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Multiple maternity and paternity in single broods of apparently monogamous eastern bluebirds (*Sialia sialis*)

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Summary. In an effort to assess genealogical relationships between breeding adults and their putative offspring, we collected and analyzed blood from 257 wild, uniquely color-banded eastern bluebirds (*Sialia sialis*). Electrophoretic analysis of the protein products of two loci, esterase-2 (*EST-2*) and nucleoside phosphorylase (*NSP*), revealed that at least 5% of adult males and 15% of adult females were caring for at least one offspring not their own. Multiple parentage was evident in 9% of all broods sampled. Twenty-five percent of complete families sampled evidenced multiple parentage. Biparental care of nestlings by adult eastern bluebirds is not necessarily a reflection of the genetic kinship between putative parents and their offspring. The consort pattern among eastern bluebirds does not completely correlate with the pattern of gametic contributions, leading to a reconsideration of their mating system as possibly polygamous. This is among the first unambiguous observations of multiple patenity in a wild bird population, and of multiple maternity in a wild passerine.

Introduction

Evolutionary theories in behavioral ecology are based on assumptions about genetic kinship (Hamilton 1964; Trivers 1972; Wilson 1975). Yet, seldom have the patterns of genetic relatedness amongst individuals in social units of vertebrates been described (McCracken and Bradbury 1977; Schwartz and Armitage 1980; Sherman 1981; Hanken and Sherman 1981; Foltz and Hoogland 1981). Here we report the first direct evidence for multiple paternity and multiple maternity in the broods of a wild population of passerine bird. Our observations suggest that actual kinship may not always be reliably inferred from patterns of "parental" care in apparently monogamous birds.

Multiple parentage (maternity and paternity) results when individuals in a single clutch or brood are offspring of more than one father or more than one mother. This will result from intraspecific nest parasitism (egg dumping) (Yom-Tov 1980), communal nesting (Vehrencamp 1978), multiple mating by (choosing) females (Ridpath 1972 I,II,III), forced copulation (Beecher and Beecher 1979), or kleptogamy of females. Kleptogamy (Gowaty 1981; May and Robertson 1980) occurs when an individual invests parental care in offspring not its own, specifically to cases in which no obvious selective benefits thus accrue to the care-taker. Selection against males that care for non-kin offspring has received theoretical attention (Trivers 1972) and many aspects of behavior have been explained as strategies to avoid such care of non-kin (Barash 1976; Zenone et al. 1979; Power et al. 1981; Power and Doner 1980; Gowaty 1981). Multiple maternity may result from egg dumping, communal nesting, or kleptogamy of females. Multiple maternity is seldom discussed theoretically.

Multiple paternity, in which individuals in a single clutch or brood are fathered by more than one male, has been described for a variety of wild vertebrates (Hjoth 1971; Simonsen and Frydenberg 1972; Birdsall and Nash 1973; Borowsky and Kallman 1976; Gibson and Falls 1975) and invertebrates (Cobb 1977; Nelson and Hedgecock 1977; Anderson 1974; Karlin et al. 1981), and has been inferred in wild bird populations (Beecher and

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Beecher 1979). Multiple maternity has been inferred in communally nesting birds (Vehrencamp 1978; Bertram 1979), and among species in which intraspecific nest parasitism regularly occurs (Yom-Tov 1980; Power et al. 1981). Observations of multiple paternity and maternity have never been directly observed in apparently monogamous birds.

Eastern bluebirds (*Sialia sialis*) are cavity nesting Turdinae which appear to be monogamous (Pinkowski 1974; Gowaty 1980); apparent polygyny and polyandry have also been reported (Verner and Willson 1969). Experimental manipulation of parental behavior (Gowaty 1981, 1983a), and resources critical for reproduction suggested that apparent monogamy – one male/one female social units – might not reflect the actual paternity of all offspring. "Apparent monogamy" is distinguished from the pattern of genetically effective mating because copulations are seen too infrequently in the field to estimate the frequency of extra-pair copulations from behavioral observations alone. Additionally, observations of extra-pair copulations would not eliminate potential biases such as sperm competition. Alternatively, electrophoretic analysis of isozyme variation of blood proteins offers a highly reliable method for the separation of putative and genetic kinship (Sherman 1981).

Materials and methods

As part of a long term field study of the behavioral ecology of eastern bluebirds (*Sialia sialis*) in northwestern South Carolina (Gowaty 1980, 1981, 1983), we sampled blood from 257 uniquely color-banded bluebirds: 20 adult males, 27 adult females, and 210 nestlings from 56 broods. Between 29 April and 22 July 1981, PAG collected blood from vessels on tibiotarsi into preheparanized capillary tubes. Samples were kept frozen (-40 to -70 deg F) until electrophoretic analysis in late September. Field observations indicate that mortality associated with this sampling was less than 0.01%. Nestlings were usually bled when they were 12 days old. An additional 20 adult individuals were sacrificed in the Fall of 1980 and their tissues (liver, muscle, skeletal muscle, heart, kidney and blood) were used to survey isozyme variation.

The 257 birds sampled may be divided into four categories based on whether or not putative parents were also sampled. 1) We sampled 16 complete families, a total of 25 broods in which we analyzed blood for both putative parents and all nestlings (families may consist of several broods); 2) 12 partial families, a total of 17 broods in which blood was analyzed for the putative mother and the nestlings; 3) 4 partial families, a total of 4 broods in which blood was analyzed for the putative father and the nestlings; and 4) 8 families, a total of 11 broods, from which only the blood of the nestlings were analyzed.

Methods of electrophoretic analysis of the blood and tissue were modified from standard techniques (Selander et al. 1971). Of 22 protein loci surveyed in liver, heart, skeletal muscle, kidney and blood, 20 isozyme protein loci were monomorphic. The 2 polymorphic loci, esterase-2 – *EST-2* – (E.C. 3.1.1.7: Dixon and Webb 1964) and nucleoside phosporylase – *NSP* – (E.C. 2.4.2.1: Dixon and Webb 1964) were resolved from blood. Each was diallelic and frequencies of homozygotes and heterozygotes agree with the Hardy-Weinberg expectation computed from observed allele frequencies ($P > 0.95$ in all cases χ^2) in the adults and juveniles sampled. This result suggests that null alleles did not occur in our samples. A lithium hydroxide, pH 8.4, gel and tray buffer was used for the protein separations in a 13.5% starch matrix (Electrostarch, lot # 392, Otto Hiller Electrostarch Co., Madison, Wisconsin). Histochemical staining for esterase-2 (*EST-2*) employed the substrate alphanapthol acetate in a Na_2HPO_4 buffer solution with fast blue RR salt as the staining agent. The staining recipe for nucleoside phosphorylase included 5 units of xanothine oxidase, 30 mg of inosine, 2 mg MTT and 1 mg PMS dissolved in 0.1 *M* sodium phosphate pH 6.5. The esterase stain was permitted to develop at room temperature (20 deg C) under fluorescent light; the *NSP* stain was incubated at 37 deg C for 30 min. Gels were read immediately, photographed, and interpreted by conventional techniques (Brewer 1970).

The esterase locus products appeared as monomers with heterozygotes demonstrating a typical two banded pattern. The nucleoside phosphorylase demonstrated three patterns, two patterns consisted of only a single band and one pattern consisted of three bands. In all cases, allemorphs were labeled from the anode.

Results

We assume that the isozyme variation we observe reflects allozymic and allelic variation. No breeding has been done to check the genetic basis of the isozyme patterns; however, the observed frequencies of blood protein loci are Mendelian. Allele frequencies for the common allele at each locus were $EST\text{-}2 = 0.687$, and $NSP = 0.67$ for adults, $EST\text{-}2 = 0.704$, for $NSP = 0.65$ for nestlings. All genotypes were in Hardy Weinberg equilibrium (*EST-2* in adults, $\chi^2 = 0.85$, $P > 0.50$; *NSP* in adults, $\chi^2 = 1.38$, $P > 0.75$; *EST-2* in nestlings, $\chi^2 = 0.131$, $P > 0.25$; *NSP* in nestlings, $\chi^2 = 1.674$, $P > 0.75$), and there were no differences in the number of alleles for either locus when the adults were compared to the nestlings (for *EST-2* $\chi^2 = 0.421$, $P > 0.25$ and for *NSP*, $\chi^2 = 0.496$, $P > 0.50$).

One nestling in our sample had an electrophoretically detectable phenotype inconsistent with its putative father's (Table 1). Six nestlings from four broods had electrophoretically detectable phenotypes inconsistent with their putative mothers' phenotypes (Table 1). That is, at least one adult male (5% of 20 males sampled) cared for at least one nestling not his own, and at least 15% of 27 adult females sampled cared for nestlings not their own (Table 1). Nine percent of all broods showed evidence of multiple parentage. In the 16 completely sampled families, 25% had evidence of multiple parentage.

Table 1. Variation at two loci for five families which demonstrated patterns among one or more offspring which were inconsistent with electrophoretically detectable patterns among the putative parents. Numbers in parentheses are color band numbers. SS, FS, FF refer to electrophoretic phenotypes. Offspring sex is indicated in ( ) by M or F

Variants	Family #	Male	Female	Offspring		
	1	(L216)	(R427)	(L211 (M))		
EST-2		*SS*[a]	FS	*FF*[a]		
NSP		FS	SS	FS		
	7	(L222)	(L938)	(L271 (F))		
EST-2		FS	*SS*[a]	*FF*[a]		
NSP		FF	FS	FF		
	5	(R039)	(R033)	(L413 (M))		
EST-2		FS	*SS*[a]	*FF*[a]		
NSP		SS	FS	FS		
	9	(R105)	(L553)	(L278 (F))		
EST-2		SS	FS	FS		
NSP		FF	*SS*[a]	*FF*[a]		
	35[b]		(L145)	(L439 (F))	(1400 (F))	(L438 (M))
EST-2			SS	FS	FS	FS
NSP			*FF*[a]	*SS*[a]	*SS*[a]	*SS*[a]

[a] Indicates inconsistencies

[b] Field observations on this family are incomplete and ambiguous. PAG observed female L145 feeding nestlings and removing fecal sacs from the nest box. However, another female was also seen at the nest site. Positive identification of the resident male was never accomplished

Discussion

Our estimates of multiple parentage must be considered minimal estimates for four reasons. 1. The observation of electrophoretically detectable phenotypes are useful for exclusion only: multiple parentage will go undetected in the event that the phenotype of the putative parent is the same as the phenotype of the actual parent. 2. Electrophoresis underestimates existing genotypic variability by about 30% (Brewer 1970; Hartl 1981) leading to the inference that multiple parentage will frequently go undetected using electrophoretic analysis of protein products of many loci even when multiple parentage exists. 3. Our initial survey (see above) revealed only two polymorphic loci which further reduced the likelihood of our observing multiple parentage even if it did exist. 4. Nestlings were bled late in nestling life, thus if clutch or brood reduction occurred in response to the detection of non-kin before our sampling, multiple parentage would again be underestimated by our study.

Note that in each case we are assuming multiple parentage. The evidence clearly indicates that excluded adults are not the parent of at least one offspring in the broods they cared for. We *assume* that they are the biological parents of the other offspring on the basis of *consistency* in electrophoretic phenotypes between these adults and other offspring.

Clutches with multiple paternity were expected on theoretical grounds (Trivers 1972) and from empirical data; therefore, we were not surprised by our observations of one such clutch. To our knowledge, this is the first direct evidence for kleptogamy of males in any passerine species. Our data do not allow discrimination of the alternative explanations for this, i.e., multiple matings by females, forced extra-pair copulations, or intraspecific nest parasitism. Females who care for clutches and broods of multiple paternity may be victims, along with their mates, of intraspecific egg dumping; they simply may have mated with more than one male to produce a multiply sired brood; or they may have been force copulated. Discrimination among these explanations is not possible with current data and it is highly unlikely that electromorphic variation alone with allow elimination of any of these explanations.

Although intraspecific nest parasitism had been suggested as an explanation for female-female aggression among eastern bluebirds (Gowaty 1981), unambiguous evidence of intraspecific nest parasitism is rare if not unique (Manwell and Baker 1975). The documented intraspecific nest parasitism among eastern bluebirds may represent kleptogamy by non-resident females of territorial females and may be a regular reproductive tactic in this nest-site limited species as it may be in other cavity nesting species (Yom-Tov 1980). Intraspecific nest parasitism of females may be an adaptive option in the event of interrupted nesting as, for example, when a nest is destroyed during egg-laying. Communal egg laying by females is a possible explanation for this phenomenon although field observations in support of this contention are rare and ambiguous (Gowaty 1983b).

The six nestlings with electrophoretically detectable phenotypes inconsistent with their putative mothers' had phenotypes consistent with their putative fathers'. However, from our data we are unable to directly discern if paternity was attributable to the territorial male, or to some other male altogether. Thus one or both parents could be caring for unrelated offspring. Nevertheless, these data indicate that uncertainty of maternity may be a significant factor in the evolution of the behavior of female birds; clearly the uncertainty of maternity for birds deserves theoretical and empirical attention (Gowaty, in press).

We conclude from these minimal estimates that: 1) Multiple paternity of eastern bluebirds occurs on study sites in South Carolina. 2) Our observations add increased credibility to speculations about males and females that engage in mixed mating strategies in apparently monogamous birds. 3) Biparental care of nestlings by adult eastern bluebirds is not necessarily a reflection of the genetic kinship between putative parents and their offspring.

Our observations that adults frequently may be unrelated to the offspring they care for raises questions and issues about the mating system. For example, the assumption that the apparent mating pattern reflects the actual pattern of gametic contributions may be unwarranted. And, if multiple parentage is explainable by multiple mating of either males or females, the mating system might be more appropriately characterized as genetically polygamous in keeping with the practice of Verner and Willson (1969) of calling a species polygynous if 5% or more of nesting attempts are apparently-polygynous (R.A. Tilson, personal communication). Calling these individuals genetically polygamous is also in keeping with usage recently favored by Wickler and Seibt (1983).

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