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MULTIPLE PATERNITY IN A WILD POPULATION OF MALLARDS

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ABSTRACT.—Blood, heart, liver, and breast muscle samples from wild Mallard (*Anas platyrhynchos*) hens and their ducklings were analyzed using starch-gel electrophoresis. We found that 17.4% of the total broods sampled ($n = 46$) involved observable multiple mating events, that 25.8% of the sample broods involved multiple mating events when broods that were unlikely to yield information on multiple paternity were excluded ($n = 31$), and that a pooled maximum-likelihood estimate indicated a 60% chance that a particular duckling came from a brood that was multiply fathered, or at least 48% of broods involved multiple mating events. Received 15 January 1987, accepted 8 June 1987.

MALLARDS (*Anas platyrhynchos*) are basically monogamous, forming new pair bonds before each breeding season. Most copulations occur between mates, but copulations have also been observed between females and males other than their mates. The behavior associated with pair-bond and extrapair copulations is very different. Copulations between paired individuals are preceded by head-pumping by both sexes, the female assumes a prone posture, the male mounts and bends his tail around the female's, and there is an obvious thrust. After a successful copulation, the male performs characteristic postcopulatory displays, usually bridling and nod-swimming (Lorenz 1951). In extrapair copulations, precopulatory displays are absent, the male pursues the female in a vigorous chase and, if he succeeds in grasping her, mounts, and copulates apparently by force. After an apparently successful copulation, the male usually departs quickly, sometimes in response to active defense by the female's mate, and often without performing postcopulatory displays. This be-

havior has been called "rape" (Titman and Lowther 1975, Barash 1977), but we prefer the more neutral term "forced copulation" (FC).

Although Heinroth (1911) described FC behavior in waterfowl and suggested that male Mallards were combining pair bonding with promiscuity via FC, many observers have since considered FC to be an aberrant behavior caused by factors such as crowding or urbanization (Bezzel 1959, Wüst 1960). This and several other hypotheses have been suggested for the occurrence of FC behavior in waterfowl (McKinney et al. 1983). The most plausible explanation for FC, however, is that it is a secondary reproductive strategy of paired males. While their mates are laying or incubating, these males attempt to fertilize more eggs by inseminating other females. This agrees with Trivers's (1972) prediction that males of monogamous species should be expected to maximize their reproductive success by helping a single female while also taking advantage of opportunities to inseminate other females that they will not help.

The hypothesis that male Mallards use a mixed male strategy of this kind is supported by several lines of evidence (McKinney et al. 1983): (1) the paired status of males involved in FC attempts has been established in both captive and wild populations, (2) FCs occur during the period when eggs are being fertilized and appear to be directed mainly at fertilizable females, (3) paired males usually defend their mates from FC attempts and may force copulation on their mates after they have been subjected to FC, and (4) studies of captives have shown that females can store viable sperm for up to 17 days, with full viability maintained for the first 8 days. In addition, in captive Mallards sperm from different males compete, and eggs can be fertilized by sperm delivered during FC (Burns et al. 1980, Cheng et al. 1983).

That males observed performing FCs are actually achieving intromission and fertilizing eggs is a crucial assumption of the hypothesis that FCs are a reproductive strategy for males. Using plumage markers in captive Mallards, Burns et al. (1980) showed that at least 8% of the progeny of one study group differed from the expected type and must have resulted from forced copulations. These proportions cannot be extrapolated to wild Mallards, however. Our purpose was to measure the incidence of multiple paternity in a wild population of Mallards and thereby to gain some understanding of the importance of FC activity in wild Mallards.

Biochemical methods of paternity testing have become an important tool in understanding fertility selection (Siegismund and Christiansen 1985) and mating systems in both plants (Ellstrand 1984) and animals (e.g. Birdsall and Nash 1973, Anderson 1974, Hanken and Sherman 1981, Ross 1986). These techniques have been applied to birds only recently and have yielded valuable insight into reproductive strategies used by several species. Until recently, parentage in apparently monogamous species of birds was not questioned. Gowaty and Karlin (1984), however, found that in Eastern Bluebirds (*Sialia sialis*) "at least 5% of adult males and 15% of adult females were caring for at least one offspring not their own." Evidence of extrapair copulations (EPCs) resulting in multiple paternity has been found in Bobolinks (*Dolichonyx oryzivorus*; Gavin and Bollinger 1985), Indigo Buntings (*Passerina cyanea*; Westneat 1987), and House Sparrows (*Passer domesticus*; Burke and Bruford 1987, Wetton et al. 1987). Multiple par-

entage also has been found in two species of cooperative breeders. Multiple paternity has been confirmed in two studies of Acorn Woodpeckers (*Melanerpes formicivorus*; Joste et al. 1985, Mumme et al. 1985), and in White-fronted Bee-eaters (*Merops bullockoides*) multiple paternity and maternity both occur as a result of EPCs, intraspecific egg parasitism, and "quasi-parasitism" (parasitism by a female that was fertilized by the male attending the parasitized nest) (Wrege and Emlen 1987). In waterfowl, multiple parentage as a result of extrapair copulations and intraspecific brood parasitism has been confirmed in Lesser Snow Geese (*Anser caerulescens caerulescens*; Quinn et al. 1987). We used biochemical techniques for testing paternity in dabbling ducks, and we present the first concrete evidence of multiple paternity in this group.

METHODS

Mallard hens and their clutches were collected in the prairie pothole region of southwestern Manitoba. Males were not collected because of the great investment of time required to determine which male was paired to each hen. Samples of blood, heart, liver, and breast muscle were taken from the hens immediately, and from the ducklings when they were 10 days old; this delay ensured the absence of fetal enzymes (Simons 1966). Brood sizes ranged from 3 to 11 ducklings. Originally, clutches of 6 or more eggs were collected. The smaller brood sizes are a result of embryo mortality during incubation.

The samples were analyzed using starch-gel electrophoresis (May et al. 1979). The genotype of each individual was determined at specific genetic loci. Eight loci had enough variability to be valuable for the analysis (Table 1): glutathione reductase (GR), adenosine deaminase (ADA), glycerol-3-phosphate (G-3-P), aspartate aminotransferase (AAT), a peptidase (PEP-GL1), isocitrate dehydrogenase (IDH), mannosephosphate isomerase (MPI), and phosphogluconate dehydrogenase (PGD). Histochemical stains were adapted from Harris and Hopkinson (1976), and gels were scored blindly with respect to the hypothesis being tested.

To determine the incidence of multiple paternity in this population, genotypes of ducklings and hens were compared for each brood, and a probability model (Williams and Evarts in prep.) was developed that used a method of maximum likelihood to estimate model parameters. The model has two parameters of interest: θ , the probability of a randomly chosen duckling having a certain allele (with all other alleles pooled), and Φ , the probability that the duckling comes from a clutch that was multiply fathered. This model

TABLE 1. Allelic frequencies for the 8 loci used in the estimate of concurrent multiple paternity.

Enzyme [E.C. no.]		Allelic frequency			
		1	2	3	Other ^a
Glutathione reductase (GR) [1.6.4.2]	Hens	0.540	0.439	0.021	
	Ducklings	0.452	0.524	0.024	
Adenosine deaminase (ADA) [3.5.4.4]	Hens	0.840	0.140	0.020	
	Ducklings	0.821	0.156	0.015	0.008 ^b
Glycerol-3-phosphate (G-3-P) [1.1.1.8]	Hens	0.970	0.030		
	Ducklings	0.963	0.026	0.011 ^b	
Aspartate aminotransferase (AAT) [2.6.1.1]	Hens	0.990	0.010		
	Ducklings	0.995	0.005		
Peptidase (PEP-GL1) [3.4.11-13]	Hens	0.880	0.020		0.100
	Ducklings	0.864	0.029		0.107
Isocitrate dehydrogenase (IDH) [1.1.1.42]	Hens	0.990	0.010		
	Ducklings	0.997	0.003		
Mannosephosphate isomerase (MPI) [5.3.1.8]	Hens	0.770	0.140		0.090
	Ducklings	0.773	0.133		0.094
Phosphogluconate dehydrogenase (PGD) [1.1.1.44]	Hens	0.979			0.021
	Ducklings	0.987			0.013

^a Alleles with small frequencies were combined.

^b Most likely paternal alleles. These loci did not contribute to the minimum estimates of multiple paternity.

is an extension of two allele models used by Arnold (1981) and Arnold and Morrison (1985) to estimate allele probabilities. The data used for the model are the number of each mother's offspring that are homozygous for the allele of interest, heterozygotes, or homozygous for the pooled other alleles. This is represented symbolically by a progeny vector $n = (n_0, n_1, n_2)$ for each mother, where n_0 is the number of progeny homozygous for the pooled other allele, n_1 is the number of progeny heterozygous for the allele of interest, and n_2 is the number of progeny homozygous for the allele of interest. The mother's allelic contribution is recorded by y_0 , which equals the number of copies of the allele of interest carried by the mother. If the mother is singly inseminated, the father's allelic contribution is recorded similarly by y_1 , while under double insemination y_1 and y_2 record the allelic status of the first and second male, respectively. The variables y_0 , y_1 , and y_2 have values of 0, 1, or 2.

Using this notation, the probability of observing a particular progeny array n , given y_0 , θ , and Φ , is:

$$P(n|\theta, \Phi) = (1 - \Phi) \sum_{y_1=0}^2 P(n|y_1)P(y_1|\theta) + \Phi \sum_y P(n|y)P(y|\theta)$$

(Williams and Evarts in prep.), where y equals either $\{y_0, y_1\}$ or $\{y_0, y_1, y_2\}$, and the probabilities $P(n|y_1)$, $P(y_1|\theta)$, $P(n|y)$, and $P(y|\theta)$ are determined from Mendelian segregation ratios. This expression can then be used to form the likelihood of the sample, on which the method of maximum likelihood is based.

The maximum-likelihood estimates were computed

on a locus-by-locus basis, and standard errors were estimated by jackknifing (Efron 1983) across loci.

RESULTS

Some instances of multiple paternity for a brood can be detected directly by examining the genotypic information from the mother and her progeny array. For example, in one brood the mother had a 11 genotype at the PEP locus, while the genotypes 11, 12, and 13 occurred among her progeny. This could happen only if more than one male mated with the mother. Among the 46 broods there are 8 such directly observable cases of concurrent multiple paternity (hereafter CMP). Thus, 8 of 46 (17.4%) sample broods must have involved multiple mating events. Nine of 298 (3.0%) ducklings appeared to have been fathered by a male other than the female's mate, assuming in each case that the largest collection of compatible genotypes in a brood were associated with her mate.

CMP was observed only in broods of six or more ducklings, however, largely because it is unlikely that CMP would be detected in small broods (Akin et al. 1984). When only broods of six or more ducklings were considered, 8 of 31 (25.8%) of the sample broods involved multiple mating events, and 9 of 238 (3.8%) ducklings appeared to have been fathered by a male other than the mate.

These percentages are most likely underestimates because of two sources of error in estimating CMP. These errors could arise where a second father has a genotype with alleles already present in the mother and first father, and when allelic diversity in multiple fathers is not seen because of small brood size.

To improve upon the empirical estimates of CMP, we decided to estimate the actual amount of CMP that occurred in this sample by calculating the probability that a randomly chosen duckling came from a brood that involved CMP.

Maximum-likelihood estimates of CMP probability at each of the eight loci were found using three methods (likelihood plots, steepest ascent, and iteratively reweighted least squares). Three loci (AAT, IDH, and PGD) had allele probabilities too close to 1 to yield information about the probability of CMP. The estimates for the other five loci were pooled, and the combined estimate of CMP for all five loci was 0.60 (SE = 0.066). That is, there was a 60% chance that a particular duckling came from a brood that was multiply fathered. The percentage of broods that had CMP events does not necessarily equal 60% because of differential brood sizes in the sample. By putting 60% of the total ducklings into the largest broods, we obtain a minimum estimate of 48% for the broods that involved CMP.

The model assumes that if a brood is multiply fathered, then it is doubly fathered; that Mendelian laws apply when a brood is singly fathered, and if doubly fathered, then random sperm mixing occurs; and that random mating occurs. Cheng et al. (1983) showed that for Mallards, the proportion of progeny from two different artificial inseminations was not significantly different if the two inseminations occurred simultaneously, 1 h apart, or 3 h apart. If competing doses of sperm were administered 6 or more hours apart, however, significantly more progeny were attributed to the second of the two inseminations. Because this does not agree with the second assumption, we performed a Chi-square test of goodness of fit on the 5 loci used in the CMP estimation. The fit ($df = 3$) for 3 of the loci was good (ADA: $\chi^2 = 4.47$, $P = 0.213$; G-3-P: $\chi^2 = 3.39$, $P = 0.335$; PEP: $\chi^2 = 2.87$, $P = 0.414$), and the fit for GR ($\chi^2 = 7.18$, $P = 0.065$) was fair. The null hypothesis was rejected at the MPI locus ($\chi^2 = 10.14$, $P = 0.017$), however. We concluded that the overall pattern is acceptable. Furthermore, given that competing doses of sperm seem to mix random-

ly if inseminations are less than 6 h apart, that both forced and pair copulations are most frequent in the morning (Cheng et al. 1982), and that mates are more likely to attempt forced pair copulations within 1 h after a forced copulation attempt has occurred (Barrett 1973), we feel the model is useful in providing an estimate of the probability of CMP.

DISCUSSION

Multiple parentage occurred in at least 17–25% of the Mallard clutches examined, and we believe this resulted from copulations between females and males other than their mates. There is no evidence that female Mallards solicit copulations from other males, and when they are approached by these males they fly long distances, dive repeatedly, and struggle constantly to get away (Titman 1973). Females that have been victims of repeated copulation attempts may be injured or even killed as a result (Huxley 1912, Titman and Lowther 1975), and often hide in cover or crouch when other Mallards approach or fly over (Titman 1973). This strongly suggests that the copulations are forced, rather than solicited by females.

In theory, however, there is another possible source of this observed multiple parentage: intraspecific brood parasitism. If this were the case, two predictions follow. First, supernormal clutches should be found. Supernormal clutches are common in Wood Ducks (*Aix sponsa*; Grice and Rogers 1965), Redheads (*Aythya americana*; Weller 1959), and Common Goldeneyes (*Bucephala clangula*; Andersson and Eriksson 1982), which show a relatively high frequency of intraspecific brood parasitism. No supernormal clutches have been reported for Mallards nesting under dispersed conditions, and none were found in the clutches we collected. Wild Mallard clutch sizes average 9 eggs but range up to 14 eggs (Coulter and Miller 1968, Dzubin and Gollop 1972). In the clutches collected for this study the average clutch size was 9.2, and clutches ranged up to 12 eggs.

Second, hens should visit other nests or follow other hens back to their nests. This behavior is known to occur in hole-nesting and over-water nesting birds in which brood parasitism is common (e.g. Wood Ducks and Redheads; Weller 1959, Grice and Rogers 1965). Such behavior has not been reported in Mallards, whose nests are usually quite dispersed (e.g. 2.3–2.6

pairs/km²; Drewien and Springer 1969, Duebbert 1969). Presumably, dispersed nests are difficult to find and make brood parasitism impractical for hens of this species.

The evidence for a relatively high incidence of multiple paternity in wild Mallards, combined with a lack of evidence for brood parasitism, strongly indicates that forced copulation is an important secondary reproductive strategy of paired males in this species. Indeed, the maximum-likelihood estimate of CMP in our sample indicated that at least half of the females in the population were involved in multiple mating events. This raises interesting questions on the tactics males use to achieve FCs and to maximize their effectiveness, and how males compromise between FC activities and minimizing the likelihood of being cuckolded.

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LITERATURE CITED

- AKIN, E., H. LEVENE, L. LEVINE, & R. ROCKWELL. 1984. A conservative procedure for the estimation of multiple insemination in *Drosophila*. *Amer. Natur.* 124: 723-737.
- ANDERSON, W. W. 1974. Frequent multiple insemination in a natural population of *Drosophila pseudoobscura*. *Amer. Natur.* 108: 709-711.
- ANDERSSON, M., & M. O. G. ERIKSSON. 1982. Nest parasitism in Goldeneyes *Bucephala clangula*: some evolutionary aspects. *Amer. Natur.* 120: 1-16.
- ARNOLD, J. 1981. Statistics of natural populations. I. Estimating an allele probability in cryptic fathers with a fixed number of offspring. *Biometrics* 37: 495-504.
- , & M. MORRISON. 1985. Statistics of natural populations. II. Estimating an allele probability in families descended from cryptic mothers. *Genetics* 109: 785-798.
- BARASH, D. P. 1977. Sociobiology of rape in Mallards (*Anas platyrhynchos*): responses of the mated male. *Science* 197: 788-789.
- BARRETT, J. 1973. Breeding behavior of captive Mallards. M.Sc. thesis, Minneapolis, Univ. Minnesota.
- BEZZEL, E. 1959. Beiträge zur Biologie der Geschlechter bei Entenvögeln. *Anz. Ornithol. Ges. Bayern* 5: 269-355.
- 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.
- BURKE, T., & M. W. BRUFORD. 1987. DNA fingerprinting in birds. *Nature* 327: 149-152.
- BURNS, J. T., K. M. CHENG, & F. MCKINNEY. 1980. Forced copulation in captive Mallards: I. Fertilization of eggs. *Auk* 97: 875-879.
- CHENG, K. M., J. T. BURNS, & F. MCKINNEY. 1982. Forced copulation in captive Mallards (*Anas platyrhynchos*): II. Temporal factors. *Anim. Behav.* 30: 695-699.
- , ———, & ———. 1983. Forced copulation in captive Mallards: III. Sperm competition. *Auk* 100: 302-310.
- COULTER, M. W., & W. R. MILLER. 1968. Nesting biology of Black Ducks and Mallards in northern New England. *Vermont Fish Game Dept. Bull.* 68-2.
- DREWEN, R. C., & P. F. SPRINGER. 1969. Ecological relationships of breeding Blue-winged Teal to prairie potholes. Pp. 102-105 in *Saskatoon Wetlands Seminar*. Can. Wildl. Serv. Rept. Ser. No. 6.
- DUEBBERT, H. F. 1969. High nest density and hatching success of ducks on South Dakota CAP land. *North Amer. Wildl. Nat. Resources Conf.* 34: 218-228.
- DZUBIN, A., & J. B. GOLLOP. 1972. Aspects of Mallard breeding ecology in Canadian parkland and grassland. Pp. 113-152 in *Population ecology of migratory birds*. *Bur. Sport Fish. Wildl., Wildl. Res. Rept.* 2.
- EFRON, B. 1983. The jackknife, the bootstrap, and other resampling plans. *Soc. Ind. Appl. Math. CBMS, Natl. Sci. Found. Monogr.* 38.
- ELLSTRAND, N. C. 1984. Multiple paternity within the fruits of the wild radish, *Raphanus sativus*. *Amer. Natur.* 123: 819-828.
- GAVIN, T. A., & E. K. BOLLINGER. 1985. Multiple paternity in a territorial passerine: the Bobolink. *Auk* 102: 550-555.
- 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.
- GRICE, D., & J. P. ROGERS. 1965. The Wood Duck in Massachusetts. Final rept., Federal Aid in Wildlife Restoration, Project No. W-19-R. Westboro, Massachusetts, Div. Fisheries Game.

- 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.
- HEINROTH, O. 1911. Beiträge zur Biologie, namentlich Ethologie und Psychologie der Anatiden. *Verh. 5 Int. Ornithol. Kongr.*: 589-702.
- HUXLEY, J. S. 1912. A "disharmony" in the reproductive habits of the wild duck (*Anas boschas* L.). *Biol. Centralblatt* 32: 621-623.
- JOSTE, N., J. D. LIGON, & P. B. STACEY. 1985. Shared paternity in the Acorn Woodpecker (*Melanerpes formicivorus*). *Behav. Ecol. Sociobiol.* 17: 39-41.
- LORENZ, K. 1951. Comparative studies on the behaviour of Anatinae. *Avicult. Mag.* 57: 157-182.
- 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. Fish. Res. Board Can.* 36: 1114-1128.
- MCKINNEY, F., S. R. DERRICKSON, & P. MINEAU. 1983. Forced copulation in waterfowl. *Behaviour* 86: 250-294.
- MUMME, R. L., W. D. KOENIG, R. M. ZINK, & J. A. MARTEN. 1985. Genetic variation and parentage in a California population of Acorn Woodpeckers. *Auk* 102: 305-312.
- QUINN, T. W., J. S. QUINN, F. COOKE, & B. N. WHITE. 1987. DNA marker analysis detects multiple maternity and paternity in single broods of the Lesser Snow Goose. *Nature* 326: 392-394.
- ROSS, K. G. 1986. Kin selection and the problem of sperm utilization in social insects. *Nature* 323: 798-800.
- SIEGISMUND, H. R., & F. B. CHRISTIANSEN. 1985. Selection component analysis of natural polymorphisms using population samples including mother-offspring combinations, III. *Theor. Pop. Biol.* 27: 268-297.
- SIMONS, J. A. 1966. The ontogeny of the multiple molecular forms of hemoglobin in the developing chick under normal and experimental conditions. *J. Exp. Zool.* 162: 219-230.
- TITMAN, R. D. 1973. The role of the pursuit flight in the breeding biology of the Mallard. Ph.D. dissertation, Fredericton, Univ. New Brunswick.
- , & J. K. LOWTHER. 1975. The breeding behavior of a crowded population of Mallards. *Can. J. Zool.* 53: 1270-1283.
- TRIVERS, R. L. 1972. Parental investment and sexual selection. Pp. 136-179 in *Sexual selection and the descent of man, 1871-1971* (B. Campbell, Ed.). Chicago, Aldine.
- WELLER, M. W. 1959. Parasitic egg laying in the Redhead (*Aythya americana*) and other North American Anatidae. *Ecol. Monogr.* 29: 333-365.
- WESTNEAT, D. F. 1987. Extra-pair fertilizations in a predominantly monogamous bird: genetic evidence. *Anim. Behav.* 35: 877-886.
- WETTON, J. H., R. E. CARTER, D. T. PARKIN, & D. WALTERS. 1987. Demographic study of a wild House Sparrow population by DNA fingerprinting. *Nature* 327: 147-149.
- WREGE, P. H., & S. T. EMLÉN. 1987. Biochemical determination of parental uncertainty in White-fronted Bee-eaters. *Behav. Ecol. Sociobiol.* 20: 153-160.
- WÜST, W. 1960. Das Problem des Reihens der Enten besonders von *Anas strepera*. *Proc. 12th Intern. Ornithol. Congr.* 2: 795-800.