# Mitochondrial DNA Variation in the Northern Flicker (*Colaptes auratus*, Aves)[1]

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The northern flicker is a common woodpecker that inhabits open woodlands throughout North America. A narrow hybrid zone occurs along the range boundaries between the eastern yellow-shafted and western red-shafted subspecies. Mitochondrial DNA (mtDNA) was isolated from 201 flickers from 27 locales, primarily along two transects that cross the hybrid zone, one across the northern United States and one across the southern United States. Analysis of restriction-fragment patterns revealed substantial mtDNA polymorphism. Three restriction endonucleases—*Hin*dIII, *Pvu*II, and *Ava*I—together define eight haplotypes. Geographic variation in haplotype frequencies is highly significant. UPGMA analysis of haplotype frequencies reveals two major clusters of populations, a northern-and-eastern cluster and a southwestern cluster; 86% of the variance in haplotype frequency occurs between the two clusters. Geographic variation in haplotype frequencies is not clearly associated with the hybrid zone. This pattern of divergence may reflect a history of isolation of southwestern populations; alternatively, it is possible that the group of related southwestern haplotypes is adapted to hot, arid conditions common in the deserts of the American Southwest. The gilded flicker is a third subspecies that occurs in the Sonoran Desert. Gilded flickers from Arizona have an mtDNA haplotype closely related to the haplotypes observed in red-shafted flickers of the Southwest.

## Introduction

Species of birds often exhibit striking patterns of geographic differentiation in morphological traits, contrasted with low levels of differentiation in the frequencies of nuclear genes as detected by protein electrophoresis (Avise and Aquadro 1982; Barrowclough 1980*a*, 1983; Barrowclough et al. 1985; Van Wagner and Baker 1986; Zink and Remsen 1986; Corbin 1987; Grudzien et al. 1987; Haig and Oring 1988; Seutin and Simon 1988). Any number of factors may combine to determine this common avian pattern. Allozyme alleles may be adaptively neutral (Barrowclough 1980*b*; Barrowclough et al. 1985) whereas morphological traits may be selected, dispersal rates and hence gene flow may be high because birds fly (Zink and Remsen 1986; Corbin 1987; Grudzien et al. 1987; Karl et al. 1987; Moore and Dolbeer 1989), and it is possible that the avian species studied are relatively young and that sufficient time has not elapsed to permit evolution of geographic structure (Sibley and Ahlquist 1982; Zink 1982; Avise 1983). Recently, a few studies of mitochondrial DNA (mtDNA) variation in avian species have been reported (Mack et al. 1986; Shields

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and Wilson 1987*b;* Tegelstrom 1987; Ball et al. 1988). Although few in number and based on small sample sizes, these studies suggest that the extent of geographic variation in mtDNA varies among avian species and that patterns of mtDNA variation are not necessarily congruent with patterns of morphological or allozyme variation (see Discussion). Patterns of geographic variation in mtDNA could differ from those of nuclear genes for additional reasons. First, the mitochondrial genome is maternally inherited and haploid. Thus, effective population sizes for the mtDNA genome are one-fourth those of nuclear allozyme genes. Second, it is possible that there is differential dispersal between males and females. Third, with regard to nucleotide base substitution in vertebrates, mtDNA appears to evolve at a higher rate than does nuclear DNA—~5–10 times as fast (for a review, see Moritz et al. 1987). Thus, it is possible that geographic structure has evolved in relatively young populations with regard to mtDNA but not with regard to allozyme-coding nuclear genes. Fourth, mtDNA and nuclear allozyme phenotypes may respond to different selection patterns. In the present paper we describe geographic variation in mtDNA of the northern flicker (*Colaptes auratus*) across much of its range in the United States and compare these results with patterns of morphological (Short 1965; Moore and Buchanan 1985) and allozyme variation (Grudzien et al. 1987).

The northern flicker comprises three major subspecies groups—the yellow-shafted flicker, the red-shafted flicker, and the gilded flicker—in North America, north of Mexico, that differ in several plumage traits and in morphometric traits related to overall size (Short 1965, 1982). The plumage differences are so conspicuous that the three forms were considered distinct species through the fifth edition of the American Ornithologists' Union's (1957) *Check-list of North American Birds.* The specific distinction of the three groups was dropped in the sixth edition (American Ornithologists' Union 1983) primarily because hybrid zones exist where their largely allopatric ranges come in contact (Short 1965). The subspecific taxonomy of the northern flicker is presently unsettled. For simplicity, we follow Short (1965, 1982) and refer to all of the minor variant forms in the respective yellow-shafted, red-shafted, and gilded groups as subspecies *Colaptes auratus auratus, C. a. cafer,* and *C. a. chrysoides.*

In the present paper we are concerned primarily with the eastern yellow-shafted (*C. a. auratus*) and the western red-shafted (*C. a. cafer*) subspecies, which freely hybridize where their ranges come together on the western Great Plains and along the eastern edge of the Canadian Rocky Mountains. The approximate U.S. ranges of these two subspecies and the hybrid zone are shown in figure 1. (For a full North American–range description, see Short 1965; one subspecies or the other occurs in open woodlands throughout most of North America.) Although this hybrid zone extends the full length of the interface between the two subspecies, it is narrow and has been stable in historical times, with regard to both position and width (Moore and Buchanan 1985). Step clines formed by individual morphological traits that distinguish the two subspecies are more or less congruent (Short 1965). Hybridization in the contact zone is rampant; that is; one rarely sees a pure parental morph, mating in the hybrid zone is random (Moore 1987), and there is no evidence of reduced reproductive success in the hybrids (Moore and Koenig 1986). The origin of the hybrid zone is unclear and may be complex (i.e., different parts of the zone arose at different times); it is ancient, predating the arrival of European colonizers in North America, and probably arose as a result of geographical isolation and secondary contact during Pleistocene glacial and interglacial episodes (Moore and Buchanan 1985). With regard to geographic variation,

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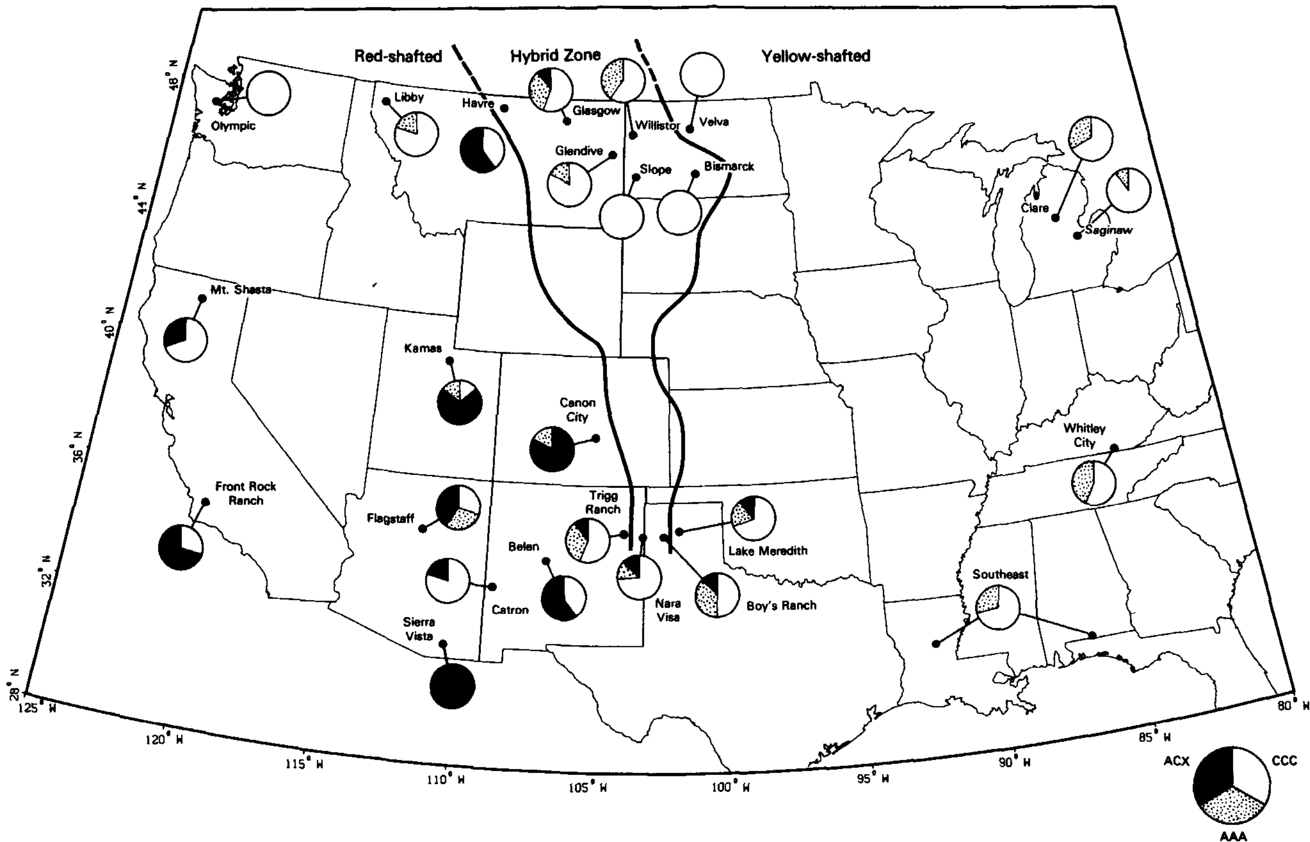


FIG. 1.—Geographic distribution of mtDNA haplotypes in red- and yellow-shafted flickers. The pie diagrams show the frequencies of haplotype groups I, II, and III at each locale. Sample sizes are given in table 2 and fig. 5. Group I comprises CCC and BCC (one individual at Libby, Mont.); group II comprises ACC, ACB, ACD, and ACE (collectively designated ACX); and group III comprises AAA and AAC (one individual at Lake Meredith, Tex.). The approximate geographic boundaries of the hybrid zone between red- and yellow-shafted flickers are indicated by the lines running north from the Texas Panhandle region; these boundaries represent the morphological transition from 20% to 80%.

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the hybrid zone highlights an abrupt and dramatic morphological transition in the architecture of the northern flicker species population.

The third subspecies, the gilded flicker (*C.a. chrysoides*), is largely restricted to the Sonoran Desert, where it excavates nest cavities in giant cacti. Its plumage is much like that of the red-shafted flicker, except that its shaft color is very similar to that of the yellow-shafted flicker (Short 1965). The gilded flicker is smaller than either the red- or yellow-shafted flicker and produces smaller clutches (Koenig 1984). The gilded flicker hybridizes with the red-shafted flicker, but hybridization is more limited than that between red- and yellow-shafted flickers (Short 1965). Hybrid populations between gilded and red-shafted flickers occur primarily along water courses flowing from montane conifer woodlands onto lowland deserts in Arizona (Short 1965). Although some data on mtDNA haplotypes in the gilded flicker will be presented in the present paper, the main focus is on geographic variation in the red- and yellow-shafted subspecies.

## Material and Methods

Yellow-shafted, red-shafted, or hybrid flickers were collected at 27 locales (fig. 1 and table 1). The compilations in figure 1 and in table 1 differ slightly in that the two samples from Covington Co., Ala. (two birds) and Grant Parish, La. (five birds) were pooled (mapped as Southeast), as were the two samples from Lake Meredith and Lake Marvin, Tex. (mapped as Lake Meredith). These samples were pooled because the locales are in close proximity and because one or both samples in each pair was small. The sample labeled "Clare, Mich." includes one specimen from Newaygo Co. and one from Benzie Co. Collections comprised only adults.

We believe that all, or nearly all, of the collected specimens were resident breeders. This belief is based on the following several facts: Examination of the U.S. Fish and Wildlife Service (USFWS) banding-recovery records show that the spring migratory period for flickers is fairly sharply delineated; most flickers stop moving by the end of the first week in April and are sedentary until late September, when fall migration begins in earnest (W. S. Moore, unpublished personal analysis). Our collections were made between May 5 and June 14, which is well within the period when flickers appear to be sedentary on breeding grounds. Second, we planned our collecting trips so that the southern collections were made earlier than the northern collections. Third, flickers were attracted by playback of recorded calls; territorial birds are expected to be most responsive to these. Finally, many of the birds were collected in clear association with nest cavities.

The collections were made in 1986, with the following exceptions: Clare and Saginaw, Mich. (1985) and Louisiana (1988). The bulk of the collections fall along either a northern transect (from Michigan to the Olympic Peninsula) or a southern transect (Alabama to southern California). The transects were sampled more intensely where they cross the hybrid zone. Additional samples were collected away from the transects to get a coarse resolution of any midlatitude transition (Whitley City, Ky.; Canon City, Colo.; Kamas, Utah; and Mt. Shasta, Calif.). Two collections of gilded flickers were made (not mapped in fig. 1): Santa Catalina Mountains, ~20 km ENE of the center of Tucson (14 birds; 10 collected in 1986 and four collected in 1988), and Picture Rocks, ~50 km WNW of the center of Tucson (five birds in 1988).

Flickers were collected with a shotgun. The liver, heart, and a piece of skeletal muscle were removed immediately from each specimen, enclosed in an airtight plastic bag, and placed on dry ice within 15 min of the time the bird was shot. Tissue samples were kept on dry ice until returned to the laboratory where they were stored in an

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**Table 1**
**Collecting Sites, Sample Sizes, and Distributions of mtDNA Haplotypes**

| Locale | Sample Size | Group I | | Group II | | | | Group III | |
|---|---|---|---|---|---|---|---|---|---|
| | | BCC | CCC | ACC | ACB | ACD | ACE | AAC | AAA |
| Kamas, Utah | 7 | | 1 | 3 | | 2 | | | 1 |
| Canon City, Colo. | 6 | | | | 2 | 2 | 1 | | 1 |
| Sierra Vista, Ariz. | 9 | | | | | 9 | | | |
| Belen, N.M. | 5 | | 2 | 3 | | | | | |
| Havre, Mont. | 5 | | 2 | 3 | | | | | |
| Front Rock Ranch, Calif. | 10 | | 3 | 2 | | | | | |
| | | | | 2 AC? (ACC–ACE) | | | | | |
| | | | | 3 A?C (ACC–AAC) | | | | | |
| Flagstaff, Ariz. | 10 | | 3 | 3 | 1 | | | | 3 |
| Lake Mererdith, Tex. | 13 | | 11 | | | 1 | | 1 | |
| Lake Marvin, Tex. | 3 | | | | 1 | | | | 2 |
| Saginaw, Mich. | 10 | | 9 | | | | | | 1 |
| Libby, Mont. | 10 | 1 | 7 | | | | | | 2 |
| Glendive, Mont. | 6 | | 5 | | | | | | 1 |
| Nara Visa, N.M. | 8 | | 6 | 1 | | | | | 1 |
| Olympic National Forest, Wash. | 7 | | 7 | | | | | | |
| Slope, N.D. | 5 | | 5 | | | | | | |
| Bismarck, N.D. | 7 | | 7 | | | | | | |
| Velva, N.D. | 6 | | 6 | | | | | | |
| Catron, N.M. | 5 | | 4 | 1 | | | | | |
| Mt. Shasta, Calif. | 10 | | 7 | 2 | 1 | | | | |
| Clare, Mich.[a] | 12 | | 8 | | | | | | 4 |
| Southeastern United States[b] | 7 | | 5 | | | | | | 2 |
| Whitley City, Ky. | 9 | | 5 | | | | | | 4 |
| Williston, N.D. | 5 | | 3 | | | | | | 2 |
| Glasgow, Mont. | 9 | | 5 | 1 | | | | | 3 |
| Trigg Ranch, N.M. | 9 | | 5 | 1 | | | | | 3 |
| Boy's Ranch, Tex. | 8 | | 4 | | | 1 | | | 3 |

[a] Includes one specimen from Benzie Co., one from Newaygo Co., and 10 from Clare Co.
[b] Includes two specimens from Alabama and two from Louisiana.

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ultracold freezer at −70°C. One shipment of specimens thawed in transit. This included the 10 gilded flickers collected in 1986 and the two California collections. mtDNA from these specimens proved difficult to isolate, purify, and resolve on gels, because of contamination with degraded DNA. Banding patterns could, nonetheless, be scored for the three diagnostic enzymes for all but two gilded flickers, one scored as AC? and one scored as A??, and five red-shafted flickers from Front Rock Ranch, Calif., two scored as AC? and three scored as A?C. (The nomenclature is as described below; here question marks denote character states that could not be scored.)

mtDNA was isolated by using the protocol summarized by Dowling et al. (1989) and based on earlier protocols described by Wright et al. (1983) and Densmore et al. (1985). The protocol uses CsCl centrifugation to separate mtDNA from nuclear DNA. Minor modifications of the Dowling et al. protocol were made to accommodate flicker tissues and as dictated by available equipment. In most isolations, 0.75 g of liver was homogenized for each flicker. This is roughly half of an adult liver. In a few cases a comparable amount of heart was used instead of liver. Other modifications are the use of a Beckman SW50.1 rotor and starting the centrifugation with a homogenous CsCl solution as in the method of Wright et al. (1983), as opposed to an initial step gradient as in the method of Dowling et al. (1989). Our centrifugation time was 48 h at 36,000 rpm, and single equilibrium centrifugations provided sufficiently "clean" mtDNA preparations.

The mtDNAs were digested by restriction endonucleases in buffers specified or supplied by the manufacturer. DNA fragments produced by the restriction digests were end-labeled with $^{32}P$ (Wright et al. 1983), separated on 1.2% horizontal agarose and 4.0% vertical acrylamide gels, and visualized by autoradiography (Brown 1980).

## Results

To obtain a preliminary estimate of geographic variation, the mitochondrial genomes of 13 red- and yellow-shafted flickers from four geographically disparate areas were surveyed with 14 restriction enzymes. Restriction enzymes with six-nucleotide-recognition sites were *Bam*HI, *Bcl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Pvu*II, *Sac*II, *Sal*I, and *Xba*I. Restriction enzymes with four-nucleotide-recognition sites were *Dde*I, *Hin*fI, and *Hpa*II. One restriction enzyme, *Ava*I, effectively has a five-nucleotide-recognition site. The locales and numbers of birds constituting the preliminary-survey sample are as follows: Michigan (four yellow-shafted flickers, a group which includes one specimen from Newaygo and three from Saginaw); Whitley City, Ky. (two yellow-shafted flickers); Flagstaff (three red-shafted flickers); Libby, Mont. (four red-shafted flickers, a group which includes one specimen from Olympic National Forest, Wash.). Eight of the enzymes—*Bam*HI, *Bcl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Sac*II, *Sal*I, and *Xba*I—revealed single unvaried fragment patterns for all 13 of the survey birds. All of the four-nucleotide-recognition-site enzymes revealed polymorphisms in the 13 survey specimens, as did *Ava*I, *Hin*dIII, and *Pvu*II.

The preliminary analysis suggested the existence of several haplotypes that have variable geographic distributions and are diagnosable by combinations of *Ava*I, *Hin*dIII, and *Pvu*II restriction patterns. Thus, the remaining 188 red- and yellow-shafted flickers from all locales were surveyed for *Ava*I, *Hin*dIII, and *Pvu*II variants. A subset of these was also surveyed for *Dde*I, *Hin*fI, and *Hpa*II variants, but this survey was not carried to completion because the number of restriction-fragment patterns was overwhelming and because little additional insight into patterns of geographic variation was apparent.

Fragment-size patterns are summarized in table 2 for each of the *Hin*dIII, *Pvu*II,

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**Table 2**
**Fragment Sizes for Variant Restriction-Enzyme Patterns**

| *Hind*III | | | | *Pvu*II | | | *Ava*I | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pattern | | | | Pattern | | | Pattern | | | | | |
| Size (kb) | C | A | B | Size (kb) | C | A | Size (kb) | C | A | B | D | E | F |
| 7.5 ..... | | + | | 9.0 ..... | + | + | 6.2 ..... | + | + | + | | | |
| 6.6 ..... | + | | + | 4.3 ..... | | + | 5.0 ..... | + | | + | + | + | + |
| 4.4 ..... | + | + | + | 3.4 ..... | + | | 3.6[a] ..... | | + | | + | + | + |
| 2.6 ..... | + | + | + | 2.5 ..... | + | + | 2.7 ..... | | | + | | | |
| 2.3 ..... | | | + | 1.3 ..... | + | + | 2.6[b] ..... | | | | + | + | |
| 2.0 ..... | + | + | + | 1.0 ..... | + | | 2.1 ..... | + | + | + | + | + | + |
| 1.3 ..... | + | + | | | | | 1.6 ..... | + | + | | + | + | + |
| 0.7 ..... | + | | | | | | 1.4 ..... | | + | | | | + |
| | | | | | | | 1.1 ..... | | | | | | + |
| | | | | | | | 1.0 ..... | + | + | | + | + | + |
| | | | | | | | 0.5 ..... | + | + | + | + | + | + |
| | | | | | | | 0.3 ..... | + | + | + | + | + | + |

NOTE.—A plus sign (+) indicates presence of fragment in pattern.
[a] Slightly larger in E pattern than in D pattern.
[b] Slightly smaller in E pattern than in D pattern.

and *Ava*I variants. The *Ava*I digests provided the best range of fragments for estimates of genome size; the average genome size, as based on the *Ava*I patterns listed in table 2, is 16.7 kb.

Mutational transformations between most restriction-enzyme patterns were readily discernible for all of the enzymes that revealed variant genomes (see table 2 and figs. 2 and 3). For example, the *Pvu*II-A pattern differs from the *Pvu*II-C pattern by the loss of a restriction site that cleaves a 4.3-kb fragment in the *Pvu*II-A pattern into 3.4- and 1.0-kb fragments in the *Pvu*II-C pattern. (The slight discrepancy in sums is probably due to imprecision in measuring migration distance on the electrophoretic gels.) Although the transformation networks become more complex as the number of fragments and patterns increase, the character transformations are no less obvious. With one exception, all of the transformations can be explained by the gain or loss of a single restriction site. The exception is the relationship between *Ava*I-E and either *Ava*I-D or *Ava*I-C. *Ava*I-E is nearly identical to *Ava*I-D, but the fragments designated 3.6 kb and 2.6 kb are slightly larger and slightly smaller, respectively, in the *Ava*I-E pattern than in the *Ava*I-D pattern (table 2 and fig. 2). Although the two patterns appear nearly identical, derivation of *Ava*I-E from *Ava*I-D (or vice versa) would require either at least one gain and one loss of restriction sites or another event, such as an asymmetrical inversion around a restriction site. Other possibilities exist. For example, the *Ava*I-E pattern could have been derived independently from the *Ava*I-C pattern (or vice versa), but, again, this requires a complex explanation, such as either a gain and a loss of restriction sites or the existence of a small fragment that we did not resolve on our electrophoretic gels. In any case, the details of the transformation among these three *Ava*I character states is unimportant to the analysis of geographic variation presented here. What is important is that these two phylogenetically unresolved *Ava*I character states occur in a cluster of related haplotypes united by identical *Hind*III and *Pvu*II character states.

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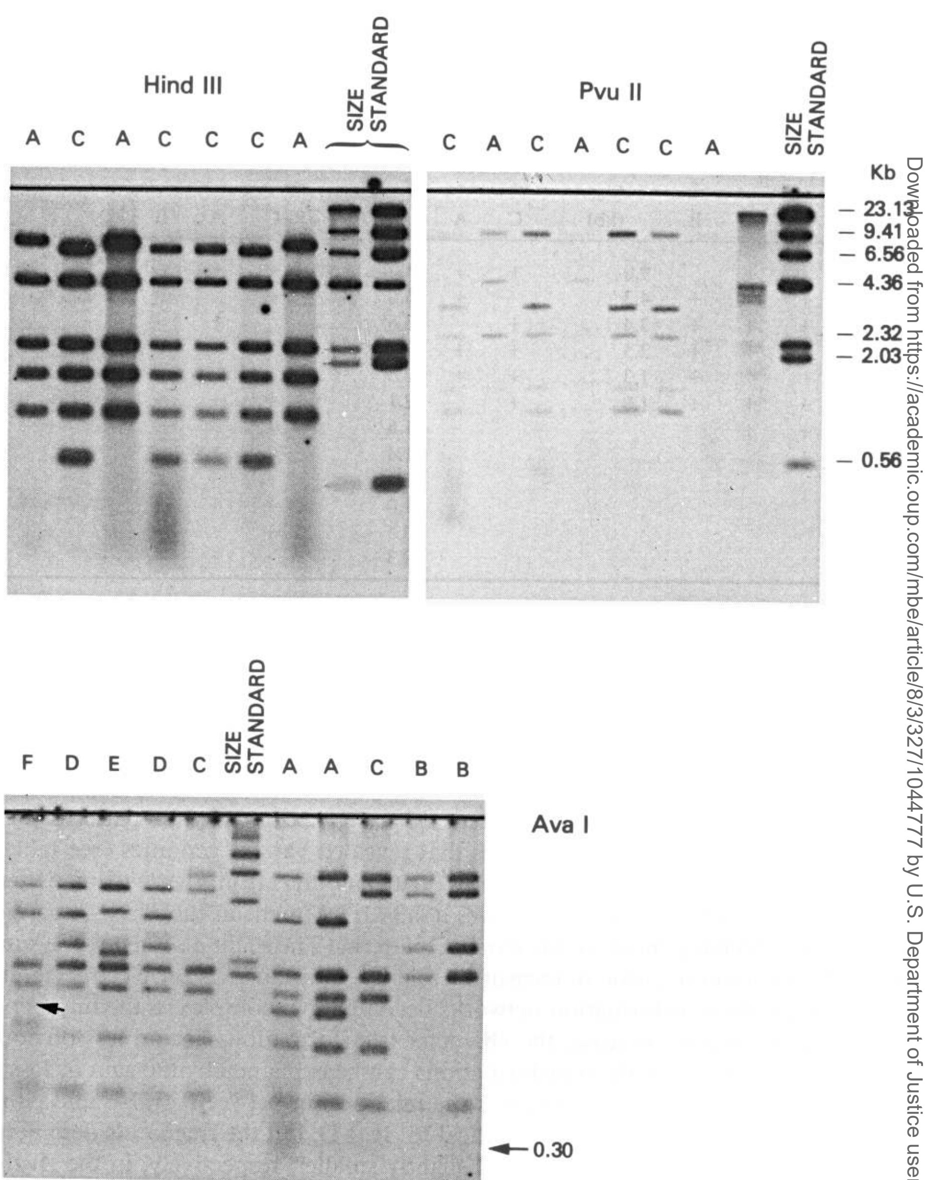


FIG. 2.—Autoradiographs of diagnostic restriction-enzyme patterns resolved on 1.2% horizontal agarose gels. The origin is at the top of each gel. The fragment sizes can be cross-indexed to table 1. The *Pvu*II bands are light because *Pvu*II produces blunt-ended fragments that end-label less readily with the Klenow enzyme. The 0.3-kb bands are only faintly visible on the *Ava*I gel; this band resolves clearly on 4.0% acrylamide gels. The arrow at the bottom right of the *Ava*I gel indicates the position of the 0.3-kb band. The 1.4-kb band in the F pattern of the *Ava*I digest is also only faintly visible; this seems to be an artifact on this gel, as its visibility is equal to that of other bands on other gels. The position of this band is indicated by an arrow. The size standards on each gel are lambda phage DNA digested with *Hin*dIII; the standard fragment sizes, in kilobases, are listed to the right of the *Pvu*II gel. The second lane from the right in the *Pvu*II gel contains partially digested lambda DNA; this can be ignored. The 0.125-kb standard band, normally seen in *Hin*dIII digests of lambda DNA, ran off the end of each gel.

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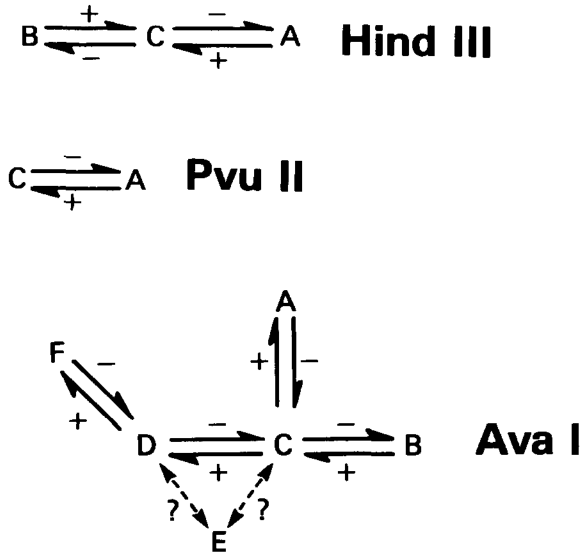


FIG. 3.—Character state transformations for diagnostic restriction-enzyme patterns. The patterns symbolized by letters correspond to the patterns in fig. 2 and table 1. With the exception of the transformation from D to E (marked with a question mark; for discussion, see text) all transformations involve gain (+) or loss (−) of single restriction sites.

The two primary *Hin*dIII patterns, the two *Pvu*II patterns, and the six *Ava*I patterns are presented in figure 2. Networks for proposed transformations between restriction patterns are summarized in figure 3.

A total of eight distinct mitochondrial haplotypes was detected among the 201 red- and yellow-shafted flickers by the *Hin*dIII, *Pvu*II, and *Ava*I digests. The mtDNAs of the 19 gilded flickers were also digested with *Ava*I, *Hin*dIII, and *Pvu*II. A novel pattern was revealed in one specimen (*Ava*I-F); all other patterns that could be scored in the gilded flickers were *Hin*dIII-A, *Pvu*II-C, and *Ava*I-C.

We adopted the convention of designating haplotypes by letter strings, where the letters symbolize enzyme patterns. The positions are ordered from left to right as six- and five-nucleotide recognition sites and alphabetically within a recognition-site class. The most common pattern for each enzyme is designated "C," and the other patterns are alphabetized according to order of discovery. Thus the haplotype ACC is *Hin*dIII-A, *Pvu*II-C, and *Ava*I-C.

The evolutionary relationship among the nine haplotypes was estimated by cladistic parsimony. Two analyses were conducted in which characters were defined in different ways. In the first analysis, restriction patterns were considered characters with specific patterns as character states. For example, *Hin*dIII is a character with three states—C, A, and B. The character-state-transformation networks illustrated in figure 3 were assumed to be true, and the haplotype network that required the fewest evolutionary steps and that was consistent with the character state transformations was deduced. The data are so simple that this could be done by hand. The resultant network, illustrated in figure 4, is devoid of homoplasy (consistency index 1.0), but the placement of ACE is ambiguous, because, as discussed above, there is uncertainty whether the *Ava*I-E pattern is related to *Ava*I-D or to *Ava*I-C. (We have illustrated ACE as attached to ACD; the other possibility would require attaching ACE directly to ACC.)

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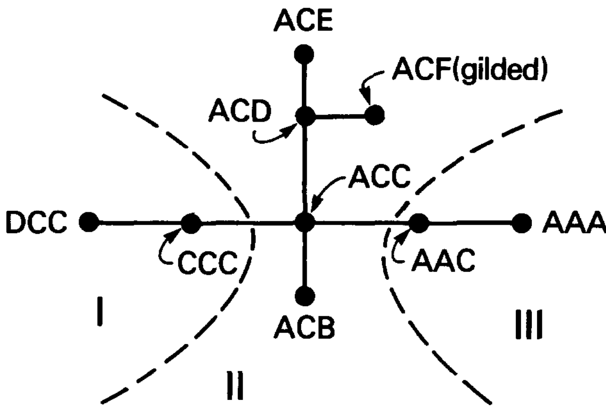


FIG. 4.—Parsimony network showing phylogenetic relationship between nine haplotypes defined by *Hin*dIII, *Pvu*II, and *Ava*I restriction patterns. The roman numerals indicate the clusters of related haplotypes as mapped in fig. 1: haplotypes in group I are synapomorphous for *Pvu*II-C and *Ava*I-C; haplotypes in group II are synapomorphous for *Hin*dIII-A and *Pvu*II-C; and haplotypes in group III are synapomorphous for *Hin*dIII-A and *Pvu*II-A. The ACF pattern was observed in only one gilded flicker; other gilded flickers are ACC.

In the second analysis, each restriction site was considered a character with two states—present or absent. This is the more conventional method, and it is the preferred method for complex restriction-fragment data; but it usually requires mapping restriction sites by a series of double enzymatic digests. However, our data are sufficiently simple that the presence or absence of restriction sites could be inferred without mapping, in all cases except where the *Ava*I-E pattern was involved. Thus, the one haplotype involving *Ava*I-E was ignored, leaving eight OTUs and seven characters (restriction sites). The most parsimonious network was found by using PAUP (version 2.4; Swofford 1985). The network is identical to that derived in the previous analysis for the eight haplotypes common to both analyses (i.e., all haplotypes except ACE).

Tissue from an appropriate out-group was not available to us, and so, with regard to the root of the haplotype network, nothing is implied by figure 4. However, the following two pieces of indirect evidence suggest that the ACC haplotype is ancestral: (1) it is the midpoint of the network, and (2) more lineages could plausibly derive from ACC than from any other haplotype, which suggests that it is the oldest haplotype.

Fifteen of 19 gilded flickers had the ACC haplotype; three others (??C, A?C, and AC?) could not be scored completely; and one had the ACF pattern, which was not observed in any other specimen. ACF differs from the common gilded flicker pattern—ACC—by two restriction sites but differs from the ACD pattern by only one site (see fig. 3 and 4). The latter pattern is fairly common in red-shafted flickers in the Southwest. The gilded flicker mtDNAs were also digested with *Hin*fI, and some were digested with *Dde*I and *Hpa*II (as were some red- and yellow-shafted flicker mtDNAs). These digests revealed several novel patterns not seen in the red- or yellow-shafted populations. Because of (*a*) the complex array of restriction patterns in the gilded flicker, (*b*) our limited samples, and (*c*) the bearing these results might have on the specific-versus-subspecific status of the gilded flicker, we decided to postpone a thorough analysis of mtDNA variation in the gilded flicker.

For the purpose of testing the statistical significance of geographic variation in haplotype frequency in red- and yellow-shafted flickers, the three haplotypes represented

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by single specimens were each combined with their most closely related haplotype; that is, BCC was pooled with CCC, ACE with ACD, and AAC with AAA, leaving ACC and ACB as classes with single haplotypes. These five classes were then tested for heterogeneity in haplotype frequency across 24 locales, in a $24 \times 5$ contingency table (Front Rock Ranch was excluded from this analysis because either *Pvu*II or *Ava*I could not be scored for five specimens). The null hypothesis was tested by a Monte Carlo resampling procedure developed by Roff and Bentzen (1989). Observations are randomly assigned to cells, with the constraints that the row totals and column totals are fixed at the observed values. We wrote a computer program that executes this resampling procedure. The largest "$\chi^2$" value in 1,000 Monte Carlo samples was 139.28, which is much less than the value of 249.13 calculated from the original table; therefore, we reject the null hypothesis.

In order to facilitate visualization of the pattern of geographic variation, the five haplotype classes were further reduced, to three, by pooling ACC, ACB, ACD, and ACE into a single group designated "ACX." Thus, group I comprises CCC (plus the one BCC specimen); group II comprises the ACX cluster; and group III comprises AAA (plus the one AAC specimen). With this grouping, it is apparent from figure 1 that ACX is most prevalent in the Southwest—and, in fact, is restricted to that region, with the exceptions of Glasgow and Havre, Mont., where one and three specimens, respectively, with the ACC haplotype were collected—whereas the CCC and AAA haplotypes are prevalent in the North and East. Although the four ACX haplotypes were grouped primarily to simplify data presentation, there is a rationale to this particular grouping. In addition to the fact that the four genotypes are largely confined to the Southwest, they differ only with regard to *Ava*I pattern, which is the most variable character. Moreover, if ACC is the ancestral haplotype, as suggested above, then ACX comprises the basal haplotype plus (*a*) two haplotypes, ACB and ACD, which cluster with ACC at the base of the "radiation," and (*b*) ACE, which may have been directly derived from ACC, although it may have been derived from ACD.

To quantify the pattern of geographic variation, Nei's (1972) genetic identity was calculated, on the basis of the frequencies of the three haplotype classes (CCC, ACX, and AAA), (BIOSYS, SIMDIS; Swofford and Selander 1981) for all pairs of locales; the locales were then clustered by the UPGMA procedure (BIOSYS, CLUSTER; Swofford and Selander 1981). This genetic identity statistic is applicable to populations of haploids as well as diploids (Nei 1972, p. 291). The UPGMA procedure clusters samples with similar haplotype frequencies, but the cluster diagram should not be construed as a phylogeny; it is simply a way of visualizing population structure. The locales form two major clusters (fig. 5); the upper cluster comprises locales from the Southwest plus one population from the northwestern Great Plains at Havre, Mont, and the lower cluster comprises mostly locales from the North and East. One of four populations from New Mexico fell into the southwestern cluster, whereas the other three fell into the northern-and-eastern cluster. Hierarchical analysis of variance (Wright 1978) indicates that 86% of the total variance in haplotype frequencies is apportioned between the two clusters (regions) and that 14% is within clusters (WRIGHT78; Swofford and Selander 1981). For this analysis, the FORTRAN code in STEP WRIGHT78 was modified for haploids by changing the sampling error correction from $q(1-q)/2n$ to $q(1-q)/n$, where $q$ is the frequency of the haplotype in question and where $n$ is the number of individuals sampled from a particular locale (Swofford and Selander 1981, p. 36). Heterogeneities of haplotype frequencies within the northern-and-eastern cluster and within the southwestern cluster were also tested

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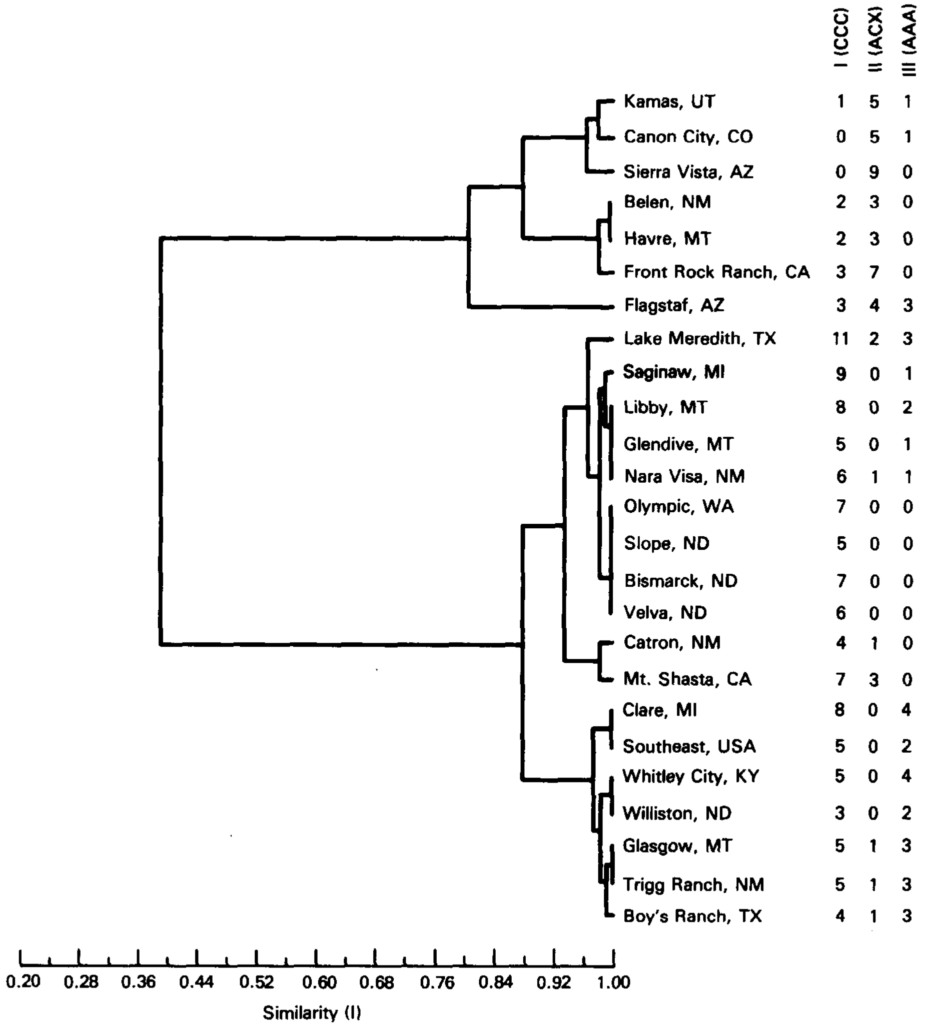


FIG. 5.—Red- and yellow-shafted flicker populations clustered by UPGMA, based on similarity (Nei 1972) of mtDNA haplotype frequencies. The columns list the numbers of specimens belonging to each haplotype class at each locale. Group I includes CCC and one individual from Libby, Mont., that is BCC; group II includes haplotypes ACC, ACB, ACD, and ACE; group III includes AAA and one individual from Lake Meredith, Tex., that is AAC.

by the Roff-Bentzen test. For the northern-and-eastern cluster, 218/1,000 replicates gave $\chi^2$ values greater than the observed value (39.15); for the southwestern cluster, 172/1,000 replicates gave $\chi^2$ values greater than the observed value (16.09). This analysis indicates that the variance between the two clusters is significant but that the variance within clusters is not. Inspection of the geographic distribution of haplotype frequencies in figure 1 does not reveal a clear association between the pattern of mtDNA divergence and the red- and yellow-shafted flicker hybrid zone.

## Discussion

Protein electrophoresis typically reveals little population structure in avian species in comparison with other vertebrates (and most invertebrates and plants as well).

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These same avian species often exhibit marked structure with regard to plumage and morphometric traits (e.g., see Barrowclough 1980*a;* Zink 1986; Gill 1987; Grudzien et al. 1987). The mitochondrial genomes of too few avian species' have been studied to make conclusive generalizations. In the most comprehensive study to date, Ball et al. (1988) did restriction-fragment analysis on mtDNA genomes of 129 red-winged blackbirds (*Agelaius phoeniceus*)—collected from 17 locales in the United States, one located at Edmonton, Alberta, and one located at Puebla, Mexico. Thirty-four distinct haplotypes were observed, but geographic variation in haplotype frequencies was slight, and no geographic trend was obvious. In contrasting the striking homogeneity of haplotype frequencies in red-winged blackbirds to the heterogeneity observed in deer mice (*Peromyscus maniculatus*), Ball et al. (1988) suggested that gene flow resulting from high dispersal rates in red-winged blackbirds may account for the difference.

More limited mtDNA studies have been reported for the Canada goose and for the black-capped and Carolina chickadees. The situation in the Canada goose is, in some respects, similar to that in the northern flicker. The Canada goose comprises numerous subspecies defined on the basis of morphological traits (particularly size) that exhibit low levels of—or, in most cases, no—allozyme divergence (Van Wagner and Baker 1986). Shields and Wilson (1987) and Shields and Helm-Bychowski (1988) reported distinct mtDNA haplotypes for each of five subspecies, but this was based on a total sample size of only 14. The black-capped and Carolina chickadees are considered separate species (American Ornithologists' Union 1983), although they hybridize in a narrow zone. The two are difficult to distinguish morphologically in the field but can be distinguished by discriminate function analysis of morphometric variables; their vocalizations are quite distinct, however (Robbins et al. 1986). Braun and Robbins (1986) reported no allozyme divergence between the two chickadees. Mack et al. (1986) did restriction-fragment analyses of mtDNA of black-capped and Carolina chickadees from just north and south, respectively, of the hybrid zone in Pennsylvania. They found apparent diagnostic differences with 11 of 14 restriction enzymes. Unfortunately, their sample sizes were not specified—but presumably were small.

The survey reported here is the most extensive survey of geographic variation of mtDNA, in an avian species, reported to date. The sample sizes are small, but this is a limitation that is difficult to overcome with birds, because collecting permits are difficult to obtain and because, even with the permits in hand, specimens are difficult to collect in large numbers. Despite small sample sizes, these few studies reveal important aspects of genetic structure that bear on understanding evolution in birds. Although, with regard to mtDNA, geographic variation in the northern flicker appears greater than that in the red-winged blackbird, our results are in agreement with the major conclusions drawn by Ball et al. (1988)—namely, that geographic variation is low compared with that in other vertebrates and that dispersal is probably a major force that mitigates geographic divergence in avian species (Moore and Buchanan 1985; Moore and Dolbeer 1989). If this is true, however, then selection would be implicated in maintaining plumage patterns which do exhibit striking patterns of geographic variation. It is clear that mtDNA will be a useful molecule for resolving forces (gene flow, drift, and selection) and historical events that have determined genetic structure in avian species (e.g., see Avise and Nelson 1989).

mtDNA variation has been studied in several hybrid zones. Although all of these studies have been very limited in scope, they suggest that patterns of mtDNA variation are quite variable relative to those of other genetic, morphological, and behavioral

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clines that define the hybrid zones. For example, an mtDNA haplotype frequency cline is concordant with other clines across a hybrid zone between two chromosomal races of the white-footed mouse (*Peromyscus leucopus*) in Oklahoma (Nelson et al. 1987). The same can be said for the hybrid zone involving fire-bellied toads (*Bombina bombina* × *B. variegata*) in Poland (Szymura et al. 1985; Szymura and Barton 1986). In contrast, mtDNA clines in hybrid zones involving species or subspecies of domestic mice (*Mus domesticus* × *M. musculus;* Ferris et al. 1983; Gyllensten and Wilson 1984), minnows (*Notropis cornutus* × *N. chrysocephalus;* Dowling et al. 1989), field crickets (*Gryllus firmus* × *G. pennsylvanicus;* Harrison et al. 1987), and pocket gophers (*Geomys bursarius major* × *G. b. knoxjonesi;* Baker et al. 1989) reveal a variety of patterns of incongruence and asymmetry, relative to other clines that define the hybrid zone. Differential introgression between nuclear genes and mtDNA often appears to reflect sexual dimorphism in the extent to which reproductive isolating mechanisms are developed. In the pocket gophers, for example, Baker et al. (1989) suggested that *G. b. knoxjonesi* females mate more often with *G. b. major* males than vice versa, resulting in asymmetrical introgression of *knoxjonesi* mtDNA into the *major* population.

The pattern of southwestern divergence in the northern flicker mtDNA haplotype frequencies is an interesting contrast to patterns of geographic variation in plumage traits (Short 1965; Moore and Buchanan 1985) and allozymes (Grudzien et al. 1987). The red- and yellow-shafted subspecies are defined by plumage differences, and a narrow hybrid zone occurs along the full length of the interface of their range boundaries on the western Great Plains (fig. 1). The divergence in mtDNA haplotype frequencies is not clearly associated with the hybrid zone. With regard to allozymes, Grudzien et al. (1987) found no pattern of geographic structure at all (although the range of samples available for the allozyme study did not include locales in Arizona, California, or Utah). That is, one sees a different pattern of geographic variation for each of the three kinds of traits—morphology, allozymes, and mtDNA.

The pattern of mtDNA variation observed in the northern flicker could be explained by several hypotheses, but the data are insufficient to test any one of them critically. Therefore, we will briefly summarize, in general terms, only two of the more plausible alternatives. One hypothesis attributes the observed pattern to the historical biogeography of the species, and the other attributes it to natural selection; the two hypotheses are not mutually exclusive.

The American Southwest is an area of complex physiography and of complex Pleistocene and Holocene climatic and biotic history. The range of the red-shafted flicker in the Southwest appears to be limited only by the availability of suitable nesting trees. Flickers excavate nest cavities in snags and are opportunistic as long as the snag is of appropriate size and state of decay. Presently, red-shafted flickers occur (and are common) in virtually all woodland communities of the Southwest where suitable snags occur (Rasmussen 1941; Snyder 1950; Marshall 1957; Balda 1970; W. S. Moore, unpublished data), but the distribution of these woodlands has changed radically through the Pleistocene to recent times (Martin and Mehringer 1965; Spaulding et al. 1983; Van Devender 1987). Since the end of the Wisconsin glacial advance, these woodlands have become scattered and isolated, on higher-elevation plateaus and mountain ranges and in the form of riparian woodlands along a few water courses. During the Wisconsin glacial advance (and presumably during other glacials), woodlands in the Southwest were more extensive than at present. Indeed, suitable flicker habitat probably occurred in a more or less continuous series of woodland types, from

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the Appalachian Mountains across the Great Plains (boreal spruce forest; Wright 1970; Watts 1983) through New Mexico and Arizona (Martin and Mehringer 1965; Spaulding et al. 1983) and into southern California (Axelrod 1981). Suitable habitat extended north from Arizona throughout much of the Great Basin, where subalpine conifers dominated to near the level of pluvial lakes (Martin and Mehringer 1965; Thompson and Mead 1982; Spaulding et al. 1983). Throughout the Holocene (i.e., from ~12,000 years ago to the present), mesic forests have retreated to higher elevations, where they persist as island refugia for more temperate species, scattered across a hot, inhospitable desert.

Another factor that impinges on the potential for divergence and diversification of the southwestern populations is the amount of gene flow both between these seemingly isolated island habitats and between the southwestern and northern-and-eastern populations as a whole. Flickers are seasonally migratory, and it is likely that much dispersal occurs in conjunction with seasonal migration. But banding-recovery records from the Southwest, of birds banded during the breeding season and recovered during the winter (or vice versa), suggest that these populations are less migratory than northern populations, although the number of records available is small (W. S. Moore, unpublished analysis of USFWS banding-recovery records). This is consistent with the notion that red-shafted flicker populations of the Southwest are relatively isolated in woodland refugia. Dispersal estimates based both on allozyme allele frequencies (Grudzien et al. 1987) and on a small number of USFWS banding recoveries (Moore and Buchanan 1985) suggest that dispersal is generally high in flickers elsewhere. The relative homogeneity of mtDNA haplotype frequencies among populations constituting the northern-and-eastern cluster in the UPGMA phenogram (fig. 5, lower cluster) is consistent with this notion.

According to the historical biogeography hypothesis, then, divergence of southwestern red-shafted populations, as an aggregate, accrues during interglacials, and the diversity that comprises the small radiation of southwestern clones results from isolation of populations in scattered montane woodlands. Presumably this was a cyclical process throughout the Pleistocene, with divergence and diversity accruing during interglacials, only to be eroded by increased gene flow during more mesic conditions correlated with glacial advances.

According to the selectionist hypothesis, the haplotypes constituting the group united by the synapomorphous *Hin*dIII-A and *Pvu*II-C patterns adapt the birds to the hot, arid conditions of the desert Southwest, whereas the CCC and AAA genomes adapt birds to more temperate and mesic conditions. The observation that suggests this, of course, is the prevalence of the ACX haplotypes in the Southwest, but an additional interesting observation is that ACX birds were observed out of the Southwest at just two locales—Havre and Glasgow, Mont. Both of these locales occur in shortgrass prairie regions where summer precipitation is low and where temperatures are high; i.e., a region that may be, effectively, a desert during the seasons when flickers are resident.

These alternative hypotheses could be tested to a considerable extent by using larger and more numerous samples, which would allow more powerful correlation of haplotype frequencies with (*a*) physiographic features and climate variables and (*b*) data on population size, dispersal, and generation time. These data are potentially available for the northern flicker.

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LITERATURE CITED

AMERICAN ORNITHOLOISTS' UNION. 1957. Check-list of North American Birds, 50th ed. American Ornithologists' Union, Washington, D.C.

———. 1983. Check-list of North American Birds, 6th ed. American Ornithologists' Union. Washington, D.C.

AVISE, J. C. 1983. Commentary. Pp. 262–270 *in* A. H. BRUSH, and G. A. CLARK, JR. Perspectives in ornithology. Cambridge University Press, New York.

AVISE, J. C., and C. F. AQUADRO. 1982. A comparative summary of genetic distances in vertebrates, patterns and correlations. Evol. Biol. **15**:151–185.

AVISE, J. C., and W. S. NELSON. 1989. Molecular genetic relationships of the extinct dusky seaside sparrow. Science **243**:646–648.

AXELROD, D. I. 1981. Holocene climatic changes in relation to vegetation disjunction and speciation. Am. Nat. **117**:847–870.

BAKER, R. J., S. K. DAVIS, R. D. BRADLEY, M. J. HAMILTON, and R. A. VAN DENBUSSCHE. 1989. Ribosomal-DNA, mitochondrial-DNA, chromosomal, and allozymic studies on a contact zone in the pocket gopher, *Geomys*. Evolution **43**:63–75.

BALDA, R. P. 1970. Effects of spring leaf-fall on composition and density of breeding birds in two southern Arizona woodlands. Condor **72**:325–331.

BALL, R. M., S. FREEMAN, F. C. JAMES, E. BERMINGHAM, and J. C. AVISE. 1988. Phylogenetic population structure of red-winged blackbirds assessed by mitochondrial DNA. Proc. Natl. Acad. Sci. USA **85**:1558–1562.

BARROWCLOUGH, G. F. 1980*a*. Gene flow, effective population sizes, and genetic variance components in birds. Evolution **34**:789–798.

———. 1980*b*. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. Auk **97**:655–668.

———. 1983. Biochemical studies of microevolutionary processes. Pp. 223–261 *in* A. H. BRUSH and G. A. CLARK, JR. Perspectives in ornithology. Cambridge University Press, New York.

BARROWCLOUGH, G. F., N. K. JOHNSON, and R. M. ZINK. 1985. On the nature of genic variation in birds. Curr. Ornithol. **2**:135–154.

BRAUN, M. J., and M. B. ROBBINS. 1986. Extensive protein similarity of the hybridizing chickadees *Parus atricapillus* and *P. carolinensis*. Auk **103**:667–675.

BROWN, W. M. 1980. Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. Proc. Natl. Acad. Sci. USA **77**:3605–3609.

CORBIN, K. W. 1987. Geographic variation and speciation. Pp. 321–353 *in* F. COOKE and P. A. BUCKLEY. Avian genetics. Academic Press, New York.

DENSMORE, L. D., J. W. WRIGHT, and W. M. BROWN. 1985. Length variation and heteroplasmy are frequent in mitochondrial DNA from parthenogenetic and bisexual lizards (Genus *Cnemidophorus*). Genetics **110**:689–707.

DOWLING, T. E., G. R. SMITH, and W. M. BROWN. 1989. Reproductive isolation and introgression between *Notropis cornutus* and *Notropis chrysocephalus* (family cyprinidae): comparison of morphology, allozymes, and mitochondrial DNA. Evolution **43**:620–634.

FERRIS, S. D., R. D. SAGE, C.-M. HUANG, J. T. NIELSEN, U. RITTE, and A. C. WILSON. 1983.

Downloaded from https://academic.oup.com/mbe/article/8/3/327/1044777 by U.S. Department of Justice user on 16 August 2022

Flow of mitochondrial DNA across a species boundary. Proc. Natl. Acad. Sci. USA **80**:2290–2294.

GILL, F. B. 1987. Allozymes and genetic similarity of blue-winged and golden-winged warblers. Auk **104**:444–449.

GRUDZIEN, T. A., W. S. MOORE, J. R. COOK, and D. TAGLE. 1987. Genetic population structure of the northern flicker (*Colaptes auratus*) hybrid zone. Auk **104**:654–664.

GYLLENSTEN, U., and A. C. WILSON. 1984. Rate of mitochondrial gene flow between *Mus domesticus* and *Mus musculus:* evidence of *Mus domesticus* mitochondrial DNA introgression 750 kilometers north of the hybrid zone. Genetics **3**(part 2): s42.

HAIG, S., and L. W. ORING. 1988. Genetic differentiation of piping plovers across North America. Auk **105**:268–277.

HARRISON, R. G., D. M. RAND, and W. C. WHEELER. 1987. Mitochondrial DNA variation in field crickets across a narrow hybrid zone. Mol. Biol. Evol. **4**:144–158.

KARL, S. A., R. M. ZINK, and J. R. JEHL. 1987. Allozyme analysis of the California gull (*Larus californicus*) Auk **104**:767–769.

KOENIG, W. D. 1984. Clutch size of the gilded flicker. Condor **86**:89–90.

MACK, A. L., F. B. GILL, R. COLBURN, and C. SPOLSKY. 1986. Mitochondrial DNA: a source of genetic markers for studies of similar passerine bird species. Auk **103**:676–681.

MARSHALL, J. T. 1957. Birds of pine-oak woodland in southern Arizona and adjacent Mexico. Pacific Coast Avifauna 32. Cooper Ornithological Society, Berkeley.

MARTIN, P. S., and P. J. MEHRINGER, JR. 1965. Pleistocene pollen analysis and biogeography of the Southwest. Pp. 433–451 *in* H. E. WRIGHT, and D. B. FREY. The Quaternary of the United States. Princeton University Press, Princeton, N.J.

MOORE, W. S. 1987. Random mating in the northern flicker hybrid zone: implications for the evolution of bright and contrasting plumage patterns in birds. Evolution **41**:539–546.

MOORE, W. S., and D. B. BUCHANAN. 1985. Stability of the northern flicker hybrid zone. Evolution **39**:135–151.

MOORE, W. S., and R. A. DOLBEER. 1989. The use of banding recovery data to estimate dispersal rates and gene flow in avian species: case studies in the red-winged blackbird and common grackle. Condor **91**:242–253.

MOORE, W. S., and W. D. KOENIG. 1986. Comparative reproductive success of yellow-shafted, red-shafted and hybrid flickers across a hybrid zone. Auk **103**:42–51.

MORITZ, C., T. E. DOWLING, and W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annu. Rev. Ecol. Syst. **18**:269–292.

NEI, M. 1972. Genetic distance between populations. Am. Nat. **106**:283–292.

NELSON, K., R. J. BAKER, and R. L. HONEYCUTT. 1987. Mitochondrial DNA and protein differentiation between hybridizing cytotypes of the white-footed mouse, *Peromyscus leucopus*. Evolution **41**:864–872.

RASMUSSEN, D. I. 1941. Biotic communities of the Kaibab Plateau, Arizona. Ecol. Monogr. **11**:229–275.

ROBBINS, M. B., M. J. BRAUN, and E. A. TOBEY. 1986. Morphological and vocal variation across a contact zone between the chickadees *Parus atricapillus* and *P. carolinensis*. Auk **103**:655–666.

ROFF, D. A., and P. BENTZEN. 1989. The statistical analysis of mitochondrial DNA polymorphisms: $\chi^2$ and the problem of small samples. Mol. Biol. Evol. **6**:539–545.

SEUTIN, G., and J.-P. SIMON. 1988. Genetic variation in sympatric willow flycatchers (*Empidonax traillii*) and alder flycatchers (*E. alnorum*). Auk **105**:235–243.

SHIELDS, G. F., and K. M. HELM-BYCHOWSKI. 1988. Mitochondrial DNA of birds. Curr. Ornithol. **5**:273–295.

SHIELDS, G. F., and A. C. WILSON. 1987. Subspecies of the Canada goose (*Branta Canadensis*) have distinct mitochondrial DNA's. Evolution **41**:662–666.

SHORT, L. L. 1965. Hybridization in the flickers (*Colaptes*) of North America. Bull. Am. Museum Nat. Hist. **129**:307–428.

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———. 1982. Woodpeckers of the world. Delaware Museum of Natural History, Greenville.

SIBLEY, C. G., and J. E. AHLQUIST. 1982. The relationships of the yellow-breasted chat (*Icteria virens*), and the alleged 'slow-down' in the rate of macromolecular evolution in birds. Postilla **187**:1–19.

SNYDER, D. P. 1950. Bird communities in the coniferous forest biome. Condor **52**:17–27.

SPAULDING, W. G., E. B. LEOPOLD, and T. R. VAN DEVENDER. 1983. Late Wisconsin paleoecology of the American southwest. Pp. 259–293 *in* S. C. PORTER, ed. Late Quaternary environments of the United States, vol. **1**: The late Pleistocene. University of Minnesota Press, Minneapolis.

SWOFFORD, D. L. 1985. PAUP: phylogenetic analysis using parsimony, version 2.4. Illinois Natural History Survey. Champaign.

SWOFFORD, D. L., and R. B. SELANDER. 1981. BIOSYS1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. **72**:281–283.

SZYMURA, J. M., and N. H. BARTON. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata,* near Cracow in southern Poland. Evolution **40**:1141–1159.

SZYMURA, J. M., C. SPOLSKY, and T. UZZELL. 1985. Concordant change in mitochondrial and nuclear genes in a hybrid zone between two frog species (genus *Bombina*). Experentia **41**: 1469–1470.

TEGELSTROM, H. 1987. Genetic variability in mitochondrial DNA in a regional population of the great tit (*Parus major*). Biochem. Genet. **25**:95–110.

THOMPSON, R. S., and J. I. MEAD. 1982. Late Quaternary environments and biogeography in the Great Basin. Quaternary Res. **17**:39–55.

VAN DEVENDER, T. R. 1987. Holocene vegetation and climate in the Puerto Blanco Mountains, southwestern Arizona. Quaternary Res. **27**:51–72.

VAN WAGNER, C. E., and A. J. BAKER. 1986. Genetic differentiation in populations of Canada geese (*Branta canadensis*). Can. J. Zool. **64**:940–947.

WATTS, W. A. 1983. Vegetational history of the eastern United States 25,000 to 10,000 years ago. Pp. 294–310 *in* S. C. PORTER. Late Quaternary environments of the United States, vol. **1**: The late Pleistocene. University of Minnesota Press, Minneapolis.

WRIGHT, H. E., JR. 1970. Vegetational history of the central plains. Pp. 157–172 *in* W. DORT, JR.,and J. K. JONES, JR. University of Kansas Press, Lawrence.

WRIGHT, J. W., C. SPOLSKY, and W. M. BROWN. 1983. The origin of the parthenogenetic lizard *Cnemidophorus laredoensis* inferred from mitochondrial DNA analysis. Herpetologica **39**: 410–416.

WRIGHT, S. 1978. Evolution and the genetics of populations, vol. **4**: Variability within and among populations. University of Chicago Press, Chicago.

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

———. 1986. Patterns and evolutionary significance of geographic variation in the *schistacea* group of the fox sparrow (*Passerella iliaca*). American Ornithologists' Monograph 40. American Ornithologists' Union, Washington, D.C.

ZINK, R. M. and J. V. REMSEN, JR. 1986. Evolutionary processes and patterns of geographic variation in birds. Current Ornithology (R. F. JOHNSTON, ed.). **4**:1–69. Plenum Press, New York.

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