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FURTHER ANALYSIS OF ALLOZYME VARIATION IN THE NORTHERN FLICKER, IN COMPARISON WITH MITOCHONDRIAL DNA VARIATION[1]

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The Northern Flicker (*Colaptes auratus*) is a common woodpecker whose range includes nearly all of the continental United States, and extends into Canada, Mexico, Central America, and the West Indies. Short (1965) recognized 15 subspecies which he divided into five subspecies "groups": *C. a. auratus* (the Yellow-shafted Flicker of eastern North America), *C. a. cafer* (the Red-shafted Flicker of western North America), *C. a. chrysoides* (the Gilded Flicker of the southwestern deserts), *C. a. chrysocaulosus* (Cuba and Grand Cayman), and *C. a. mexicanoides* (highland southern Mexico to Nicaragua; Short 1965, 1982). Although presently considered subspecies groups (American Ornithologists' Union 1983), the Red-shafted, Yellow-shafted, and Gilded Flickers are so distinct in plumage and size that they were formerly considered species (American Ornithologists' Union 1957).

A hybrid zone between the Red- and Yellow-shafted groups occurs where the ranges of the two come into contact (Short 1965, Moore and Buchanan 1985). The hybrid zone existed when explorers of European ancestry reached the Great Plains (see June, 1843 entry in the journal of Edward Harris, McDermott 1951), and it has been historically stable (Moore and Buchanan 1985). The origin of the hybrid zone is not fully resolved, and it may be that segments of it arose at different times under different circumstances (see Short 1965; Moore and Buchanan 1985; and Moore and Price, in press, for discussion). It is probable that major portions of the present hybrid zone formed at the end of the Wisconsin Glacial Advance or during the Holocene Altithermal, at the latest. The hybrid zone extends the length of the western Great Plains, from northwestern Texas to southern Alaska (Short 1965; Moore and Buchanan 1985; Moore and Price, in press). No evidence of assortative mating between the subspecies groups has been found (Bock 1971, Moore 1987).

Previous allozyme surveys (Grudzien and Moore 1986, Grudzien et al. 1987) indicated that there is little if any genic population structure corresponding with features of the flicker hybrid zone and that gene flow across the zone and between the subspecies groups is high. In contrast, a recent mitochrondrial DNA (mtDNA) survey of the three subspecies groups (Moore et al. 1991) indicated that there is a significant geographic structure in mtDNA, not associated with the hybrid zone, that involves divergence of populations in the southwest from northern and eastern populations.

The allozyme surveys did not include samples from the western and southwestern U.S. where divergence in mtDNA haplotype frequencies was most apparent. Consequently, the objective of this study is to investigate the geographic distribution of nuclear genes in the southwest for comparison with the mtDNA data; the comparison bears on questions concerning the differential evolution of mitochondrial and nuclear genomes in flickers (see Discussion).

We electrophoretically surveyed three loci, Lactate Dehydrogenase (LDH-2, E.C. no. 1.1.1.27), Phosphoglucomutase (PGM, E.C. no. 5.4.2.2), and Alpha-glycerophosphate Dehydrogenase (AGP, E.C. no. 1.1.1.8), in 215 Northern Flickers. These loci are among those previously identified as polymorphic in this species (Grudzien and Moore 1986, Grudzien et al. 1987), and based on the limited geographic range of samples, were most suggestive of divergence in southwestern populations. The sample surveyed includes individuals from both Red-shafted and Yellow-shafted subspecies groups, hybrids between the two, and 19 Gilded Flickers from Arizona.

MATERIALS AND METHODS

Specimens were collected in 1985, 1986, and 1988. Collecting sites were in 11 states and represent major regions of the continental United States (Table 1). Birds were shot and partially dissected in the field, where skeletal muscle, heart, and liver were immediately placed on frozen CO_2 for transport. Samples were maintained in the laboratory at −80°C.

The majority of Red- and Yellow-shafted Flicker samples was surveyed for LDH and PGM using the horizontal starch-gel electrophoresis techniques of Turner (1983). However, samples from 1988 and Gilded Flickers were surveyed for LDH and PGM, and all samples were surveyed for AGP, using cellulose acetate gel electrophoresis and the methods of Hebert and Beaton (unpubl. manuscript). Results were identical at all loci on both media, but we completed the survey using cellulose acetate because it provided greater resolution and proved less labor-intensive than use of starch-gel.

Allele frequencies and hierarchical *F*-statistics were calculated using the computer program BIOSYS-1 (Swofford and Selander 1981). The significance of the estimates of between-population variance (F_{st}) was tested using the methods of Roff and Bentzen (1989).

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TABLE 1. Sample sites and allele frequencies for the three subspecies groups of the Northern Flicker and hybrids between the Red- and Yellow-Shafted groups.

	County	State	Collection year-locale (*n*)	LDH-2 (1.1.1.27)		PGM (5.4.2.2)		AGP (1.1.1.8)		
				100	73	100	127	100	114	91
Yellow-shafted	Newaygo	MI	85-1 (10)	1.00		0.850	0.150	1.00		
	Clare & Missaukee	MI	85-2 (10)	1.00		1.00		1.00		
	Benzie	MI	85-3 (10)	0.950	0.050	1.00		1.00		
	Oscoda	MI	85-4 (10)	0.950	0.050	1.00		1.00		
	Saginaw	MI	85-5 (8)	0.938	0.063	0.875	0.125	1.00		
	McCreary	KY	86-1 (9)	1.00		1.00		1.00		
	Covington	AL	86-2 (2)	1.00		1.00		1.00		
Red-shafted	Quay	NM	86-8 (8)	0.875	0.125	1.00		1.00		
	San Miguel	NM	86-9 (9)	1.00		1.00		1.00		
	Coconino	AZ	86-10 (10)	1.00		1.00		1.00		
	Cochise	AZ	88-2 (9)	1.00		0.833	0.167	1.00		
	San Luis Obispo	CA	86-13 (10)	0.950	0.050	0.950	0.050	1.00		
	Siskiyou	CA	86-14 (10)	1.00		1.00		0.950	0.050	
	Mason	WA	86-15 (7)	0.857	0.143	1.00		1.00		
	Lincoln	MT	86-16 (10)	1.00		1.00		1.00		
	Slope	ND	86-20 (5)	1.00		1.00		1.00		
	Ward	ND	86-23 (6)	1.00		1.00		1.00		
	Summit	UT	83-5 (5)	1.00		1.00		1.00		
Hybrid	Potter	TX	86-4 (6)	0.833	0.167	1.00		1.00		
	Oldham	TX	86-7 (8)	1.00		1.00		0.875		0.125
	Hill	MT	86-17 (5)	1.00		1.00		1.00		
	Valley	MT	86-18 (9)	0.944	0.056	1.00		1.00		
	Dawson	MT	86-19 (6)	1.00		1.00		1.00		
	Burleigh	ND	86-21 (7)	1.00		1.00		1.00		
	McKenzie	ND	86-22 (5)	0.900	0.100	1.00		0.900	0.100	
Gilded	Pima	AZ	86-11 (10)	1.00		1.00		1.00		
	Pima	AZ	88-4 (4)	1.00		1.00		1.00		
	Pima	AZ	88-5 (5)*	1.00		1.00		1.00		

* This population was combined with 88-4 (4) for calculation of *F*-statistics.

TABLE 2. Summary of F-statistics at all loci.

Locus	F_{IS}	F_{IT}	F_{ST}
AGP	−0.114	−0.008	0.095
LDH-2	−0.124	−0.031	0.083
PGM	−0.162	−0.019	0.123
Mean	−0.134	−0.023	0.098

The Roff-Bentzen test is analogous to a χ^2 test for heterogeneity of allele frequencies among populations but compares the observed χ^2 value with a probability distribution based on computer re-sampling. This test eliminates problems inherent in χ^2 analysis resulting from small sample sizes. The distribution of the test statistic is actually a close approximation of χ^2.

RESULTS

Two LDH alleles were observed in Northern Flicker tissue, with relative migration distances of 100 (common) and 73 (rare) (Table 1). Two alleles were likewise identified for PGM, scored as 100 (common) and 127 (rare) (Table 1). AGP displayed three alleles, two rare and one common, with the relative migration distances of 114, 91, and 100, respectively.

The data confirm that LDH, PGM, and AGP are polymorphic in the Northern Flicker in several of the populations surveyed (Table 1). However, the frequency of the rare alleles surpasses 5% in only three major geographic regions: Michigan (PGM; five populations, 50 birds), New Mexico (LDH; two populations, 17 birds), and Washington (one population, 7 birds, data not shown).

The mean F_{st} value for all three loci indicates that only 9.8% of the maximum possible between-population variance in allele frequencies actually exists across the area surveyed (Table 2). The F_{st} values reported here for the entire United States are similar to those reported for the same enzymes by Grudzien et al. (1987) for the region of the hybrid zone, including the order observed when the three values are ranked by magnitude.

The results of the Roff-Bentzen test for heterogeneity of allele frequencies indicate that significant spatial heterogeneity is present only in the case of PGM ($P < 0.01$). The data for this locus suggest that the rare allele is more common than expected at four locales: 85-1 and 85-5, both in Michigan; 86-13 in California, and 88-2 in Arizona. None of these locales represents specimens of Gilded Flicker, which were monomorphic for the common allele at all three loci (Table 1).

DISCUSSION

The allozyme data presented here suggest that the Northern Flicker has very little geographic variation across three subspecies groups and the entire continental United States. No fixed allelic differences or subspecies-specific alleles were found, and the heterogeneity test indicated significant spatial heterogeneity only in the case of PGM. However, the distribution of the locales at which PGM has a higher than expected frequency of its rare allele (two sites in Michigan, one in Arizona, and one in California) reveals no clear geographic trend.

These results agree with previous findings (Grudzien and Moore 1986, Grudzien et al. 1987) that the hybrid zone between Red- and Yellow-shafted Flickers does not act as a barrier to nuclear gene flow. In addition, they are consistent with the conclusion reached by Grudzien et al. (1987) that allozymic variation in flickers is selectively neutral, whereas morphological characters are apparently subject to fairly intense selection pressure, a hypothesis suggested previously for Canada Geese (Van Wagner and Baker 1986).

Widespread allozymic homogeneity in flickers is not unexpected. Studies of other avian hybrid zones (Corbin et al. 1979, Barrowclough 1980a, Seutin and Simon 1988) similarly uncovered neither subspecies-specific alleles nor clinal variation in allele frequency. Further, birds in general are known to display little allozymic differentiation, even between recognized species (Barrowclough 1980a, 1980b; Johnson and Zink 1983, Braun and Robbins 1986). The F_{st} values observed here are comparable to those reported for other avian species (Evans 1987).

Perhaps most interesting is that, with the inclusion of allozyme data for collecting sites in the far west (notably Arizona and California), the Northern Flicker does not have the population structure in nuclear genes that is apparent in mtDNA (Moore et al. 1991). This disparity has been noted previously in birds (Mack et al. 1986, Ball et al. 1988, Moore et al. 1991). It also bears on the two hypotheses suggested by Moore et al. (1991) as the "most plausible" explanations for the trend they observed in Northern Flicker mtDNA, although it does not conclusively disprove either. First, they suggested the pattern could reflect past geographic isolation, citing evidence which suggests that populations in the southwest are both less migratory and more isolated than populations from other parts of the continental United States. Second, the mitochondrial and nuclear genomes in the flicker may experience different selection pressures, and the southwestern divergence may be the result of selection for mitochondrial haplotypes which increase fitness in hot, arid regions.

If a pattern of divergence in nuclear gene frequencies were congruent with that observed in mtDNA haplotype frequencies, the geographical isolation hypothesis would be strongly supported. This was not observed; thus, to that extent, the selection hypothesis is favored. However, the geographical isolation hypothesis can not be conclusively rejected because mtDNA evolves more rapidly than nuclear DNA (Brown et al. 1979, Brown 1985) and mitochondrial haplotypes have a smaller effective population size than nuclear genes. Thus, it is possible that the southwestern populations have been isolated long enough to have diverged significantly with regard to mtDNA but not nuclear genes.

Finally it is interesting that no significant divergence was found in Gilded Flickers. Allozyme data do not reflect the sharper morphological distinctions apparent between Gilded Flickers and the other two subspecies groups (Short 1965). In addition, limited mtDNA data suggest that they are "closely related" to Red-shafted Flickers of the southwest (Moore et al. 1991). However, more data are needed before taxonomic considerations

regarding this subspecies group can be adequately addressed.

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LITERATURE CITED

AMERICAN ORNITHOLOGISTS' UNION. 1957. Checklist of North American Birds. 5th ed., The Lord Baltimore Press, Baltimore, MD.

AMERICAN ORNITHOLOGISTS' UNION. 1983. Checklist of North American Birds. 6th ed., Allen Press, Lawrence, KS.

BALL, R. M., S. FREEMAN, F. C. JAMES, E. BERMINGHAM, AND J. C. AVISE. 1988. Phylogeographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. Proc. Nat. Acad. Sci. USA 85:1558–1562.

BARROWCLOUGH, G. F. 1980a. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. Auk 97:655–668.

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

BOCK, C. E. 1971. Pairing in hybrid flicker populations in eastern Colorado. Auk 88:921–924.

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. 1985. The mitochondrial genome of animals, p. 95–130. *In* R. J. MacIntyre [ed.], Molecular evolutionary genetics. Plenum Press.

BROWN, W. M., M. GEORGE, AND A. C. WILSON. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967–1971.

CORBIN, K. W., C. G. SIBLEY, AND A. FERGUSON. 1979. Genic changes associated with the establishment of sympatry in orioles of the genus *Icterus*. Evolution 33:624–633.

EVANS, P. G. H. 1987. Electrophoretic variability of gene products, p. 105–162. *In* F. Cooke and P. A. Buckley [eds.], Avian genetics. Academic Press.

GRUDZIEN, T. A., AND W. S. MOORE. 1986. Genetic differentiation between the Yellow-shafted and Red-shafted subspecies of the Northern Flicker. Biochem. Syst. Ecol. 14:451–453.

GRUDZIEN, T. A., W. S. MOORE, J. R. COOK, AND D. TAGLE. 1987. Genic population structure and gene flow in the Northern Flicker (*Colaptes auratus*) hybrid zone. Auk 104:654–664.

JOHNSON, N. K., AND R. M. ZINK. 1983. Speciation in sapsuckers (*Sphyrapicus*): I. Genetic differentiation. Auk 100:871–884.

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.

MCDERMOTT, J. F. [ed]. 1951. Up the Missouri with Audubon. The journal of Edward Harris. Univ. of Oklahoma Press, Norman, OK.

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 in historical times: implications for adaptive speciation theory. Evolution 39:135–151.

MOORE, W. S., J. H. GRAHAM, AND J. PRICE. 1991. Mitochondrial DNA variation in the Northern Flicker (*Colaptes auratus*, Aves). Mol. Biol. Evol. 8:327–344.

MOORE, W. S., AND J. T. PRICE. In press. The nature of selection in the Northern Flicker hybrid zone and its implications for speciation theory. *In* R. G. Harrison [ed.], Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

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

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

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

SHORT, L. L. 1982. Woodpeckers of the World. Monograph Series No. 4, Delaware Museum of Natural History, Greenville, DE.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Heredity 72: 281–283.

TURNER, B. J. 1983. Genic variation and differentiation of remnant natural populations of the desert pupfish, *Cyprinodon macularius*. Evolution 37: 690–700.

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