

# SPERM COMPETITION AFTER SEQUENTIAL MATING IN THE RINGED TURTLE-DOVE<sup>1</sup>

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**Abstract.** Male Ringed Turtle-Doves (*Streptopelia risoria*) were sequentially mated, two to a single female, using albinism as a genetic marker. We determined that the second male had a higher probability of fertilizing the ova. This advantage is contingent on the female's reproductive stage at the time of pairing. Females which ovulate within two days of encountering the second male lay eggs fathered by the first male, but subsequent eggs are fathered by the second male if he was able to copulate with the female. Thus, in this domesticated columbiform, as has been reported in domesticated Galliformes and Anseriformes, there seems to be a second male precedence in competitive mating situations. This finding is consistent with the utility of reported behavioral paternity guards.

**Key words:** Sperm competition; mate guarding; extra-pair copulation; paternity guards; reproductive behavior; follicular development; Ringed Turtle-Dove; *Streptopelia risoria*.

## INTRODUCTION

Over 90% of all avian species are thought to be monogamous (Lack 1968, Silver et al. 1985). As a consequence of this, paternity has traditionally been viewed as a simple by-product of mating and the outcome of sperm competition in birds has been little explored. However, several recent field studies have shown that, in "apparently" monogamous species (Gowaty 1983) extra-pair copulations are routinely observed (see McKinney et al. 1984 for review). Also, it has been shown that the occurrence of these extra-pair copulations is not random. For example, in a captive population of Green-winged Teal (*Anas crecca carolinensis*), it was found that paired males preferentially attempted to force copulations on females in the laying or prelaying condition (McKinney and Stolen 1982). In the closely related Mallard (*Anas platyrhynchos*) Burns et al. (1980) demonstrated that before and during laying, forced copulations can result in the fertilization of eggs and the consequent kleptogamy, sometimes called cuckoldry (see Gowaty 1982 for discussion), of the female's mate.

Trivers (1972) extending the work of Bateman (1948), argued that in species with a substantial

male parental investment, the male can be expected to protect his investment by adopting a variety of tactics to insure that he has fathered the offspring. Since Trivers first discussed these issues, a number of behavioral paternity guards have been proposed for males in monogamous avian species. Among the hypothesized paternity guard mechanisms are: (1) escorting the mate just prior to and during the egg laying period (Beecher and Beecher 1979; Birkhead 1979, 1982; Power and Doner 1980; Carlson et al. 1985); (2) immediately copulating with the mate subsequent to her mating with another male (McKinney and Stolen 1982); (3) delaying incubation until clutch completion to facilitate guarding (Power et al. 1981); (4) discriminating male from female territorial intruders during the laying period (Power and Doner 1980, but see Gowaty 1981); and (5) discriminating previously courted females (which may be carrying another male's sperm) from noncourted ones (Erickson and Zenone 1976, Zenone et al. 1979). An understanding of the dynamics of sperm competition which has been so useful in other taxa (e.g., Parker 1970) is essential for a proper analysis of the utility of any of these avian paternity guards.

In this study we examine the consequences of multiple mating and the resultant sperm competition for captive Ringed Turtle-Doves (*Streptopelia risoria*). The male Ringed Turtle-Dove, like many male birds, devotes a considerable amount of time and energy in the rearing of the eggs and young. It has been described as showing two of the paternity guards listed above: (1) sur-

<sup>1</sup> Received 24 February 1986. Final acceptance 11 July 1986.

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veillance or mate guarding during the female's fertile period (Lumpkin et al. 1982); and (2) the discrimination of a previously courted female which may be carrying another male's sperm, in that male doves direct less courtship and more aggressive behavior toward females that exhibit premature nest-soliciting behavior as a result of their prior exposure to other males (Erickson and Zenone 1976, Zenone et al. 1979). Zenone et al. (1979) interpret this second behavioral paternity guard as the male's attempt, by means of aggressive behavior, to postpone ovulation beyond the effective life time (six days) of any sperm previously deposited by another male. However, the degree to which the dynamics of sperm precedence make such paternity guards appropriate has not hitherto been determined. If the sperm deposited last is favored in a competition, then these hypothesized guards are useful; if the sperm deposited first is favored, then a reinterpretation of their utility is necessary. We examine the importance of mating order on reproductive success, using the phenotypic marker of albinism, by sequentially mating male doves and ascertaining who fathered the progeny.

#### MATERIALS AND METHODS

Albino female, albino male, and wild-type male doves were raised in the laboratory. To insure that wild-type males were homozygous, breeding lines were traced back at least seven generations. This examination of lineage confirmed that albino females (XO) and wild-type males (XX) matings always produce wild-type offspring as was previously reported by Hollander (1959). Albino female (XO) and albino male (XX) matings always result in albino offspring. All birds had previous breeding experience with birds not included in the study. Each was kept in visual, but not auditory, isolation from other birds for a minimum of three weeks prior to use. In one group, each albino female was paired to a wild-type male in a standard breeding cage (80 × 34 × 35 cm) with food, grit, water, nesting material, and a glass nest-bowl. Observations of each pair were made the first hour of pairing. Spot checks for copulations were made daily for four days. On the fifth day of pairing, the male and female were separated by an opaque partition for 1 hr, then the partition was removed and each pair was observed for 1 hr. The male and female were again separated by an opaque partition and the wild-type male was removed and exchanged with

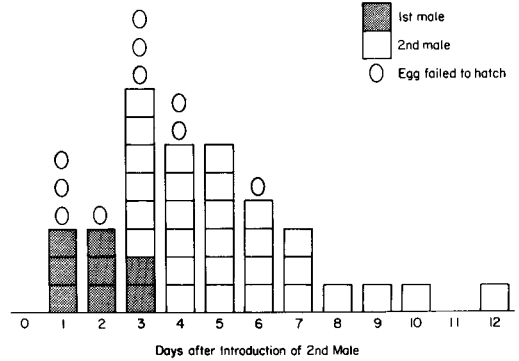


FIGURE 1. Paternity of offspring in reference to day of egg laying. For example, on day 1 after introduction of the second male, six eggs were laid. Three were fertilized by the first male and three others failed to hatch. On day 3 a total of 11 eggs were laid. In two cases the eggs were fertilized by the first male, and in six cases the eggs were fertilized by the second male. The remaining three eggs failed to hatch.

an albino male. The albino male was permitted 1 hr behind the partition to adjust to the new surroundings. The partition was then removed and the female was observed for 1 hr with the albino male ( $n = 12$ ). Observations were conducted between 09:30 and 14:00. Spot checks for copulation were continued daily until completion of the clutch (two eggs). The same procedure was repeated with a reversal of the mating order. Albino females were each paired for four days with albino males. On the fifth day, each albino male was exchanged for a wild-type male ( $n = 12$ ). Paternity of the offspring was established by phenotype.

#### RESULTS

##### LAYING LATENCY AND RATIO OF PROGENY BY FIRST AND SECOND MALE

Figure 1 shows the paternity of offspring with respect to the day the first egg was laid from the time of mate exchange. For eggs laid four or more days after mate exchange, all offspring were fathered by the second male regardless of his genotype. The first males fathered offspring from eggs laid within two days after mate exchange, but only two of the eight offspring from eggs laid three days after mate exchange. These two cases are particularly interesting as illustrations that paternity may be mixed within a single clutch. The young hatched from the second eggs, laid on day 5, were fathered by the second male. In both cases, copulations with the second male were not

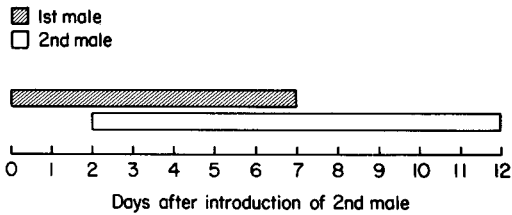


FIGURE 2. Respective fertilizing potential of first and second males in reference to day of egg laying. An egg laid on day 7 is ovulated in the early morning of day 6. The stored sperm of the first male is viable at least until this time. The second male has his first opportunity to fertilize an egg laid on day 2, which is ovulated early on the day after his introduction.

observed until day 2 after mate exchanges. All other females laying on day 3 or later were observed copulating with second males, but not always on the day of his introduction. The minimum time period between copulations of the first and second males was 4 hr.

Of the eight females laying on day 1 and 2 that successfully hatched young, only two were observed copulating with the second male. Although the male had the time and opportunity to fertilize the second egg, most of the females did not appear receptive. Paternity of the second egg could not be established, since all were either cracked or thrown out of the nest. The other four females that laid eggs on the first two days did display behavioral receptivity to the male, but in each case the eggs were abandoned prior to hatching. These females laid a new clutch of eggs which were fathered by the second male.

#### FEMALE'S FOLLICULAR DEVELOPMENT: A CONTRIBUTING FACTOR IN THE OUTCOME OF SPERM COMPETITION

The fertilizing potential of the first and second male, respectively, is depicted in Figure 2. The potential of the first male is limited by the female's capacity to store viable sperm in the oviduct which can be as long as six days after insemination (Zenone et al. 1979). In contrast, the second male's ability to fertilize the eggs is limited by the amount of time he has to inseminate the female prior to ovulation. Ovulation precedes egg laying by 36 to 40 hr (Riddle and Behre 1921) and fertilization usually occurs within the first few hours after ovulation (Bohr et al. 1964). The first egg appears in the early evening coincident with the second ovulation (Sims 1983, Sims et al. 1983). Hence the second male had no

opportunity to fertilize an egg laid on day 1 after his introduction, since the ova had already been fertilized by the first male. With respect to an egg laid on day 2, the male had only a few hours in which to inseminate the female which had ovulated in the early morning hours of day 1. This means the second male had only the previous afternoon to achieve fertilization. Egg laying on day 3 allowed the male about 1.5 days to inseminate the female. The fertilizing potential of the first and second males, therefore, depends on how soon ovulation takes place after the second male's copulation. In other words, a male's fertilizing potential depends on the female's stage of follicular development at the time of his insemination. Follicular growth proceeds slowly but steadily until five or more days of pairing. It then proceeds in an exponential fashion during approximately the last two days until ovulation (Sims 1983, Sims et al. 1983). Application of the present data to this pattern of follicular growth indicates that the second male has a high probability of fathering the offspring if he mates before the exponential growth phase. After this critical time, the second male's fertilizing potential may be limited not only by there being less time to copulate before ovulation, but also by a reduction in the female's behavioral receptivity. The second male's potential for paternity of the offspring is thus determined by the female's reproductive stage.

#### DISCUSSION

The results of the present study demonstrate that a second male has the capacity and even the advantage in fathering the offspring of a previously inseminated female. This finding agrees with what is known about the timing of insemination in birds and the probability of fertilization from studies of domesticated fowl and ducks. For example, it has been shown in domestic hens (*Gallus domesticus*) by Warren and Gish (1943) and in domestic turkeys (*Meleagris gallopavo*) by Payne and Kahrs (1961) that, if females are inseminated on successive days with semen from different phenotypes, the second insemination takes precedence over the first. This advantage to the second insemination is maintained even if the inseminations occur within hours of each other, as is illustrated by Compton et al.'s (1978) study where domestic hens were sequentially inseminated at 4-hr intervals with semen from two different male phenotypes. About 80% of the re-

sulting progeny had the phenotype of the second insemination. Cheng et al. (1983) also found an advantage to the second male, when they sequentially inseminated Mallard hens at an interval of 6 hr or more with semen from drakes with different phenotypes.

Compton et al. (1978) argue that this advantage occurs because the uterovaginal sperm-host gland of the hen fills sequentially, with the most recent semen having first access to the eggs. The data reported here on doves and those of Cheng et al. (1983) on ducks are consistent with this hypothesis. All studies in birds to date have reported a mating advantage to the second male in a competitive mating situation. However, one must be cautious when extrapolating from data on domesticated forms (representing only three orders) to birds in general, especially with the diversity of mating-order effects reported in other taxa such as rodents (Dewsbury 1984). However, if this second male advantage is at all generalizable, then it confirms the utility of the hypothesized avian paternity guards. All of the guards enumerated in the "Introduction" function to insure that the mate's semen is the most recent or the only semen in the female's reproductive tract. What is needed now are studies on sperm storage and multiple mating on truly feral species, for which much is known about their copulatory and mate-guarding behavior in the field. As a step toward this goal, Hatch (1983) has recently identified sperm-storage glands in the uterovaginal region of the oviduct in three feral seabird species, the Northern Fulmar (*Fulmarus glacialis*), the Horned Puffin (*Fratercula corniculata*), and Leach's Storm-Petrel (*Oceanodroma leucorhoa*).

The finding of second-male sperm precedence in Ringed Turtle-Doves is consistent with the utility of paternity guards proposed for this species. The mate guarding activity of the male Ringed Turtle-Dove observed by Lumpkin et al. (1982) can be of critical importance in the prevention of kleptogamy. When a male is courting a nonreceptive female, he should mate guard to insure that copulations with other males do not occur and he should frequently copulate during the prelaying period to insure that the progeny are his. Both surveillance and frequent copulation have been shown to occur (Lumpkin et al. 1982, Cheng et al. 1981).

The effectiveness of a second male strategy may be contingent on the female's reproductive

stage at the time of their encounter. Zenone et al. (1979) observed male aggression toward females which show behavior indicative of advanced ovarian development and contact with a prior male. They hypothesized that male aggression functions as a paternity guard by delaying ovulation beyond the viable time of sperm deposited by the previous male. Such a delay in ovulation may occur until a critical phase in the female's ovarian growth i.e., when she enters the exponential phase (Sims 1983, Sims et al. 1983). Until this time, a strategy of delayed ovulation by male aggression may operate in conjunction with a mechanism of second male sperm precedence to insure the paternity of the second male (Erickson 1985).

When a female is very close to ovulation the second male has little opportunity to father the clutch. It appears that the exponential growth phase cannot be retarded, leaving only a brief window of potential fertilizability. Also, many females do not exhibit behavioral receptivity at this time. In this situation, an appropriate strategy would be for the male to determine how soon the eggs were laid after his initial encounter with the female. Any eggs laid within two days should be abandoned and a new clutch started, which did occur in several cases in this study. This would assure the male that he is rearing only his own offspring.

In the final analysis, the question of the "adaptiveness" of these different male behaviors cannot be answered by studies of captive birds. Captive studies such as this one can elucidate relevant underlying physiological mechanisms and suggest behavioral adaptations, but field studies are necessary to show if these phenomena are important in nature.

#### ACKNOWLEDGMENTS

Contribution number 403 of the Institute of Animal Behavior. We thank C. Banas for graphics and W. Cunningham for secretarial assistance. We also thank P. Marler and S. Clark for reading and commenting on the manuscript. This research was supported by NSF Grant BNS-8121495 and Research Scientist Development Award KO2-MH-70897 (to M.-F. Cheng).

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