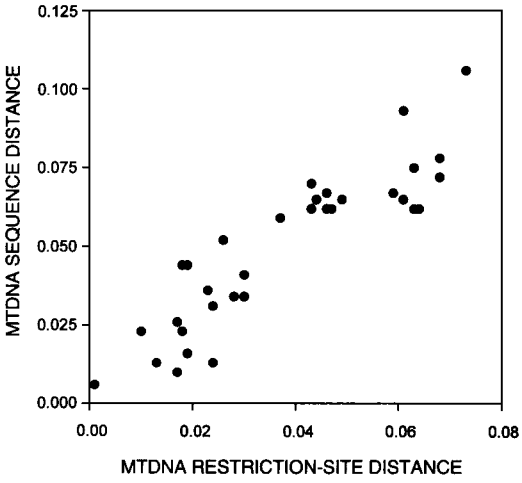

Fig. 7. Plot of molecular distances derived from restriction sites and cytochrome-*b* sequences.

Total evidence and phylogenetic conclusions.—The "congruence" school advocates comparing phylogenetic trees derived from different data sets as a measure of confidence (e.g. Swofford 1991). If topologies of two independent trees are statistically congruent, there is confidence that phylogenetic signal has been recovered. Conflicting phylogenetic signals require further study. One should determine if the disagreement is between nodes on each tree that are strongly supported by the data; that is, if the conflicting trees differ at nodes that are weakly supported in one or both trees, perhaps the "conflict" is illusory. In our study, sequence data from mtDNA and allelic variation at protein (nuclear) loci represent presumably genetically independent sources of information. Our comparisons of trees derived from the two sources of data indicate significant congruence. The reason for such congruence likely is that both data sets reflect phylogenetic history. Disagreement occurs primarily in the placement of the White-throated Sparrow and Fox Sparrow, neither of which was clearly resolved by the sequence data alone (Fig. 5); hence, the degree of congruence might be even greater.

When two phylogenetic trees disagree, as ours do somewhat, it would seem that analysis of all available evidence (Fig. 6) offers the best phylogenetic hypothesis (Eernisse and Kluge 1993). This might especially be true when one of the data sets reflects a single gene tree, as that for mtDNA does (Avise 1994). In our study, even after combining our allozyme and cytochrome-*b* data, there is uncertainty, as indicated by the occurrence of two equally-parsimonious trees. Our total-evidence tree, therefore, requires additional data to determine which regions of the topology might not be stable. We limit phylogenetic conclusions to those based on the nodes in Figure 6 that are most consistently supported by our data. The sister-species relationship of Swamp and Lincoln's sparrows, and the basal position of the Song Sparrow in *Melospiza* clarify evolutionary history in this genus. Mayr and

Short's (1970) conclusion that "The Fox Sparrow seems closely related to *P.* [*M.*] *melodia*" is refuted.

Patterns of evolutionary history within *Zonotrichia* have been controversial (Mayr and Short 1970). Consequently, we explored phylogenetic patterns further by adding the restriction-site data from Zink et al. (1991) to the allozyme plus cytochrome-*b* data (we found that there are very few restriction sites in our cytochrome-*b* data; therefore, the two data sets basically are independent mtDNA estimates). The single most-parsimonious tree ($l = 177$, $ci = 0.87$, $ri = 0.79$) differed from that in Figure 6 by the switching of the White-throated Sparrow and the Harris' Sparrow, resulting in the same topology as Figure 2; however, the arrangement shown in Figure 6 requires only 179 steps, revealing the need for additional data.

The view (Mayr and Short 1970) that the White-throated Sparrow is most closely related to the Golden-crowned Sparrow is not supported by allozyme data, mtDNA restriction-site data, cytochrome-*b* sequence data, or the combined data sets. Golden-crowned and White-crowned sparrows are sister taxa and, in fact, differ by only 1 of 432 (0.2%) nucleotide positions at cytochrome *b* (we comment further on this extreme similarity elsewhere). Cytochrome-*b* sequence data confirm the basal position of the Rufous-crowned Sparrow within *Zonotrichia*, although the bootstrap support is relatively low. We determined the length of the total-evidence tree—in which the sister haplotypes of the Rufous-crowned Sparrow were switched with Harris' Sparrow—to be 190 steps. The Rufous-crowned Sparrow therefore does not belong within the North Temperate *Zonotrichia*. Although the plumage of the Harris's Sparrow differs markedly from the general pattern of its congeners, all genetic data suggest that it is not atypical, although it is basal to the North Temperate species.

Junco and *Zonotrichia* consistently appear to be sister taxa as implied by Mayr and Short (1970). The close relationship of these latter genera was inferred from documented hybridization between White-throated Sparrow and Dark-eyed Junco (Jung et al. 1994). However, inspection of the phylogenetic hypothesis reveals that this is an instance of nonsister taxa hybridizing, and that there are many sympatric combinations of more closely related species that do not appear to hybridize. Such observations support the notion that the pattern of hybridization is a poor taxonomic indicator (Zink et al. 1991). Disagreement over the relationships of the other genera exists in our data, but molecular evidence does not indicate a close relationship between *Passerella* and *Melospiza* (Mayr and Short 1970).

Comparative biology.—Mapping behavioral and life-history traits on phylogenetic hypotheses has become an important endeavor (Brooks and McLennan 1991). The evolution of song dialects was addressed by Zink et al. (1991), who concluded that song dialects had evolved independently in *Z. leucophrys* and *Z. capensis*. According to our phylogenetic hypothesis (Figs. 2 and 6), this is most parsimonious (two independent gains). This suggests that "song dialects" per se are not homologous (McKitrick 1993), and, in fact, are attributes (Mickevich and Weller 1990). Careful study likely would reveal differences in the song dialects of the two species, meaning that they should not be considered as systematic characters and "mapped" onto phylogenetic trees in the standard parsimony fashion (Brooks and McLennan 1991). One could search for ecological or behavioral factors involved in the origin of "song dialects" in *Z. leucophrys* or *Z. capensis*, although different correlates might be predicted. However, if song dialects were homologous, one could only study their maintenance in the latter species, not their origin (Brooks and McLennan 1991), and one might postulate the evolution of song dialects at the base of the *Zonotrichia* clade, and three "loses". Hence, the origin of song dialects would have occurred early in *Zonotrichia* evolution, and not recently in *leucophrys*.

Morphometric evolution.—Inspection of morphometric distances (Fig. 6) reveals instances of apparent rate heterogeneity, suggesting that morphometric evolution has proceeded unevenly, and is decoupled from phylogeny. To explore this idea further, we examined the branching pattern derived solely from morphometric distances (Zink 1982: fig. 2). The pattern of skeletal variation in *Melospiza* is the same as the molecular pattern (Fig. 6), suggesting that skeletal evolution tracks phylogeny in this genus (we recognize that, by chance alone, there is a one-third probability of the two patterns matching). Morphometric patterns for the other taxa do not mirror molecular ones, the reason being that rates of differentiation in the two data sets differ. Within *Zonotrichia* it is clear that

skeletal evolution has proceeded more rapidly in *capensis, querula,* and *atricapilla.* Also, given the very recent separation of Golden-crowned and White-crowned sparrows, it appears that skeletal evolution proceeded rapidly in the Golden-crowned Sparrow, roughly twice the rate in its sister species. Notably, Harris' Sparrow is distinctive both in plumage and skeletal variation, owing apparently to an accelerated rate of evolution relative to molecular variation. Instances where phenotypic evolution seems to "outdistance" molecular evolution might be useful contexts to search for evolutionary processes that accelerate phenotypic evolution such as sexual selection (e.g. Rohwer 1985).

Systematic considerations.—Several authors (e.g. Donoghue et al. 1989, Weller et al. 1992) have commented on the "exemplar effect" in which an insufficient number of representatives per taxon can affect branching sequence. We discovered such an effect in our sequence data. For example, parsimony analysis using a single individual of each species (including the Fox Sparrow) produced a single most-parsimonious tree (not shown) with a topology different from those with two or more individuals per species. Although this tree is only one step shorter than one with the pattern in Figure 6, it reveals the dangers inherent in insufficient taxon representation, especially when some taxa are similar and polymorphism becomes interpreted as phylogenetic signal. Sequence data are not immune to general problems inherent in characters used in all phylogenetic analyses.

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LITERATURE CITED

AVISE, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

BROOKS, D. R., AND D. L. MCLENNAN. 1991. Phylogeny, ecology, and behavior. Univ. Chicago Press, Chicago.

BROWN, E. K., AND W. H. E. DAY. 1984. A computationally efficient approximation to the nearest neighbor interchange metric. J. Classif. 1:93–124.

BUTH, D. G. 1984. The application of electrophoretic data in systematic studies. Annu. Rev. Ecol. Syst. 15:501–522.

CAETANO-ANOLLES, G., AND P. M. GRESSHOFF. 1993. Staining with silver. Promega Notes 45:13–18.

CARPENTER, J. M. 1992. Random cladistics. Cladistics 8:147–153.

CARR, S. M., AND O. M. GRIFFITH. 1987. Rapid isolation of animal mitochondrial DNA in a small fixed-angle rotor at ultrahigh speed. Biochem. Genet. 25:385–390.

DITTMANN, D. L., AND R. M. ZINK. 1991. Mitochondrial DNA variation among phalaropes and allies. Auk 108:771–779.

DODGE, A. G., A. J. FRY, R. C. BLACKWELL, AND R. M. ZINK. 1995. Comparison of phylogenies derived from two molecualr data sets in the avian genera *Pipilo* and *Spizella.* Wilson Bull. 107:641–654.

DONOGHUE, M. J., J. A. DOYLE, J. GAUTHIER, A. G. KLUGE, AND T. ROWE. 1989. The importance of fossils in phylogeny reconstruction. Annu. Rev. Ecol. Syst. 20:431–460.

DOWLING, T. E., C. MORITZ, AND J. PALMER. 1990. Nucleic acids II. Restriction site analysis. Pages 250–319 *in* Molecular systematics (D. M. Hillis and C. Moritz, Eds). Sinauer Associates, Sunderland, Massachusetts.

EERNISSE, D. J., AND A. G. KLUGE. 1993. Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules, and morphology. Mol. Biol. Evol. 10:1170–1195.

FAITH, D. P., AND P. S. CRANSTON. 1991. Could a cladogram this short have arisen by chance alone?: On permutation tests for cladistic structure. Cladistics 7:1–28.

FARRIS, J. S. 1988. HENNIG86. Computer program published by author. Port Jefferson, New York.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

FELSENSTEIN, J. 1994. PHYLIP, version 3.55c. Computer program distributed by author. Univ. Washington, Seattle.

HACKETT, S. J. 1992. Phylogenies and biogeography of Central American birds. Ph.D. dissertation, Louisiana State Univ., Baton Rouge.

HILLIS, D. M. 1991. Discriminating between phylogenetic signal and random noise in DNA sequences. Pages 278–294 *in* Phylogenetic analysis of DNA sequences (M. M. Miyamoto and J. Cracraft, Eds.). Academic Press, New York.

HILLIS, D. M., A. LARSON, S. K. DAVIS, AND E. A. ZIMMER. 1990. Pages 318–370 *in* Molecular systematics (D. M. Hillis and C. Moritz, Eds.). Sinauer Associates, Sunderland, Massachusetts.

JUNG, R. E., E. S. MORTON, AND R. C. FLEISCHER. 1994. Behavior and parentage of a White-throated Sparrow × Dark-eyed Junco hybrid. Wilson Bull. 106:189–202.

KESSLER, L. G., AND J. C. AVISE. 1985. A comparative

description of mitochondrial DNA differentiation in selected avian and other vertebrate genera. Mol. Biol. Evol. 2:109–125.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PAABO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in mammals: Amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86:6196–6200.

KUMAR, S., K. TAMURA, AND M. NEI. 1993. MEGA: Molecular evolutionary genetics analysis, version 1.01. Pennsylvania State Univ., University Park.

LANSMAN, R. A., R. O. SHADE, J. F. SHAPIRA, AND J. C. AVISE. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations III. Techniques and potential applications. J. Mol. Evol. 17:214–226.

MAYR, E., AND L. L. SHORT, JR. 1970. Species taxa of North American birds. Publ. Nuttall Ornithol. Club 9.

MCKITRICK, M. C. 1993. Phylogenetic constraint in evolutionary theory: Has it any explanatory power? Annu. Rev. Ecol. Syst. 24:307–330.

MICKEVICH, M. F., AND S. J. WELLER. 1990. Evolutionary character analysis: Tracing character change on a cladogram. Cladistics 6:137–170.

PAGE, R. D. M. 1993. Component, version 2.0. Natural History Museum, London.

NEI, M., AND W. H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269–5273.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. Texas Studies Genet. 7:145–153.

ROHWER, S. 1985. Dyed birds achieve higher social status than controls in Harris' Sparrows. Anim. Behav. 33:1325–1331.

RZHETSKY, A., AND M. NEI. 1992. A simple method for estimating and testing minimum-evolution trees. Mol. Biol. Evol. 9:945–967.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

SIDDALL, M. E. 1994. Random cladistics, version 2.1. Univ. Toronto, Toronto, Ontario.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy. W. H. Freeman Co., San Francisco.

SWOFFORD, D. 1990. PAUP. Phylogenetic analysis using parsimony, version 3.0. Illinois Natural History Survey, Champaign.

SWOFFORD, D. L. 1991. When are phylogeny estimates from molecular and morphological data incongruent? Pages 295–333 *in* Phylogenetic analysis of DNA sequences (M. M. Miyamoto and J. Cracraft, Eds.). Academic Press, New York.

WELLER, S. J., T. P. FRIEDLANDER, J. A. MARTIN, AND D. P. PASHLEY. 1992. Phylogenetic studies of ribosomal RNA variation in higher moths and butterflies (Lepidoptera: Ditrysia). Mol. Phylogenet. Evol. 1:312–337.

WENINK, P. W., A. J. BAKER, AND M. G. TILANUS. 1994. Mitochondrial control-region sequences in two shorebird species, the Turnstone and the Dunlin, and their utility in population genetic studies. Mol. Biol. Evol. 11:22–31.

WILSON, A. C., E. A. ZIMMER, E. M. PRAGER, AND T. D. KOCHER. 1989. Restriction mapping in the molecular systematics of mammals: A retrospective salute. Pages 407–419 *in* The hierarchy of life (B. Fernholm, K. Bremer, and H. Jornvall, Eds.). Elsevier Science Publishers B.V., Amsterdam, The Netherlands.

ZINK, R. M. 1982. Patterns of genic and morphologic variation among sparrows in the genera *Zonotrichia, Melospiza, Junco,* and *Passerella.* Auk 99: 632–649.

ZINK, R. M. 1994. The geography of mitochondrial DNA variation, population structure, hybridization, and species limits in the Fox Sparrow (*Passerella iliaca*). Evolution 48:96–111.

ZINK, R. M., AND J. C. AVISE. 1990. Patterns of mitochondrial DNA and allozyme variation in the avian genus *Ammodramus.* Syst. Zool. 39:148–161.

ZINK, R. M., AND D. L. DITTMANN. 1991. Evolution of Brown Towhees: Mitochondrial DNA evidence. Condor 93:98–105.

ZINK, R. M., AND D. L. DITTMANN. 1993. Gene flow, refugia, and evolution of geographic variation in the Song Sparrow (*Melospiza melodia*). Evolution 47:717–729.

ZINK, R. M., D. L. DITTMANN, AND W. L. ROOTES. 1991. Mitochondrial DNA variation and the phylogeny of *Zonotrichia.* Auk 108:578–584.