MOLECULAR GENETIC DIVERGENCE BETWEEN AVIAN SIBLING SPECIES: KING AND CLAPPER RAILS, LONG-BILLED AND SHORT-BILLED DOWITCHERS, BOAT-TAILED AND GREAT-TAILED GRACKLES, AND TUFTED AND BLACK-CRESTED TITMICE

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ABSTRACT.—Surveys of electrophoretic variation in proteins, and restriction site variation in mitochondrial DNA (mtDNA), were conducted to assess the resolving power of these molecular genetic techniques to distinguish four pairs of avian sibling taxa. Samples of rails (*Rallus elegans* and *R. longirostris*), dowitchers (*Limnodromus scolopaceus* and *L. griseus*), grackles (*Quiscalus major* and *Q. mexicanus*), and titmice (*Parus bicolor bicolor* and *P. b. atricristatus*) were assayed for allozymes encoded by 34–37 nuclear loci, and for an average of 77 mtDNA restriction sites per individual by 19 endonucleases. MtDNA's of the two rail species showed large-scale size polymorphism and individual heteroplasmy, the first such findings of these molecular features in an avian species. Genetic distances based on allozyme comparisons were small for all assayed taxa (Nei's $D \leq 0.063$). The mtDNA assays offered consistently greater resolving power, providing at least five fixed restriction site differences for samples of any taxon pair. The Long-billed and Short-billed dowitchers were especially divergent, differing by at least 24 assayed mtDNA restriction sites and an estimated nucleotide sequence divergence of $p = 0.082$. We compared these results to previous reports of genetic distances within and among closely related bird species. The mtDNA divergence among dowitchers is near the high end of the scale of such estimates for avian congeners. The mtDNA distances between the pairs of rails ($p = 0.006$), titmice ($p = 0.004$), and grackles ($p = 0.016$) were typical for extremely closely related species, and overlap maximum values reported for some avian conspecifics. *Received 13 November 1987, accepted 21 March 1988.*

VARIOUS laboratory techniques in molecular evolution offer differing resolution of genetic differentiation and phylogeny along a taxonomic hierarchy (Avise 1986a). For example, methods of nuclear DNA/DNA hybridization (Sibley and Ahlquist 1986) or of protein immunology such as microcomplement fixation (Wilson 1985) are usually best applied to higher taxonomic levels, because the observed genetic differences within and between closely related species are typically negligible. Conversely, multilocus protein electrophoresis (Ayala 1976) or restriction enzyme analysis of mitochondrial DNA (mtDNA; Avise 1986b) have proved best suited for phylogenetic comparisons within and among closely related species, because beyond the genus or family level, the maximum genetic distance observable by these techniques typically has been reached (see, however, Lanyon and Zink 1987). By the criterion of focus alone, it should not be claimed that any technique is necessarily "better" than another for systematics purposes. Rather, the situation can be likened to microscopy, where use of different lenses can bring into focus distinct and important aspects of structure at multiple tiers.

As a general rule, birds at all taxonomic levels commonly exhibit less genetic divergence than do many of their counterparts in other vertebrate classes (Avise and Aquadro 1982; Barrowclough et al. 1985; Prager et al. 1974). Although this situation has facilitated molecular analysis of major groupings across the class Aves (Prager and Wilson 1980; Sibley and Ahlquist 1983), it has also exacerbated attempts genetically to differentiate and study entities at the lower end of the avian taxonomic scale. For example, many conspecific populations and congeneric avian species appear difficult to distinguish even when assayed by the sensitive and commonly employed protein electrophoretic methodology (e.g. Braun and Robbins 1986; Zink 1986).

We characterized genetic divergence in each of 4 pairs of avian sibling "species" with two of the powerful molecular approaches of allozymes and mtDNA analysis. The taxa (*Rallus*

elegans and *R. longirostris* [Rallidae], *Limnodromus scolopaceus* and *L. griseus* [Scolopacidae], *Quiscalus major* and *Q. mexicanus* [Emberizidae], and *Parus bicolor bicolor* and *P. b. atricristatus* [Paridae]) include non passerines as well as passerines, and involve systematically controversial taxonomic pairs that have all been considered conspecific and specifically distinct at one time or another. We were interested in the relative sensitivities of allozyme and mtDNA assay techniques to disclose genetic markers that may distinguish avian sibling species, and what the genetic results reveal about the particular evolutionary histories of the taxa.

Materials and Methods

Specimens were collected in Louisiana and Texas (precise locales available from RMZ on request) during the winter and spring of 1987. Voucher specimens are deposited in the Museum of Zoology, LSU. Allozyme data for titmice were obtained from Braun et al. (1984). For mtDNA analyses, samples of liver, heart, and kidney were immersed in MSB-Ca-EDTA buffer (Lansman et al. 1981), and transported on wet ice to the laboratory within 7 days. For protein electrophoresis, samples of liver and muscle were frozen until time of assay.

Protein electrophoresis followed standard protocols (Selander et al. 1971, Johnson et al. 1984). Between 34 and 37 loci were scored for each species pair. Sixteen of these loci are listed in Table 1; the remainder (which proved monomorphic and incapable of discriminating members of any species pair in which they were assayed) are ethanol dehydrogenase (Enzyme Commission number 1.1.1.1), α-glycerophosphate dehydrogenase (1.1.1.8), lactate dehydrogenase-1 and 2 (1.1.1.27), malate dehydrogenases-1 and 2 (1.1.1.37), malic enzyme (1.1.1.40), isocitrate dehydrogenase-2 (1.1.1.42), glutamate dehydrogenase (1.1.1.47), glutathione reductase (1.6.4.2), superoxide dismutase (1.15.1.1), glutamic-pyruvic transaminase-2 (2.6.1.2), creatine kinases-1 and 2 (2.7.3.2), acid phosphatases-1 and 2 (3.1.3.2), leucineamino peptidase (3.4.11), fumarate hydratase (4.2.1.2), aconitate hydratase (4.2.1.3), and non-enzymatic proteins-1 and 2. Mean observed heterozygosity in each species was calculated from genotypic counts. These counts were also used to determine allele frequencies, from which genetic distances were computed by Nei's (1978) method.

MtDNA in closed-circular form was isolated from pooled tissues of each specimen by ultracentrifugation in cesium chloride gradients. Following dialysis, the purified mtDNA was digested by each of 19 restriction endonucleases. Fifteen of these enzymes are listed in Table 2; the remaining 4 (which proved not to distinguish any of the sibling species assayed) are *Kpn*I (recognition site GGTACC), *Pst*I (CTGCAG), *Sst*II (CCGCGG), and *Xba*I (TCTAGA). MtDNA fragments were end-labeled with ^{35}S-radionuclides, separated by molecular weight through 1.0, 1.2, or 1.8% agarose gels, and revealed by autoradiography as bands in gel profiles (Brown 1980; Lansman et al. 1981; Maniatis et al. 1982). Fragment sizes were compared against a 1-kilobase ladder standard purchased from Bethesda Research Labs. MtDNA genetic distances in terms of estimated base substitutions per nucleotide were calculated by the fragment method of Nei and Li (1979), which involves appropriate weighting of distance estimates calculated separately for endonucleases recognizing 4-, 5-, and 6-base sites.

Many of the mtDNA fragment profile differences between sibling taxa were attributable to gains (or losses, depending on which state was ancestral) of single restriction sites. Such instances are recognized when two digestion profiles (A and B) are identical at all except three fragments, and in which the sum of the molecular weights of the two unique fragments in A equals the weight of the third unique fragment in B (for a detailed description of the gel scoring procedure, see Fig. 2 in Avise et al. 1987). Thus, although sites were not formally mapped in this study, we were often able to interpret gel patterns with respect to restriction site as well as restriction fragment changes. We observed a total of more than 100 distinct fragment profiles. (Avise will gladly supply diagrams of any gel patterns upon request.)

Results

King and Clapper rails.—These species are of uncertain status, with some (e.g. Ripley 1977) favoring conspecific status and extensive intraspecific variation, and others (e.g. A.O.U. 1983) advocating recognition as distinct species. There is little doubt that these saltwater (*R. longirostris*)/freshwater (*R. elegans*) "replacements" are closely related and may hybridize at least occasionally where sympatric (Meanley and Wetherbee 1962). *Rallus longirostris* was from coastal Louisiana where hybrids have occasionally been reported (B. Meanley *in* Ripley 1977), and the sample of *R. elegans* was from inland marshes away from apparent hybrid zones. We found no morphological evidence of hybridization of our samples.

For 37 allozyme loci (Table 1), mean heterozygosities for *R. longirostris* and *R. elegans* were 0.04 $\pm$ 0.02 (SE) and 0.03 $\pm$ 0.01, respectively. We observed no fixed allelic differences between samples, so the same loci that were polymorphic were also the sole contributors to the

TABLE 1. Frequencies of alleles at polymorphic loci (common allele <0.95 in frequency) within or between at least some of the sibling avian taxa.[a] Allelic designations apply only to comparisons made between members of a taxon pair, such as Long-billed vs. Short-billed dowitchers. Open positions in the table indicate that members of the taxon pair were monomorphic for the same allele at the locus in question.

Enzyme commission number	Locus	Allelic designation	Rails		Dowitchers		Grackles		Titmice[b]	
			King (n = 10)	Clapper (n = 7)	Long-billed (n = 8)	Short-billed (n = 5)	Boat-tailed (n = 8)	Great-tailed (n = 11)	Tufted (n = 12)	Black-crested (n = 12)
1.1.1.1	Octanol dehydrogenase	a							1.00	0.92
		b							—	0.08
1.1.1.14	Sorbitol dehydrogenase	a			0.12	—				
		b			0.88	1.00				
1.1.1.42	Isocitrate dehydrogenase-1	a							—	0.38
		b							1.00	0.62
1.1.1.44	6-Phosphogluconate dehydrogenase	a			0.06	—			0.04	—
		b			0.06	—			0.92	1.00
		c			0.88	1.00			0.04	—
1.1.1.-	Unknown dehydrogenase	a							—	0.79
		b							1.00	0.21
2.4.2.1	Purine nucleotide phosphorylase	a			0.31	0.10				
		b			0.13	0.10				
		c			0.56	0.70				
		d			—	0.10				
2.6.1.1	Aspartate aminotransferase-1	a							0.71	1.00
		b							0.29	—
2.6.1.2	Glutamic-pyruvic transaminase-1	a	0.05	0.43						
		b	0.95	0.57						
2.7.5.1	Phosphoglucomutase	a			—	0.10			0.92	1.00
		b			0.94	0.90			0.08	—
		c			0.06	—			—	—
3.1.1.1	Esterase	a			—	1.00			0.12	—
		b			0.06	—			0.88	1.00
		c			0.94	—			—	—
3.4.11	Leucylglycylglycine peptidase	a			0.06	—	0.09	—	0.08	0.08
		b			0.56	1.0	0.82	1.00	0.92	0.92
		c			0.38	—	0.09	—	—	—
3.4.11	Leucylalanine peptidase-1	a			1.00	—	—	0.12	0.17	—
		b			—	1.00	1.00	0.88	0.71	0.09
		c			—	—	—	—	0.12	0.17
		d			—	—	—	—	—	0.74

TABLE 1. Continued.

Enzyme commission number	Locus	Allelic designation	Rails		Dowitchers		Grackles		Titmice[b]	
			King ($n = 10$)	Clapper ($n = 7$)	Long-billed ($n = 8$)	Short-billed ($n = 5$)	Boat-tailed ($n = 8$)	Great-tailed ($n = 11$)	Tufted ($n = 12$)	Black-crested ($n = 12$)
3.4.11	Leucylalanine peptidase-2	a	0.15	0.07					0.12	—
		b	0.80	0.86					—	0.04
		c	0.05	0.07					0.88	0.96
3.5.4.4	Adenosine deaminase	a	0.20	0.29					0.04	0.92
		b	0.80	0.71					0.96	0.08
5.3.1.8	Mannose phosphate isomerase	a	0.85	1.00	0.88	1.00	—	0.06		
		b	0.15	—	0.12	—	1.00	0.94		
5.3.1.9	Phosphoglucose isomerase	a	1.00	0.79						
		b	—	0.21						

[a] Other loci assayed are listed in Materials and Methods.
[b] Data from Braun et al. (1984).

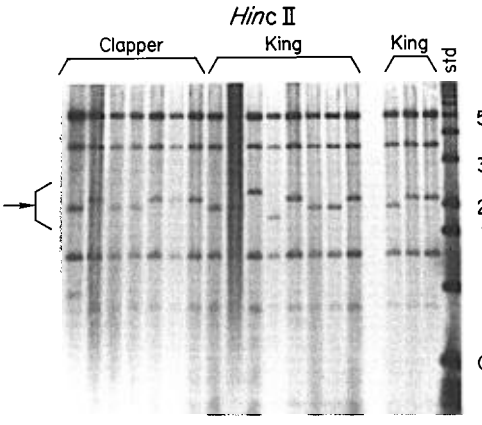

Fig. 1. *Hin*cII digests of mtDNA from King and Clapper rails. Arrow marks region of mtDNA size polymorphism and heteroplasmy. A molecular weight standard with selected fragment sizes (in kilobase pairs) in right lane.

extremely small genetic distance observed: $D = 0.004 \pm 0.006$ (SE). The largest and only statistically significant allele frequency difference ($P < 0.05$ in a two-way Chi-square test using Yates' correction for small sample size; Sokal and Rohlf 1969) involved glutamic-pyruvic transaminase-1, where the "b" electromorph frequencies were 0.95 and 0.57 in the rail samples.

We estimate *Rallus* mtDNA to be of approximate mean size 17.9 kilobases (kb). A somewhat surprising molecular-level finding was that *Rallus* mtDNA exhibits large-scale intraspecific mtDNA size polymorphism, and individual heteroplasmy. In *R. longirostris*, we found two size classes of mtDNA that differed by about 180 base pairs, and 4 of 7 individuals were detectably heteroplasmic (i.e. carried genomes of both sizes). In *R. elegans*, we found 4 mtDNA size classes differing in increments of about 180 base pairs, and at least 4 of 10 assayed individuals were heteroplasmic (one of them for 3 mtDNA size classes). The two most common size classes in *R. elegans* mtDNA appeared to be the same as those found in *R. longirostris* (Fig. 1).

The most compelling evidence for mtDNA size polymorphism and heteroplasmy in *Rallus* consists of concordant patterns of fragment size differences across digestion profiles produced by separate restriction endonucleases. The size differences of *Ava*I and *Hin*cII fragments (Fig. 2) provide an example. They exhibit concordant

TABLE 2. Frequencies of mtDNA digestion profiles produced by endonucleases whose restriction sites were polymorphic within or between at least some of the sibling avian taxa.[a] Fragment profiles labeled by adjacent letters in the alphabet differ by a single restriction site; non-adjacent letters denote 2 or more site differences. Profile designations apply only to comparisons made between members of a taxon pair, such as Long-billed vs. Short-billed dowitchers. Open positions in the table indicate that the taxon pair did not differ in position of sites for the endonuclease in question.

Restriction endonuclease	Recognition site (5′ to 3′)	Fragment profile designation	Rails		Dowitchers		Grackles		Titmice	
			King (n = 10)	Clapper (n = 7)	Long-billed (n = 8)	Short-billed (n = 6)	Boat-tailed (n = 8)	Great-tailed (n = 6)	Tufted (n= 3)	Black-crested (n = 5)
*Ava*I	CPyCGPuG	C			1.00	—				
		X			—	1.00				
*Ava*II	GG(A or T)CC	C	—	1.00	1.00	—	1.00	—	1.00	—
		D	1.00	—	—	—	—	—	—	0.80
		E	—	—	—	—	—	—	—	0.20
		X	—	—	—	1.00	—	1.00	—	—
*Bam*HI	GGATCC	C			1.00	—				
		D			—	0.83				
		E			—	0.17				
*Bcl*I	TGATCA	C			1.00	—				
		X			—	1.00				
*Bgl*I	GCCN$_5$GGC	C			1.00	—	1.00	—	1.00	—
		D			—	1.00	—	—	—	—
		X			—	—	—	1.00	—	1.00
*Bgl*II	AGATCT	C	—	1.00						
		X	1.00	—						
*Bst*EII	GGTNACC	C			1.00	—			1.00	—
		D			—	1.00			—	1.00
*Cla*I	ATCGAT	C			1.00	—				
		X			—	1.00				
*Eco*RI	GAATTC	C			1.00	—				
		D			—	1.00				
*Hin*cII	GTPyPuAC	C			1.00	—	1.00	0.83	1.00	—
		D			—	—	—	0.17	—	1.00
		X			—	1.00	—	—	—	—
*Hin*dIII	AAGCTT	C			1.00	—	1.00	—	0.67	1.00
		D			—	—	—	1.00	0.33	—
		X			—	1.00	—	—	—	—
*Msp*I	CCGG	C	—	1.00	1.00	—	1.00	—	1.00	0.80
		D	1.00	—	—	—	—	—	—	—
		X	—	—	—	0.67	—	1.00	—	0.20
		Y	—	—	—	0.33	—	—	—	—

TABLE 2. Continued.

Restriction endonuclease	Recognition site (5' to 3')	Fragment profile designation	Rails		Dowitchers		Grackles		Titmice	
			King (n = 10)	Clapper (n = 7)	Long-billed (n = 8)	Short-billed (n = 6)	Boat-tailed (n = 8)	Great-tailed (n = 6)	Tufted (n = 3)	Black-crested (n = 5)
*Nde*I	CATATG	C	—	1.00	1.00	—	1.00	—		
		D	1.00	—	—	—	—	1.00		
		X	—	—	—	1.00	—	—		
*Pvu*II	CAGCTG	C			1.00	—				
		X			—	1.00				
*Stu*I	AGGCCT	C	0.90	1.00	1.00	—	1.00	—		
		D	0.10	—	—	—	—	—		
		X	—	—	—	1.00	—	1.00		

* Other endonucleases employed are listed in Materials and Methods.

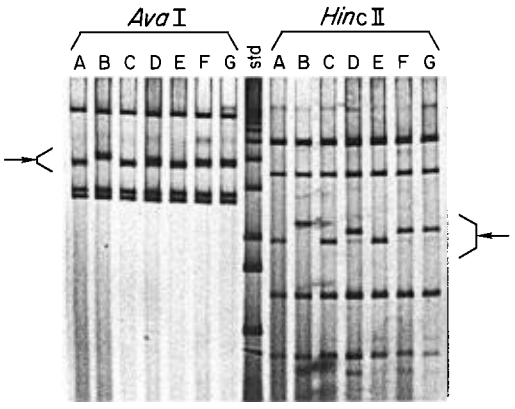

Fig. 2. *Ava*I and *Hin*cII digests of mtDNA from Clapper Rail (lanes F) and six King Rails (lanes A-E and G). Arrows indicate concordant shifts across the digestion profiles in the fragments that contain the region of mtDNA size polymorphism and heteroplasmy. Molecular weight standard (center lane) fragment sizes can be determined by comparison to Fig. 1.

patterns of variation among the 7 pictured individuals. The patterns suggest that the addition/deletion variation is probably discrete, tandemly arranged, and confined to one portion of the genome (perhaps the D-loop area, which is thought to harbor most known instances of size variation in non-avian species). Bermingham et al. (1986) suggest that these phenomena may be more prevalent in lower vertebrates than in birds and mammals. To our knowledge, this is the first reported instance of these mtDNA features in birds.

The 15 endonucleases employed produced a total of 68–69 scored mtDNA fragments in each *Rallus* specimen. The size-variable region was counted only once in any profile. Sixty-two of these fragments were shared by representatives of *R. elegans* and *R. longirostris*, yielding an estimated sequence divergence (in terms of base substitutions per nucleotide) of $p = 0.006$. No correction for intraspecific variation was required because, with a single exception (a *Stu*I restriction site change in one King Rail), all conspecific individuals in the sample appeared identical with respect to restriction sites (Table 2). Four enzymes (*Ava*II, *Bgl*II, *Msp*I, and *Nde*I) produced gel profiles that clearly distinguished all King Rail from Clapper Rail specimens. Three profile differences each involved a single restriction site; the fourth (*Bgl*II), entailed at least 2 site differences.

Long-billed and Short-billed dowitchers.—These species were considered conspecific until Pitelka (1950) substantiated their distinctness. As in the rails, the two species tend to be respectively associated with fresh water and salt water, at least in nonbreeding periods. Other differences include voice and details of plumage (Hayman et al. 1986). The breeding ranges appear not to overlap, and the degree of reproductive isolation is unknown.

Mean allozyme heterozygosity for *L. scolopaceus* was 0.05 ± 0.02, and for *L. griseus* 0.02 ± 0.02. Samples of the 2 species shared no alleles at 2 loci (an esterase and a peptidase) and exhibited a statistically significant allele frequency difference at a third locus (another peptidase, Table 1). Nei's genetic distance, based on 36 loci, was 0.06 ± 0.04.

The mtDNA's of *L. scolopaceus* and *L. griseus* proved highly divergent (Table 2). Fragment profiles for 14 of 19 endonucleases were consistently distinct in samples of the two species, and for each of 10 enzymes, at least 2 mtDNA restriction site changes were involved. Nonetheless, it is unlikely that major gene order rearrangements were responsible for the profile changes, because multifragment digestion patterns in the 2 species were identical for *Bgl*II, *Pst*I, *Sst*II, and *Xba*I, and differed by a single restriction site gain/loss for *Bam*HI, *Bgl*I, *Bst*EII, and *Eco*RI. Under the reasonable (and, in many other species, directly verified) assumption that base substitutions leading to restriction site gains and losses were primarily responsible for mtDNA differences, we estimated the mtDNA genetic distance between Long-billed and Short-billed dowitchers at $p = 0.082$. The estimate was based on an average of 77 restriction fragments scored per individual (representing 418 base-pairs of information in recognition sequences).

MtDNA genome size in *Limnodromus* was roughly 18.1 kb. Because of the nonlinearity of migration rates for larger fragments (greater than about 7 kb), and the difficulty of detecting small fragments (less than about 0.3 kb), estimates of total mtDNA genome size from fragment profiles are often crude. Nonetheless, the mtDNA's of dowitchers (and rails) were about 1 kb larger than those of titmice and grackles (see beyond) and of several other avian species assayed previously (Shields and Helm-Bychowski 1988).

Boat-tailed and Great-tailed grackles.—These grackles were considered conspecific until Selander and Giller (1961) found that they breed sympatrically without introgression. This interpretation was challenged by Phillips et al. (1964). Occasional hybrids occurred in southwestern Louisiana in the mid-1970's, when the two species were establishing sympatry. The current status appears to be genetic isolation (Pratt 1973, unpubl. data). Our specimens, collected outside the presumptive hybrid zone, did not show phenotypic or other evidence of introgression. Prevailing opinion currently regards the forms as distinct biological species (A.O.U. 1983).

Mean allozyme heterozygosity, based on 37 loci, was only about 0.01 for both species. Furthermore, there were no significant allele frequency differences between samples (Table 1), so that Nei's genetic distance was negligible ($D = 0.001 \pm 0.002$).

In contrast, samples of Boat-tailed and Great-tailed grackles differed by several mtDNA markers. *Ava*II, *Bgl*I, *Hind*III, *Msp*I, *Nde*I, and *Stu*I all exhibited fixed restriction site differences (Table 2), and the overall estimate of sequence divergence, based on an average of nearly 80 restriction sites scored per individual, was $p = 0.016$. MtDNA genome size in *Quiscalis* appeared to be about 16.7 kb.

Tufted and Black-crested titmice.—These taxa have been viewed both as subspecies (A.O.U. 1983) and species (A.O.U. 1957). They meet in a narrow hybrid zone in central Texas where interbreeding occurs (Dixon 1955). The sample of *P. b. bicolor* from Louisiana was well outside the hybrid zone, and our sample of *P. b. atricristatus* was adjacent to but outside the hybrid zone. None of the specimens showed phenotypic signs of introgression. Braun et al. (1984) suggested that the narrowness of the hybrid zone might imply a genetic barrier to introgression outside the limits of the zone.

The allozyme data (Table 1) were taken from Braun et al. (1984), who surveyed protein products of 36 loci. Mean heterozygosity for both forms was 0.06 ± 0.02, and Nei's genetic distance was 0.063. Five loci (isocitrate dehydrogenase-1, an unknown dehydrogenase, aspartate aminotransferase-1 (their glutamic-oxaloacetic transaminase-1), leucylalanine peptidase-1, and adenosine deaminase) exhibited significant allele frequency shifts between samples of the titmice forms, though no locus had fixed allelic differences.

The mtDNA data for small samples of titmice

(Table 2) showed fixed restriction profile differences for four endonucleases (*Ava*II, *Bgl*I, *Bst*EII, and *Hin*cII). Genetic distance, based on 83 restriction fragments scored per individual, was approximately $p = 0.004$. We estimated mtDNA genome size in *Parus bicolor* at about 16.6 kb, which agrees closely with reports for other *Parus* species (Mack et al. 1986, Tegelstrom 1987).

Discussion

For certain problems in evolutionary biology, such as construction of taxonomic keys or documentation of hybridization in contact zones, available diagnostic genetic markers may be sufficient to distinguish the forms under surveillance. For other issues, such as estimation of phylogenetic relationships or times since common ancestry, the magnitude and pattern of genetic divergence are necessary. These aspects of genetic differentiation were compared for the allozyme vs. mtDNA data sets in the assayed samples of rails, dowitchers, grackles, and titmice.

Diagnostic genetic markers.—The mtDNA assays offered consistently greater distinguishing power for this sample of avian sibling species than did the allozyme assays (Table 3). At least four mtDNA restriction fragment profiles, that involved a minimum of 5 fixed restriction site differences, discriminated members of each taxonomic pair. In the comparison of Long-billed and Short-billed dowitchers, more than 24 mtDNA restriction sites and 14 fragment profiles exhibited fixed differences. In contrast, there were no diagnostic allozyme differences between members of 3 of the 4 pairs of taxa. In one comparison (*Quiscalus major* vs. *Q. mexicanus*), statistically significant allele frequency shifts were also lacking.

These results parallel two previous studies of the relative discriminatory capacities of conventional allozyme and mtDNA approaches for avian species. Kessler and Avise (1984, 1985) noted many more diagnostic mtDNA markers than had been found in earlier allozyme surveys of congeneric waterfowl, sparrows, and warblers. Mack et al. (1986) reported a large number of mtDNA restriction site differences between two *Parus* species (*atricapillus* and *carolinensis*) that were indistinguishable in a survey of 35 allozyme loci (Braun and Robbins 1986). Apparently, a large number of readily

TABLE 3. Summary of genetic differences between avian sibling taxa.

Taxon pair	Allozymes				MtDNA				
		Allele freq. differences					No. fixed differences		
	No. loci	Significant[a]	Fixed	Genetic distance (*D*)	No. endonucleases	Mean no. restriction sites per ind.	Fragment profiles	Restriction sites[b]	Genetic distance (*p*)
Rallus elegans vs. *R. longirostris*	38	1	0	0.004	19	68	4	5	0.006
Limnodromus scolopaceus vs. *L. griseus*	36	3	2	0.060	19	77	14	24	0.082
Quiscalus major vs. *Q. mexicanus*	38	0	0	0.001	19	80	6	10	0.016
Parus bicolor bicolor vs. *P. b. atricristatus*	36	5	0	0.063	19	83	4	5	0.004

[a] $P \leq 0.05$.

[b] Conservative estimate, assuming that fragment profile differences not due to a single restriction site gain/loss are due to only 2 such changes.

TABLE 4. Rank ordering of genetic distances (p, base substitutions per nucleotide) in mtDNA reported for comparisons between avian congeneric taxa. Taxa assayed in the present study are in bold. Published estimates of protein divergence are in righthand column.

Taxa	No. pairwise species comparisons	Mean no. of: Restriction endonucleases	Mean no. of: Fragments or sites scored per individual	mtDNA genetic distance (p)	mtDNA reference	Protein genetic distance[1] (D)
Parus	2	14	39	0.09	Mack et al., 1986	—
Anas	5	15	54	0.083–0.088	Kessler and Avise, 1984	0.118–0.183
Limnodromus scolopaceus* vs. *L. griseus	**1**	**19**	**77**	**0.082**	**present study**	**0.060**
Branta canadensis vs. *B. bernicla*	1	14	75	0.061	Shields and Wilson, 1987a	—
Parus atricapillus vs. *P. carolinensis*	1	14	39	0.04	Mack et al., 1986	0.001
Anas	29	15	54	0.024–0.081	Kessler and Avise, 1984	0.002–0.186
Dendroica	10	17	71	0.031–0.055	Kessler and Avise, 1985	0.000–0.032
Aythya	6	15	54	0.025–0.043	Kessler and Avise, 1984	0.004–0.055
Melospiza	3	18	59	0.026–0.030	Kessler and Avise, 1985	0.028
Anser	2	14	75	0.027–0.028	Shields and Wilson, 1987a	0.001–0.003
Platycercus	15	6	≈156	0.017–0.064	Ovenden et al., 1987	≈0.040
Quiscalus major* vs. *Q. mexicanus	**1**	**19**	**80**	**0.016**	**present study**	**0.001**
Anser rossi vs. *A. caerulescens*	1	14	75	0.008	Shields and Wilson, 1987a	0.001
Anas fulvigula vs. *A. platyrhynchos*	1	15	54	0.007	Kessler and Avise, 1984	0.011
Rallus elegans* vs. *R. longirostris	**1**	**19**	**68**	**0.006**	**present study**	**0.004**
Parus bicolor bicolor* vs. *P.b. atricristatus	**1**	**19**	**83**	**0.004**	**present study**	**0.063**
Anas discors vs. *A. cyanoptera*	1	15	54	0.004	Kessler and Avise, 1984	0.061

[1] Based on multilocus protein-electrophoretic comparisons, and taken from citations which can be found in the appropriate mtDNA references. All protein distances involved Nei's (1972 or 1978) D measures.

assayable mtDNA markers may distinguish samples of even closely related avian taxa. Use of mtDNA genotypes should aid the study of gene flow, biogeography, and phylogeny on a microevolutionary scale previously beyond the reach of protein electrophoresis.

In vertebrates, mtDNA is a non-recombining, maternally transmitted molecule, and the multiple genetic markers along its length are tightly linked. In a diagnostic sense, this implies some redundancy of information. Unlike allozyme markers which are typically encoded by unlinked nuclear loci with separate evolutionary histories through male and female ancestors, mtDNA markers record historical events occurring in one "supergene" transmitted along a matriarchal component of an organismal phylogeny (Avise 1986b, Avise et al. 1987, Wilson et al. 1985). Nonetheless, there remains a diagnostic significance to the observation of numerous mtDNA genetic differences between taxa. Specifically, the likelihood of misclassification of a specimen due to convergent evolutionary changes at many restriction sites is minimal.

Evolutionary implications.—The species status of closely related taxa cannot be determined unequivocally solely by reference to some arbitrary standard of genetic divergence, but information on genetic distance can contribute to systematic inference. The allozyme literature for avian taxa has been reviewed elsewhere (Barrowclough and Corbin 1978, Avise and Aquadro 1982, Avise 1983, Barrowclough 1983, Barrowclough et al. 1985). Suffice it to say that the protein distances between *Limnodromus scolopaceus* vs. *L. griseus* ($D = 0.060$) and *Parus bicolor bicolor* vs. *P. b. atricristatus* ($D = 0.063$), although small, are of a magnitude frequently observed between some avian taxa whose status as species has not been subject to debate. This led Braun et al. (1984) to suggest that species status for the 2 forms of *Parus bicolor* should be investigated through studies of genetic introgression near their contact zone. On the other hand, the available allozyme data provide no confirmation of genetic differences between *Rallus elegans* vs. *R. longirostris* or *Quiscalus major* vs. *Q. mexicanus*.

Fewer data are available on mtDNA differences in birds. We have listed (Table 4) by rank order, mtDNA genetic distances reported previously between avian congeners. Values range from a low of 0.004 between *Anas discors* and *A. cyanoptera*, to a high of 0.09 between titmice and

TABLE 5. Rank ordering of *maximum* observed genetic distances (p, base substitutions per nucleotide) in mtDNA reported for comparisons between avian conspecific individuals (when two or more locales or subspecies were surveyed).

Species	No. of surveyed		Geographic scale	mtDNA *maximum* genetic distance	Reference
	Individuals	Locales			
Platycercus eximius	4	3	southern Australia	0.018	Ovenden et al., 1987
Platycercus elegans	8	4	eastern Australia	0.012	Ovenden et al., 1987
Branta canadensis	14	7	Alaska, California, North Dakota	0.011	Shields and Wilson, 1987b
Agelaius phoeniceus	127	19	continental USA, Canada, Mexico	0.008	Ball et al., 1988
Dendroica coronata	5	2	Texas and Georgia	0.005	Kessler and Avise, 1985
Platycercus adscitus	6	4	Queensland, Australia	0.005	Ovenden et al., 1987
Platycercus caledonicus	3	2	Tasmania	0.005	Ovenden et al., 1987
Parus major	18	3	Sweden	0.005	Tegelstrom, 1987
Melospiza georgiana	3	2	Texas and Georgia	0.000	Kessler and Avise, 1985

chicadees *Parus bicolor* vs. *P. atricapillus*, and *P. bicolor* vs. *P. carolinensis*. The distance between *Limnodromus scolopaceus* and *L. griseus* ($p = 0.082$) falls near the higher values. Based on mtDNA, the Long-billed and Short-billed dowitchers appear more divergent from each other than any of 5 species of *Dendroica* warblers, 3 species of *Melospiza* sparrows, 4 species of *Aythya* diving ducks, 6 species of *Platycercus* rosellas, or 31 of 36 comparisons among 9 species of *Anas* puddle ducks (Table 4). By these criteria, the current recognition of the dowitchers as distinct species appears appropriate. If mtDNA in dowitchers has evolved at the rate suggested for other vertebrates (Brown et al. 1979), including birds (Shields and Wilson 1987a), *L. scolopaceus* separated from *L. griseus* about 4 million years ago.

By contrast, mtDNA divergence between the pairs of rails and titmice (and, to a lesser extent, grackles) falls toward the lower end of the scale for avian congeners (Table 4). Other avian species that exhibit a comparable divergence all involve taxonomic pairs traditionally thought to be extremely closely related: Ross' and Snow geese; Mallard and Mottled ducks; and Blue-winged and Cinnamon teals.

Throughout this paper, we claim only that the genetic distances reported strictly apply to the particular samples assayed and not necessarily to the entire taxonomic population from which they were drawn. Particularly in the range of smaller mtDNA distances, the issue of intraspecific mtDNA divergence cannot be ignored. Unfortunately, few data are available on mtDNA geographic variation within avian species (Table 5). Only one species, the Red-winged Blackbird (*Agelaius phoeniceus*), has been sampled extensively. The maximum distance between mtDNA genotypes ($p_m = 0.008$) was slightly greater than the mean distance between our samples of *R. elegans* and *R. longirostris* or *P. bicolor bicolor* and *P. b. atricristatus*. The largest reported mtDNA distance within an avian species ($p_m = 0.018$ in *Platycercus eximius* [Table 5]) is slightly greater than that between *Q. major* and *Q. mexicanus*. It also exceeds observed distances between several avian "species" pairs (Table 4). The resolution of conventional mtDNA assay methodology extends to some of the lowest levels of the avian taxonomic hierarchy.

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100 Years Ago in The Auk

From "Great Auk Notes" by Frederic A. Lucas
(1888, Auk 5: 278-283):

"I cannot close this paper without referring to the published figures of the Great Auk, for this bird has suffered grievously at the hands of nearly every artist (Audubon is an exception) who has been called upon to portray it.

"It is true that the artists may plead extenuating circumstances in the shape of the stuffed—it were flattery to say mounted—specimens that have served as models, most of which are from two to eight inches longer than they should be. I trust Mr. Hancock will pardon me for including his figure in this criticism, for his bird is too long, too slender, and with too pronounced a crop.

"Artists have evidently recognized the fact that the stuffed Auks are too slender, and endeavored to make amends for the shortcomings of the taxidermist, for obesity is the general trouble with figures of the Great Auk, although the neck is usually as much too thin as the body is too stout. Could the bird have seen himself portrayed as he is even on the cover of his namesake, he might, like Wolfe, have exclaimed, 'Now I die content.'

"The question might naturally be asked what right has one who never saw the Great Auk alive to criticise him dead, and the answer is this, having just compared three mounted skeletons with one of the Razorbill: the conclusion is unavoidable that the two species resembled one another very closely in outward contour.

"As for internal structure, I must plead guilty to a belief that the two species should be included in the genus *Alca*, and with this bit of cis-Atlantic heresy bring these notes to a close."