The Condor 92:520–521

ADDITIONAL EVIDENCE OF MULTIPLE PARENTAGE IN EASTERN BLUEBIRDS[1]

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Key words: *Multiple parentage; Eastern Bluebirds;* Sialia sialis.

Cavity-nesting Eastern Bluebirds (*Sialia sialis*) were thought to be monogamous (Pinkowski 1974, Gowaty 1980), but Verner and Willson (1969), Gowaty and Karlin (1984), and later Gowaty (1985) and Gowaty and Bridges (unpubl.) found genetic evidence to the contrary. Because selective pressures on behavioral traits may differ greatly between populations, it is necessary to know whether geographically distant populations exhibit similar behavioral characteristics. Here we report on a sample of Eastern Bluebirds from a different population and provide evidence of nondescendent offspring of at least one putative parent.

METHODS AND MATERIALS

As part of a nonrelated project, 45 bluebird nest boxes were distributed along fence posts throughout a 200-ha pasture in Durham, Washington County, Arkansas during the summer of 1986. Blood samples were obtained from vessels on the tibio-tarsi of 48 individuals between May and July 1988. All sampled individuals were color-banded for future reference. Blood was collected directly onto 3- × 7-mm filter paper wicks and maintained individually on dry ice; they were later transferred to laboratory facilities and stored at −80°C until used for electrophoretic analysis. Electrophoretic methods followed those described previously by Gowaty and Karlin (1984).

RESULTS AND DISCUSSION

As previously reported (Gowaty and Karlin 1984), electrophoretic variants (F = fast and S = slow forms) for both nucleoside phosphorylase (Nsp, E.C. 2.4.2.1) and esterase-2 (Est-2, E.C. 3.1.1.7) can be resolved. Although only seven adults were sampled (gene frequencies for Est-2 [$F = 0.357$, $S = 0.643$]), genotype frequencies showed not significant departure from Hardy-Weinberg equilibrium ($H = 3.33$, $P > 0.05$). Similarly, Est-2 gene frequencies in 41 juveniles ($F = 0.24$, $S = 0.76$) were in close agreement with adult gene frequencies. Similar results were obtained for nucleoside phosphorylase. Although we did not detect the Nsp F allele in the adults, it was present among nestlings ($F = 0.08$, $S = 0.92$).

In eight of 10 nest boxes used by bluebirds, only nestlings were sampled because putative parents could not be collected. In four of 10 families sampled, all

TABLE 1. Genotypes for two protein loci in families of Eastern Bluebirds exhibiting multiple parentage. F = fast and S = slow forms.

Nest box	Individual	Sex	Est-2	Nsp
May 1988 nesting attempts:				
Box 5	JUV 534	F	*FS*	*SS*
	JUV 535	M	*SS*	*FS*
	JUV 536	F	*FS*	*SS*
Box 10	JUV 506	M	*SS*	*SS*
	JUV 507	M	*FS*	*SS*
	JUV 508	F	*SS*	*SS*
	JUV 509	M	*SS*	*SS*
	JUV 510	F	*SS*	*SS*
Box 39	JUV 523	M	*SS*	*SS*
	JUV 524	F	*SS*	*SS*
	JUV 525	F	*FS*	*SS*
	JUV 526	F	*SS*	*SS*
	JUV 527	M	*SS*	*SS*
July 1988 nesting attempts:				
Box 10	JUV 576	F	*SS*	*SS*
	JUV 577	F	*SS*	*FS*
	JUV 578	M	*SS*	*SS*
	JUV 579	M	*SS*	*SS*
Box 19	JUV 555	F	*FS*	*SS*
	JUV 556	F	*SS*	*SS*
	JUV 557	M	*FS*	*SS*
	JUV 558	F	*FS*	*SS*
	JUV 559	M	*FS*	*SS*

[1] Received 17 July 1989. Final acceptance 27 November 1989.

TABLE 2. Calculation of probability of detection at two loci, Est-1 and Nsp (see Gowaty and Davies 1986). F = fast and S = slow forms.

Observed mating types	Observed frequency offspring genotypes			f_i
	FF	*FS*	*SS*	
Est-2:	0.5122	0.4878	0.0000	
Female × male				
SS × ??	0.0000	—	—	0.0000
SS × *FF*	0.0000	0.4878	0.0000	0.4878
			Est-2 total	0.4878
Nsp:	0.0000	0.1707	0.8293	
SS × ??	0.0000	—	—	0.0000
SS × *FF*	0.0000	0.1707	0.0000	0.1707
			Nsp total	0.1707

nestlings appeared to be related to all other nestlings in the nest box; there was no obvious evidence of multiple parentage. In nest box #22, where we captured both putative parents, one of the four nestlings (Est-2: *SS*) could be the offspring of the attending female (Est-2: *SS*), but the male (Est-2: *FF*) could not be the father of that offspring (Table 1). This case is unambiguous because the male's genotype is inconsistent with that of one nestling.

In the remaining five families (Est-2 in four families; and, Nsp in one family), we do not know genotypes of putative parents and thus cannot establish definitive genotypic inconsistencies. However, we can calculate the probability of detecting multiple parentage from the observed genotype distributions (Gowaty and Davies 1986). Making the assumptions that: (1) parasites' (nestlings with genotypes inconsistent with caretakers') genotypes are distributed at random with respect to caretakers' genotypes, and (2) parasites are nonlinearly related to *both* female and male caretakers, we estimate our probability of detection for Est-2 to be approximately 0.4878 (Table 2). Because we observed one nondescendent individual in Box 22 (Table 1, JUV 503), and we had about a 50% chance of observing any, we suggest that at least one parasitic nestling went undetected in our study (1/0.4878 = 2.05 detected and undetected nondirectly descendant nestlings). For Nsp, the probability of detection (Table 2, 0.1707) was so low that failure to observe inconsistent genotypes for Nsp was not surprising. Thus, we estimate two (i.e., one detected and one undetected) nestlings of the 41 sampled to represent parasites; a total of almost 5% of the nestlings in our sample are nondirectly descendant from caretakers.

We conclude from these limited data that alternative nesting strategies in Eastern Bluebirds occur not only in South Carolina, but also in northwestern Arkansas. These findings support the notion that biparental care of nestlings by adult bluebirds is not necessarily a reflection of the genetic kinship between putative parents and their offspring. Additional studies should focus attention on nest-box density and availability, as well as, food and habitat characteristics in determining the roles of these variables in nesting behavior in Eastern Bluebirds.

We thank M. Cassidy for the use of his property. P. Gowaty reviewed early drafts of the manuscript and contributed computer assistance. N. Kilambi, D. Clapp, and K. Tarvin helped in the field. This study was funded under the NSF Research Experiences for Undergraduates Program as an extension of NSF grant BSR-8408090.

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