Numbers of Sperm-storage Tubules in the Zebra Finch (*Poephila guttata*) and Bengalese Finch (*Lonchura striata*)

T. R. Birkhead and F. M. Hunter
Department of Animal and Plant Sciences, The University, Sheffield S1O 2TN, United Kingdom

Female birds are known to store sperm for prolonged periods after copulation or artificial insemination (Howarth 1974, Birkhead 1988). The primary storage sites are sperm-storage tubules (SSTs) located at the junction of the uterus and vagina (the uterovaginal junction; UVJ). A secondary sperm-storage site is believed to be in the distal infundibulum which is also the site of fertilization (Van Drimmlem 1946, Fuji and Tamura 1963, Bobr et al. 1964, Bakst 1987).

Sperm storage and sperm-storage tubules have been examined in domestic poultry, mainly chickens (*Gallus*) and turkeys (*Meleagris*) (Gilbert et al. 1968, Burke et al. 1969, Bakst 1987), but there have been relatively few studies in other birds (see Hatch 1983; Bakst and Bird 1987; Birkhead 1987, 1988; Shugart 1988). Although SSTs have been reported in only a small number of species, they may prove to be ubiquitous. The size and form of SSTs differ between and within bird species (Shugart 1988), but the number of SSTs within individual birds is virtually unknown. One estimate is 20,000 in turkeys (Goodrich-Smith and Marquez 1978).

We estimated the number of SSTs in two estrildine finches, the Zebra Finch (*Poephila guttata*, but see 1989, Auk 106: 750) and the Bengalese Finch (*Longchura striata*), and compared SST characteristics between domesticated and wild forms of these species. Recent work has shown that female Zebra Finches can produce fertile eggs for a median duration of 10 days and a maximum of 13 days after the last mating (Birkhead et al. 1989). Similar studies are currently in progress with Bengalese Finches, which indicate slightly longer maximum sperm storage duration in this species (Birkhead and Price unpubl.).

We examined the oviducts of six domesticated Zebra Finches and five domesticated Bengalese Finches. Most birds had been paired and allowed to breed naturally. They were sacrificed just before or during egg laying (where day 0 is the day the first egg is laid; negative values refer to estimated days before laying, and positive values refer to days after laying). Zebra Finches 1–6 were examined on days +1, +2, −2, $-n$, 0, and +1, respectively, where n is an unknown number of days before laying (this was the only unpaired bird examined, although it had bred previously). Bengalese Finches 1–5 were examined on days 0, +2, 0, +1, and +1, respectively.

We examined the oviducts of two wild Zebra Finches (WZ7 and WZ8), and two wild Bengalese Finches (munias 1 and 2; also known as the Sharp-tailed Finch or White-rumped Munia, see Eisner 1957). All four birds laid eggs in captivity and were examined during egg laying. Zebra Finches 7 and 8 were sacrificed on days +2 and 0, and munias 1 and 2 on days +1 and +2, respectively.

Oviducts were removed immediately after death and the section containing the shell gland, vagina, and cloaca was placed in phosphate buffered saline (PBS). The material was then pinned at each end and the connective tissue surrounding the vagina and shell gland removed. This allows the vagina, which is coiled and "concertinaed," to be straightened (Bakst and Bird 1987). A longitudinal incision was made along the segments to expose the luminal mucosa. The flattened tissue was then pinned to a dissecting board to reveal the folds of the shell gland and vagina (Fig. 1). The material was examined (Goodrich-Smith and Marquez 1977, Bakst and Bird 1987) as fresh, unfixed and unstained tissue. Briefly, our procedure was as follows.

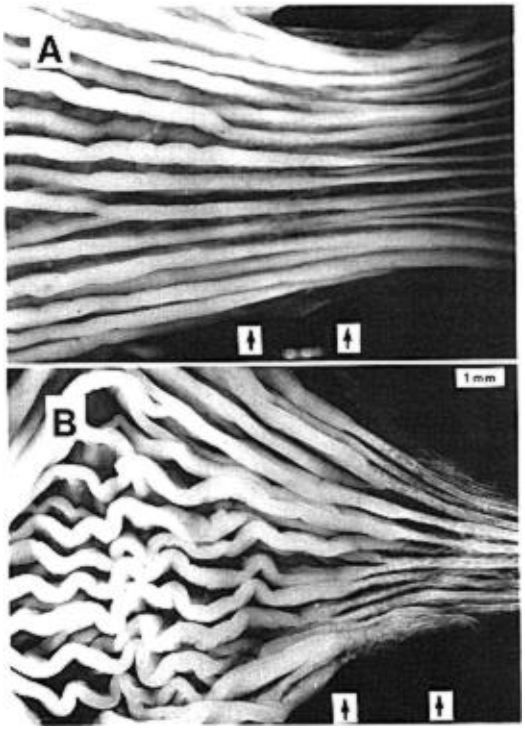

Fig. 1. Oviduct of (A) domesticated Zebra Finch and (B) Bengalese Finch. The primary folds are in the uterovaginal region where the sperm-storage tubules lay. The SSTs are seen as the gray shapes in the zone between the two arrows. The scale = 1 mm.

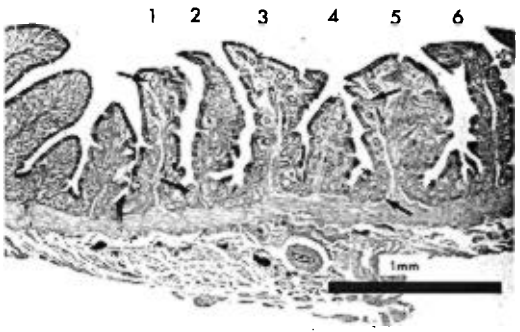

Fig. 2. Transverse section through the uterovaginal region of a domesticated Zebra Finch oviduct. Six primary folds (numbered) are shown and SSTs (arrows) can be seen within and between the folds. Scale = 1 mm.

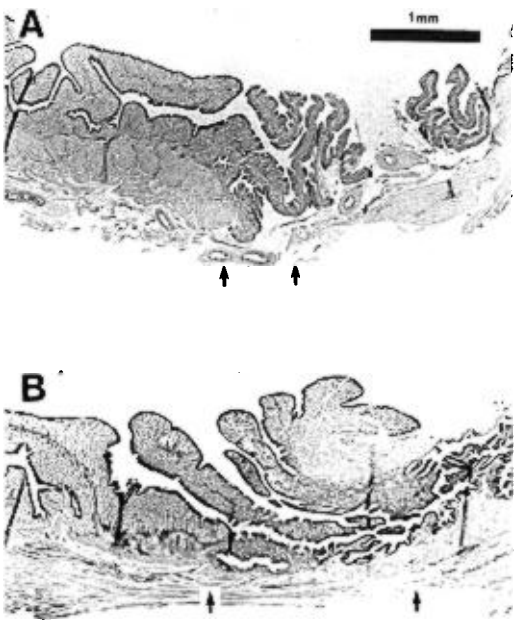

Fig. 3. Longitudinal section through the uterovaginal region of (A) domesticated Zebra Finch and (B) Bengalese Finch oviducts to show the location of the SSTs. In both (A) and (B), the large lobes on the left are the shell gland. The zone containing the SSTs lies between the arrows. Scale = 1 mm.

The reproductive tract was examined under low power (magnification 15× to 20×) binocular microscope to identify the region in which SSTs were located (Fig. 1). Five separate mucosal folds were removed from each bird and examined. The lamina propria of folds that run through the region of SSTs (from the vagina through to the shell gland) was removed from the underlying connective tissue. The connective tissue within the fold was also removed. The lamina propria of each fold was placed on a microscope slide, with the surface epithelium against the slide, and it was spread open. A coverslip with a drop of PBS was placed on the specimen. The SSTs were counted using a Kyowa light microscope, with low power (40× magnification) using either transillumination (see Bakst and Bird 1987) or transmitted light with the iris diaphragm closed down. These two lighting systems give opposite effects but allow the SSTs to be seen and counted (see below). The uterovaginal junction was examined using standard histological techniques. We stained 10 μm thick longitudinal and transverse sections with Heidenhain's Azan (see Birkhead 1987).

In both species the SSTs were restricted to a discrete band ca. 3–5 mm wide at the uterovaginal junction (Fig. 1). Sperm-storage tubules were distributed evenly across the entire tissue in the folds and the troughs between them (Fig. 2). The width of this band depends upon how much the tissue is stretched during preparation; in life, the zone of SSTs is much shorter. The oviduct at the uterovaginal junction is "concertinaed" to form the UV sphincter (see Gilbert 1979). The SSTs are located within one of several ridges of tissue that form this sphincter. This is seen in a longitudinal section through the uterovaginal junction (Fig. 3). The infundibulum of both species was examined, but no sperm stores were detected.

We found qualitative differences in the tissue of the two species. In the Bengalese Finch, the SSTs were more clearly defined and the underlying connective tissue was less dense than in the Zebra Finch. As a result, mucosal folds were more easily removed and prepared for examination in the Bengalese Finch.

The mean number of primary, mucosal folds was 15.3 ± 1.03 (range: 14–17) in the Zebra Finch and 12.8 ± 0.45 (range: 12–13) in the Bengalese Finch. This difference was significant ($t = 4.97$, df = 9, $P < 0.02$). The number of primary mucosal folds was similar in wild and domesticated birds (Table 1).

In both species, the SSTs appeared to be smaller and most densely distributed in the narrow region immediately adjacent to the shell gland (Fig. 4). In the Zebra Finch, the mean number of SSTs per fold varied from 83 and 118 between birds, but this difference was not significant ($F = 1.30$, df = 2, 24, NS; Table 1). Similarly in the Bengalese Finch, the mean number of SSTs per fold varied from 105 to 139 but did not differ between individuals ($F = 1.36$, df = 4, 20, NS). However, the number of SSTs per fold was significantly higher in the Bengalese Finch (118.0 ± 30.6) than in the Zebra Finch (97.8 ± 25.3) (see Table 1). The mean number of SSTs per fold in the wild

TABLE 1. Numbers of primary mucosal folds, sperm-storage tubules (SSTs), and branches in the uterovaginal junction of Zebra and Bengalese finches. Bird numbers refer to Zebra Finches (Z), Wild Zebra Finches (WZ), Bengalese (B), and munias (M).

Birds	Folds (n)	SST/fold ($\bar{x} \pm$ SD)	Branches[a]					No SSTs	No SST-ends
			1	2	3	4	5		
Z1	17	82.6 ± 32.0	—	—	—	—	—	1,534	—
Z2	15	89.6 ± 17.4	0.80	0.14	0.03	0.02	0.01	1,252	1,645
Z3	16	98.4 ± 35.5	0.96	0.03	0.004	0	0	1,574	1,640
Z4	15	92.2 ± 28.5	0.81	0.15	0.03	0.01	0	1,384	1,677
Z5	15	117.6 ± 9.0	—	—	—	—	—	1,764	—
Z6	14	106.2 ± 12.9	0.73	0.18	0.07	0.02	0	1,487	2,038
WZ7	15	114.6 ± 32.3	0.84	0.15	0.01	0	0	1,719	2,021
WZ8	15	98.2 ± 15.9	0.87	0.12	0.01	0	0	1,473	1,671
B1	13	107.0 ± 16.0	0.94	0.04	0.01	0	0	1,391	1,485
B2	13	104.8 ± 24.8	0.78	0.15	0.05	0.005	0.005	1,362	1,764
B3	12	109.2 ± 12.0	0.87	0.11	0.02	0	0	1,310	1,505
B4	13	139.4 ± 48.9	0.78	0.16	0.04	0.01	0	1,812	2,336
B5	13	129.4 ± 31.6	0.70	0.16	0.08	0.04	0.02	1,682	2,540
M1	13	149.6 ± 34.0	0.89	0.07	0.03	0.003	0	1,945	2,221
M2	11	98.0 ± 18.5	0.56	0.34	0.09	0.01	0.005	1,078	1,687

[a] Proportion of all SSTs. Dash (—) indicates no data.

Zebra Finches (WZ7: 114.6 ± 32.26, and WZ8: 98.2 ± 15.90) fell within the range of domesticated birds. Values for the two munias were higher (munia 1: 149.6 ± 33.99) and lower (munia 2: 98.0 ± 18.51) than recorded for domesticated Bengalese Finches (Table 1).

We calculated the total number of SSTs per bird in each species from the mean number per fold (from the five folds examined) multiplied by the number of folds. In the Zebra Finch, we estimated from 1,252 to 1,763 between individuals, with an overall mean of 1,499 ± 174.17 (coefficient of variation: 11.62 [Table 1]). In the Bengalese Finch, the mean number of SSTs was 1,511 ± 221.74 (range for individuals: 1,310–1,812; coefficient of variation: 14.67). The difference between species in the mean number of SSTs was not significant ($t = 0.10$, df = 9, NS). For the wild birds, SST numbers were WZ7: 1,719, WZ8: 1,473; munia 1: 1,945 and munia 2: 1,078.

In both species some SSTs were branched. We recorded the extent of branching in four Zebra Finches (2, 3, 4, and 6), and all Bengalese Finches. The extent of branching was determined by examining the tissue in different focal planes. In both species the majority of SSTs were unbranched (Table 1). In both species, most branched tubules had two branches, but some had three; and up to eight were recorded in one Zebra Finch. The proportion of branched SSTs did not differ significantly between Zebra Finches and Bengalese Finches (Mann-Whitney U-test, $U = 8$; NS). Within species the proportion of branched SSTs differed between individuals. In the Zebra Finch, values ranged from 73 to 96% ($\chi^2 = 80.3$, df = 3, $P < 0.001$), and in the Bengalese Finch from 70 to 94% ($\chi^2 = 122.5$, df = 4, $P < 0.001$).

The extent of branching in the two wild Zebra Finches was similar and did not differ intraspecifically ($\chi^2 = 1.5$, df = 2, NS). The values were similar to domesticated birds. The extent of branching differed significantly in the two munias ($\chi^2 = 126.3$, df = 2, $P < 0.001$; Table 1). Munia 2 had a higher proportion of branched SSTs than any of the Bengalese Finches.

The total number of SST branches may be more important than the number of SSTs for the numbers of spermatozoa stored. We estimated the mean number of SST-endings and found 1,750 ± 192.70 in the Zebra Finch, and 1,926 ± 485.5 in the Bengalese Finch. This difference was not significant (Mann-Whitney U-test, $U = 9$). However, the number of SST-endings was significantly more variable for Bengalese Finches (Variance ratio test, $F = 6.35$, df = 4, 5, $P < 0.05$). For both the wild Zebra Finches and the munias, the number of SST-endings was within the range of the domesticated birds (Table 1).

The factors that affect the duration of sperm storage in different bird species are poorly understood. One possibility is that storage duration is related to the number of SSTs or SST-branches, and thus it is related to the amount of space available to store sperm. Our results and those of Goodrich-Smith and Marquez (1978) are consistent with this idea. The median sperm-storage duration in the Zebra Finch is 10 days (Birkhead et al. 1989). The duration of sperm storage in the Bengalese Finch is a few days longer than this (unpubl. data), and this species has slightly more SST-endings. Turkeys can store sperm for a mean duration of 40–50 days (Lorenz 1950, McCartney 1951, Hale 1955) with ca. 20,000 (mainly unbranched) SSTs (Goodrich-Smith and Marquez 1977, Bakst 1987). Data from other species are needed to test this idea more rigorously.

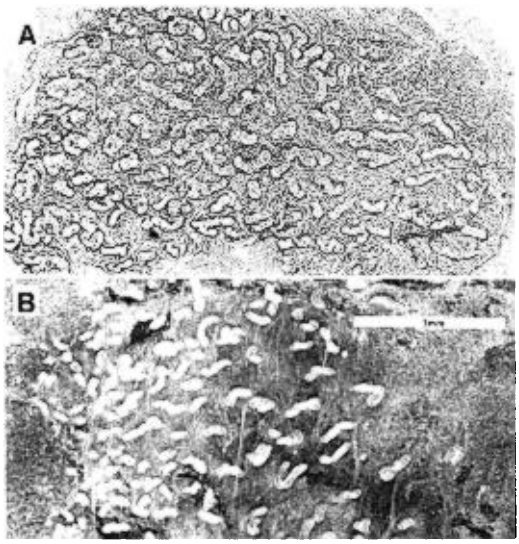

Fig. 4. Flattened lamina propria from a primary mucosal fold of (A) domesticated Zebra Finch, photographed with the microscope iris diaphragm closed down. (B) Bengalese Finch, photographed by transillumination. The SSTs are the sausage-shaped structures. Note the smaller SSTs at the LHS at the junction with the shell gland, and the reduced density of SSTs at the vaginal end of the piece of tissue. Scale = 1 mm.

Almost all the information on the duration of sperm storage in birds comes from domesticated species (Birkhead 1988). Extrapolation to wild birds must be made with care since the process of domestication may have increased their sperm-storage ability (Lake 1975, see also Clayton 1972). We found no differences in SST numbers or form between domesticated and wild forms of two finches, which does not preclude the possibility that the duration of sperm storage differs between wild and domesticated forms of the same species.

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Age-related Pair Bonding by Male Eurasian Wigeons in Relation to Courtship Activity

JUAN A. AMAT

Estación Biológica de Doñana, Apartado 1056, E-41013 Sevilla, Spain

Mate choice by ducks occurs during a communal courtship in which several males display to a single female. For most ducks, pair formation occurs months before breeding, and pair bonds generally last a single season. By pairing early, female ducks may increase their ability to breed early and lay large clutches (Rohwer and Anderson 1988). *Parental investment theory* (Trivers 1972) predicts that females should be the "choosier" sex in these species (Wishart 1983, Rohwer and Anderson 1988, but see Afton and Sayler 1982). Male qualities important in acquiring mates include age, courtship behavior, plumage color, size, and body condition (e.g. Wishart 1983).

Young male ducks pair later than do adults (Wishart 1983, Hepp 1986, McKinney 1986). Given that the sex-ratio is male biased in most duck populations (Bellrose et al. 1961, Campredon 1983, Wishart 1983), the later a male pairs, the greater the probability of remaining unpaired. Because young males often develop alternate plumage later than adults (McKinney 1965, Weller 1965, Wishart 1985), the probability of remaining unpaired should be greater for the younger cohort. Indeed, Blohm (1982) and Wishart (1983) found that adult male Gadwalls (*Anas strepera*) and American Wigeons (*A. americana*) paired more frequently than yearling males. Wishart (1983) suggested that energetic constraints would affect pairing chronology (see also Afton and Sayler 1982, Brodsky and Weatherhead 1985), and Blohm (1982) speculated that age-related differences in neuroendocrine development, plumage growth, or courtship behavior (see also Bruggers and Jackson 1981) conferred a competitive advantage to older males. Although adult dabbling ducks predominate in pair bonds, it has not been shown whether this is actually due to female choice, as Blohm (1982) suggested. I present evidence that in a dabbling duck species male age per se is not a criterion used by females when choosing a mate during courtship.

During the winters of 1986/1987-1988/1989, I observed communal courtships of Eurasian Wigeons (*A. penelope*) in the Marismas (marshes) of the Guadalquivir, southwestern Spain. I noted the number and age (yearling or adult) of participating males. Yearling males can be distinguished from adults under field conditions because their greater upper wing-coverts are gray, which contrasts with the white of adults (Cramp and Simmons 1977).

On 20 February 1987 and 17 February 1989, I recorded the pairing status of male Eurasian Wigeons. I chose those dates because by mid-February most females are paired (pers. obs.). I randomly positioned a telescope and began to count males from right to left, recording whether or not the first 50 adult and 50 yearling males I watched were paired. Individuals were categorized as paired if they were close to, synchronized activities with, and defended females, or if females incited males (Afton and Sayler 1982, Hepp and Hair 1984).

The sex ratio of Eurasian Wigeons wintering in the Marismas was 100 males to 88 females, and the age ratio of males was 100 adults to 109 yearlings (Campredon 1983). Although there are annual differences in age ratio of wintering Eurasian Wigeon populations, differences were small (usually <10%; Campredon 1983).

I found no annual differences (G-test, $P > 0.05$) in the pairing frequency of males according to age, and therefore I pooled data. By late February, adult males were paired in 84 of 100 cases. Yearlings were paired in 25 of 100 cases. The difference in the pairing frequency by both categories of wigeon males is highly significant ($\chi^2 = 37.3$, $P < 0.001$) if pairing frequency is adjusted for male age ratio in the wintering population.

I recorded the composition of 27 communal courtships and found that 18.3% of the male ($n = 115$) participants were yearlings. Assuming that males pair according to frequency of participation in communal courtships, there is no difference ($\chi^2 = 1.6$, $P > 0.05$) in the frequency of occurrence of adult and yearling males in pair bonds. Consequently, I conclude that male age was not a criterion used by wigeon females when they chose mates.

Female dabbling ducks mainly choose mates according to male behavioral dominance (Brodsky et al. 1988). Females paired to a dominant male foraged