

MULTIPLE PATERNITY IN A TERRITORIAL PASSERINE: THE BOBOLINK

THOMAS A. GAVIN AND ERIC K. BOLLINGER

Department of Natural Resources, Cornell University, Ithaca, New York 14853 USA

ABSTRACT.—Electrophoretic and behavioral evidence corroborate the conclusion that Bobolink (*Dolichonyx oryzivorus*) females occasionally copulate with more than one male, which results in the insemination of a single clutch of eggs by those males. In 2 of 12 families from which blood samples were obtained from the mother, the putative father, and the nestlings, at least 2 males were responsible for fertilization of the clutch. We believe this is only the second bird species in which multiple paternity has been documented conclusively in the wild. Our speculation that it may not be uncommon leads to a caution for evolutionary biologists who use apparent reproductive success in birds to address questions about fitness.

Received 17 September 1984, accepted 18 December 1984.

AVIAN biologists generally assume that the number of young found in a nest represents the potential reproductive success of adults associated with that nest. Multiple paternity, defined as the genetic siring of one female's brood by more than one male, is a phenomenon that can result in disagreement between apparent and actual reproductive success for males. Multiple paternity is difficult to document unless specific laboratory techniques are performed on blood or tissue collected from the offspring and at least one of the parents, and therefore, its frequency and importance have been underestimated generally in avian population biology. In fact, Mock (1983: 57) believes the question of genetic paternity in birds is "the most crucial hurdle lying in the path of future progress in avian mating system research."

Among vertebrates, multiple paternity has been described in wild populations of fish (e.g. Hjorth 1971, Borowsky and Kallman 1976, Darling et al. 1980), salamanders (Tilley and Hausman 1976), snakes (Gibson and Falls 1975), and mammals (e.g. Birdsall and Nash 1973, Hanken and Sherman 1981). In birds, multiple paternity was intentionally encouraged in captive populations of chickens (*Gallus gallus*, Compton et al. 1978), domestic turkeys (*Meleagris gallopavo*, Payne and Kahrs 1961), and Mallards (*Anas platyrhynchos*, Burns et al. 1980). These studies, using plumage characteristics with known patterns of inheritance, established that multiple insemination of a single clutch is physiologically possible in birds. Bray et al. (1975) found that mates of vasectomized territorial male Red-winged Blackbirds (*Agelaius phoeniceus*) laid fertile eggs up to 40 days following the vasecto-

mies. These females must have participated in extrapair copulations, which suggests that multiple paternity may occur in this species.

Recently, biochemical evidence for multiple paternity in wild, free-ranging birds has been presented for Eastern Bluebirds (*Sialia sialis*, Gowaty and Karlin 1984) and Acorn Woodpeckers (*Melanerpes formicivorus*, Joste in press), using the technique of starch-gel electrophoresis. In this paper, we present behavioral and electrophoretic evidence of multiple paternity in Bobolinks (*Dolichonyx oryzivorus*) and discuss the potential biological significance of this phenomenon in birds.

METHODS

Since 1982 we have been studying two Bobolink populations, Bald Hill and Shackleton Point, located in discrete habitat "islands" 95 km apart in upstate New York (Gavin 1984). Bobolinks are found at many sites throughout the region that includes our study areas. Males return from South America in early May to establish contiguous territories that average 0.66 ha (± 0.23); females return about one week later than males. Breeding site fidelity is pronounced in this sexually dimorphic species (Martin 1974, Wittenberger 1978): 18 of 35 males (51%) and 13 of 43 females (30%) marked in 1982 returned to our study sites to breed in 1983. Males can be polygynous, and 0-4 females nest on each territory. Females build an open grass nest on the ground, lay 1 egg/day until the clutch is complete (4-7 eggs), and begin incubation with the laying of the penultimate egg. The female incubates the eggs alone for 12-13 days, but the male usually shares in the feeding of young during the 10-day nestling period (Martin 1974, Wittenberger 1982). Females are generally single-brooded (Gavin 1984).

TABLE 1. Enzymes and polymorphic loci used in electrophoresis of blood in Bobolinks. (Frequency of alleles among resident and transient adults at Shackelton Point in 1983.)

Enzyme	EC No. ^a	Locus	Allele (frequency)
Peptidase with glycyl-leucine	3.4.11 or 13	PEP-1 GL	1 (0.84)
			2 (0.10)
			3 (0.04)
			4 (0.02)
		PEP-2 GL	1 (0.98)
			2 (0.01)
Mannosephosphate isomerase	5.3.1.8	MPI	3 (0.00)
			4 (0.01)
			1 (0.94)
			2 (0.05)
Phosphogluconate dehydrogenase	1.1.1.44	PGD	3 (0.01)
			1 (0.84)
			2 (0.07)
			3 (0.04)
			4 (0.04)

^a Enzyme Commission number.

In 1983, adults in both populations were captured, fitted with a U.S. Fish and Wildlife Service leg band (if not banded from a previous year), and color-marked for easy identification by painting the tail. A male was defined as a resident if he was territorial for at least 7 days; a female was resident if she exhibited nest-building activity. A transient bird was one that did not meet the sex-specific criterion for residency. Nestlings were temporarily removed from the nest at 7 days of age to be banded. A blood sample (0.1 ml) was collected from the alar vein of each captured bird (adult and nestling), transferred to a small, plastic tube, and immediately placed into liquid nitrogen (-195°C). Blood samples were removed weekly from liquid nitrogen for storage in a freezer (-80°C) and maintained there for 1–3 months prior to analysis.

A preliminary survey of 48 presumptive genetic loci using liver, kidney, heart, muscle, and blood from 22 Bobolinks collected at 6 sites in New York but away from our study sites revealed 12 polymorphic loci (i.e. loci with two or more different alleles present in the population) using starch-gel electrophoresis (Harris and Hopkinson 1976, May et al. 1979). Each allele at a locus was assigned a number, with the most common allele designated as number 1. Four polymorphic loci were detected and could be interpreted unambiguously in blood (Table 1). None of the observed phenotypic distributions departed significantly from Hardy-Weinberg expectation in either of our study populations in 1983 ($P > 0.25$ for each of the 8 Chi-square tests). In addition, the phenotypic expression of banding patterns on the gels for heterozygous individuals was consistent with the quaternary structure expected for these particular enzymes (Darnall and Klotz 1976). Therefore, we concluded that these four loci behaved in a fashion consistent with predictions of Mendelian genetics and

that the electrophoretic phenotype corresponded to the bird's assigned genotype at each locus. (All gels were photographed with 35-mm Ektachrome 160 film; copies are available upon request from TAG.)

RESULTS

Electrophoresis.—Blood was obtained from all 7 Bobolink families ($n = 40$) at Shackelton Point and from 5 of 26 families ($n = 25$) at Bald Hill. Twenty-one nests failed at Bald Hill before blood could be sampled from nestlings. For each family, blood samples were taken from the female associated with that nest, the putative father (i.e. the male whose territory included that nest and who exhibited pairing behavior toward the female), and all nestlings alive 7–8 days post-hatching. Blood samples also were taken from 68 resident and transient adults that were not associated with surviving nestlings. That is, blood samples were obtained from all resident birds at Shackelton Point and from all but one resident at Bald Hill, as well as many transients at each site.

Matching the genotypes indicated that in two families at Shackelton Point (1) all of the nestlings in each nest could not have been sired by the putative father and (2) all nestlings in each clutch could not have been sired by any one male in the population. In one family, WR male and YY female were color-marked on 13 May and 24 May in 1983, respectively. By 28 May they were observed together regularly (pair-bonded) on WR's territory; these birds were paired also in 1982. We observed one copula-

TABLE 2. Genotypes at selected loci of two Bobolink families exhibiting multiple paternity at Shackelton Point in 1983.

Relationship	Locus ^a			
	PEP-1 GL	PEP-2 GL	MPI	PGD
Putative father (WR)	11	11	11	14
Mother (YY)	11	11	11	11
Nestlings				
1	11	14	11	11
2	11	11	11	14
3	11	11	11	14
4	11	11	11	11
5	11	11	12	11
6	11	14	11	11
Putative father (BY)	11	11	11	13
Mother (YW)	12	11	11	12
Nestlings				
1	— ^b	11	11	13
2	23	11	11	14

^a Boldfaced genotypes are those incompatible with male × female matings listed in table.

^b Locus could not be read unambiguously.

tion between these individuals on 2 June, but we rarely observed copulations by any pair. YY female built her nest, however, on the territory of an unpaired male, WY; the nest was placed about 125 m from WR's territory. When we found the nest on 7 June, it contained a full clutch of six eggs. The eggs hatched on 17 June, so egg-laying must have commenced about 31 May.

The genotypes of nestlings 1, 5, and 6 indicated they could not have been conceived by a mating between WR male and YY female because of the presence of rare alleles (i.e. a frequency of less than 5% in the population) at the MPI and PEP-2 GL loci found in neither parent (Table 2). The "bachelor" male WY, however, did exhibit the appropriate genotype to have sired these nestlings, and he was the only resident male that could have contributed allele 4 at the PEP-2 GL locus (Table 3). BW, Y, WW, and GY males each had allele 2 at the MPI locus (Table 3), but BW's genotype at the PEP-1 GL locus was inappropriate, Y and WW disappeared from the study area shortly after marking and before the nest-building period, and GY male did not appear at the site until mid-June, which was after YY female's eggs had hatched. Even if Y, WW, or GY male had sired nestling 5, then at least three males would have had to copulate with YY female to produce

nestlings with allele 4 at the PEP-2 GL locus and allele 4 at the PGD locus. In short, the behavioral observations that identify WR male as YY female's consort and the placement of her nest on the territory of WY support the conclusion that nestlings 2 and 3 were sired by WR male and nestlings 1, 5, and 6 by WY male. Nestling 4 was homozygous for the common allele at all four loci and could have been sired by either male.

In the other family, the nest of BY male and YW female produced 3 nestlings, 2 of which were bled (1 escaped). Nestling 2 could not have been sired by BY male, with whom YW female was pair-bonded and on whose territory she nested, because it is impossible to produce the genotypes at either the PEP-1 GL or PGD locus of that nestling from these two adults (Table 2). Again, only one male in the population, WG male, had the appropriate alleles to have sired nestling 2 (Table 3). WG male, however, could not have sired nestling 1 because of the presence of allele 3 at the PGD locus in that nestling. BY male, the pair-bonded mate of YW female, was the only resident male to carry this rare allele; the transient male WB also had allele 3 at PGD, but he was not seen after 13 May. WG male was pair-bonded to another female that successfully fledged two young.

Feeding behavior.—Nests were observed during randomly selected 30-min periods to determine the number of feeding trips made by each parent; insect prey was actually observed in the mouth of the attendant adult in 137 of 146 visits (94%) over all 7 nests at Shackelton Point. The rate that adults fed nestlings was determined during 36 sampling periods at this study site.

There was no significant difference in the rate of feeding trips by males (WR and BY) visiting nests containing some young not sired by them relative to males visiting nests in which multiple paternity was not documented (47% vs. 48%, $\chi^2 = 0.04$, $P > 0.75$). WR male made 33 of 76 (43%) feeding trips to YY female's nest, and BY male made 25 of 48 (52%) feeding trips to YW female's nest. In each case the attendant female made all of the remaining feeding trips. Males WY and WG were never observed to visit the nests of YY and YW, respectively.

DISCUSSION

Our evidence of multiple paternity in Bobolinks is based on behavioral observations of

consorts and electrophoretic data for each resident bird in the population. These data indicate that YY and YW females must have copulated with at least two males each. Electrophoretic data simultaneously excluded each male from paternity of at least one chick in the broods described.

Our conclusion that eggs in the same clutch were sired by more than one male follows directly from the electrophoretic data, but it is based in each case on the critical assumptions that (1) all eggs in a nest were laid only by the female tending that nest and (2) a transient male that we did not capture, but which had the appropriate alleles at each locus, did not copulate with the female and fertilize all her eggs.

First, although we cannot exclude the possibility of intraspecific nest parasitism or "egg dumping," we think parasitism is unlikely in these birds. Every resident female at Shackelton Point had an active nest containing 4-6 eggs in 1983, and hatching within each nest was synchronous (i.e. hatched within a 2-day period), which indicates that no eggs were laid in the nest after completion of the clutch. Also, for 9 nests that we found during the egg-laying period and checked every day, we did not observe more than 1 egg appearing in a nest per day; we have never found an abnormally large clutch ($n = 229$ nests). Both of these observations are consistent with the absence of nest parasitism (Yom-Tov 1980). Finally, nest sites do not appear to be a limiting resource for Bobolinks, as in cavity-nesting species in which intraspecific nest parasitism is more common (Yom-Tov 1980).

Second, every adult Bobolink resident at Shackelton Point in 1983 was captured, marked, and bled ($n = 23$), as were 24 transient adults. The opportunity for a transient male to copulate with a resident female seemed quite remote to us. When present, transient males were "escorted" from one territory to another by resident males, usually until the transient left the study site. Occasionally, a transient persisted, found an area not strongly defended, and developed his own territory. However, these males were always captured and bled.

We believe this is only the second conclusive example of multiple paternity among birds in the wild. The potential for multiple paternity to occur in Red-winged Blackbirds was illustrated by the experiment of Bray et al. (1975), but females may have sought or allowed extra-pair copulations due to subtle changes in the

TABLE 3. Genotypes of each adult Bobolink captured at Shackelton Point in 1983, other than those birds homozygous for the common allele at every locus. (Every resident bird and most transients found at this site were captured.)

	Locus			
	PEP-1 GL	PEP-2 GL	MPI	PGD
Resident males				
YY	22	11	11	11
RG	11	11	11	22
WR	11	11	11	14
WY	11	14	12	11
YG	12	11	11	11
G	12	11	11	11
WG	13	11	11	14
YW	11	11	11	11
BY	11	11	11	13
Resident females				
YW	12	11	11	12
WBk	11	12	11	14
WW	12	11	11	12
YB	11	11	13	11
Transient males				
BW	23	11	12	11
GY	11	11	12	11
Y	11	11	12	11
WW	11	11	12	11
WB	— ^a	11	11	13
RB	12	11	11	11
GR	14	11	11	14
Transient females				
WB	23	11	11	11
BB	11	11	11	23
W	11	11	11	12
Y	11	11	11	13
B	11	11	11	12
R	13	11	11	11

^a Locus could not be read unambiguously.

behavior of their vasectomized mates. That is, it is still not known whether Red-winged Blackbird females actually lay clutches fertilized by more than one male. Gowaty and Karlin (1984) found one example of "mixed paternity" in Eastern Bluebirds, meaning that a male was helping to raise a nestling not of his siring. Their data do not substantiate multiple paternity (our definition) because the genetic inconsistency in this case could have been due to intraspecific nest parasitism, which was occurring in their population. We believe the only other data on birds that adequately document multiple paternity are those of Joste (in press). She simultaneously excluded each of two males from paternity of all nest mates in one multi-

male family of Acorn Woodpeckers, a cooperative-breeding species.

The existence of multiple paternity in birds has important implications for studies that utilize avian reproductive "success" to address evolutionary questions (Sherman 1981). First, reproductive fitness commonly is assigned to mated pairs based on the number of young that fledge from their nest (e.g. Weatherhead and Robertson 1977, Wittenberger 1978, Butler and Jones-Butler 1982, Petrinovich and Patterson 1983). If multiple paternity is occurring, then this observational method can provide incorrect results. WR male and WY male would have been assigned fitness values of 6 and 0 for 1983, respectively, using this approach; their respective fitness values based on electrophoretic data were 2 and 3. Second, reproductive "success" often is found to be positively correlated with site fidelity in migratory birds (e.g. Harvey et al. 1979, Oring and Lank 1982). Should we have predicted that WY male would return to Shackelton Point in 1984 because he copulated at least once and sired several young, even though he was not paired behaviorally and provided no parental care? Third, female choice of mates must be based on some attribute of the male (e.g. rate of singing, display behavior) or characteristics of his territory (Searcy 1982, Bateson 1983), unless mate choice is random. By mating with more than one male, a female may obtain heritable, desirable traits from one male for some of her offspring, yet place her nest on another male's territory that has better resources for successful fledging of all her young (e.g. food). Fourth, natural selection may favor a reduced level of intraspecific competition if, for example, male A interacts with male B or B's mate, both of whom are raising young sired by male A. After their eggs hatched, we regularly observed Bobolink females and males foraging on the territories of other males without any aggressive interactions.

It was surprising to find multiple paternity in Bobolinks, because males defend territories for much of the breeding season and they closely guard their mates during the egg-laying period. It is important to realize that using electrophoretic data to detect multiple paternity necessarily results in a conservative estimate of its occurrence. We found multiple paternity in 2 of 12 (16.7%) Bobolink families, and at only 1 site. There was no suggestion that it

occurred in the other families. However, any of several males in either population could have participated in extrapair copulations that resulted in fertilized eggs within other families. Unless the electrophoretic phenotypes of these males differed from those of the putative fathers, we would not find offspring that were genetically inconsistent with their putative parents. Our results with Bobolinks, which we chose as a study organism prior to our interest in genetic paternity, prompt our speculation that the occurrence of multiple paternity within bird species may be substantial. If it is common, then interpretation of observational data commonly collected in ecological and behavioral studies may contain significant error. More interestingly, the factors that cause (or allow) multiple paternity and the benefits obtained by the participating female in noncooperative breeding species should be resolved. This will require samples from a large number of families within a population so that the frequency and pattern of its occurrence can be quantified.

ACKNOWLEDGMENTS

Electrophoresis was conducted at the Cornell Laboratory for Ecological and Evolutionary Genetics under the direction of B. P. May. P. B. Bollinger, D. C. McIntyre, and M. Read assisted with the fieldwork. R. C. Beason, D. K. Dawson, S. T. Emlen, P. A. Gowaty, C. A. Haas, M. E. Richmond, P. W. Sherman, and J. L. Zimmerman offered insightful discussion and constructive criticism of the manuscript. Our appreciation goes to J. L. Forney, Director of the Cornell Biological Field Station at Shackelton Point, and to F. Liddington, owner of the Bald Hill hayfields. Funding was provided by Hatch Grants 147426 and 147429, a graduate fellowship to E.K.B. from Cornell University, and the American Wildlife Research Foundation.

LITERATURE CITED

- BATESON, P. (Ed.). 1983. *Mate choice*. Cambridge, England, Cambridge Univ. Press.
- BIRDSALL, D. A., & D. NASH. 1973. Occurrence of successful multiple insemination of females in natural populations of deer mice (*Peromyscus maniculatus*). *Evolution* 27: 106-110.
- BOROWSKY, R., & K. D. KALLMAN. 1976. Patterns of mating in natural populations of *Xiphophorus* (Pisces: Poeciliidae). I. *X. maculatus* from Belize and Mexico. *Evolution* 30: 693-706.
- BRAY, O. E., J. J. KENNELLY, & J. L. GUARINO. 1975.

- Fertility of eggs produced on territories of vasectomized Red-winged Blackbirds. *Wilson Bull.* 87: 187-195.
- BURNS, J. T., K. M. CHENG, & F. MCKINNEY. 1980. Forced copulation in captive Mallards. I. Fertilization of eggs. *Auk* 97: 875-879.
- BUTLER, R. G., & S. JONES-BUTLER. 1982. Territoriality and behavioral correlates of reproductive success of Great Black-backed Gulls. *Auk* 99: 58-66.
- COMPTON, M. M., H. P. VAN KREY, & P. B. SIEGEL. 1978. The filling and emptying of the uterovaginal sperm-host glands in the domestic hen. *Poultry Sci.* 57: 1696-1700.
- DARLING, J. D. S., M. L. NOBLE, & E. SHAW. 1980. Reproductive strategies in the surfperches. I. Multiple insemination in natural populations of the shiner perch, *Cymatogaster aggregata*. *Evolution* 34: 271-277.
- DARNALL, D. W., & I. M. KLOTZ. 1976. Protein subunits: a table (revised edition). *Arch. Biochem. Biophys.* 149: 1-14.
- GAVIN, T. A. 1984. Broodedness in Bobolinks. *Auk* 101: 179-181.
- GIBSON, A. R., & J. B. FALLS. 1975. Evidence for multiple insemination in the common garter snake, *Thamnophis sirtalis*. *Can. J. Zool.* 53: 1362-1368.
- GOWATY, P. A., & A. A. KARLIN. 1984. Multiple maternity and paternity in single broods of apparently monogamous Eastern Bluebirds (*Sialia sialis*). *Behav. Ecol. Sociobiol.* 15: 91-95.
- HANKEN, J., & P. W. SHERMAN. 1981. Multiple paternity in Belding's ground squirrel litters. *Science* 212: 351-353.
- HARRIS, H., & D. A. HOPKINSON. 1976. *Handbook of enzyme electrophoresis in human genetics*. New York, American Elsevier.
- HARVEY, P. H., P. J. GREENWOOD, & C. M. PERRINS. 1979. Breeding area fidelity of Great Tits (*Parus major*). *J. Anim. Ecol.* 48: 305-313.
- HJORTH, J. P. 1971. Genetics of *Zoarcetes* populations. I. Three loci determining the phosphoglucomutase isoenzymes in brain tissue. *Hereditas* 69: 233-242.
- JUSTE, N. E. In press. Shared paternity in the Acorn Woodpecker. *Behav. Ecol. Sociobiol.*
- MARTIN, S. G. 1974. Adaptations for polygynous breeding in the Bobolink, *Dolichonyx oryzivorus*. *Amer. Zool.* 14: 109-119.
- MAY, B., J. E. WRIGHT, & M. STONEKING. 1979. Joint segregation of biochemical loci in Salmonidae: results from experiments with *Salvelinus* and review of the literature on other species. *J. Fisheries Res. Board Can.* 36: 1114-1128.
- MOCK, D. W. 1983. On the study of avian mating systems. Pp. 55-84 in *Perspectives in ornithology* (Alan H. Brush and George A. Clark, Jr., Eds.). Cambridge, England, Cambridge Univ. Press.
- ORING, L. W., & D. B. LANK. 1982. Sexual selection, arrival times, philopatry and site fidelity in the polyandrous Spotted Sandpiper. *Behav. Ecol. Sociobiol.* 10: 185-191.
- PAYNE, L. F., & A. J. KAHRS. 1961. Competitive efficiency of turkey sperm. *Poultry Sci.* 40: 1598-1604.
- PETRINOVICH, L., & T. L. PATTERSON. 1983. The White-crowned Sparrow: reproductive success (1975-1980). *Auk* 100: 811-825.
- SEARCY, W. A. 1982. The evolutionary effects of mate selection. *Ann. Rev. Ecol. Syst.* 13: 57-85.
- SHERMAN, P. W. 1981. Electrophoresis and avian genealogical analyses. *Auk* 98: 419-422.
- TILLEY, S. G., & J. S. HAUSMAN. 1976. Allozymic variation and occurrence of multiple inseminations in populations of the salamander *Desmognathus ochrophaeus*. *Copeia* 1976: 734-741.
- WEATHERHEAD, P. J., & R. J. ROBERTSON. 1977. Harem size, territory quality, and reproductive success in the Red-winged Blackbird (*Agelaius phoeniceus*). *Can. J. Zool.* 55: 1261-1267.
- WITTENBERGER, J. F. 1978. The breeding biology of an isolated Bobolink population in Oregon. *Condor* 80: 355-371.
- . 1982. Factors affecting how male and female Bobolinks apportion parental investments. *Condor* 84: 22-39.
- YOM-TOV, Y. 1980. Intraspecific nest parasitism in birds. *Biol. Rev.* 55: 93-108.