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SPERM STORAGE AND THE FERTILE PERIOD IN THE BENGALESE FINCH

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ABSTRACT.—Female Bengalese Finches (*Lonchura striata*) stored sperm in sperm storage tubules and were capable of producing fertile eggs for a median of 8 days (maximum 16 days) following the last copulation. The age of stored sperm had no effect on the hatching success of eggs. More sperm were present in a female's sperm storage tubules after copulation that occurred before ovulation than after one that occurred after ovulation. On average only about 1% of the sperm transferred during copulation were stored in the sperm storage tubules. Similar "selection" of sperm in the female reproductive tract has been recorded in other vertebrates. Its mechanism and functional significance are discussed. *Received 31 May 1991, accepted 19 February 1992.*

FEMALE BIRDS are known to store sperm for days or weeks following copulation or artificial insemination, prior to using it to fertilize their eggs (Howarth 1974, Birkhead 1988). Sperm storage probably evolved primarily because in birds ovulation, fertilization and oviposition occur sequentially and each egg is fertilized separately during a very short period (<30 min) following ovulation. Without sperm storage, copulation would have to occur immediately prior to each ovulation. In species laying multiple-egg clutches the duration of sperm storage is positively correlated with the number of days over which egg laying occurs (Birkhead and Møller 1992a). In species that produce only a single-egg clutch, such as many seabirds, sperm storage allows females to forage away from their partner for prolonged periods of unpredictable duration prior to egg laying and still ensure fertilization (Imber 1976, Hatch 1983, Birkhead and Møller 1992a). A further explanation for sperm storage in birds is that it allows a population of sperm to be "selected" from those inseminated (Bakst and Bird 1987).

The fertile period is the period during which a copulation potentially can fertilize one or more eggs (Lake 1975). The fertile period is determined by the duration of sperm storage and the spread of laying (i.e. clutch size multiplied by the interval between successive eggs; Birkhead 1988). A prolonged fertile period enhances the opportunities for sperm competition because it increases the likelihood of ejaculates from different males overlapping (Parker 1970). As several recent studies have shown, sperm competition among birds is widespread, despite monogamy being the predominant mating system (Birkhead et al. 1987, Westneat et al. 1990, Birkhead and Møller 1992b).

The duration of sperm storage and, hence, the fertile period is known for relatively few birds, mainly domesticated species (for review, see Birkhead and Møller 1992a) and in detail for only a single passerine, the Zebra Finch (*Taeniopygia guttata*; Birkhead et al. 1989). The mean or median sperm-storage duration in birds varies from 6 to 45 days (Birkhead and Møller 1992a). The aim of the present study was to determine the duration of the fertile period in another passerine, the Bengalese Finch (*Lonchura striata*), for comparison. In addition, some aspects of the mechanism of sperm storage were investigated by examining the pattern of sperm uptake by the sperm storage tubules (referred to hereafter as SSTs; Birkhead and Hunter 1990, Birkhead et al. 1990).

METHODS

Study species.—The Bengalese Finch is the domesticated form of the Sharp-tailed Munia (Eisner 1957). In the present type of study, it is virtually essential to use domesticated birds since, unlike their wild counterparts, they breed readily in captivity (unpubl. data). Some aspects of the reproductive biology of the Sharp-tailed Munia have been studied in the wild in southeastern Asia (Avery 1978, 1980) and, therefore, it is possible to make some cautious extrapolations from the studies of the Bengalese Finch. In the wild, the Sharp-tailed Munia is an opportunistic breeder that breeds whenever conditions are suitable. Bengalese Finches in captivity also breed throughout the year given suitable conditions. In this respect they are similar to the Zebra Finch and some other estrildid finches (Sossinka 1980a, b). In the present study, Bengalese Finches were maintained with *ad libitum* food and water on a 14:10 L:D cycle. Birds bred in cages (44 × 50 × 42 cm high) with a nest box (15 × 15 × 15 cm) mounted on the outside of the cage.

Sperm storage.—The duration of sperm storage was determined by pairing females to a male and allowing them to copulate. The pair male was removed after the female had laid three eggs. Females usually continued to lay the rest of their clutch after the removal of the male. They then were paired to a vasectomized male (Samour and Markham 1987, Birkhead and Pellatt 1989) and allowed to produce subsequent clutches. Only females that produced fertile eggs from the first (intact) male were used to determine the sperm-storage duration.

Twenty-five different females were used in three groups. The first group of 13 females was used only once, while the second and third groups of 6 females each were used three and four times, respectively. Groups 2 and 3 were used several times in an attempt to determine whether the sperm-storage duration was repeatable (Lessells and Boag 1987), as it is in ducks (Olver et al. 1977). However, in contrast to ducks and domestic poultry that typically lay one egg each day for prolonged periods, many other birds (including the Bengalese Finch) lay in discrete clutches with long intervals between clutches.

In my study, Bengalese Finches laid a modal clutch of six eggs ($\bar{x}$ = 5.75 eggs ± SD of 0.78, n = 40 pairs), with one egg laid each day. Since the initiation of egg laying following pairing was variable ($\bar{x}$ = 7.45 days ± 2.73, n = 40), there were relatively few instances (n = 4) in which egg laying covered the same time period in two or more trials, thus allowing us to check for repeatability. The available data on the sperm-storage duration (i.e. when the last fertile egg was laid part way through a clutch) for four females in two different trials provided no indication of repeatability ($r = -0.243$, df = 2, $P > 0.05$). Therefore, I pooled data for all birds and all trials to produce a fertility curve and used the median value as the duration of sperm storage (see Birkhead et al. 1989).

Eggs were taken from nests the day they were laid and incubated in a commercial incubator at 38.5°C. After four days of incubation the fertility of eggs was determined by "candling." The eggs then were returned to the incubator and the proportion that subsequently hatched was recorded. For eggs where no obvious development occurred after four days, I used Kosin's (1944) method for distinguishing fertile and infertile eggs. Hatching success was determined, since some studies have shown that eggs fertilized by "old" sperm are less likely to hatch (for review, see Birkhead 1988). If this occurred, from a functional point of view the fertile period would be shorter than that determined simply from the fertility of eggs (Birkhead 1988).

The duration of sperm storage for a particular female was the interval between the second day after the intact male was removed and the day the last fertile egg was laid. Days were numbered from two days after the intact male was removed, because the eggs of Bengalese Finches are fertilized 24 h before being laid; the egg laid on the day after male removal would have been fertilized during the time the intact male was with the female.

Sperm uptake by sperm storage tubules.—Following copulation, female birds store sperm in SSTs located at the uterovaginal junction (Bakst 1987, Birkhead and Hunter 1990, Birkhead et al. 1990). The mechanisms by which sperm enter, are maintained, and are released from the SSTs have been examined extensively in poultry, but are not well understood (Bakst 1987, Zavaleta and Ogasawara 1987, Wishart 1987). In most birds, pair copulations are most frequent before the first egg of the clutch is ovulated (Birkhead and Møller in press). Therefore, I wished to determine whether copulations prior to the first ovulation resulted in sperm accumulating within the SSTs. To do this I compared the mean numbers of sperm per SST for females either before or after the first ovulation, following a single insemination. I defined "before ovulation" as days -5 to -2, where day -1 is the day the first ovulation occurs and day 0 is the day the first egg is laid (oviposition occurs 24 h after ovulation; unpubl. data). "After ovulation" was considered to be the day the first egg was laid (i.e. day 0). In an attempt to control for male effects, the same males were used both before and after ovulation. To determine whether there was any repeatability for the number of sperm transferred, each male was used twice before and twice after the females' first ovulation. Females were dissected, their SSTs examined and the number of sperm in each of 100 SSTs was counted by examining the SSTs under high power (400×) with a compound microscope (see Birkhead and Hunter 1990, Birkhead et al. 1990).

Results

Sperm storage and hatching success.—The decline in fertility of eggs following removal of the intact male is shown in Figure 1. The median duration of sperm storage was 8 days, and the maximum (one instance) was 16 days. There was no evidence that eggs fertilized towards the end of the duration of sperm storage were less likely to hatch than those fertilized earlier. This was examined in three ways. First, if hatching success declined with the age of stored sperm, one would predict a negative correlation between the proportion of fertile eggs that hatched and the number of days of sperm storage. No such effect was apparent ($r = 0.461$, df

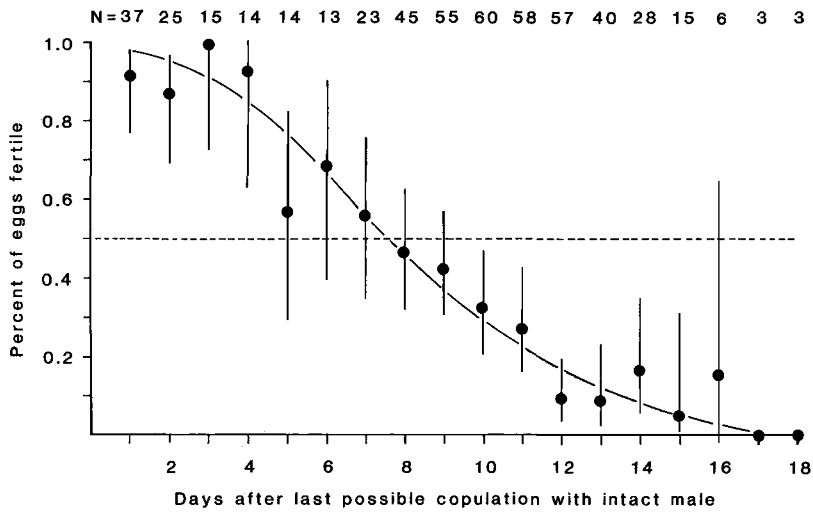

Fig. 1. Fertility curve for female Bengalese Finches. Decline seen in proportion of fertile eggs with time since last possible copulation with an intact male. Values are mean proportions with 95% confidence intervals (see Rohlf and Sokal 1981). Sample sizes (number of eggs) shown at top. Line drawn by eye. There were nine further eggs laid on days 19 to 23, none of which were fertile.

$= 13$, $P > 0.05$). Second, I checked whether the last-laid fertile egg for any particular female in any particular trial was less likely to hatch than earlier eggs: 8 (26%) out of 31 last eggs failed to hatch compared with 22 (32%) of 68 other eggs (fertilized with sperm stored for between 1 and 10 days). This difference was not significant ($X^2 = 0.43$, df $= 1$, $P > 0.05$). These two comparisons include more than one egg from individual females. Therefore, in order to avoid pseudoreplication, I made an additional comparison of the first and last egg laid by the same female (using each female only once). Of 23 fertile first eggs, 20 (86.9%) subsequently hatched, compared with 19 of 23 last eggs (82.6%), a difference that is not significant ($X^2 = 0.17$, $P > 0.05$). Therefore, there was no evidence for any decrease in hatching success with increasing age of sperm. This result is similar to that found in the Zebra Finch (Birkhead et al. 1989), but differs from that recorded in poultry (Elder and Weller 1954, Hale 1955, Sittman and Abplanalp 1965, Lodge et al. 1971).

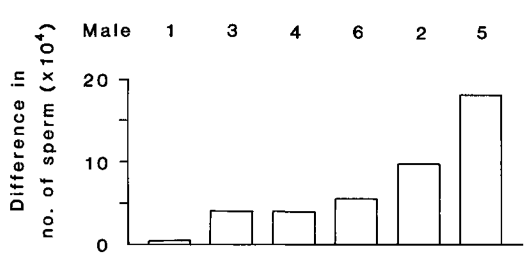

Fig. 2. Sperm storage tubules (SSTs) sequester more sperm from copulations occurring before first ovulation than those occurring after first ovulation for six males. Difference in number of sperm before and after first ovulation for each male (values calculated more sperm in the SSTs when copulation occurred before ovulation. Difference before and after ovulation is significant ($P < 0.05$).

Are sperm stockpiled?—Copulations occurring before the first ovulation resulted in there being significantly more sperm in the SSTs than copulations occurring after the first ovulation (Fig. 2). A two-way ANOVA revealed a significant effect of before versus after ovulation ($F_{1,23} = 5.44$, $P = 0.038$), but no effect of males ($F_{1,5} = 1.93$, $P > 0.05$). Overall, copulations occurring before the first ovulation resulted in there being just over twice as many sperm in the sperm storage tubules than copulations occurring after the first ovulation (Fig. 2). These results confirm that copulations before ovulation result in greater numbers of sperm being retained in the SSTs compared with copulations occurring after the onset of egg laying.

Proportion of sperm retained in SSTs.—The number of sperm transferred during a normal insemination in birds is difficult to determine. Elsewhere, I estimated that male Bengalese Finches transfer in the order of 2.93×10^6 sperm during a single copulation (Birkhead 1991). The number of sperm in the SSTs 24 h after a single copulation and insemination was estimated by multiplying the number of sperm per SST by 1,511 (the mean number of SSTs per female; Birkhead and Hunter 1990). To produce estimates of the proportion of sperm retained in the SST 24 h after one copulation, I used the mean (12) and maximum (33) values for the mean number of sperm per SST. The estimates are:

$$(12 \times 1{,}511)/2.93 \times 10^6 = 0.6\%$$

and

$$(33 \times 1{,}511)/2.93 \times 10^6 = 1.7\%$$

of sperm transferred during copulation are retained by the SSTs.

Discussion

This study has shown that female Bengalese Finches can store sperm for a median of 8 days (maximum 16 days). The hatching success of eggs fertilized by sperm stored for different periods did not differ. Females accumulate and store sperm in sperm storage tubules prior to their first ovulation. The proportion of sperm from an ejaculate found in the SSTs 24 h after copulation is low, about 1% of those inseminated.

Duration of sperm storage.—The duration of sperm storage in the Bengalese Finch is similar to that in the Zebra Finch, where the median and maximum duration were 10 and 13 days, respectively. The sperm storage of the Bengalese Finch also falls within the range known for nonpasserines (for a review, see Birkhead and Møller 1992a). The modal clutch size of Sharp-tailed Munias in the wild is 5 eggs ($\bar{x} = 4.34 \pm 0.96$, $n = 16$; Avery 1978). Assuming the median duration of sperm storage to be the same as in the Bengalese Finch, the fertile period would span day −9 until the day the penultimate egg was laid (i.e. day 4). However, if Sharp-tailed Munias lay their eggs in the early morning, as in most passerines (Schifferli 1979) including the Bengalese Finch (pers. observ.), then in practice the fertile period would terminate at the end of day 3.

Are sperm stockpiled?—All birds start to copulate with their partner some days or weeks (depending upon species) before the first egg is laid, and the majority of species copulate most frequently a few days before laying starts, after which copulation declines markedly (Birkhead et al. 1987, Birkhead and Møller in press). Therefore, it seems logical that, during the copulation period prior to ovulation, females should accumulate sperm in their SSTs. In other words, one might expect the SSTs to accept, but not to release sperm during this period. Despite extensive work on sperm storage in poultry, there is little information on whether the "behavior" of SSTs varies through the prelaying and laying periods. This is because most poultry studies have used females that were laying continuously and, under such conditions, sperm are released from the SSTs continuously (Wishart 1987). However, McIntyre and Christensen (1983), working with domestic turkeys (*Meleagris gallopavo*), found that the uptake of sperm by the SSTs was greater prior to the onset of laying than once egg laying had started. This result has been confirmed by Brillard and Bakst (1990), who found that the uptake of sperm by SSTs was both greater and more rapid prior to the first ovulation. This result could have arisen if the SSTs accumulated, but did not release sperm prior to the onset of ovulation. It is not known what the "trigger" is that stimulates sperm release from the SSTs just prior to the first ovulation (Bakst 1989).

Proportion of sperm getting to SSTs.—The results in the present study show that only a small proportion of sperm (ca. 1%) transferred during copulation are retained by the SSTs. The rest are probably ejected from the cloaca, possibly with faecal material (see Howarth 1971). Similar results for the proportion of sperm taken up by the SSTs have been obtained in domestic poultry using artificial insemination (Brillard and Bakst 1990). The results presented here, which were based on natural copulation, show that the low uptake of sperm by the SSTs is not a consequence of artificial insemination. It has been thought for some time that SSTs are selective, since they do not, for example, take up dead sperm (e.g. Ogasawara et al. 1966; also see Bakst and Bird 1987). However, the selection of sperm is now thought to be mediated by the vagina and is based on sperm surface proteins (Wishart and Steele (1990). Sperm "selection" also has been recorded in mammals (e.g. Cohen and Tyler 1980, Overstreet 1983). This raises some interesting questions concerning both the underlying mechanism of this selection (Wishart and Steele 1990) and its functional significance from the standpoint of the female. M. R. Bakst (pers. comm.) has suggested that the basis for sperm selection may differ between mammals and birds. In the former, the process selects sperm capable of immediate fertilization, whereas in birds part of the selection at least must be on the basis of the ability of sperm to survive a prolonged period of storage in the female reproductive tract. Since sperm competition is widespread in the animal kingdom (Smith 1984) and the probability of any male fertilizing a female's eggs depends to some extent on the numbers of sperm reaching the ova (Martin et al. 1974), one would expect strong selection on males to produce a high proportion of sperm that could penetrate any "filter" in the female reproductive tract.

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