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MITOCHONDRIAL DNA PHYLOGEOGRAPHIC DIFFERENTIATION AMONG AVIAN POPULATIONS AND THE EVOLUTIONARY SIGNIFICANCE OF SUBSPECIES

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ABSTRACT.—Phylogeographic population structures revealed by restriction analyses of mitochondrial (mt) DNA were assessed within each of six avian species with continentwide distributions in North America. The magnitude and geographic pattern of mtDNA variation differed considerably among species. The Downy Woodpecker (*Picoides pubescens*) and Mourning Dove (*Zenaida macroura*) exhibited little mtDNA polymorphism and a shallow phylogeographic structure. The Brown-headed Cowbird (*Molothrus ater*) and Song Sparrow (*Melospiza melodia*) showed somewhat higher nucleotide diversity, but no evidence of long-standing population separations. For each of these four species, evolutionary effective sizes of female populations estimated from mtDNA were substantially smaller than population sizes at the present time, suggesting historical demographic constraints on the numbers of females through which mtDNA lineages successfully have been transmitted. In contrast, the Rufous-sided Towhee (*Pipilo erythrophthalmus*) and Common Yellowthroat (*Geothlypis trichas*) showed relatively deep mtDNA separations (mean nucleotide sequence divergence $p = 0.008$ and $p = 0.012$, respectively) between populations in Washington versus those in the central and eastern states. In the case of the Rufous-sided Towhee, the mtDNA clades may correspond to morphological and behavioral differences distinguishing the western "Spotted Towhee," which was formerly recognized as a distinct species. Overall, however, most of the taxonomic subspecies currently recognized within the six assayed species were genetically very close, and showed no obvious mtDNA differences. These results raise questions concerning the population-genetic and evolutionary significance of current subspecies designations in ornithology. *Received 5 June 1991, accepted 23 February 1992.*

MANY AVIAN SPECIES are taxonomically subdivided into multiple subspecies. For example, among approximately 800 North American birds in the 1957 AOU *Check-list* (the latest AOU list to include subspecies names), an average of about 2.1 subspecies per species was recognized. Several researchers have argued that intraspecific categories are evolutionarily important and deserve taxonomic recognition (e.g. Johnson 1982, Parkes 1982), while many authors nonetheless question the validity of subspecies designations as they have been employed in the ornithological community (Mayr 1942, Wilson and Brown 1953, Cracraft 1983, McKitrick and Zink 1988, Zink 1989; also see forum on subspecies moderated by Wiens 1982). Reasons for skepticism about particular subspecies epithets are varied, but include failures of genetic data to corroborate distinctions among morpho-subspecies (Barrowclough 1980, Ball et al. 1988, Avise and Nelson 1989), and indications that rearing environment can directly influence the development of some morphological traits of traditional taxonomic importance (James 1983).

A general feature of avian classification at supraspecific levels is a pattern of molecular-genetic conservatism relative to many nonavian vertebrates of comparable taxonomic rank (Prager et al. 1974, Avise and Aquadro 1982). Such relative conservatism in magnitude of genetic divergence in birds also may extend to the intraspecific level. Thus, in a review of allozyme literature, Barrowclough (1983) reported a mean F_{st} (a measure of the among-population component of genetic variance) in birds of only 0.02, compared to average estimates ranging from 0.11 to 0.38 for conspecific populations within four nonavian vertebrate classes. One likely explanation is comparatively high contemporary gene flow among most avian populations (consistent with birds' high dispersal potential through flight), but another contributing factor may be a recent evolutionary age for most avian species. On the other hand, many bird species also show strong tendencies for

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nest site philopatry (Greenwood 1980, Greenwood and Harvey 1982), and commonly exhibit considerable geographic differentiation in various morphological or behavioral attributes that have prompted the subspecies descriptions. Thus, it remains possible that more sensitive molecular-genetic assays will reveal geographic differentiation within avian species that allozyme methods failed to detect. If so, the coincidence between the major intraspecific subdivisions identified by molecular characters and the subspecies identified by morphological or behavioral evidence becomes an issue. According to Avise and Ball (1990), concordance among genetically independent attributes should be a deciding criterion upon which to base formal taxonomic recognition of subspecies.

Because of its rapid evolution and uncomplicated (maternal) inheritance, mitochondrial (mt) DNA offers a sensitive molecular probe of intraspecific evolutionary processes (Wilson et al. 1985, Avise et al. 1987, Shields and Wilson 1987b, Avise 1989). In many instances, mtDNA analyses have revealed dramatic population-level differentiation undetected with other methods. This led us to expect that mtDNA techniques might also reveal extensive population structure in birds. However, in the first large-scale mtDNA study of an avian species, the Red-winged Blackbird (*Agelaius phoeniceus*), ample mtDNA genetic variation was found, but the diversity was not strongly partitioned among geographic or subspecific populations (Ball et al. 1988).

In the present study, we spot survey mtDNA differentiation in six common species of North American birds: Downy Woodpecker (*Picoides pubescens*, Picidae); Mourning Dove (*Zenaida macroura*, Columbidae); Brown-headed Cowbird (*Molothrus ater*, Emberizidae); Song Sparrow (*Melospiza melodia*, Emberizidae); Rufous-sided Towhee (*Pipilo erythrophthalmus*, Emberizidae); and Common Yellowthroat (*Geothlypis trichas*, Emberizidae). All of these species are distributed over a large portion of North America, and each has been subdivided into multiple subspecies (7, 2, 3, 31, 16, and 12, respectively; AOU 1957). Our samples were taken from two or more recognized subspecies within each species. Because of its relatively sedentary lifestyle (Staebler 1949), the Downy Woodpecker was targeted as a species likely to show geographic or subspecies differences, and was sampled most extensively. Nonetheless, the purpose of this study was not to provide a comprehensive examination of the mtDNA population genetics of any one species, but rather to assess whether current subspecies reflect genetic boundaries as might be revealed in conventional mtDNA assays. In general, what are the magnitudes of mtDNA divergence among avian populations, and are the genetic differences concordant with the traditionally-recognized subspecies boundaries?

Fig. 1. Downy Woodpecker collection locales (numbered as in Table 1).

Materials and Methods

During the breeding seasons of 1986–1988, heart, liver, and pectoral muscle tissues were collected from a total of 145 birds from the locations listed in Table 1 (see also Fig. 1). Samples were stored in MSB-EDTA buffer on ice until they could be transported to the laboratory, where mtDNA was extracted according to the procedures of Lansman et al. (1981). The mtDNA from each individual was digested with a battery of 16 or more restriction enzymes (Table 1), and radioactively end-labeled. After electrophoresis through 1.0 to 1.6% agarose gels, restriction-fragment profiles were scored from autoradiographs on *X*-ray film. The mtDNA fragment sizes were estimated by comparison with migration distances of size standards in a 1-kb ladder purchased from Bethesda Research Labs. Enzymes producing only zero or one cut in all mtDNAs of a species were disregarded in subsequent analyses.

Genetic distances between samples, expressed as percent substitutions per nucleotide site, were estimated from the fragment profiles using the method of Nei and Tajima (1983). Cluster analysis was then performed by UPGMA (Sneath and Sokal 1973). Bootstrap-parsimony analysis of restriction-fragment data was also performed, using version 3.0 of the computer program PAUP provided by David Swofford.

TABLE 1. Collection locales, sample sizes, and restriction enzymes employed in mtDNA assays of six avian species.

Species	No. individuals	Location[a] (and no. individuals per location)	Enzymes[b]
Downy Woodpecker	51	1(5), 2(3), 3(1), 4(4), 5(1), 6(3), 7(2), 8(2), 9(2), 11(2), 12(3), 13(5), 15(1), 17(5), 18(6), 19(4), 20(2)	a-k, n-t
Mourning Dove	17	4(7), 11(6), 18(4)	a-g, i-k, n-t
Brown-headed Cowbird	26	4(9), 11(4), 14(1), 16(8), 18(4)	a-t
Song Sparrow	11	1(6), 10(5)	a-g, i-l, n-t
Rufous-sided Towhee	19	2(5), 4(4), 11(8), 14(2)	a-g, i-k, m-t
Common Yellowthroat	21	1(8), 2(3), 4(1), 9(4), 10(5)	a-k, m-t

[a] (1), Anoka Co., MN; (2), Barnwell Co., SC; (3), British Columbia, Canada; (4), Clarke, Greene, Madison, and Oglethorpe cos., GA; (5), Covington Co., AL; (6), Douglas Co., KS; (7), Douglas Co., WA; (8), Hemphill Co., TX; (9), Iron Co., MI; (10), King and Snohomish cos., WA; (11), Kittitas Co., WA; (12), Lancaster Co., PA; (13), Morgan Co., WV; (14), Okanogan Co., WA; (15), Potter Co., TX; (16), Sandusky Co., OH; (17), Valencia Co., NM; (18), Winona Co., MN; (19), Cameron Parish, LA; (20), Fairbanks, AK.

[b] a, *Ava*I; b, *Bam*HI; c, *Bcl*I; d, *Bgl*II; e, *Bgl*II; f,*Bst*EII; g, *Cla*I; h, *Eco*RI; i, *Hinc*II; j, *Hind*III; k, *Kpn*I; l, *Mbo*I; m, *Msp*I; n, *Nde*I; o, *Pst*I; p, *Pvu*II, q, *Sac*I; r, *Sst*II; s, *Stu*I; t, *Xba*I.

Estimates of genotypic diversity (analogous to heterozygosity in nuclear genes) and nucleotide diversity (mean number of nucleotide substitutions per nucleotide across all pairwise comparisons of individuals) were calculated for each species from formulas presented by Nei (1987; see footnote to Table 2). Estimates of evolutionary effective population sizes of females were derived from the approach described in Avise et al. (1988). The variance of effective population size was estimated through the use of computer simulations of the coalescent process similar to those outlined by Hudson (1990). Our simulations differed from those of Hudson in that the time at which each coalescence occurs is determined by using a transition matrix based on the Wright-Fisher model (Tavare, 1984) and a random-number generator to pick the generation at which two lineages coalesce. The transition matrix was calculated using the estimated effective female population size. The evolutionary effective female population sizes for 100 simulated populations of each species were calculated as above, and the variance of these values was used as an estimate of the variance of this statistic. These simulations were written in Lightspeed C and run on a MacIntosh SE/30. The program is available on request from R.M.B.

For purposes of discussion, each sample was provisionally assigned to a traditionally recognized subspecies on the basis of particular collection locale, and the breeding distributions of subspecies as described in the 1957 AOU *Check-list*.

RESULTS

Estimated sizes of the mtDNA molecules ranged from 16.2 kb in the Rufous-sided Towhee to 20.1 kb in some Mourning Doves (see Table 2). In five of the six species, each individual appeared homoplasmic (carried a single detectable mtDNA genotype) with regard to restriction profile, and only one mtDNA size class was observed per species. In contrast, the Mourning Dove exhibited heteroplasmy (multiple mtDNA genotypes within an individual) for mtDNA size variants, with some individuals

TABLE 2. Summary of molecular features and genetic diversity measures in six avian species.

Species	mtDNA size (kb)	No. haplotypes observed	Genetic diversity		$N_{f(e)}$[c] (and SE)
			Genotypic[a]	Nucleotide[b]	
Downy Woodpecker	16.7	5	0.15	0.0003	6,500 (2,756)
Mourning Dove	19.3–20.7	4[d]	0.32[d]	0.0011[d]	27,500 (15,056)
Brown-headed Cowbird	17.0	6	0.66	0.0017	42,500 (21,043)
Song Sparrow	16.7	5	0.73	0.0023	57,500 (42,327)
Rufous-sided Towhee	16.2	5	0.69	0.0034	85,000 (51,833)
Common Yellowthroat	17.0	10	0.76	0.0048	120,000 (60,180)

[a] $(n/[n-1])(1-\Sigma f_i^2)$, where f_i is frequency of ith mtDNA haplotype.

[b] Mean $p = (n/[n-1])(\Sigma f_i f_j p_{ij})$, where f_i and f_j are frequencies of ith and jth haplotypes in sample of size n, and p_{ij} is estimated sequence divergence between ith and jth sequences (Nei 1987: 256).

[c] Estimated from inbreeding approach described in Avise et al. (1988). Estimates for Rufous-sided Towhee and Common Yellowthroat included here for sake of completeness; technically, however, these two estimates are inappropriate due to evident mtDNA phylogeographic subdivision, which violates an assumption of the models. Thus, estimates for these two species may be inflated artificially.

[d] Does not include size variants.

TABLE 3. Descriptions of mtDNA clones observed in six avian species.

Haplotype description[a]																			Clone	No. individuals
a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	
Downy Woodpecker																				
C	C	C	C	C	1	C	C	C	C	1	—	—	1	C	C	C	C	C	C	D1	47
D/C	C	C	C	C	1	C	C	C	C	1	—	—	1	C	C	C	C	C	C	D2	1
C	C	D/C	C	C	1	C	C	C	C	1	—	—	1	C	C	C	C	C	C	D3	1
C	C	C	C	C	1	C	C	C	C	1	—	—	1	C	C	D/C	C	C	C	D4	1
C	C	C	C	C	1	C	C	C	C	1	—	—	1	C	C	C	C	D/C	C	D5	1
Mourning Dove																				
C	C	C	C	C	C	C	—	C	C	1	—	—	C	C	C	1	C	C	C	M1	14
D/C	C	D/C	C	C	C	C	—	C	C	1	—	—	C	C	C	1	C	C	C	M2	1
C	C	C	C	C	D/C	C	—	C	C	1	—	—	C	C	C	1	C	C	C	M3	1
C	C	C	C	C	C	C	—	C	B/B	1	—	—	C	C	D/D	1	C	C	C	M4	1
Brown-headed Cowbird																				
C	C	1	C	1	C	C	C	C	C	C	C	C	C	C	C	1	C	C	C	B1	13
B/C	C	1	C	1	C	C	C	C	C	C	B/B	C	B/B	B/B	C	1	C	C	C	B2	1
C	C	1	C	1	C	C	C	C	C	C	B/C	C	B/B	B/B	C	1	C	B/B	C	B3	8
C	C	1	C	1	C	C	C	C	C	C	C/C	C	B/C	B/C	C	1	C	B/C	C	B4	2
C	C	1	C	1	C	C	C	C	C	C	B/C	C	C/C	C/C	C	1	C	C/C	C	B5	1
D/D	C	1	C	1	C	C	C	C	C	?	B/B	C	C	C	C	1	C	C	C	B6	1
Song Sparrow																				
C	C	C	C	C	1	1	—	C	C	C	C	—	C	1	C	1	C	C	C	S1	5
C	C	C	C	C	1	1	—	C	C	B/C	C	—	C	1	C	1	C	C	C	S2	3
C	C	B/C	C	C	1	1	—	C	C	C/C	B/C	—	B/C	1	C	1	C	C	C	S3	1
C	C	C/C	C	C	1	1	—	C	C	C	C/C	—	B/C	1	C	1	C	C	C	S4	1
C	C	D/D	C	C	1	1	—	C	C	C	C	—	C/C	1	C	1	C	C	C	S5	1
Rufous-sided Towhee																				
C	C	C	C	C	1	C	—	C	C	C	—	C	C	C	C	C	C	C	C	R1	8
C	C	D/C	C	C	1	C	—	C	C	C	—	C	C	C	C	B/C	C	B/C	C	R2	1
D/D	C	C/C	C	C	1	B/B	—	B/B	C	C	—	C	C	C	C	C/C	C	C/C	C	R3	7
D/D	C	C	C	C	1	B/B	—	B/B	B/C	C	—	C	C	C	C	C	C	C	C	R4	2
D/D	C	C	C	C	1	B/B	—	B/B	C	C	—	C	C	C	C	C	C	C	B/B	R5	1

TABLE 3. Continued.

Haplotype description[a]																						No. individuals
a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	Clone	
Common Yellowthroat																						
C	C	C	C	1	C	1	C	C	C	C	—	C	C	1	C	C	C	C	C	C1	10
C	C	C	C	1	C	1	C	C	C	C	—	C	D|C	1	C	C	C	C	C	C2	1
C	C	C	C	1	C	1	C	C	C	C	—	C	C	1	B|C	C	C	C	C	C3	1
C	C	C	C	1	C	1	C	D|C	C	C	—	C	C	1	C	C	C	C	C	C4	1
C	C	C	C	1	C	1	B|C	C	C	C	—	C	C	1	C	C	C	C	C	C5	2
D|C	C	C	C	1	C	1	C	C	D|C	C	—	C	C	1	C	C	D|C	C	B	C7	2
C	E|C	C	C	1	C	1	C	E|C	D|C	C	—	X|C	C	1	C	C	D|C	D|C	B	C8	1
C	D|C	C	C	1	C	1	C	E|C	D|C	C	—	X|C	C	1	C	C	C	D|C	B	C9	1
C	D|C	C	C	1	C	1	A|C	E|C	D|C	C	—	X|C	C	1	C	C	C	D|C	B	C10	1

[a] Lower-case letters refer to restriction endonucleases listed in footnote to Table 1. A "1" in body of table indicates only one mtDNA fragment observed.

Fig. 2. UPGMA dendrograms (plotted on common scale of mtDNA sequence divergence) for four assayed avian species in which there was no evidence for distinction among conventionally recognized subspecies (names indicated at left). MtDNA clones labeled as in Table 3. Parsimony networks similar or identical to these dendrograms, although most branches not strongly supported statistically. Among 500 bootstrap replicates, only one branch (see the Brown-headed Cowbird) identified more than 95% of time.

clearly showing two or three length classes. The range of estimated genome sizes was 19.3 to 20.7 kb. The variants always occurred in one series of homologous fragments, no matter which restriction enzyme was used, indicating that the length variation was confined to one region of the mtDNA genome. Thus, observations on mtDNA size variation and heteroplasmy in the Mourning Dove are consistent with similar observations in several other vertebrates (Bermingham et al. 1986, Moritz et al. 1987), including at least two avian species (Avise and Zink 1988).

The following sections summarize the magnitude and geographic distribution of mtDNA restriction-site differences in the six species. Various measures of genetic diversity are compiled in Table 2, and the mtDNA clonal descriptions are summarized in Table 3. Figures 2 and 3 summarize the genetic relationships among the mtDNA clones within each species.

Downy Woodpecker.—Five mtDNA haplotypes were observed among the 51 individuals sampled. The most common haplotype (D1) was found in 47 individuals, while the remaining four specimens showed unique mtDNA haplotypes each differing from D1 by a single, independent restriction-site change. The unique haplotypes occurred in four separate locations (South Carolina, Kansas, Texas, and New Mexico), each of which also contained individuals with the common genotype.

Samples of the Downy Woodpecker came from 13 states of the United States and 1 Canadian province. They represent five described subspecies (*nelsoni, medianus, pubescens, turati,* and *leucurus*) and a significant fraction of the species' range in North America. Despite the immense geographic area sampled, mtDNA polymorphism and differentiation in the Downy Woodpecker were exceptionally low. Estimates of genotypic and nucleotide diversity for the pooled samples were 0.15 and 0.0003, respectively (Table 2), among the lowest such values reported in any vertebrate species (Avise et al. 1987, Avise 1992). From the estimate of nucleotide diversity, the evolutionary effective size of the female population was estimated by the approach of Avise et al. (1988; see also Ball et al. 1990) to be $N_{f(e)} \cong 6{,}500$ (SD = 2,756; Table 2). To generate this value, we assumed for this (and all subsequent) species a generation length of two years, and a "conventional" rate for mtDNA evolution of 2% sequence divergence between lineages per million years (Brown et al. 1979, Shields and Wilson 1987a).

Mourning Dove.—Based on restriction-site (rather than length) differences, the 17 sampled Mourning Doves yielded only four mtDNA clonal types, three of which were confined to single individuals (Table 3). The most common genotype, M1, predominated at all three locales (Georgia, Minnesota, and Washington) and in both of the subspecies represented (*carolinensis* and *marginella*). Thus, genotypic and nucleotide diversities were again very low—0.32 and 0.0011, respectively (Table 2). The nucleotide-diversity value yields an estimate of $N_{f(e)} \cong 27{,}500$ (SD = 15,056) for the species.

Brown-headed Cowbird.—Our mtDNA samples of this species showed considerably greater restriction-site diversity than was observed within either the Downy Woodpecker or Mourning Dove. The most common haplotype, B1, was found in 13 individuals from three of the four collection locales (Georgia, Minnesota, Ohio). The next most common haplotype, B3, was shared by eight individuals from three locales (Georgia, Minnesota, Washington). Of the remaining four haplotypes, one occurred in two individuals (Ohio), and the remainder were confined to single specimens. Genotypic diversity was 0.66. Nucleotide diversity was 0.0017, from which a value of $N_{f(e)} \cong 42{,}500$ (SD = 21,043) was obtained. In the Brown-headed Cowbird, two conventionally recognized subspecies (*ater* and *artemisiae*) were represented among the four sample locales.

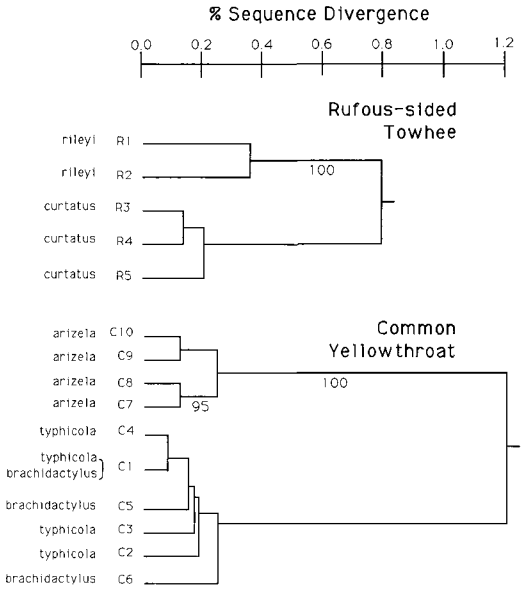

Fig. 3. UPGMA dendrograms (plotted on common scale of mtDNA sequence divergence) for two assayed avian species in which there was evidence for distinction among some conventionally recognized subspecies (names indicated at left). MtDNA clones labeled as in Table 3. Parsimony networks nearly identical, and levels of bootstrapping support among 500 replicates indicated (only values greater than 95% shown).

Song Sparrow.—Five mtDNA genotypes were observed among the 11 sampled specimens from Minnesota and Washington. The two common haplotypes, S1 and S2, were observed at both locales, while the other three genotypes were confined to single specimens. Genotypic diversity was 0.73, nucleotide diversity 0.0023, and $N_{f(e)} \cong 57{,}500$ (SD = 42,327). The samples from Minnesota and Washington represent the subspecies *euphonia* and *morphna*, respectively. Zink (1991) and Hare and Shields (1992) have

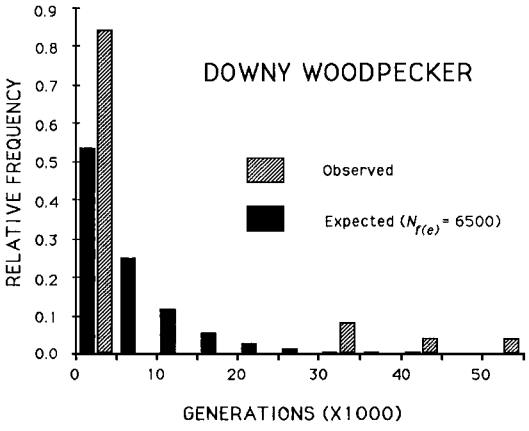

Fig. 4. Frequency distributions of times to shared ancestry of mtDNA haplotypes in the Downy Woodpecker. Hatched bars represent observed times calculated from mtDNA data, assuming generation length of two years and rate of evolution of 2% sequence divergence per million years; solid bars represent expected times generated from inbreeding theory with $N_{f(e)} = 6{,}500$.

recently surveyed additional Song Sparrow populations from the western United States, and reached similar conclusions about a relative paucity of mtDNA phylogeographic structure.

Rufous-sided Towhee.—In sharp contrast to the species covered above, the Rufous-sided Towhee showed a clear pattern of mtDNA phylogeographic differentiation. None of the five mtDNA haplotypes observed was shared between the eastern (Georgia and South Carolina, representing *rileyi*) and western (two sites in Washington, representing *curtatus*) locales. Furthermore, the mtDNA haplotypes found in Georgia and South Carolina were more similar to one another than to any of the haplotypes in Washington, and the samples from Washington were more similar to one another than to any haplotypes in the east (Fig. 2). The eastern and western genotypic assemblages differed at a mean level of sequence divergence $p \cong 0.008$, a value higher than the *maximum* sequence divergence between conspecific mtDNA haplotypes in any of the four species considered above.

Common Yellowthroat.—This species exhibits a phylogeographic pattern generally similar to that for the Rufous-sided Towhee. A total of 10 haplotypes was observed among 21 assayed specimens. Six haplotypes observed in the eastern and midwestern locales (Minnesota, Michigan, South Carolina, Georgia) clustered together, and differed from the four haplotypes present in the western (Washington) collections by an average sequence divergence of $p \cong 0.012$ (Fig. 2). Thus, when the data are considered in composite, the Common Yellowthroat exhibited relatively high levels of both genotypic and nucleotide diversity: 0.76 and 0.0048, respectively (Table 2). In contrast, mtDNA differentiation among the eastern and midwestern locales was minimal. Thus, the most common genotype, C1, was present in the South Carolina, Michigan, and Minnesota samples, and the single individual assayed from Georgia exhibited a haplotype (C2) only one mutation step removed from C1. The sample of Common Yellowthroat from Washington is referable to the subspecies *arizela*, those from Minnesota and Michigan to *brachidactylus*, and those from Georgia and South Carolina to *typhicola*.

DISCUSSION

Among the 16 traditionally recognized subspecies considered in this report, only two (*curtatus* in the Rufous-sided Towhee, and *arizela* in the Common Yellowthroat) proved to be readily distinguished from other conspecifics in terms of mtDNA genotype. In the remaining comparisons, particular mtDNA genotypes and/or closely related assemblages of genotypes were shared among subspecies, even when the samples came from distant locales (Figs. 2 and 3). Clearly, for at least two reasons, such results do not "prove" the null hypothesis that these subspecies lack genetic differentiation: (a) within each organismal pedigree, only one "gene" genealogy (the matriarchal phylogeny) is assessed by mtDNA assays; and (b) only about 2.2% of the mtDNA genome (360 nucleotide positions in recognition sequence per individual) was assayed with the endonucleases employed in this study. Nonetheless, when interpreted against the proven sensitivity of comparable mtDNA assays in revealing dramatic phylogeographic differentiation within many other vertebrate species (Avise et al. 1987, Moritz et al. 1987), the lack of differentiation among these avian conspecifics remains impressive.

The most striking example of a shallow mtDNA phylogeographic structure involved the Downy Woodpecker. Spot samples from across North America, including Georgia, South Carolina, Louisiana, Kansas, New Mexico, Pennsylvania, West Virginia, Michigan, Minnesota,

Washington, and Alaska, contained specimens that proved to be identical in our mtDNA assays, and all observed variants differed from this common genotype by only single mutation steps (and estimated $p \cong 0.002$). The Downy Woodpecker is generally nonmigratory and relatively sedentary (Staebler 1949). Nonetheless, our results suggest that populations throughout the species' range may be very closely related to one another in a historical sense. Even if more sensitive mtDNA assays were to reveal geographically localized polymorphisms within the species (suggestive of contemporary restrictions on gene flow), the phylogeographic "depth" of the species would likely remain very shallow in comparison to most other vertebrates surveyed to date (see discussions of the distinction between contemporary gene flow and historical connectedness in Slatkin [1987] and Avise [1989]).

One way to quantify and interpret depth in an mtDNA phylogeny involves comparison of empirically estimated times of lineage coalescence with expected times generated under neutrality theory as a function of evolutionary effective size of female populations ($N_{f(e)}$; Avise et al. 1988). If we assume a generation length of two years for the Downy Woodpecker, and further assume a "conventional" rate of mtDNA evolution of 2% sequence divergence per million years, then $N_{f(e)}$ is about 6,500 (Fig. 4). In other words, the empirically estimated times of mtDNA lineage separation in the Downy Woodpecker are roughly similar to those expected for a single panmictic population whose effective size was a only few thousand females. Downy Woodpeckers are currently one of the most abundant and widespread avian species in North America. The very small $N_{f(e)}$ relative to census N_f could be attributable in principle to either of two general classes of possibility: (a) historical demographic factors, such as large variances in progeny production among females, periodic fluctuations in population size (perhaps including population bottlenecks), or rapid colonization of the continent from a limited population source; or (b) the recent occurrence of a favorable mtDNA mutation, which might have swept through the species (assuming the populations were historically connected by gene flow) with the net effect of cleansing the mtDNA genome of much pre-existing genetic differentiation. In the Downy Woodpecker, the finding that $N_{f(e)}$ is much less than N_f qualitatively parallels results for many other vertebrate species, although the magnitude of the disparity is unusually large (Avise et al. 1988, Avise 1992).

Although the Mourning Dove, Brown-headed Cowbird, and Song Sparrow were sampled less extensively than was the Downy Woodpecker, preliminary mtDNA results were similar. Thus, populations belonging to different subspecies, collected from locales often separated by thousands of kilometers, shared particular mtDNA haplotypes and closely related haplotype assemblages (Fig. 2). In each species, mtDNA-inferred estimates of $N_{f(e)}$ again were considerably smaller than the current-day census sizes of these abundant species (Table 2), although not as dramatically so as in the Downy Woodpecker. The magnitude of the disparity between $N_{f(e)}$ and current population size in these birds may be biased upward by the fact that the species in this study were chosen, in part, precisely because of their present abundance, a state that may well be temporary over evolutionary time scales.

In contrast, the Rufous-sided Towhee and Common Yellowthroat exhibited marked mtDNA phylogeographic differentiation by avian standards (Fig. 3). In the towhee, the samples from Washington (referable to subspecies *curtatus*) differed cleanly from samples in Georgia and South Carolina (referable to *rileyi*) by a mean estimated sequence divergence of $p = 0.008$. No mtDNA clones or clonal assemblages were shared between these collections, and the various genotypes in the two respective regions differed in digestion profiles at 3 to 7 of the 17 informative endonucleases employed (Table 3). In the yellowthroat, the samples from Washington (referable to *arizela*) differed sharply from those in Minnesota, Michigan, Georgia, and South Carolina (referable to *brachidactylus* [north] and *typhicola* [south]) by a mean estimated sequence divergence of $p = 0.012$. No mtDNA clones or clonal assemblages were shared between the Washington and other collections, and the various genotypes in these two regions differed in digestion profiles at 6 to 8 of the 16 informative endonucleases employed (Table 3).

The mtDNA distinctions in the Rufous-sided Towhee may be of special interest, given an earlier taxonomy in which western populations ("Spotted Towhee," *P. erythrophthalmus*) were considered specifically distinct from those in the eastern United States ("Eastern Towhee," *P.*

maculatus; see AOU 1983). The two forms appear to intergrade in overlap zones in Saskatchewan and Nebraska and, hence, are currently regarded as conspecific. Nonetheless, the Spotted Towhee is phenotypically quite distinct from eastern forms, with evident white spotting on the scapulars and wing coverts, less sexual dimorphism in plumage, and browner eye color. The songs of the eastern and western forms also are very different. Although additional geographic sampling will be required to confirm that the mtDNA differences are concordant with the formerly recognized Spotted/Eastern towhee dichotomy, current data strongly suggest a considerable genetic distinction. The average mtDNA distance between the two Rufous-sided Towhee clades (Fig. 3) is somewhat greater than that found between some species pairs such as the King and Clapper rails (*Rallus elegans* and *R. longirostris*), or the Mallard and Mottled Duck (*Anas platyrhynchos* and *A. fulvigula*; Avise and Zink 1988). Of course, mtDNA genetic distances cannot be used alone to decide the specific or subspecific status of populations, but they can be very useful in identifying sets of populations that likely had been separated for a significant period of evolutionary time, particularly when such differences are concordant with those registered by nuclear genes and their products (including morphological or behavioral traits; Avise and Ball 1990).

Similarly, the distinction between mtDNA clades characteristic of Washington versus South Carolina, Georgia, Minnesota, and Michigan populations of Common Yellowthroats (Fig. 3) suggests a long-standing historical population separation. Without further geographic sampling, we hesitate to speculate further as to the present-day distributions of these clades, but the species appears to be an excellent candidate for further phylogeographic analysis.

Overall, we have found that some avian species exhibit dramatic mtDNA disjunctions across their ranges, while others do not (see also Avise and Ball 1991). While this kind of idiosyncrasy in phylogeographic outcome makes it difficult to make sweeping generalizations about the magnitude and pattern of molecular genetic structure among avian conspecifics, the idiosyncrasy itself remains of interest. The differences in mtDNA phylogeography in the Rufous-sided Towhee and Common Yellowthroat versus the Downy Woodpecker and Mourning Dove, for example, indicate that distinct evolutionary histories have been recorded in the genetics of these species. Further comparative studies involving both additional species and other genes (in the cell nucleus) should prove highly informative in assessing the major genetic subdivisions within particular species. To the extent that subspecies designations are interpreted to reflect long-term historical gene-pool separations, the results should also be highly relevant to intraspecific taxonomy.

Recognition of deep historical separations may not be the only rationale for subspecies descriptions. For example, many birds migrate, and it is of interest to identify the breeding locales of individuals observed during migration or on wintering grounds. Any mutations serving as genetic markers of breeding populations (including those underlying particular morphological or behavioral traits) can be of great utility in such endeavors, even if the mutations are of recent origin and do not reflect long-term population separations or genome wide patterns of differentiation. Elsewhere we have argued that short-term population separations should not be sufficient to justify formal taxonomic recognition of subspecies (in part, because sensitive and refined genetic assays will likely reveal significant structure even at demic and family levels in most species; Avise and Ball 1990). Under this perspective, subspecies names should be reserved for the major subdivisions of gene-pool diversity within species. Such subdivisions might best be indicated by concordant subdivisions at multiple independent loci and, therefore, some other means for cataloging geographic distributions of individual genetic markers should be implemented. Overall, an enlightened perspective on intraspecific differentiation would recognize the great variety of evolutionary depths and patterns likely to be represented among populations, and the various taxonomic and population applications to which these levels of genetic separation might be applied.

Authors of the latest AOU *Check-list* (1983) dropped the former use of subspecies names solely on practical grounds, and emphasized "we wish to make clear that the omission of separate listings of subspecies in this edition is not a rejection of the validity or utility of this systematic category." However, the heterogeneity in population structure revealed in the current study suggests that avian species may exhibit a wide variety of demographic and phy-

logenetic histories. The population-genetic and evolutionary significance of current subspecies designations may have to be reevaluated on a case-by-case basis.

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