Wilson Bull., 100(1), 1988, pp. 60–69

THE FREQUENCY OF CUCKOLDRY IN THE EUROPEAN STARLING (*STURNUS VULGARIS*)

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ABSTRACT.—Cuckoldry was studied in a New Jersey population of European Starlings (*Sturnus vulgaris*). Biopsy of both blood and pectoral muscle was done on 550 wild birds, including both adults and all chicks at 95 nests between 1983–1985. Vertical thin-layer polyacrylamide gel electrophoresis (PAGE) was employed rather than starch gel electrophoresis because we found through using both that PAGE has greater resolution for the enzymes we examined. Thirty-three loci were screened, but only three were both resolvable and found to have bona fide polymorphism. Two unambiguous cases of cuckoldry were discovered, each involving two chicks. Another six cases may have been either cuckoldry or intraspecific brood parasitism; they involved only one chick each. If only the two unambiguous cases are counted, the frequency of cuckoldry was 2.1%. If the six ambiguous cases are included, the frequency was 8.4%. Low measured frequencies do not necessarily imply a low risk of cuckoldry because they may reflect the conservatism of electrophoresis and the effectiveness of anticuckoldry behaviors. Cuckoldry may be a serious, but contained, risk in our population. *Received 18 Mar. 1987, accepted 15 June 1987.*

Cuckoldry is defined as "a male's involuntary rearing of another male's offspring as a result of the latter male (the "cuckolder") having inseminated the mate of the former male (the "cuckold")" (Power et al. 1981). This can occur as a result of extrapair copulation(s) by a female when she is unguarded by her mate, with or without her cooperation. Although there has been some disagreement regarding its usage (Gowaty 1982, 1984; Power 1984), the term "cuckoldry" seems to be the best term to describe the above defined phenomenon. Cuckoldry specifically refers to the situation in which males are the victims of an act of mate infidelity, and distinguishes between that phenomenon and brood parasitism. In this paper we use the term "cuckoldry" as it is this specific phenomenon that we wish to address.

Cuckoldry is significant from an evolutionary standpoint, because it negatively affects the reproductive success of the cuckold. Thus, males should evolve anticuckoldry behavior, and this behavior should evolve in tandem with the evolution of male parental investment. Without cuckoldry avoidance behavior, cuckoldry should impede the evolution of male parental investment, because males who do not provide parental investment will be rewarded genetically while males who do provide it will be

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punished genetically (Trivers 1972, Power and Doner 1980, Power et al. 1981).

Because birds comprise a group of animals in which male parental care is prevalent and highly developed, we expected to find behavior associated with cuckoldry in European Starlings (*Sturnus vulgaris*). In fact, many observations suggesting that cuckoldry is indeed a serious threat to the male who makes a substantial parental investment have been made in recent years in various avian species. For example:

(1) Mate guarding, the close association between males and their mates occurs with the highest frequency during the fertilizable period (Birkhead 1979, Power et al. 1981, Lumpkin et al. 1982).

(2) Extrapair copulations (EPCs) have also been observed in many apparently monogamous species of birds (McKinney et al. 1984), indicating the widespread existence (or at least possibility) of cuckoldry.

(3) EPCs have elicited various responses from males towards their mates. In some species, particularly ducks, EPCs are sometimes accomplished by force (McKinney et al. 1984), and in some of these the female's mate has been observed to copulate with her immediately afterward, apparently in response to the forced copulation of the intruder male(s). This response is consistent with the threat of cuckoldry as well as the notion of sperm competition and the "last male advantage." Female birds of some species are known to be able to store sperm for substantial periods of time (McKinney et al. 1984) with the most recently introduced sperm being the most likely to fertilize the eggs, both because of viability due to the age of sperm and their location in the female reproductive tract.

(4) In Ring Doves (*Streptopelia risoria*), males were found to reject females forcibly as prospective mates when these females had actually or apparently already been inseminated by other males (Erickson and Zenone 1976, Zenone et al. 1979).

Because the European Starling is an apparently monogamous species in which the male provides a good deal of parental care (Kessel 1957, Feare 1984), we looked for cuckoldry and associated behavior in this species. Certain apparently anticuckoldry behavioral patterns have, in fact, been documented in our Piscataway, New Jersey, population of starlings suggesting that cuckoldry is a threat to the male birds in this population. Male starlings have been found to monitor carefully the movements of their mates during the egg-laying period and to delay the onset of their own incubation duties, apparently so that they may continue to monitor their mates closely until the end of this vulnerable period (Power et al. 1981).

Until recently the existence of cuckoldry and the actual frequency with which extrapair copulations resulted in fertilization of eggs could only be

estimated crudely. Here we used electrophoresis to provide unequivocal evidence of genealogical relationships.

Evans (1980) had already used these methods to examine genetic variation in the European Starling in Britain in relation to its population biology. Other investigators have applied these methods to other species and have shown the existence of multiple parentage (Eastern Bluebirds [*Sialia sialis*], Gowaty and Karlin 1984; Bobolinks [*Dolichonyx oryzivorus*], Gavin and Bollinger 1985; Acorn Woodpeckers [*Melanerpes formicivorus*], Joste et al. 1985; Mumme et al. 1985).

MATERIALS AND METHODS

We studied starlings on the Kilmer Campus of Rutgers—The State University of New Jersey, in Piscataway, Middlesex County, New Jersey. The campus supports a population of starlings that has been monitored since 1975 (Power et al. 1981). Starlings nest in wooden nestboxes that are mounted on telephone poles along roadways. The area delineated by the roadways included some scattered buildings, fields in various stages of succession, and some large areas of mowed lawn.

Censuses were made regularly and were begun a few days before the date on which we expected laying to begin. In 1983 and 1984, nests were visited twice daily during the laying period, once in the morning and once again in the afternoon. In 1985, nests were visited three times daily during the laying period. Censuses began at 07:00, 11:00, and 16:00 EST.

Eggs were numbered in sequential order with a waterproof marking pen. Censusing ceased at any particular nest when no additional eggs were laid for three consecutive days. In order to identify hatchlings as to egg number, we modified a technique developed by Rotterman and Monnett (1984) that enabled us to dye the embryos before hatching. The dyes used were *Durkee* and *McCormick* brand food colors in red, blue, green, and yellow.

Embryos were colored by injecting dye into the air chamber of the egg when pipping was observed. Subsequently, the egg number of each hatchling was preserved by clipping its toenails according to a scheme in which each toe represented a number (Romagnano 1987). The clipped toe remained discernible for some time, enabling us to band and identify each chick in a nest by its egg number at the time of biopsy.

Because the use of electrophoresis required that we obtain tissue samples, we captured all adults and young. Upon capture, all birds were given numbered aluminum U.S. Fish and Wildlife Service bands. In addition, adults were given sex-specific color bands. Sex was determined according to the color of the iris and the base of the bill (Kessel 1951, Evans 1980, Feare 1984).

The young were captured before they fledged and were prevented from fledging prematurely by the placement of restrictors over box entrance holes. Adults could feed young through the small hole of the restrictor, but the young were too large to escape through this opening. No chick mortality was attributed to the use of restrictors.

Adult females were captured at night when they brooded chicks, the sixth day after hatching of the last chick. Adult males were captured by means of a radio-control trap (modified from a design used by Lombardo and Kemly [1983]) the following morning when they went to feed chicks.

Our biopsy procedure was modified from Baker (1981) and Seidensticker (1970) (Romagnano 1987). A small portion of pectoral muscle was removed and placed in a tube with homogenizing solution. This preparation was then placed on ice. Next, blood was drawn from the bird's brachial vein. Heparinized microhematocrit tubes were filled with blood

and then emptied into a plastic tube. The tube was immediately placed on ice. Only one of the 365 chicks biopsied died as a result of this procedure (Stangel 1986, Westneat et al. 1986).

Upon returning to the lab, muscle tissue was immediately frozen at −80°C. Blood was refrigerated at 4°C and spun for 10 min in a 13,750-rpm centrifuge (within 48 h of collection). After separation, the plasma was decanted and frozen at −80°C. The cellular fraction remaining in the tube was reserved and frozen. Plasma was then ready for use and could be thawed and loaded into a gel. Previously frozen pectoral muscle, however, had to be thawed and ground manually in the homogenizing fluid already present in the tube. The sample was centrifuged, and the supernatant decanted to separate it from cellular material. The supernatant was refrozen at −80°C until needed.

Electromorphs were determined for each bird at the three loci found to be resolvable and genetically polymorphic among the 33 that were screened. The three genetically polymorphic loci were amylase in plasma, and esterase zones 1 (more anodal) and 2 (less anodal) in pectoral muscle. An electromorph, for purposes of parental exclusion, was presumed to represent a genotype. A male was considered to have been cuckolded if his electromorph was not consistent with that of all of his putative chicks, provided there was no evidence of brood parasitism. If there was evidence of the latter, a judgement as to whether a male was cuckolded or the victim of brood parasitism was made based on field observations (see below).

A total of 550 birds were captured and biopsied at 178 nests, including both adults and all chicks at 95 nests in 1983–1985. Vertical thin-layer polyacrylamide gel electrophoresis (PAGE) was employed rather than starch because it has greater resolving power for the enzymes we examined. The procedures used for PAGE are described by Taggart et al. (1978). Recipes for stains were taken from Steiner and Joslyn (1979). Separation (pH 8.8), stacking (pH 6.8), and electrode buffer (pH 8.3) solutions were made in accordance with recipes for System A in Taggart et al. (1978). The gels themselves were mixed to obtain a 7% separation gel and a 5% stacking gel (Taggart et al. 1978).

Upon completion, gels were scored and photographed for our permanent records using black-and-white film. Scoring was according to Brewer (1970).

RESULTS

Each of the three electrophoretically resolvable polymorphic loci used was found to conform to Hardy-Weinberg equilibrium (Romagnano 1987). The adult allelic frequencies for amylase were 0.42 for the allele we designated F and 0.58 for S. The allelic frequencies for esterase 1 were 0.04 for F, 0.92 for S, and 0.04 for D. Those for esterase 2 were 0.94 for A and 0.06 for B.

Table 1 lists the genetic mismatches that were detected by electrophoresis in 1984 and 1985. The final assignment of any particular mismatch to one of three categories (cuckoldry, parasitism, or unassigned) depended ultimately on a careful review of the field notes.

Cuckoldry could be detected only electrophoretically, but a mismatch between the male resident at a nest box and his putative chicks could not be taken by itself as sufficient evidence of cuckoldry, as mismatch could arise if intraspecific brood parasitism had occurred. Field evidence of intraspecific brood parasitism included (1) the discovery of two new eggs

TABLE 1

GENETIC MISMATCHES DETECTED BY ELECTROPHORESIS AND ASSIGNED TO CUCKOLDRY OR BROOD PARASITISM ACCORDING TO COMPARATIVE ELECTROMORPH AND CORROBORATIVE BEHAVIORAL OBSERVATIONS

Cuckoldry	Parasitism	Unassigned
(1) Clutch size = 4 (1985)[a] System = Amylase Female = FS Male = SS Chick 1 = FF Chick 3 = FF	(1) Clutch size = 5 (1985) System = Amylase Female = SS Male = FS Chick 5 = FF	(1) Clutch size = 5 (1984) System = Amylase Female = FF Male = FF Chick 5 = FS
(2) Clutch size = 4 (1985) System = Amylase Female = SS Male = FF Chick 1 = SS Chick 2 = SS	(2) Clutch size = 5 (1985) System = Amylase Female = FF Male = unknown Chick 2 = SS	(2) Clutch size = 3 (1984) System = Esterase 2 Female = AA Male = AA Chick 2 = AB
	(3) Clutch size = 5 (1985) System = Amylase Female = SS Male = SS Chick 2 = FS	(3) Clutch size = 5 (1984) System = Esterase 2 Female = AA Male = AA Chick 1 = AB
	(4) Clutch size = 6 (1985) System = Amylase Female = FS Male = FF Chick 4 = SS Chick 5 = SS	(4) Clutch size = 6 (1985) System = Esterase 1; Esterase 2 Female = SS; AA Male = SS; AA Chick 4 = SD; AB

TABLE 1
CONTINUED

Cuckoldry	Parasitism	Unassigned
	(5) Clutch size = 4 (1985) System = Amylase; Esterase 2 Female = FS; AA Male = SS; AA Chick 1 = FF; AB	(5) Clutch size = 7 (1985) System = Esterase 1 Female = SS Male = FS Chick 3 = SD (6) Clutch size = 4 (1985) System = Esterase 1 Female = SS Male = SS Chick 4 = FS

[a] Year.

in a nest in one 20-h interval, (2) removal of previously marked eggs, and (3) the discovery of unmarked eggs at the base of a nestbox pole. Points (2) and (3) were considered evidence of brood parasitism because egg removal and dumping are tactics of brood parasites (Lombardo, unpubl. data). Because eggs are more or less female specific (Feare 1984), egg color, size, and shape were also noted in field notes as possible indicators of parasitism. Consequently, field notes were reviewed carefully for all nests at which mismatches were detected before a final determination was made. Cuckoldry was considered to have occurred if a mismatch with the male was detected and none of the above signs of parasitism had been observed. (For a detailed account of determinations, see Hoffenberg [1986] and Romagnano [1987].)

Cuckoldry was clearly detected at two nests in 1985 in second broods (Table 1). Five of the genetic mismatches were attributable to brood parasitism. The remaining mismatches were unassigned due to ambiguous evidence. They can be regarded as potential additional cases of cuckoldry. If only the two unambiguous cases are considered, then the frequency of cuckoldry was 2.1% (2/95 complete families). If the six ambiguous cases are added, the frequency of cuckoldry was 8.4% (8/95 complete families).

In order to detect cuckoldry through electrophoresis, the cuckold must have a genotype that differs from that of the putative father. Otherwise cuckoldry will go undetected. For two of our enzyme systems (esterases 1 and 2), the allelic frequencies indicated that the birds were nearly monomorphic (i.e., the most common alleles at each locus were 0.92 and 0.94, respectively), making it highly probable that a cuckold and a cuckolder would have the same genotype. Therefore the cuckoldry frequencies detected by this study yield conservative estimates of the actual frequency of cuckoldry.

DISCUSSION

It is noteworthy that each of the two unambiguous cases of cuckoldry occurred in second broods (all other genetic mismatches occurred in first or replacement broods). Second broods were not as closely attended by males as first broods (pers. obs.) and we believe that females were likewise not as carefully guarded.

Each of the two unambiguous cases involved two chicks, suggesting that cuckoldry may frequently involve more than one egg per nest. In contrast, usually only single parasite eggs are found in nests (Power, unpubl. data; Romagnano 1987). This difference probably reflects the different logistics of cuckoldry and brood parasitism. Cuckoldry is logistically simple because a single insemination can potentially fertilize several eggs, given that starlings can store sperm. Bullough (1942) described uterovaginal folds similar to those later found to be sperm storage organs in other

species (Compton et al. 1978, McKinney et al. 1984). Brood parasitism, however, is logistically more difficult because (1) a female starling can lay only one egg in a day (Romagnano 1987) and (2) parasites must get past host defenses each time they try to dump an egg. This makes the occurrence of more than one parasite egg/nest uncommon.

The detection of cuckoldry in our population supports our interpretation that the close guarding of females by males during the fertilizable period functions to protect males against cuckoldry. The range of frequencies (2.1–8.4%) that we measured is lower than we had expected based on our observations of cuckoldry avoidance behaviors, but this does not necessarily imply a low risk of cuckoldry as it might simply reflect the conservatism of electrophoresis, or the effectiveness of anti-cuckoldry behavior. Thus while the behavior of males suggests that cuckoldry is a serious risk in our population, it may be a contained one.

The low frequency of cuckoldry that we detected is consistent with similarly low frequencies of multiple paternity detected by electrophoresis in other species (Gowaty and Karlin 1984, Gavin and Bollinger 1985, Joste et al. 1985, Mumme et al. 1985). However, we do not know whether these low frequencies imply that cuckoldry (or multiple paternity) actually occurs at low levels, or whether this finding is only an artifact of electrophoresis and its associated techniques. The various difficulties we encountered (low variability in enzyme systems, nongenetic patterns of polymorphism) call into question whether electrophoresis is actually a good technique for this kind of study. Mumme et al. (1985) were quite explicit about the problems they encountered, and emphasized the low return that is obtained in relation to the great effort expended in such a study.

Other techniques must be explored for detecting cuckoldry in birds. Some possibilities that may be worth exploring are radiotracers (Scott and Tan 1985) and DNA marker analysis (Burke and Bruford 1987, Quinn et al. 1987, Wetton et al. 1987). We agree with Mumme et al. (1985) that electrophoresis, in its current state, is simply too time-consuming and expensive, considering the data it yields.

ACKNOWLEDGMENTS

We would like to thank K. Cybulski, H. Heintz, T. Vail, and P. C. Stouffer for their assistance in the lab and field, as well as the students in Animal Social Behavior Lab 444 at Rutgers University for their time and effort in the field. This study was supported by NSF grant BSR 8316361 to H. W. Power and T. R. McGuire, and two Busch Memorial Grants from Rutgers University to H. W. Power.

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