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Breeding-cycle Patterns of Sperm Storage in the Pied Flycatcher (*Ficedula hypoleuca*)

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Breeding females of all bird species examined so far have possessed sperm-storage tubules at the utero-ovaginal junction of the oviduct (Fuji and Tamura 1963; Bobr et al. 1964; Bray et al. 1975; Hatch 1983; Bakst 1987; Bakst and Bird 1987; Birkhead 1987; Shugart 1988; Birkhead and Møller 1992a, b; Briskie and Montgomerie 1993). The median duration of storage of viable sperm in these tubules varies from 6 to 45 days in different species (Birkhead and Møller 1992a, 1993). This variation in the duration of sperm storage is related to the number of days over which a clutch is laid (Birkhead and Møller 1992b) and to the timing and frequency of copulations.

Most wild birds have specific breeding seasons during which their reproductive organs increase in size (Gilbert 1979, Lake 1981). Within a breeding cycle, sperm-storage tubules reach maximum size just before or at the start of egg laying (Briskie 1994, Pellatt 1998). The period during which sperm-storage tubules contain viable sperm differs between species. This variation presumably determines variation in length of the fertile period. For example, some seabirds store sperm for several weeks prior to the onset of egg laying (e.g. Hatch 1983), whereas many passerines appear to store sperm for much shorter periods (Birkhead 1988, 1992; Birkhead et al. 1989). Few studies have examined changes in the size and content of sperm-storage tubules relative to egg laying. The aim of the present study was to describe these changes in the Pied Flycatcher (*Ficedula hypoleuca*).

Methods.—The Pied Flycatcher (body mass 12 to 15 g) is a migratory passerine that breeds in temperate regions of Eurasia. It nests in natural tree cavities or nest boxes and produces a clutch of 5 to 7 eggs in a single brood each year (Lundberg and Alatalo 1992). Pair copulations are relatively infrequent and appear to be confined to a relatively short period immediately before the start of egg laying (Haartman 1956, Alatalo et al. 1987, Chek et al. 1993).

Nineteen female Pied Flycatchers were collected (under license) from nest-box study sites near Oslo, Norway, at different stages of the breeding cycle in 1988 (n = 4), 1991 (n = 4), and 1996 (n = 11). The birds were not previously marked so were of unknown age. From each bird the ovary and oviduct were removed immediately after death and fixed in 5% formalin. All females were paired and could be assigned to a specific nest. The stage of the breeding cycle of females collected before the start of egg laying was estimated from the size of follicles in the ovary relative to those in the oviduct, and these were classified according to the day on which the first egg would have been laid (e.g. day −1, −2, etc.). For birds that had already initiated egg laying, the day the first egg was laid was designated day zero, and subsequent days as day +1, +2, etc. Overall, our samples spanned the period from day −6 to day +11. Methods used to examine sperm-storage tubules followed Briskie and Birkhead (1993). Briefly, primary mucosal folds were removed, placed on a microscope slide, and examined at high (400×) magnification to count, measure (outside length and width), and record contents of the sperm-storage tubules. Measurements were made using a calibrated ocular micrometer (± 3.3 μm). Sperm that numbered less than 30 were counted individually; more than 30 sperm were estimated to the nearest 10. A total of 50 sperm-storage tubules from five primary mucosal folds was examined for each bird. Means are presented ± 1 SE.

Results.—The number of primary mucosal folds ($\bar{x}$ = 15.0 ± 0.28, range 13 to 17, n = 19) was independent of the stage in the breeding cycle (as in other species; Briskie 1994, Pellatt 1998). The mean number of sperm-storage tubules per primary mucosal fold in eight birds examined in detail was 57.8 ± 7.34, which translates to an estimate of 849.6 ± 95.2 (range 525 to 1,287) sperm-storage tubules per female. Birds differed in: (1) the number of sperm per sperm-storage tubule (F = 33.84, df = 18 and 949, $P < 0.001$); (2) tubule length (F = 32.43, df = 18 and 949, $P < 0.001$); and (3) tubule width (F = 54.75, df = 18 and 949, $P < 0.001$), reflecting differences in the stage of the breeding cycle and among females.

Patterns over the breeding cycle relative to the onset of laying (day zero) were analyzed using two-factor polynomial regression. For all variables, a significant inverted U-shaped pattern was apparent: sperm number per tubule (r^2 = 0.456, F = 6.70, df = 2 and 18, $P < 0.01$; Fig. 1A); proportion of tubules containing sperm (r^2 = 0.485, F = 7.52, df = 2 and 18, P = 0.005; Fig. 1B); tubule length (r^2 = 0.634, F = 13.86, df = 2 and 18, $P < 0.001$; Fig. 1C); and tubule

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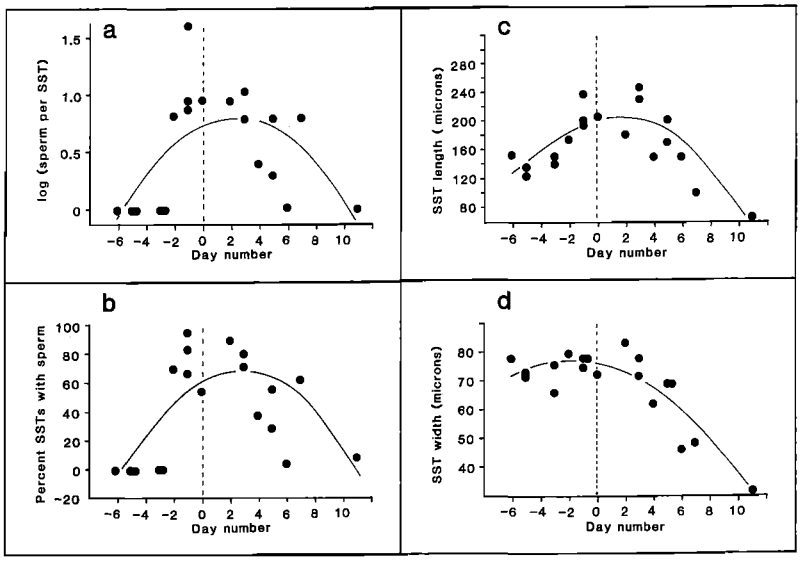

FIG. 1. Relationships between day in the female's cycle (day 0 = day first egg laid) and: (A) sperm number per tubule, (B) percentage of sperm-storage tubules containing one or more sperm, (C) tubule length, and (D) tubule width. All relationships are significant based on two-factor polynomial regressions (see text).

width ($r^2 = 0.790$, $F = 30.17$, df = 2 and 18, $P < 0.001$; Fig. 1D). Sperm-storage tubules increased in length from about 150 μm several days before laying to a maximum of about 240 μm during the early laying period. Tubule length then decreased (Fig. 1C) over the laying period (days 0 to +6), although the decline was not significant (Pearson correlation, $r = -0.464$, df = 6, $P = 0.247$). Tubule width was similar (ca. 80 μm) over the prelaying period and declined rapidly during and after the laying period (Fig. 1D). The decline in tubule width over the laying period approached significance ($r = -0.646$, df = 6, $P = 0.083$). Only birds from day −2 onward contained sperm in their sperm-storage tubules. The proportion of tubules containing sperm declined through the laying period, from 96% at the start of laying to less than 10% after day +5 ($r = -0.679$, df = 6, $P = 0.064$).

Birds examined after laying was completed had some sperm in their tubules, but many of these sperm were disintegrated and presumably no longer viable. This phenomenon has been recorded in other species (see Birkhead et al. 1993, Briskie 1994, Pellatt 1998). The mean number of sperm per tubule (Fig. 1A) showed a similar pattern to the proportion of tubules containing sperm, except that one bird (collected on day −1) contained very high numbers of sperm; with a mean of 40.5 sperm per tubule, this bird was estimated to have stored 34,425 sperm in her 850 tubules. In contrast, another bird also collected on day −1 contained an estimated 6,600 sperm. Similar variation in sperm numbers between individuals has been found in the Zebra Finch (*Taeniopygia guttata*; Birkhead et al. 1990). Overall, the number of sperm per tubule declined significantly over the laying period ($r = -0.777$, df = 6, $P = 0.023$).

Discussion.—The size of the sperm-storage tubules in the Pied Flycatcher and the number of sperm they contained show a marked change over just 10 to 12 days of the breeding cycle, first increasing over the prelaying period and then decreasing during egg laying. Similar patterns occurred in the two other passerines that have been examined, the Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*; Briskie 1994) and the Zebra Finch (Pellatt 1998).

The estimated number of sperm-storage tubules in the Pied Flycatcher (850) is very close to that predicted (851) from its body mass (see Briskie and Montgomerie 1993). This suggests that the number of sperm-storage tubules in this species primarily is a function of allometric constraints rather than being associated with the low level of sperm competition in this species (Gelter 1989, Lifjeld et al. 1991, Ratti et al. 1995). The mean length of tubules (181.40 ± 11.84 μm) also is close to that predicted (203 μm) from the mean length of sperm in the Pied Flycatcher (i.e. 98.30 μm; Briskie and Montgomerie 1992, 1993; J. V. Briskie unpubl. data).

One of the most striking aspects of this and other studies is the rapid decline in both tubule dimensions and the number of spermatozoa following the onset of egg laying. Sperm-storage tubules appear to start to decline in length from day +2 (the day the third egg is laid). These declines, which also occur in Yellow-Headed Blackbirds (Briskie 1994) and Rock Doves (*Columba livia*; Lovell-Mansbridge 1995), pre-

cede the end of the female's ovulatory cycle (Pied Flycatchers lay 6 to 7 eggs in our study area) and hence the regression of the oviduct as a whole. Apparently, then, the maintenance of sperm-storage tissue is costly.

Sperm first appeared in the sperm-storage tubules two days before the first egg was laid. Although some sperm remained in the tubules after the end of egg laying, these sperm showed obvious signs of deterioration, and it is unlikely that they were capable of fertilizing eggs. Thus, a female Pied Flycatcher probably stores sperm for a period lasting 7 to 9 days on average. Because copulations that occur outside this period of active sperm storage are not likely to lead to fertilizations, it is not surprising that copulation frequency in Pied Flycatchers is highest between days −1 and 0 (Haartman 1956, Alatalo et al. 1987). Copulations occasionally may occur as early as day −9 in this species (Haartman 1956; Lifjeld et al. 1997a,b), but our observations of sperm-storage tubule morphology suggest that sperm from such early copulations will not be stored, and therefore will not play a role in subsequent fertilizations. Indeed, experiments by Lifjeld et al. (1997b) have shown that only inseminations occurring around day −2 resulted in fertilization, which is consistent with the pattern of sperm presence in tubules in our study.

The decline in sperm numbers per tubule over the laying period is part of the process in which sperm are released from the tubules and transported to the infundibulum, the site of fertilization. In addition, the steady decline in tubule sperm numbers through the laying period suggests that sperm numbers are not replenished by further copulations. This is consistent with the lack of observed copulations once egg laying has started (Alatalo et al. 1987). If we assume that copulations do not occur during laying, then the decline in the number of sperm per tubule can be used to calculate the instantaneous per capita rate of sperm loss from the female tract (see Lessells and Birkhead 1990). In other bird species, the instantaneous per capita rate of sperm loss has been calculated from the $\log_e$ numbers of sperm trapped on the vitelline layers of successively laid eggs (see Wishart 1987, Birkhead et al. 1993). Studies of domestic fowl (*Gallus domesticus*) have shown that the numbers of sperm in the sperm-storage tubules and in the perivitelline layers of laid eggs are positively correlated (Brillard and Antoine 1990, Brillard 1993). Therefore, we have used the numbers of sperm in the tubules of Pied Flycatchers over the laying period to estimate the instantaneous per capita rate of loss; i.e. 0.027 $\log_e$ (sperm) $h^{-1} \pm 0.010$. This value is similar to that in the Zebra Finch (0.025; Birkhead et al. 1993), and some other passerines (0.02 to 0.03; Birkhead et al. 1994), although it is lower than that in some other bird species (Birkhead et al. 1996). The instantaneous per capita rate of sperm loss from the female reproductive tract is one of the most important variables in predicting the outcome of sperm competition in birds (Birkhead et al. 1995, Colegrave et al. 1995). Thus, it would be worth verifying the value for the Pied Flycatcher by counting sperm on successive eggs from several different clutches.

Our observations suggest that the process of sperm storage in the Pied Flycatcher is a dynamic one. Because females appear to store sperm for only a very limited period each breeding attempt, males must time their copulations (both pair and extrapair) to coincide with this brief period of opportunity. Why female Pied Flycatchers store sperm for so short a period is not clear, but presumably it reflects some tradeoff between the costs and benefits of storing sperm for extended durations. Whatever the reason, such a short fertile period may in turn limit the opportunities for both sexes to pursue extrapair copulations and is perhaps associated with the relatively low rate of extrapair paternity in this species (Lifjeld et al. 1991). It is tempting to speculate that females might even control the extent of sperm competition by varying the duration over which they actively store sperm. Whether such a process occurs in Pied Flycatchers, or in any other species, is unknown. However, the fact that sperm storage occurs in the female tract, and thus is under her control, suggests that it would not be surprising if selection favored patterns of sperm storage that increased this control.

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Carotenoid Availability and Plumage Coloration in a Wild Population of Northern Cardinals

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Bright red, orange, and yellow ornamental plumage and colors of other exposed tissues in birds are produced by deposition of various carotenoid pigments. Because these pigments cannot be synthesized by birds, they must be obtained through dietary sources (Goodwin 1950). As a consequence, the expression of carotenoid-based color in tissues must result from numerous physiological and behavioral processes that determine carotenoid intake, absorption, transport, and deposition (Brush 1990).

The presence of carotenoid pigments in secondary sexual characteristics in birds and fish has evoked much recent interest in their function as signals of individual quality. Carotenoid deposition may be an indicator of genetic quality (Hamilton and Zuk 1982, Kodric-Brown and Brown 1984, Hudon 1994), foraging ability (Kodric-Brown 1989, Hill 1992), presence of parasites or other diseases (Schaeffer et al. 1988, Houde and Torio 1992), and hormone levels (Brush 1967, Temple 1974).

Hypotheses for the function of carotenoid-based ornaments in sexual selection center on the availability of these pigments in natural food sources. In the absence of direct measurements, researchers have suggested that carotenoids are limiting for some wild populations of birds (e.g. Miskimen 1980; Slagsvold and Lifjeld 1985; Hill 1993 a, b; 1994) and fish (Kodric-Brown 1989), and that differences in expression of color are the result of differential foraging ability. In contrast, Hudon (1994) proposed that carotenoids generally are not limiting in the environment and that the expression of color results from physiological condition and not foraging ability (see Burley et al. 1992). Carotenoids are ubiquitous pigments and are synthesized by nearly all plants, photosynthetic algae, and some fungi and bacteria. In avian food sources, carotenoids are most abundant in animals (e.g. insects) and fruits.

Despite their different assumptions concerning carotenoid availability, the "foraging hypothesis" (Hill 1992) and the "health hypothesis" (Hudon 1994) make predictions that are not separable under nonexperimental conditions. Significant fluctuations in the availability of food sources that contain carotenoids may affect not only foraging efficiency but also the health or physical condition of individuals dependent on these food sources. Indeed, the predictions of these hypotheses may be difficult to distinguish even under experimental conditions (Hill 1994, Hudon 1994). However, under any circumstances, a significant decline in availability of important, carotenoid-rich food sources without an accompanying decrease in ornament expression would call into question both hypotheses.

A natural event that dramatically reduced fruit availability enabled us to determine if fruit scarcity resulted in a decrease in plumage brightness in a wild population of Northern Cardinals (*Cardinalis cardinalis*). Cardinals are sexually dichromatic and socially monogamous. Both sexes possess orange bills and dull red-brown remiges and rectrices that are nearly invariant in color. Males also possess carotenoid-based bright red plumage with especially

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