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PATTERNS OF MITOCHONDRIAL DNA DIVERGENCE IN NORTH AMERICAN CRESTED TITMICE[1]

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Abstract. This survey of patterns of mitochondrial DNA variation in the Tufted Titmouse (*Parus bicolor*), Plain Titmouse (*P. inornatus*), and Bridled Titmouse (*P. wollweberi*) provides a critical assessment of genetic divergence in a well-defined taxonomic hierarchy of passerine birds. Maximum divergence between coexisting haplotypes was 0.16% and no more than two haplotypes were found in a local population sample. Divergence distances between taxa were calculated from matrices of 191 shared restriction fragments and 128 shared restriction sites. Distances calculated from proportions of shared sites tended to be higher than distances calculated from shared fragments.

Divergence distances between species suggest origins in the Pliocene (*wollweberi* vs. *bicolor* and *inornatus*) and in the early Pleistocene (*bicolor* vs. *inornatus*). Comparisons of allopatric, conspecific populations confirm the low divergence value reported by Avise and Zink (1988) for *P. b. bicolor* versus *P. b. atricristatus*. Our calculated distance (0.006) for these two semispecies was the same as the distance between *P. w. wollweberi* versus *P. w. phillipsi*. In contrast, coastal *inornatus* (*transpositus*) exhibit species level divergence ($p = 0.05$) from interior *inornatus* (*ridgwayi*). This result supports the conclusions of other recent studies that suggest a long historical separation of coastal California populations from sister taxa in the Great Basin.

Key words: Parus; *titmouse; genetics; mtDNA; speciation; taxonomy; distance analysis.*

INTRODUCTION

Our growing library of mtDNA divergence distances between bird species provides a new source of comparative data pertinent to research on species taxonomy, timing of past isolation events, changes in nuclear versus mitochondrial DNA genomes, and reticulate evolution. Recent comparisons of North American bird populations, for example, reveal the initial patterns of genetic divergence including unprecedented clues to biogeographic history (Avise 1989, Avise et al. 1990, Zink 1991, Zink and Dittmann 1991, Quinn et al. 1991). The utility or value of published divergence distances, however, depends on a critical understanding of their limitations and especially on improved biochemical and analytical standardization of the comparisons.

This paper is one of a planned series on the evolutionary relationships of taxa included in the genus *Parus*. The two previous papers concerned relationships of subgenera based on analyses of allozymes (Gill et al. 1989) and DNA × DNA hybridization data (Sheldon et al. 1991). To set the stage for future analyses of evolutionary relationships among species of *Parus* based on restriction fragment and site analyses of mitochondrial DNA, we examine here divergence distances and relationships among a limited set of taxa, the North American crested titmice. Specifically, we assessed mtDNA divergence and variation within and between populations of *P. bicolor* (Tufted Titmouse, Black-crested Titmouse), *P. inornatus* (Plain Titmouse), and *P. wollweberi* (Bridled Titmouse). These constitute a well-defined lineage of parids in which *wollweberi* positions as the sister species of the *bicolor-inornatus* species group, i.e., the subgenus *Baeolophus* (Gill et al. 1989, Sheldon et al. 1991). These taxa were chosen to provide a clearly defined taxonomic hierarchy that included three pairs of morphologically-defined subspecies or semispecies, a pair of unambiguous sister species (*bicolor* and *inornatus*), and a divergent outgroup species (*wollweberi*). Furthermore, we wished to compare the results of different analytical options in this controlled taxonomic setting. We wished particularly to compare calculations of divergence distances based on shared restriction fragments versus shared restriction sites inferred from fragment profiles.

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TABLE 1. Mitochondrial DNA variation within populations of North American crested titmice (*Parus*).

Taxon	*n*	Sites		Haplotypes		Diversity	
		RS	RS/Ind	No.	$p(\times 10^{-2})$	Geno.[1]	Nucl.[2]
bicolor							
bicolor (PA)	10	47	46.1	2	0.16	0.19 (0.11)	1.6 (2.7)
bicolor (LA)	9	46	46.0	1	0	0	0
bicolor (ALL)	19	47	46.0	2	0.16	0.10 (0.06)	0.85 (1.6)
atricristatus	8	44	44.0	1	0	0	0
inornatus							
ridgwayi	10	54	54.0	1	0	0	0
transpositus	7	55	54.6	2	0.14	0.53 (0.06)	4.0 (1.9)
wollweberi							
phillipsi	9	45	45.0	1	0	0	0

[1] Unbiased estimates of genotype diversity (see Nei 1987, formula 8.4; standard error from formula 8.12).
[2] Nucleotide diversity ($\times 10^{-4}$) calculated as: $(n/n - 1)\ \Sigma p_{ij} f_i f_j$; where n is the number of different haplotype sequences, p_{ij} is the estimated mean number of base substitutions per nucleotide site between mtDNA genotypes i and j, standard error from Nei (1987) formula 10.7.

METHODS

We surveyed restriction site variability based on RFLP analysis of populations of *P. b. bicolor* (PA—Berks and Delaware Counties, LA—Iberville Parish), *P. b. atricristatus (sennetti)* (TX—Kinney County), *P. inornatus ridgwayi* (NM—Bernalillo County), *P. i. transpositus* (CA—Los Angeles County), *P. w. wollweberi* (MX—Michoacán), and *P. w. phillipsi* (AZ—Cochise County). Sample sizes are indicated in Table 1, except for *P. w. wollweberi* of which only two individuals were examined. Voucher specimens are preserved in the collections of the Academy of Natural Sciences of Philadelphia.

Tissues (liver, heart, pectoral muscle) were preserved on dry ice or in liquid nitrogen in the field for transfer to ultracold (−80°C) laboratory storage. We extracted mtDNA by homogenizing the tissues in cold STE buffer (0.25 M sucrose, 0.03 M Tris, 0.1 M EDTA), spinning the homogenate at 3,000 rpm (700 g) for 5 min to remove large fragments including nuclei, and respinning the supernatant at 12,000 rpm (11,220 g) for 20 min to pellet the mitochondria. We further isolated the mitochondria on a 0.9/1.8 M sucrose step gradient in the ultracentrifuge at 26,000 rpm for 1 hr. We repelleted the mitochondria in KTE (0.55 M KCl, 0.03 M Tris, 0.01 M EDTA), lysed the mitochondria with 60 μl of 10% SDS, precipitated protein with 5 M KAc, and then purified the released mtDNA with two phenol and chloroform extractions, precipitated the DNA in 95% ethanol at −80°C, washed the pellet in 70% ethanol, and resuspended the vacuum dried mtDNA in 100 μl of 0.1 M TE (0.001 M Tris, 0.0001 M EDTA).

We cut 20–40 ng aliquots of purified mtDNA for 4–6 hr with 5- and 6-base restriction endonucleases (3–5 units/digest) using reaction conditions and buffers specified by the manufacturer. Fragments were separated on 1% agarose gels in TBE buffer (0.1 M Tris, 0.002 M EDTA, 0.1 M boric acid) and visualized by endlabelling with ^{32}P using DNA polymerase (Klenow fragment). We estimated fragment and genome sizes by direct comparison to the mobilities of known size standards (1 Kb DNA ladder and high molecular weight ladders [BRL]). Our gels did not reliably resolve fragments smaller than 300 bp. In the surveys of variability within populations we used 12–15 restriction enzymes which located 45–55 restriction sites per taxon. In the broader comparison of populations and species we used 19 enzymes which produced 191 restriction fragments by cutting a minimum of 128 restriction sites. The 19 enzymes were *Apa I, Ava I, BamH I, Bgl II, BstE II, Cla I, EcoR I, EcoR V, Hae II, Hinc II, Hind III, Hpa I, Nci I, Nde I, Pvu II, Sal I, Sph I, Sst I,* and *Sst II.*

We converted shared fragment matrices to estimates of nucleotide divergence using Upholt's (1977) formula 6b: $p = 1 - [1/2(-F + (F^2 + 8F)^{1/2})]^{1/n}$, where F is the proportion of shared fragments, or $2F_{xy}/(F_x + F_y)$, and n specifies the

TABLE 2. MtDNA divergence between populations of three species of North American crested titmice (*Parus*): *P. bicolor, P. inornatus,* and *P. wollweberi.* Values in upper half of matrix are estimates of nucleotide sequence divergence (p) based on shared restriction fragments. Values in the lower half of the matrix are estimates of nucleotide sequence divergence (p) based on shared restriction sites (S) inferred from the fragment profiles.

	1	2	3	4	5	6
1. *P. b. bicolor*	0.0000	0.0057	0.0544	0.0522	0.0839	0.0948
2. *P. b. atricristatus*	0.0055	0.0000	0.0563	0.0568	0.0837	0.0901
3. *P. i. transpositus*	0.0709	0.0749	0.0000	0.0428	0.0893	0.0915
4. *P. i. ridgwayi*	0.0776	0.0859	0.0502	0.0000	0.0844	0.0895
5. *P. w. phillipsi*	0.0954	0.1011	0.1114	0.1039	0.0000	0.0059
6. *P. w. wollweberi*	0.1008	0.1065	0.1167	0.1092	0.0056	0.0000

number of base pairs in each category (e.g., 5- versus 6-base pair) of restriction sites. This formula assumes that $F = S^2/(2-S)$. Many studies have used equations 20 and 21 from Nei and Li (1979) as their stated basis for the calculation of distances. These two equations yield essentially the same result as Upholt's (Kaplan 1983), but Upholt's formula is analytically simpler. Restriction site changes responsible for the differences among fragment profiles were inferred without formal, double-digest mapping. The additive relationships of fragment lengths allowed us to infer the fewest sites responsible for the combined fragment profiles of compared taxa, the spatial relations of sites to one another on the mtDNA genome, and their presence or absence in each taxon. Partials, or bands resulting from incomplete digests, aided the definition of sites. We could not define the absolute positions of restriction sites in the mtDNA genomes by this procedure. We converted matrices of inferred shared sites to estimates of nucleotide divergence using Upholt's (1977) formula (1c): $p = -\ln S/n$, which underestimates divergence when base substitutions are not random, i.e., when base substitutions are biased towards the third positions of codons.

RESULTS

The mtDNA genomes of these titmice were 16.5–16.7 kb in size based on direct comparisons of linearized mtDNA with comigrating size standards and from the sums of moderate size fragments. This genome size may be typical of passerine birds (Shields and Helm-Bychowski 1988). We detected no heteroplasmy nor any length variations due to insertions or deletions.

Genotype and nucleotide variations within populations were low (Table 1). Local samples of six or more individuals revealed a maximum of two haplotypes, fewer than the mode of five (range 3–8) reported for Red-winged Blackbirds (Ball et al. 1987). We found no variant haplotypes in our samples of four populations (*P. b. bicolor* (LA), *P. b. atricristatus, P. i. ridgwayi, P. w. phillipsi*). One of nine *P. b. bicolor* from eastern Pennsylvania differed by a single (*Hind* III) site gain. Our sample of *P. i. transpositus* from southern California, however, divided (3/4) into two haplotypes which differed by a single (*Bgl* II) site gain. The unbiased estimate of genotype diversity in this sample was moderately high (0.527), but nucleotide diversity remained low (7×10^{-4}) because only a single site difference was involved. Maximum divergence (p) between coexisting haplotypes was 0.16%.

MtDNA genetic divergence in the taxonomic hierarchy of North American titmouse populations ranged from 0.6% for comparisons of subspecies (*bicolor* and *wollweberi*) to 10–11% for interspecific comparisons involving *P. wollweberi* (Table 2). The values we obtained for titmice span the range described for *Anser* and *Branta* geese (Shields and Wilson 1987a, 1987b; Wagner and Baker 1990) and conform to published estimates of mtDNA divergence between birds of different taxonomic rank (Kessler and Avise 1985, Shields and Helm-Bychowski 1988, Tegelström and Gelter 1990).

Little mtDNA divergence was evident between populations of *P. bicolor,* which includes a morphologically uniform subspecies (*P. b. bicolor*) in the southeastern U.S. and morphologically variable populations of the semispecies *P. b. atricristatus.* We found no significant differences between our samples of *P. b. bicolor* from Pennsylvania and Louisiana. Divergence between *P. b. bicolor* and *P. b. atricristatus* was slight ($p = 0.006$), reflecting differences at only three of 128 sites. This result is close to the dis-

tance $p = 0.004$ reported by Avise and Zink (1988) who used a slightly different array of restriction enzymes. The two *wollweberi* subspecies exhibited a similar level of divergence ($p = 0.006$). In contrast, divergence between the coastal and interior subspecies of *P. inornatus* was pronounced, e.g., $p(F) = 0.043$, $p(S) = 0.050$, and only slightly less than our estimate of divergence between the species *bicolor* and *inornatus*, e.g., $p(F) = 0.052–0.057$, $p(S) = 0.071–0.086$. *P. wollweberi* differed substantially from the other species of North American titmice, e.g., $p(F) = 0.084–0.095$, $p(S) = 0.101–0.117$.

Across taxa, the total number of inferred sites will be less than the total number of observed fragments because some site gains produce two new fragments. Tallies of shared sites, however, were consistently lower than expected from Upholt's (1977) assumed relationship $F = S^2/(2 - S)$, some substantially so (Fig. 1). For example, *P. i. ridgwayi* and *P. b. bicolor* shared 29 of their combined total 134 fragments ($F = 0.43$) but they shared only 43 of their combined total of 134 sites ($S = 0.64$); the Upholt formula projects 50 shared sites. Accordingly, the distances based on shared sites were higher than those based on shared fragments (Table 2). The analysis of fragment profiles revealed six cases of fragment size convergence, i.e., doublets, which were distinguished in the analyses. Absolute mapping of all sites with double digest procedures might reveal a few more cases of such convergence and thereby reduce slightly the tally of shared fragments; it also would reduce further the proportions of shared sites. The slight potential error here could not explain the observed difference in distances based on sites versus fragments.

Distance (PHYLIP-Kitsch, Fitch) and character (PAUP-Wagner parsimony, PHYLIP-Dollo parsimony) analyses of genetic relationships produced the same, unambiguous arrangement of taxa in which conspecific populations paired with each other and in which *wollweberi* was the sister species of *bicolor* and *inornatus* (Fig. 2). This arrangement matches both classical intuition based on morphology and a more-limited distance Wagner analysis of allozyme data (Gill et al. 1989). In the distance analyses, site data produce slightly higher sums of squares than did fragments, namely 0.00028 vs. 0.00008 in FITCH and 0.001 vs. 0.000 in KITSCH. Statistical comparison of FITCH vs. KITSCH trees revealed no significant differences between lineages in their relative rates of mtDNA divergence ($P < 0.025$). Site and fragment data produced single most parsimonious trees with nearly identical consistency indices (0.93, 0.90) in the PAUP Wagner parsimony analysis. Corresponding to the large distance between them, the two races of *inornatus* exhibited about five times more autapomorphies (34, 34 fragments; 16, 18 sites) than the corresponding races of *bicolor* (5, 7 fragments; 1, 3 sites) and *wollweberi* (4, 8 fragments; 0, 4 sites). Dollo parsimony allowed 40/191 (21%) fragment character reversals versus 21/128 (16%) site character reversals.

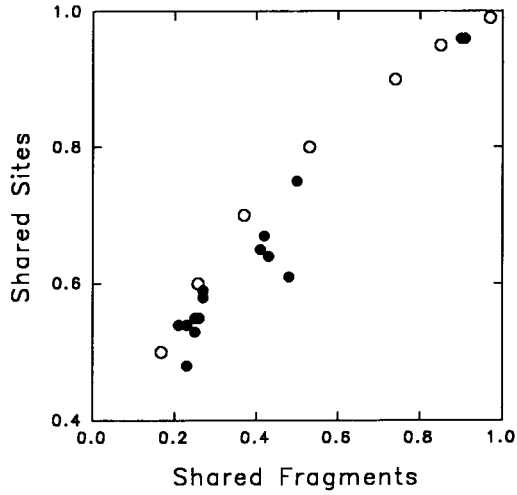

FIGURE 1. Relationship of observed proportions of shared sites to shared fragments for six taxa of titmice (*Parus*). Unfilled circles indicate relationship predicted by Upholt's (1977) formula $F = S^2/(2 - S)$.

DISCUSSION

EVOLUTION AND BIOGEOGRAPHY OF NORTH AMERICAN CRESTED TITMICE

Increasingly available for comparison are molecular data for sets of passerine bird species whose geographical distributions lie in the southern unglaciated portions of continental North America. If the prevailing estimate of 2% sequence divergence per million years is roughly correct (Shields and Wilson 1987a), the substantial divergence distances between the three species of North American crested titmice suggest origins in Pliocene (*wollweberi* versus *bicolor* and *inornatus*) and the early Pleistocene (*bicolor* versus *inornatus*). Our interspecific divergence distances for titmice were similar to those obtained

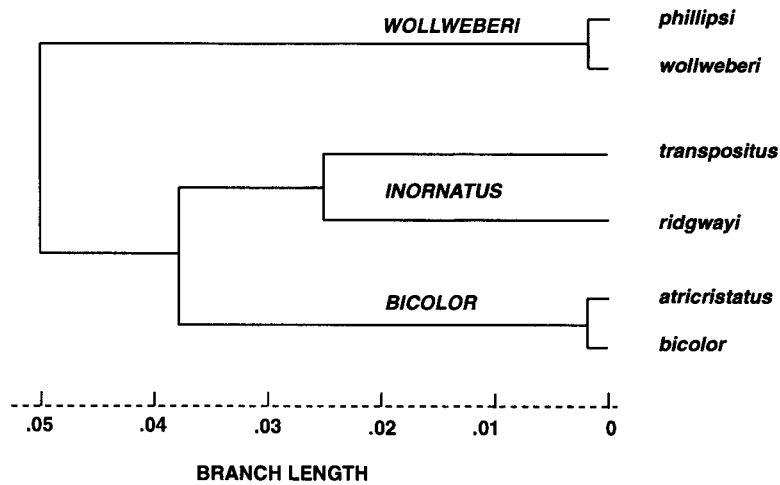

FIGURE 2. KITSCH phenogram of distances among taxa of North American titmice based on 128 restriction sites. Note that branch lengths must be doubled to obtain the estimates of nucleotide divergence between taxa presented in Table 2. 123 trees were examined. Sum of squares = 0.001; exponent p was set to 0 to render the algorithm equivalent to UPGMA clustering techniques. A cladistic analysis of shared restriction sites results in a single most parsimonious, midpoint-rooted tree (length 106) with the same topology and a consistency index of 0.934.

by Zink and Dittmann (1991) for the brown towhee species complex of the southwestern U.S. and Mexico, but were lower than those obtained by Zink et al. (1991a) for species of *Zonotrichia* of more northern latitudes. The distributions of the southern latitude species (titmice and towhees) presumably have been more stable during the glacial cycles of the Pleistocene than those of other sets of species which now inhabit large expanses of the northern part of the continent. However, fewer clones were present and genotype diversity was lower in these population samples of titmice than in Red-winged Blackbirds (Ball et al. 1987), Song Sparrows and Fox Sparrows (Zink 1991) and Common Grackles (Zink et al. 1991b). Significant polymorphism was limited to two similar haplotypes in our sample of *P. i. transpositus* from southern California. The prevalence of few haplotypes and thus of low genotype diversity suggests that the titmouse populations have been subject to stringent sorting of maternal lineages, probably during severe periodic historical bottlenecks (Avise et al. 1987). In apparent contrast, Tegelström (1987) found 13 different maternal lineages (haplotypes) in a sample of 18 *Parus major* from three neighboring localities in Sweden. Although not directly comparable to ours because it was based on the use of restriction enzymes that recognize 4-base pair sites, Tegelström's study suggests potentially interesting variability either at a different level of resolution or in other lineages of *Parus*.

Perhaps the most surprising result of this study was the high divergence between two subspecies of *inornatus* in contrast to the low divergence between subspecies of *bicolor* and *wollweberi*. We examined one population each of two decidedly different subspecies of *P. inornatus* (*transpositus, ridgwayi*) recognized by the American Ornithologists' Union (1957). These subspecies differ in coloration and bill size. *P. i. transpositus* is morphologically similar to three other dark, sooty, large-billed Pacific coastal races of *P. inornatus* (*mohavensis, cineraceus, amabilis*) (Phillips 1986). None of these coastal races comes into contact with the paler gray interior race *ridgwayi*, which reaches southeastern California (Grinnell and Miller 1944). Separated by the Mojave desert habitats, coastal *transpositus* and interior *ridgwayi* apparently have diverged from a common ancestor for at least two million years. These two allopatric populations exhibit a 5% mtDNA sequence divergence similar to that of congeneric species (Shields and Helm-Bychowski 1988). This result is consistent with past recognition and recent reaffirmation of biogeographic distinctions

between sister taxa on opposite sides of the Sierra Nevada mountains (Miller 1956, Johnson 1978, Zink 1991). Further study of morphology, vocalizations, and allozymes will likely provide a basis for separating interior and coastal *inornatus* as distinct species.

P. b. bicolor and *P. b. atricristatus* are hybridizing semispecies (Mayr and Short 1970, Braun et al. 1984, American Ornithologists' Union 1983) which differ less in their mtDNA genomes than do some variant haplotypes in populations of Red-winged Blackbirds (Ball et al. 1987) and Canada Geese (Wagner and Baker 1990). Our estimate of divergence ($p = 0.006$) between this pair of taxa corresponds closely to that reported by Avise and Zink (1988), who used 15 informative enzymes, six of which differed from our selection; two of the six recognized 4-base pair sites. In support of Dixon's (1978) hypothesis of separation during the late Pleistocene, this level of mtDNA divergence suggests that *bicolor* and *atricristatus* have been isolated for about 250,000 years. Curiously, these two titmice exhibit substantial protein (allozyme) divergence ($D = 0.06$, Braun et al. 1984) in conflict with the general rule that mtDNA distances usually exceed protein distances (Mack et al. 1986, Avise and Zink 1988). Possibly *atricristatus* and *bicolor* actually are older taxa which exchanged mtDNA genomes through hybridization events in the late Pleistocene. Current hybridization between the two results in some mtDNA gene flow across the hybrid zone in eastern Texas (Braun, pers. comm.). The mtDNA of the southernmost Mexican populations of *atricristatus,* e.g., "*atricristatus*" from San Luis Potosí, would be pertinent because this population may not have been influenced by past hybridization events to the north.

ESTIMATING DIVERGENCE DISTANCES

Overall the shared fragment and shared distance data gave closely corresponding results, both in the calculation of divergence distances and in the parsimony analyses of phylogenetic relationships. However, better standardization than is now evident in the ornithological literature is needed for critical comparison of distances from different studies and for the use of distance data for reconstructions of biogeographical history. Past RFLP analyses of birds have calculated distances based primarily on shared fragment data (Kessler and Avise 1984; Mack et al. 1986; Ovenden et al. 1987; Shields and Wilson 1987a, 1987b; Tegelström 1987; Tegelström and Gelter 1990; Wagner and Baker 1990; Zink 1991; Zink and Avise 1990; Zink and Dittman 1991; Zink et al. 1991b), and less frequently on shared inferred sites (Avise et al. 1990, Zink et al. 1991a) or both (Avise and Zink 1988, Avise and Nelson 1989). The prevailing use of shared fragment data is based on (few) published demonstrations of the validity of this method vis-a-vis mapped sites (e.g., for mammals, Ferris et al. 1983) with the recognition that use of fragments tends to underestimate distances when divergence levels are greater than 5% (Shields and Wilson 1987a). Theoretically, distances calculated from site data have greater reliability to divergences over 20% (Nei 1987). Discrepancies between fragment- and site-based distances will affect estimates of the timing of biogeographical events. For example, the divergence distances we calculated for *P. b. bicolor* versus *P. i. ridgwayi,* namely 5% (fragment) and 7% (site), extrapolate to a common ancestor 2.5 mya ago versus 3.5 mya. The differences between fragment versus site distance data do not stem simply from the site inference procedures, which economize the number of restriction sites and thereby maximize the tally of shared sites relative to the tally of shared fragments. Occasional errors in site inference will not severely affect the distance calculations when the total number of sites and fragments scored is over 100 as in this study. Instead, the problems appear to lie deeper in the theoretical foundations for estimating divergence distances from restriction enzyme data. Of particular concern are the expected Poisson distributions used by Upholt (1977) and Nei and Li (1979) in their models. We are now investigating how non-Poisson patterns of restriction site changes in the mtDNA genome affect the expected proportions of shared fragments versus shared sites.

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APPENDIX. Character states for mtDNA restriction fragments and inferred restriction sites of six taxa of North American crested titmice. The number of base pairs/restriction site are indicated at the top of each set of data.

I. RESTRICTION FRAGMENT CHARACTERS (191)

6666666666666666655555555555555666666666666666666
6666666666666666666666666666666666666666555555555555555555555556666
6666666666666666555555555555555556666666666666666666666666666666666
66666666666

P. bicolor

BICOLOR 00001100000101101111000011010000100000001111000000
00010100000110001100011000000000010010100011010000111000001011001
00000000010101111000100011100000000101010000010100000100011001111
00001010111

ATRICRISTATUS 00001100000101101111000011010000100000001111000000
00010001010110001100010100000001010010100011100000111000001001001
00000000011001101000100011100000000101010000010100000100011001111
00001010111

P. inornatus

TRANSPOSITUS 10000000000101000100111100100100010010000010010001
01111001100000100001000001101010000000000110000101110101000010000
01010000010101111000010100110000010001101100100110100101000001111
00001011001

RIDGWAYI 00100000101100001010101110000101000011100001010010
00010010000100001001000000111000000000000001100010011100110000001
01001101110101111000100000111001100110000100010100000100011001110
01101011001

P. wollweberi

PHILLIPSI 00010010100000010111100110000000000101001001000110
00010000000001000000101000000000001100010100001100110101010000100
10000011011010100010001000000110000001111000000101011010101110000
10010101001

WOLLWEBERI 00010010100000010111100110000000000101001001000110
00010000000001000000100010000100001001011100001001110101010100100
10000011011010100010001000000110000001101011000101011010101110000
10010101001

II. RESTRICTION SITE CHARACTERS (n = 128)

6666666655555555556666666666666666666666666666666
6666666666555555555555556666666666666655555555556666666666666666
6666666666

P. bicolor

BICOLOR 11010101111100011101100011100000100110100011000110
00000001110011100001110101100100001111111010011000110100101000101
1011110011111

ATRICRISTATUS 11010101111100011101100011100000101110100011000110
00000101110011000001110101100100001101111010011000110100101000101
1011110011111

P. inornatus

TRANSPOSITUS 10000101011111111000101000111101110110000010000100000
11110000000011100101110100100101001111111011101000111110101101100
0011110011101

RIDGWAYI 10101110011101110011010101110000110001000100100000
11100000000011101001110100101101111111111010101101010110101000101
1011110111101

P. wollweberi

PHILLIPSI 11100100111100110001011001100100100000010000001001
00000011100100111001101010110110001101011100001010111100111010111
1100011010101

WOLLWEBERI 11100100111100110001011001100100100000010000001001
00001011101100111011101010110110001101011100001010111101111010111
1100011010101