The Condor 98:414–417

LACK OF SPERM STORAGE BY FEMALE MIGRANTS AND THE SIGNIFICANCE OF COPULATIONS EN ROUTE[1]

James V. Briskie[2]
Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada

Key words: Sperm storage; sperm storage tubules; migrant warblers; copulation behavior; Manitoba.

In all species studied thus far, female birds store sperm in specialized sperm storage tubules (SSTs) located at the junction of the uterus and vagina (Fujii 1963, Shugart 1988, Birkhead et al. 1990, Briskie and Montgomerie 1993). SSTs appear necessary for the long-term survival of spermatozoa within the female reproductive tract, because sperm not stored within SSTs soon die or are displaced by the passage of subsequent eggs (Lorenz 1966). The length of time that sperm can remain viable in storage varies from species to species, ranging from about six days in the Ring Dove (*Streptopelia roseogrisea*; Zenone et al. 1979) to over 72 days in the domestic Turkey (*Meleagris gallopavo*; Lorenz 1950). Why sperm storage duration varies among species is not clear, but it may be related to the proximity of males and females during pre-laying (Birkhead and Møller 1992a). For example, sperm storage durations are particularly long in seabirds, in which females are separated from their mates for prolonged periods prior to laying. In contrast, males of many passerine species typically remain close to females throughout pre-laying and these species have correspondingly shorter durations of sperm storage (Birkhead and Møller 1992a).

In most birds, copulations are generally restricted to a brief prelaying period, with the greatest frequency occurring just prior to the laying of the first egg. For migratory species, pairing and mating are thought to take place largely on the breeding grounds after the end of spring migration. However, by using lavages to census sperm within the cloacae of male and female migrant passerines, Quay (1985a, 1989) found that many Tennessee Warbler (*Vermivora peregrina*) females and possibly some Blackpoll Warbler (*Dendroica striata*) females had been inseminated during migration and south of their nearest breeding grounds. In the Tennessee Warbler, about 25% of females contained sperm in their cloacae, indicating that copulatory behavior during migration may be widespread (Quay 1989). Recently, Moore and McDonald (1993) suggested that en route copulations may have an adaptive function, such as minimizing the time and costs of mate choice or ensuring females have an adequate supply of sperm should they not find a mate on the breeding grounds. Unfortunately, the role that en route inseminations play in the eventual fertilization of eggs remains unknown.

If copulations during migration play a role in the fertilization of eggs, then females must be able to store sperm acquired en route and to arrive on the breeding grounds with this sperm still viable (Quay 1989, Moore and McDonald 1993). Since sperm can remain viable for extended periods only if stored within the SSTs, migratory birds must have active and developed SSTs if en route copulations are to have any functional significance. In this study, I examined the reproductive tracts and SSTs of four species of migratory passerines, including Tennessee and Blackpoll Warblers, to determine if females can store sperm prior to their arrival on the breeding grounds. My results suggest that sperm are unlikely to remain viable from inseminations occurring en route, and that such behavior may be simply an epiphenomenon of the recrudescence of the reproductive organs during migration.

METHODS

Six female Tennessee Warblers and three female Blackpoll Warblers were collected during the last week of May in 1992 and 1994 at Delta Marsh, Manitoba, Canada. Both species are common migrants in this area and were moving through in large numbers on the days they were collected. Neither species nests at Delta Marsh; the southernmost breeding location for Tennessee Warblers is about 100 km north of the collecting site, while the nearest record for Blackpoll Warblers lies about 450 km farther north (Godfrey 1986). I also salvaged one female Black and White Warbler (*Mniotilta varia*) on 14 May 1992 and one female Ovenbird (*Seiurus aurocapillus*) on 22 May 1992 from window kills in the south end of the city of Winnipeg, Manitoba. The salvage site for these species lies within their respective breeding ranges, but it is likely that both were migrants because of the early date and the unsuitability of the salvage site (parking lot and apartment complexes) for breeding. For comparison with migrants, I collected one additional Blackpoll Warbler in breeding condition at Churchill, Manitoba on 10 June 1994. This female had an egg in the oviduct and several enlarged follicles in the ovary. Although sample sizes were small, it is likely the results presented here are representative, as previous work has shown little vari-

---

[1] Received 28 August 1996. Accepted 26 January 1996.

[2] Current address: Department of Zoology, South Parks Road, University of Oxford, Oxford OX1 3PS, United Kingdom, e-mail: james.briskie@zoology.oxford.ac.uk

ation in SST morphology between females of the same species relative to that observed among species (Birkhead and Hunter 1990, Briskie and Montgomerie 1993).

Within an hour of obtaining the specimens, the oviduct was removed, pinned straight to a cork board, and then immersed in 10% formalin. After fixing for 24 h, the tissue was transferred to a labeled vial and stored in 10% formalin for a further four to six months before processing. The method used for preparing oviduct tissue for the examination of SSTs has been described in detail by Briskie and Birkhead (1993). Briefly, I first exposed the oviductal lumen by making a longitudinal incision through the vagina and uterus and pinning the tissue flat onto a cork dissecting tray. The folds at the uterovaginal junction were counted, and a random sample of three folds was removed. Each fold was then cut along the mid-ridge, and each half was laid flat onto a glass slide with the lumenal surface facing down. A drop of phosphate-buffered saline was added, and the tissue was covered with a glass cover slip.

Sperm storage tissue was examined at high magnification (×400) with a light microscope. For each fold, I counted the SSTs; the mean number of SSTs from the three folds was then multiplied by the total number of folds to estimate the total number of SSTs for that individual. Next, I measured the outside length of 30 SSTs per individual (10 SSTs/fold) with a calibrated ocular micrometer. SSTs for measuring were selected by running a transect across the fold from the uterine to vaginal end. This ensured that about equal proportions of SSTs from all regions of the uterovaginal junction were sampled. Finally, I examined each SST for the presence or absence of sperm. Passerine spermatozoa have a characteristic spiral-shaped head and thus are readily recognizable within the lumen of a SST (Briskie and Montgomerie 1993).

RESULTS AND DISCUSSION

SSTs were observed in all individuals of each species examined (Fig. 1). The estimated number of SSTs varied from 327 ± 39 ($n = 6$ females) in the Tennessee Warbler to 492 SSTs ($n = 1$ female) in the Black and White Warbler (Table 1). The number of SSTs in each of the four species of warblers studied here was less than half that expected for their body mass (Birkhead and Møller 1992b, Briskie and Montgomerie 1992, 1993). The reason for this difference is not known but could be either a characteristic of parulid warblers in general or the result of a seasonal change in the number of SSTs as egg laying approaches, as all birds in the other studies were in breeding condition. Since all the SSTs I observed in migrating birds were typically small (see below), it is also possible that I missed some of the very small SSTs in my census. However, seasonal

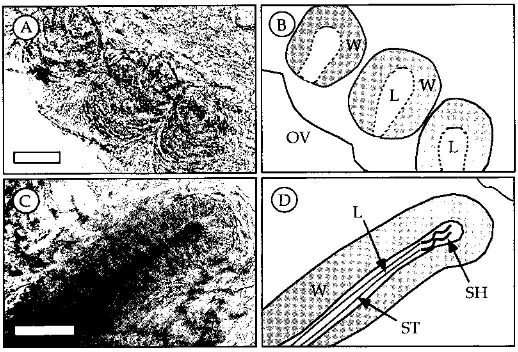

FIGURE 1. Photographs of sperm storage tubules (SSTs) in a (A) migrant and a (C) breeding female Blackpoll Warbler and line tracings (B, D) to illustrate structural features. SSTs appear as small, round structures embedded in the mucosa of the oviduct in migrating birds (A, B); the lumens of SSTs at this time are indistinct (dotted lines in B) and no sperm were observed. By breeding (C, D), SSTs have elongated about seven-fold and contain many sperm. The line tracings were made directly from the photographs but each was enhanced to show features not readily visible in a single plane. Bar is 50 μm. Symbols: W = wall of SST, L = lumen of SST, SH = head of sperm, ST = tail of sperm, OV = lumen of oviduct.

TABLE 1. Number and length of sperm storage tubules (SSTs) in individuals of four species of migrant passerines collected in southern Manitoba, Canada. SST length calculated from 30 SSTs per bird.

		SST length (μm)	
Species	Number of SSTs	Mean ± SE	Range
Tennessee Warbler	256	118.3 ± 6.9	47.5–180.0
	476	96.9 ± 5.3	42.5–167.5
	380	143.0 ± 6.0	82.5–217.5
	360	123.0 ± 5.9	57.5–187.5
	260	148.8 ± 8.1	52.5–275.0
	232	96.6 ± 3.6	52.5–132.5
Blackpoll Warbler	415	140.5 ± 7.6	67.5–210.0
	292	75.8 ± 3.4	42.5–110.0
	225	74.4 ± 4.1	40.0–137.5
Black and White Warbler	492	57.1 ± 4.3	22.5–102.5
Ovenbird	377	58.1 ± 3.2	27.5–102.5

increases or small size are unlikely to explain the low number of SSTs in Blackpoll Warblers, as the number of SSTs in the single female collected on the breeding grounds (368 SSTs) was very similar to that observed in migrants of this species (331 ± 56 SSTs, $n = 3$ females; Table 1).

Despite the presence of SSTs, no spermatozoa were observed in any migrating female of the four species examined (Fig. 1). SST length ranged from 121.2 ± 9.0 μm ($n = 6$ females) in the Tennessee Warbler to only 57.1 μm ($n = 1$ female) in the Black and White Warbler (Table 1). SST length for migrating Blackpoll Warblers (96.9 ± 21.8 μm, $n = 3$ females) was less than one-seventh that observed during the breeding season (725.8 ± 45.8 μm, $n = 1$ female). Although I was unable to measure the length of SSTs in breeding Tennessee Warblers, Black and White Warblers, and Ovenbirds, SSTs in these species on migration were generally much smaller than those measured by Briskie and Montgomerie (1993) in other passerine species collected during the breeding season. This suggests that SSTs in all the migrants I sampled were not developed nor potentially capable of storing sperm if inseminated.

This lack of development is particularly evident when SST length is compared against sperm length. In the Tennessee Warbler, sperm length averages 187.8 μm ($n = 2$ males; unpubl.) or about 55% greater than the average length of the SSTs during migration (121.2 μm; Table 1). Of the 180 SSTs measured in the six Tennessee Warbler females, only seven (3.9%) SSTs were sufficiently large enough to accommodate the entire length of a spermatozoa. Since sperm not completely protected within a SST are unlikely to survive (Lorenz 1966), it appears that most SSTs are too short and undeveloped to store sperm during spring migration.

The same pattern was evident in the three Blackpoll Warblers examined. In this species, sperm length (269.5 μm, $n = 1$ male; unpubl.) was almost three times the average length of the SSTs (96.9 μm; Table 1) and none of the 90 SSTs examined in the three migrating females were large enough to accommodate even a single spermatozoa. No information is available on the length of sperm in either Black and White Warblers or Ovenbirds; however, both species also had very short SSTs, suggesting that they too would not be able to store sperm if inseminated at this stage.

The lack of sperm storage by female migrants is not a product of the lack of sperm production in males. Two male Tennessee Warblers collected on migration were estimated to have approximately 1.3 and 5.8 million sperm, respectively, in their seminal glomera (the site of sperm storage in male birds). The seminal glomera of a single Blackpoll Warbler salvaged from a window kill also contained numerous sperm, although the exact number was not estimated. In a more extensive survey of sperm production by migratory birds, Quay (1985a, 1985b, 1986a, 1986b) found that males in a wide variety of passerines initiated sperm production while on migration and far south of their nearest breeding grounds. Thus, if copulations do occur en route in some migratory passerines, it is likely that males are capable of inseminating females with viable sperm, even though these inseminations do not appear to lead to subsequent sperm storage.

In a study of seasonal changes in SST morphology in the Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*), I found that SSTs were present in all females and in roughly equal number throughout the season (Briskie 1994). As with the migratory warblers examined in this study, SSTs in blackbirds collected on the day they arrived on the breeding grounds (and thus at the end of their spring migration) were small and regressed and did not contain any sperm. After females arrived on the breeding grounds, their SSTs rapidly doubled in size and reached maximum length in about eight to fourteen days, although sperm were not observed within SSTs until three to five days before clutch initiation (Briskie 1994). However, it is interesting to note that SSTs in newly arrived birds were almost double the length of those found in post-breeding birds (Briskie 1994). This suggests that some development of the SSTs must occur while on migration, although not nearly enough to allow for sperm storage.

Although I examined SSTs in only a few species and a small number of individuals, it was clear that none of the females collected on migration either stored sperm or appeared capable of storing sperm if inseminated. Why then do some species copulate en route to their breeding grounds if such behavior does not lead to the subsequent fertilization of eggs? Quay (1989) suggested that en route copulations may provide some physiological or behavioral advantage to the individual in a kind of "inseminatory practice run" before the breeding territory is reached. However, why this should be necessary in some species and not others is not clear. It is also possible that en route copulations may function in pair formation (Moore and McDonald 1993). Such an function should be most advantageous in northern species with a limited breeding season, and indeed, the two species known to copulate on migration (Tennessee and Blackpoll Warblers) are two of the most northerly breeding species of warblers. Nonetheless, it seems unlikely that extensive pairing occurs during migration in most species of passerines because of the differential timing of migration between the sexes (Francis and Cooke 1986).

If females participate in en route copulations only if their reproductive tracts are physiologically ready to receive and store sperm, then the lack of sperm storage I observed may have been due to inadequate sampling. This hypothesis suggests that females have two strategies, with some individuals pairing, copulating and storing sperm on migration (SSTs developed), while other individuals remain sexually inactive and do not mate until they arrive on the breeding grounds (SSTs undeveloped). If females using the former strategy are uncommon, then it is possible they were missed as my sample size was small ($n = 11$ females in total). Instead, only females of the latter strategy were collected and they would therefore not be expected to store sperm. Determining whether such a dicotomy exists among females would be relatively easy to test but will require a more extensive survey of birds during migration.

Perhaps the most parsimonious explanation for why some birds copulate en route is that such behavior results from migration occurring during the recrudescence of reproductive tissues. In other words, the hormonal system necessary to stimulate the re-growth of the genitals may also inadvertently trigger the corresponding sexual behaviors, such as singing or copulations. This may be especially true for northern birds, which face a relatively short window of favorable conditions in which to pair, mate, and raise offspring and would be selected to arrive on the breeding grounds ready to breed. Thus, en route copulations may be an epiphenomenon of the need for both males and females to arrive on the breeding grounds in reproductive condition and not as an adaptation to fertilize eggs.

I thank the University of Manitoba Field Station at Delta Marsh for providing accommodation during my collections in the area. This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC). Collections were made under a permit from the Canadian Wildlife Service. Bob Montgomerie, Stephen Yezerinac and two anonymous reviewers made several useful suggestions in the preparation of this manuscript.

LITERATURE CITED

Birkhead, T. R., and F. M. Hunter. 1990. Numbers of sperm-storage tubules in the Zebra Finch (*Poephila guttata*) and Bengalese Finch (*Lonchura striata*). Auk 107:193–197.

Birkhead, T. R., and A. P. Møller. 1992a. Sperm competition in birds: evolutionary causes and consequences. Academic Press, London.

Birkhead, T. R., and A. P. Møller. 1992b. Numbers and size of sperm storage tubules and the duration of sperm storage in birds: a comparative study. Biol. J. Linn. Soc. 45:363–372.

Birkhead, T. R., J. E. Pellatt, and F. M. Hunter. 1990. Numbers and distribution of sperm in the uterovaginal sperm storage tubules of the Zebra Finch. Condor 92:508–516.

Briskie, J. V. 1994. Seasonal patterns of sperm storage in the Yellow-headed Blackbird *Xanthocephalus xanthocephalus*. Ibis 136:323–330.

Briskie, J. V., and T. R. Birkhead. 1993. A review of the methods used to study the anatomy of avian sperm storage. Ornis Scand. 24:323–329.

Briskie, J. V., and R. Montgomerie. 1992. Sperm size and sperm competition in birds. Proc. R. Soc. Lond. B 247:89–95.

Briskie, J. V., and R. Montgomerie. 1993. Patterns of sperm storage in relation to sperm competition in passerine birds. Condor 95:442–454.

Francis, C. M., and F. Cooke. 1986. Differential timing of spring migration in wood warblers (Parulinae). Auk 103:548–556.

Fujii, S. 1963. Histological and histochemical studies on the oviduct of the domestic fowl with special reference to the region of uterovaginal juncture. Arch. Histol. Japan 23:447–459.

Godfrey, W. E. 1986. The Birds of Canada. Natl. Mus. Canada, Ottawa.

Lorenz, F. W. 1950. Onset and duration of fertility in turkeys. Poul. Sci. 29:20–26.

Lorenz, F. W. 1966. Behavior of spermatozoa in the oviduct in relation to fertility. *In* C. Horton-Smith and E. C. Amorose [eds.], Physiology of the domestic fowl. Oliver and Boyd, Edinburgh.

Moore, F. R., and M. V. McDonald. 1993. On the possibility that intercontinental landbird migrants copulate en route. Auk 110:157–160.

Quay, W. B. 1985a. Sperm release in migrating wood-warblers (Parulinae) nesting at higher latitudes. Wilson Bull. 97:283–295.

Quay, W. B. 1985b. Cloacal sperm in spring migrants:occurrence and interpretation. Condor 87: 273–280.

Quay, W. B. 1986a. Timing and location of spring sperm release in northern thrushes. Wilson Bull. 98:526–534.

Quay, W. B. 1986b. Cloacal protuberance and cloacal sperm in passerine birds:comparative study of quantitative relations. Condor 88:160–168.

Quay, W. B. 1989. Insemination of Tennessee Warblers during spring migration. Condor 91:660–670.

Shugart, G. W. 1988. Uterovaginal sperm-storage glands in sixteen species with comments on morphological differences. Auk 105:379–384.

Zenone, P. G., M. E. Sims, and C. J. Erickson. 1979. Male Ring Dove behavior and defense of genetic paternity. Am. Nat. 114:615–626.