The Condor 95:442–454

PATTERNS OF SPERM STORAGE IN RELATION TO SPERM COMPETITION IN PASSERINE BIRDS[1]

JAMES V. BRISKIE AND ROBERT MONTGOMERIE
Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada

Abstract. Using a technique for observing sperm storage tubules (SSTs) in whole-mount preparations of oviduct tissue, we estimated the number and length of SSTs across 20 species of passerine birds in seven subfamilies. The average number of SSTs per female varied nine-fold across the species examined and was correlated positively with body mass and relative egg size, suggesting that much of the interspecific variation in the number of SSTs may be a consequence of allometry. However, after controlling for body mass, the number of SSTs per female was also negatively correlated with the average length of SSTs and we suggest this pattern could result from selection for greater sperm length when access to sperm storage sites is limited by females. SST length varied eight-fold across the species examined but unlike the number of SSTs, SST length was not correlated with body mass. Instead, SST length was strongly and positively correlated with sperm length, suggesting a history of coevolution between male gametes and sperm storage sites in females that is independent of body size. Neither mating system nor any of four other morphological variables (testis length, oviduct length, clutch size, sperm storage capacity) was consistently correlated with either the number or size of SSTs. Variation between species in the potential for sperm layering within an SST, however, indicates that the pattern of sperm precedence (i.e., the pattern of paternity relative to mating order) may vary from species to species. We propose that a conflict of interest between the sexes with respect to sperm storage could lead to an arms race between sperm length and SST length and may help explain both the diversity in sperm storage patterns among species and the influence of sperm storage on the evolution of mating behavior in birds.

Key words: Sperm storage; sperm competition; sperm precedence; sperm; reproductive strategies; passerines.

INTRODUCTION

Poultry scientists have long known that female domestic fowl can store sperm for several weeks (e.g., Van Drimmelen 1946, Lorenz 1950, Lodge et al. 1971). In poultry, sperm are stored primarily in specialized sperm storage tubules (SSTs) located at the junction of the uterus and vagina (e.g., Bobr et al. 1964, Mero and Ogasawara 1970) although some storage may also occur in the infundibulum (Van Drimmelen 1946). Recent studies of SSTs in several species of wild birds (Hatch 1983; Bakst and Bird 1987; Shugart 1988; Birkhead 1987, 1988; Birkhead et al. 1990) suggest that sperm storage may be a universal feature of avian reproduction.

The significance of sperm storage for the evolution of mating patterns in wild birds was recognized by behavioral ecologists only recently (Hatch 1983, Birkhead 1988). One consequence of sperm storage by females is that sperm from different males are more likely to co-occur in the female reproductive tract. Competition between ejaculates from two or more males over the fertilization of ova of a single female was called sperm competition by Parker (1970) and subsequent work has shown that this process has played a large role in the evolution of a variety of mating behaviors (e.g., copulation frequency, duration of mate-guarding; Birkhead and Møller 1992a).

Despite a variety of studies of avian SSTs (Mero and Ogasawara 1970, Shugart 1988, Birkhead and Hunter 1990, Lessells and Birkhead 1990), the proximate mechanisms of sperm storage and their effect on sperm competition are poorly understood. Fertilization in birds typically occurs in the infundibulum during a brief "fertilization window" of only 15–30 minutes duration, shortly after ovulation (Howarth 1974). The ovum then begins its descent down the oviduct where albumin and shell membranes are deposited. Unless protected in SSTs, sperm are unlikely to survive in the oviduct during egg formation and laying (Lorenz 1966). Thus, specialized sperm storage sites may have evolved in birds so that

[1] Received 22 July 1992. Accepted 1 February 1993.

inseminations do not have to be synchronized with the fertilization window to be successful at fertilization (Hatch 1983).

Several recent experiments on captive birds have shown that the last male to copulate before fertilization enjoys considerably higher fertilization success than previous males, even if copulations from the last male are greatly outnumbered by those of the previous male (e.g., Compton et al. 1978, Cheng et al. 1983, Sims et al. 1987, Birkhead et al. 1988). For example, Birkhead et al. (1988) found that, in Zebra Finches (*Taeniopygia guttata*), the last male to mate before eggs were fertilized fathered approximately 80% of the subsequent offspring. Such observations have suggested that sperm may be stored as stratified layers within SSTs and possibly that the last insemination is stored nearest to the oviductal lumen, thus giving it the advantage of being the first out during ovulation (Compton et al. 1978). On the other hand, Lessells and Birkhead (1990) have used the available data on poultry to model the sperm storage and fertilization process but did not find clear support for this sperm layering hypothesis. Instead their analysis showed that displacement of a previous male's sperm by that of the last-copulating male could better account for the observed patterns of last-male sperm precedence. Nevertheless, sperm precedence experiments in birds have been limited to only a few species to date. It may thus be premature to generalize sperm storage mechanisms from this small sample, particularly since the pattern of sperm precedence is known to vary widely from species to species in mammals (Dewsbury 1984, Birkhead 1988).

Although SSTs seem to be important in sperm competition, little is known about variation in sperm storage capacity among species and how such variation may be related to differences in mating system or behavior patterns. Shugart (1988) measured the lengths of SSTs in a dozen species of birds but he did not attempt to account for variation among species. Recently, Birkhead and Møller (1992b) compared the number and length of SSTs across 11 species of domestic birds to determine if the duration of sperm storage could be predicted from SST morphology. Although a weak relationship was found, they observed a strong relationship between the number of SSTs and body mass.

In this paper, we estimate both the number and length of SSTs for a variety of wild passerine species. We then examine how interspecific variation in the number and length of SSTs is related to body size, sperm length, and the degree of sperm competition expected from the mating system of these species. Finally, we describe sperm arrangement patterns within SSTs and show that differences among species may have the potential to affect sperm precedence patterns.

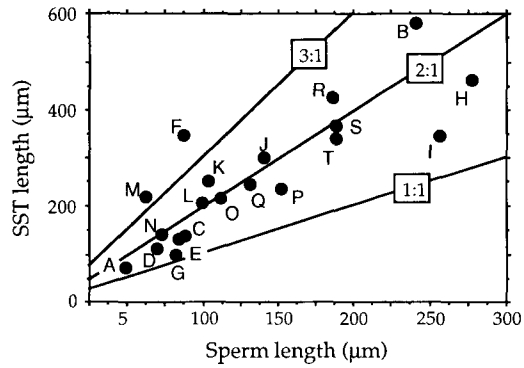

FIGURE 1. Variation in the potential for sperm stratification among 20 species of passerine birds. For each species, mean sperm length is plotted against mean SST length. Lines delineate different SST length : sperm length ratios. Species (no. of females, males examined) are: A, Least Flycatcher, *Empidonax minimus* (3, 3); B, Tree Swallow, *Tachycineta bicolor* (4, 4); C, Barn Swallow, *Hirundo rustica* (3, 5); D, American Pipit, *Anthus rubescens* (3, 3); E, Horned Lark, *Eremophila alpestris* (3, 1); F, American Robin, *Turdus migratorius* (2, 1); G, Red-eyed Vireo, *Vireo olivaceus* (2, 1); H, Yellow Warbler, *Dendroica petechia* (4, 1); I, American Redstart, *Setophaga ruticilla* (2, 1); J, Red-winged Blackbird, *Agelaius phoeniceus* (3, 1); K, Yellow-headed Blackbird, *Xanthocephalus xanthocephalus* (3, 4); L, Brewer's Blackbird, *Euphagus cyanocephalus* (3, 2); M, Brown-headed Cowbird, *Molothrus ater* (4, 3); N, Western Meadowlark, *Sturnella neglecta* (1, 1); O, Lapland Longspur, *Calcarius lapponicus* (5, 2); P, Smith's Longspur, *C. pictus* (3, 4); Q, Snow Bunting, *Plectrophenax nivalis* (4, 2); R, Common Redpoll, *Carduelis flammea* (2, 2); S, Hoary Redpoll, *C. hornemanni* (1, 2); T, American Goldfinch, *C. tristis* (4, 2).

METHODS

Oviducts from 59 females of 20 passerine species representing seven different subfamilies were collected in Manitoba, Ontario and the Northwest Territories, Canada during the 1988, 1989 and 1990 breeding seasons (see Fig. 1 for list of species and scientific names). Within an hour of collecting each female, we removed the entire reproductive tract (ovary to vent). Oviducts were placed immediately in 10% buffered formalin in separate labelled vials and stored 6 to 18 months

before examination. Only females that were laying or close to laying eggs (as evidenced by advanced developing ova) were used. All birds were collected under appropriate federal and territorial permits.

To prepare an oviduct for examination, all mesenteries, skin and other obstructing tissues were carefully removed to expose the lower reproductive tract. Oviduct length was determined by tracing the outline of each oviduct with a measuring thread. If present, developing eggs were removed and the diameter of the yolk measured. The oviduct was then straightened, pinned to a dissecting board and a single longitudinal incision was made through the cloaca, vagina and uterus. The exposed mucosa was pinned flat and keep moist with water. Under a dissecting microscope, the uterovaginal (UV) junction appears as a distinct band of thin, convoluted folds (see Bakst and Bird 1987, Birkhead and Hunter 1990, Birkhead et al. 1990). For each specimen, we counted the number of folds and measured the approximate width of the UV band with a ruler. To estimate the number of SSTs, three individual folds (out of a total of 15–20) were removed in their entirety, beginning from the middle of the vagina and extending through the entire UV region up to and including about 0.5 mm of the uterine fold containing the shell glands. Any remaining connective tissue was carefully teased away and the lamina propria of each fold was spread open onto a clean glass slide so that the medial surface of each half faced up. Each fold was placed under a cover slip and prepared as a wet mount. In some instances the preparation was flattened by gently tapping the cover slip with a blunt probe.

We counted the number of SSTs per fold using a phase contrast microscope (40 × magnification) calibrated with a stage micrometer. Individual SSTs could be distinguished and followed along their lengths by fine adjustments of the focus and iris diaphragm (see Fig. 2). SSTs could also be observed at high magnification with a dissecting microscope and transillumination (see Bakst 1987, Bakst and Bird 1987) but we found the boundaries of individual SSTs more distinct and more easily measured under a phase contrast microscope. Although some SSTs were branched (< 15% of SSTs per individual, unpubl. data), sperm were stored primarily in the distal end of each tubule or tubule branch (pers. observ., Birkhead and Hunter 1990, Birkhead et al. 1990). As we were most interested in the evolution of the number of sperm storage sites rather than their configuration, we counted each branch as a separate sperm storage site for our analyses. Next, we measured the length of five haphazardly chosen, clearly visible SSTs per fold for a total of 15 per individual bird. Branched SSTs were measured by following the length of one randomly chosen branch beginning from its distal end and continuing to its opening into the lumen of the oviduct (i.e., the distance sperm travel to enter or leave an SST). In most preparations, sperm could be easily discerned within tubules by examining the tissue in various focal planes at high magnification. Passerine sperm are readily recognizable by their characteristic spiral-shaped heads (see Fig. 2A). We also attempted to determine if sperm were present in SSTs by carefully opening a few tubules. Photographs of sperm and SST structure (Fig. 2) were taken using Neumarski optics on a Leitz Dialux 20 microscope.

To calculate the number of SSTs per female, we multiplied the mean number of SSTs per fold studied by the total number of folds. Birkhead and Hunter (1990) and Birkhead et al. (1990) applied similar techniques to estimate the number of SSTs in two species of estrildid finches. When more than one specimen of a species was available, we calculated a species mean using mean values from each female.

To determine the relationship between SST length and sperm length, we collected sperm either by gentle massage of the cloacal protuberance of live birds (see Wolfson 1952) or by salvaging semen from the seminal glomera of freshly-killed specimens. We also measured sperm coaxed out of SSTs in a few cases, avoiding those that were obviously damaged (e.g., broken tails) in the process. Sperm collected from tubules did not differ significantly in length from that collected directly from males so data from both sources were combined for analyses. Permanent mounts were made by placing a small droplet of semen on a clean glass slide and lightly smearing it by passing a second glass slide over the first. The slide was allowed to air dry and then stored in a dry slide box for later examination. Total sperm length (head plus tail) was measured under a phase contrast microscope (100× magnification; Fig. 2A) by averaging the length of 10 haphazardly chosen sperm. As be-

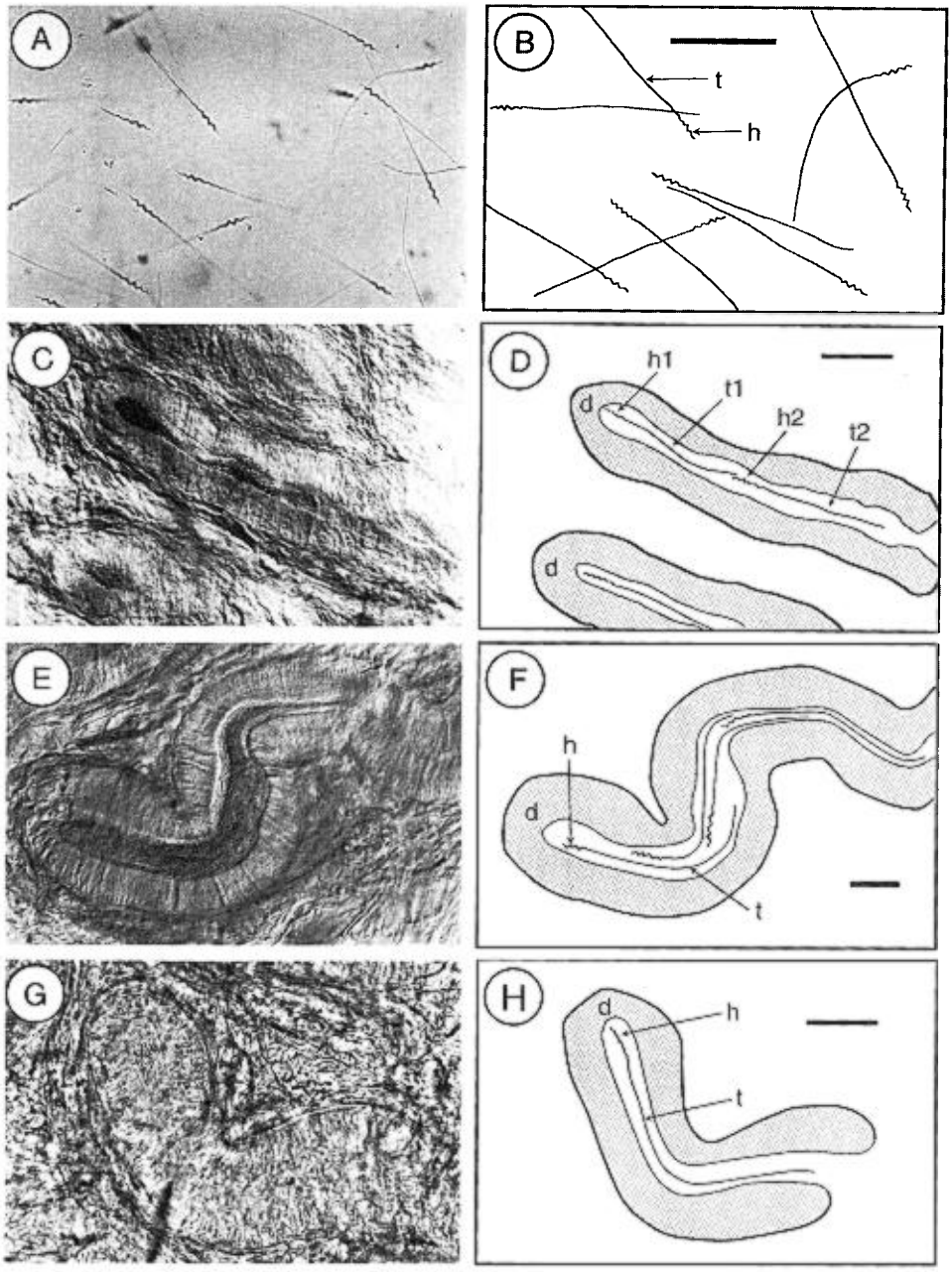

FIGURE 2. Photomicrographs of sperm (A) and sperm storage tubules (C, E, G), with line tracings (B, D, F, H) to illustrate structural features. The line tracings were made directly from the photographs but were enhanced to show features that are not readily visible in a photograph of a single plane. Sperm heads (h) and tails (t) and the distal ends (d) of SSTs are indicated. In D, F and H the walls of the SSTs are shaded and only one sperm in each layer is drawn. The scale bar on each drawing is 50 μm long. (A–B) Sperm of Yellow-headed Blackbird. (C–D) SSTs of Lapland Longspur showing two distinct layers of sperm filling the tubule where the heads of the second layer abut the tails of the first. (E–F) SST of Tree Swallow showing several layers of sperm filling the tubule—in this case the layers are not discrete. (G–H) SST of American Redstart showing a single layer of sperm filling the tubule.

fore, a single mean was calculated for each species from the mean values for all individual males studied.

We used relative testis length as an index of sperm production and the intensity of sperm competition. Interspecific comparisons of primates (Harcourt et al. 1981), birds (Cartar 1985, Møller 1991a) and mammals (Kenagy and Trombulak 1986) have shown that non-monogamous animals have relatively larger testes than monogamous species. Large testes in polygamous and promiscuous species are thought to be advantageous when the risk of sperm competition is high because they enable a male to produce large quantities of sperm to dilute or displace competing ejaculates (Harcourt et al. 1981).

We obtained data on testis size from fresh material collected by us in the field or taken from labels on museum skins housed in the Royal Ontario Museum, Toronto, the National Museum of Canada, Ottawa and the Manitoba Museum of Man and Nature, Winnipeg. When possible, we used data only from the same geographical region as the oviduct sample to avoid any bias due to variation in testis size over the range of a species (e.g., Rising 1987). At least 25 measures of testis size were used for each species. To estimate mean testis length at the peak of breeding, we fitted a second-order polynomial regression between testis length and date and used the maximum of the regression for each species. Body masses were taken from Dunning (1984).

For all comparative analyses, we used the methods described by Harvey and Pagel (1991) to control for phylogenetic effects. Such methods are required because traits may not have evolved independently among closely related species. Thus traits may be similar in closely related species simply because of common ancestry rather than independent evolution and a failure to control for this artificially weights sample sizes and the influence of some traits on the analysis. We used Sibley and Ahlquist (1990) to construct a phylogeny for the species studied. Then, using Pagel's Evolutionary Covariance Regression (unpubl. software, see Harvey and Pagel 1991), we calculated unique linear comparisons or "contrasts" within each taxon comprising two or more descendent taxa. These linear contrasts effectively reduce the information at each node in the phylogeny to a single datum for each variable being studied.

To test for relations between variables across taxa, we determined whether linear contrasts for one variable were related to those of another. A variety of statistical methods can be used to assess such relations (see Harvey and Pagel 1991) but we used simple and multiple linear regressions forced through the origin (presented as "contrast analysis"). In no case did the use of rank correlation or binomial tests on the contrasts (see Harvey and Pagel 1991) provide different results.

RESULTS

Sperm storage tubules were observed in all females of the 20 species examined. SSTs were confined to a 2–5 mm band (oviduct not stretched) at the junction of the uterus and vagina; no tubules were found in the infundibulum as has been reported for domestic chickens (Van Drimmelen 1946). The estimated mean number of SSTs per female varied among species from a low of 603 in the Tree Swallow ($n = 4$ individuals) to a high of 5,640 in the Western Meadowlark ($n = 1$), a more than nine-fold difference (see Fig. 3). Most (64.8%) of the variation in the number of SSTs occurred at the species level with lesser amounts explained by individuals within species (24.4%) and folds within individuals (10.9%; nested ANOVA, $P < 0.00001$ at all levels). The mean length of SSTs also varied greatly among species (see Fig. 1), with an eight-fold difference in size from the Least Flycatcher (71.7 μm) to the Tree Swallow (581.5 μm). Most (60.1%) of the variation in the length of SSTs occurred at the species level with lesser amounts explained by individuals within species (10.3%), folds within individuals (2.6%), and samples within folds (27.0%; nested ANOVA, $P < 0.002$ at all levels). Sperm (Figs. 2A, B) were visible in the tubules of all individuals examined (Figs. 2C–H). In all tubules studied, sperm were oriented with their heads into the blind (distal) end of the SST (Fig. 2C–H).

VARIATION IN LENGTH OF SSTs

An analysis of independent contrasts revealed that SST length was not related to female body mass (b = 0.03, $t = 0.12$, $P = 0.91$, $n = 16$ contrasts, see Table 1 for details). Consequently, we did not control for body mass in subsequent analyses of SST length.

As found in a previous analysis (Briskie and Montgomerie 1992), SST length was strongly and positively correlated with sperm length across

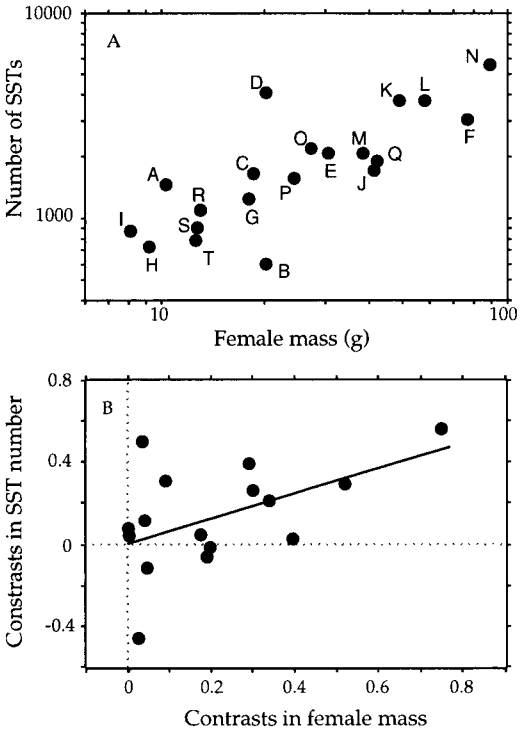

FIGURE 3. (A) Relation between the number of SSTs and female body mass across 20 species of passerine birds (species labelled as in Fig. 1) and (B) relation between independent contrasts for these variables, controlling for phylogeny (see text for statistics).

species (Table 1, Fig. 4). On average, SST length was twice that of sperm length so that each SST could hold about two layers of sperm when lined up head to tail. However, it is instructive to look at the exceptions to this pattern. In Figure 1 we have redrawn the relationship from Briskie and Montgomerie (1992) but with various SST length : sperm length ratios indicated. The pattern that emerges is that females in some species cannot accommodate much more than a single layer of sperm in their SSTs (e.g., Least Flycatcher, Red-eyed Vireo) while three or more layers of sperm are possible in others (e.g., American Robin). Indeed, sperm does form layers within single SSTs (Figs. 2C–F) when tubules are long enough to accommodate them, otherwise only a single layer may form (Figs. 2G, H).

Next, we looked for a correlation between SST length and mating system because sperm competition is thought to differ among avian mating systems (Birkhead 1988; Birkhead and Møller 1992a), being more intense in polygamous than monogamous species. We classified mating systems based on field observations but we also used testis size as an independent measure of the genetic mating system. However, testis length (controlled for male body mass) was not related to SST length (Table 1). We did not have enough independent evolutionary events to compare SST

TABLE 1. Relation between SST length and various traits related to sperm storage and fertilization. Results presented here are from multiple regression analyses (forced through the origin) of independent contrasts used to control for phylogeny (Harvey and Pagel 1991). In each case, SST length was regressed against the test variable with either male mass (for testis size and sperm length) or female mass (for oviduct length) held constant. Sample sizes (n) are the number of independent contrasts from an analysis of 20 species.

Test variable	Partial b	t	P	n
Sperm length (μm)	1.21	8.80	0.001	16
Testis length (mm)	0.48	0.68	0.91	16
Oviduct length (mm)	−0.20	0.24	0.82	16

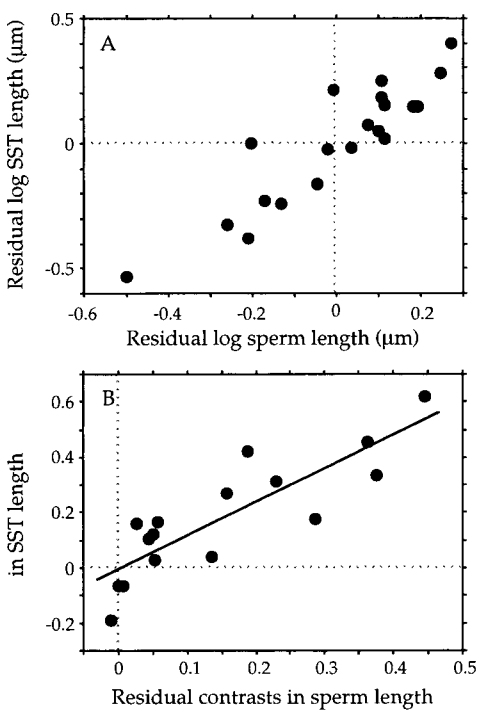

FIGURE 4. (A) Relation between residual log SST length and residual log sperm length (residuals for both from regressions on female body mass) across 20 species of passerine birds and (B) relation between independent contrasts for these variables, controlling for phylogeny (see Table 1 for statistics).

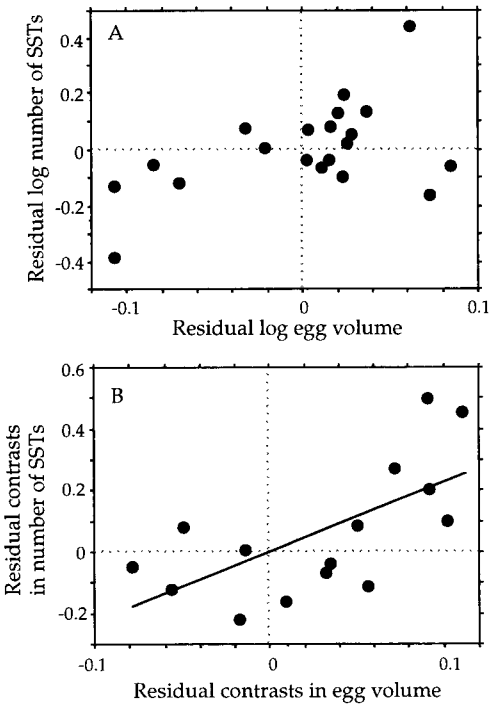

FIGURE 5. (A) Relation between the residual log number of SSTs (i.e., from regression of log number of SSTs on log female body mass) and residual log egg volume (from regression of log egg volume on female body mass) and (B) relation between independent contrasts for these same variables, controlling for phylogeny (see Table 2 for statistics).

length among mating systems but mean SST length in polygamous species (306.6 μm, $n = 7$) was about 30% longer than mean SST length in monogamous species (238.5 μm, $n = 13$). This suggests that SST length is greater in species with greater polygamy although further study is needed to determine whether this relationship holds up when the effects of phylogeny can be controlled statistically.

Oviduct length (controlled for female body mass) also was not related to SST length (Table 1). A relation between these variables might be expected if SST size were related to oviduct size through simple allometry.

VARIATION IN NUMBER OF SSTs

The number of SSTs per female was strongly and positively related to body mass across species (b = 0.65, $t = 13.7$, $P = 0.002$, $n = 16$ contrasts; Fig. 3). Thus, in all subsequent analyses, we controlled female body mass statistically when testing for correlations between the number of SSTs and other variables.

TABLE 2. Relation between number of SSTs and various traits related to sperm storage and fertilization. Results presented here are from multiple regression analyses (forced through the origin) of independent contrasts used to control for phylogeny (Harvey and Pagel 1991). In each case, the contrasts in numbers of SSTs were regressed against the test variable with female body mass controlled. Sample sizes (n) are the number of independent contrasts from an analysis of 20 species.

Test variable	Partial b	t	P	n
Egg volume (mm^3)	2.27	3.44	0.004	15
Oviduct length (mm)	0.99	1.69	0.11	16
Clutch size	0.03	0.28	0.78	15
SST length (μm)	−0.59	4.61	0.0004	16
SST capacity index[a]	−0.56	1.43	0.18	16
Testis length (mm)	0.08	0.15	0.88	16

[a] SST length/sperm length.

Interspecific variation in the number of SSTs might be related to egg size if relatively larger eggs require more sperm to ensure fertilization. For example, Wishart (1987) found that fertility in chickens decreased rapidly once the density of sperm trapped on the egg membrane dropped below a certain threshold. If larger eggs dilute the number of sperm per unit area of egg membrane, more SSTs may be required to insure that enough sperm are available to maintain this threshold density and hence maximal fertility. To test this hypothesis we compared the number of SSTs per female to egg volume (calculated from measures given in Harrison [1978]). Ideally, the number of SSTs should be compared to ovum size at fertilization but data on ovum size are unavailable for the species we studied. Egg size, however, is directly correlated with ovum size across species (Ar and Yom-Tov 1978, Sotherland and Rahn 1987). Among the species we studied, for example, egg volume was highly correlated with yolk volume ($r = 0.98$, $P < 0.0001$, $n = 13$). As predicted, the relative number of SSTs was positively related to relative egg size (both variables controlled for female body mass) across species (Fig. 5, Table 2).

Oviduct length could also influence the number of SSTs through a dilution effect. Thus, species with relatively large oviducts may require larger sperm stores (and hence more sperm) to ensure that adequate numbers of sperm reach

the ova. However, the number of SSTs was not related to oviduct length across species (Table 2).

Next, we examined the relationship between number of SSTs and clutch size reasoning that species with larger clutches might be expected to store larger quantities of sperm since they have more eggs to fertilize. The relation between the number of SSTs and clutch size, however, was not significant (Table 2).

The number of SSTs could also be affected by SST length, either positively, due to positive allometry, or negatively, as a result of an energetic trade-off. In fact, the number of SSTs was strongly and negatively correlated with SST length across species (Table 2, Fig. 6). Thus, species with relatively many SSTs had small SSTs while species with relatively few SSTs had SSTs that were relatively large.

If species capable of storing sperm in several layers can store a greater absolute quantity of sperm per SST than species which store sperm in single layers only, then fewer SSTs in total may be needed to insure an adequate sperm storage capacity. To determine if storage capacity is related to the number of SSTs, we calculated a layering index for each species by dividing SST length by sperm length. Although sperm may overlap extensively within a single SST (Figs. 2E, F), SSTs holding several layers of sperm are probably capable of storing a greater absolute number of sperm per SST than single layered SSTs. However, the number of SSTs was not significantly related to our sperm capacity index (Table 2). Thus, the capacity of single SSTs to store sperm is not related to their number and females did not compensate for fewer SSTs by making larger ones that could each potentially accommodate more sperm.

Finally, we looked for a relation between the number of SSTs and mating system. Testis length (as an index of mating system) was not significantly related to the number of SSTs across species (Table 2). As we had only seven species in our sample that were not classified as monogamous, our sample of independent events was too small to permit statistical analysis of the number of SSTs against mating system. However, it is worth noting that the average residual number of SSTs (i.e., SST number controlling for female body mass) in polygamous species (-187.1, $n = 7$) was less than that found in monogamous species (101.1, $n = 13$). These values suggest that a reduction in the number of SSTs is correlated with increasing sperm competition.

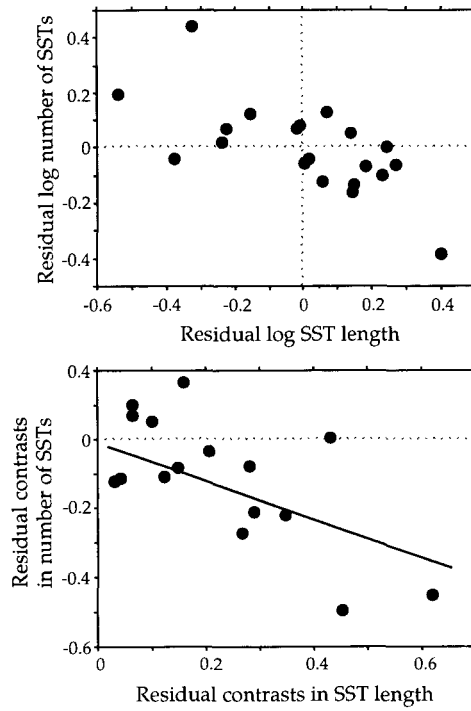

FIGURE 6. (Top) Relation between the residual log number of SSTs and residual log SST length (both variables regressed on female body mass) across 20 species of passerine birds and (Bottom) relation between independent contrasts for these same variables, controlling for phylogeny (see Table 2 for statistics).

DISCUSSION

This study provides some of the first estimates for the number and length of SSTs in free-living birds, relating differences between species to variation in behavioral and other morphological characters. We found that the number of SSTs varied considerably across a sample of 20 species of passerines but that most of this variation was attributable to interspecific differences in female body mass. Birkhead and Møller (1992b) found similar results within a smaller sample of domesticated species. These findings are not too surprising since many morphological and physiological attributes of organisms are related to body size (Peters 1983). For example, larger birds may require a greater number of SSTs to ensure successful fertilization simply as compensation for the higher likelihood of sperm "getting lost" or becoming too diluted in a larger body.

In a similar manner, selection for increased egg size (for whatever reason) also may have favored the production of more SSTs to ensure that fertility does not decline from the dilution of an otherwise limited number of sperm spread over an increased area of ovum membrane. Indeed, we found that species with larger eggs had a greater number of SSTs even when body mass was controlled. However, arguing against this dilution hypothesis was the lack of a similar relationship between oviduct length (corrected for body mass) and the number of SSTs. Nonetheless, because sperm are transported from the SSTs to the infundibulum largely via the contractions of the uterus (Allen and Grigg 1957), it is possible that few sperm are lost or diluted by increased oviduct size relative to those losses that occur when sperm transport is dependent upon sperm motility (e.g., when swimming up the vagina to the SSTs or penetrating the ovum membrane; Allen and Grigg 1957).

An unexpected result of our analysis was the inverse correlation between the number and length of SSTs, even when body mass was held constant statistically. Initially, this suggests a trade-off between the number of SSTs and the capacity of each SST to store sperm, but there was no relationship between the number of SSTs and the amount of sperm each could store (as measured by our sperm capacity index). Alternatively, the number of SSTs might decrease with increased SST length if it were energetically costly to maintain large numbers of large SSTs. Although it is not known how much females invest energetically in maintaining sperm, such costs are probably minimal because the number of sperm stored per female is equivalent to only 5% or less of a single ejaculate. Thus, relatively little sperm is stored despite the fact that most birds copulate repeatedly for each clutch of eggs (Birkhead 1992).

In a recent study of interspecific variation in sperm size, we found a strong inverse correlation between the number of SSTs and sperm length (Briskie and Montgomerie 1992). This relationship is not surprising since sperm length is strongly correlated with SST length (see above). However, it does suggest a possible adaptive explanation for the relationship between the number of SSTs and SST length that we found in this study. In mammals, it is known that longer-tailed sperm can swim faster (Gomendio and Roldan 1991) and should therefore be favored whenever there is intense competition to reach either the ova (Gomendio and Roldan 1991) or the sites of sperm storage (Briskie and Montgomerie 1992). In birds, most of the variation among species in sperm length is due to differences in the length of the sperm's tail (Briskie and Montgomerie 1992); thus, females could conceivably induce greater sperm competition by reducing the number of sperm storage sites available, with the result that selection would favor longer-tailed sperm that could swim faster and would reach the SSTs more quickly than short-tailed sperm. Since sperm that are not held completely within an SST are liable to be swept out by eggs passing down the oviduct (Lorenz 1966), females, in turn, will be favored to match the length of their SSTs to that of the male's sperm. Thus, we suggest that sperm competition for limited storage sites will result in an inverse relationship between the number of SSTs and SST length via selection for greater sperm tail-length.

Although the inverse relation between the length and number of SSTs can be interpreted as a product of sperm competition, the number of SSTs was not related to differences in sperm competition as measured by relative testis length as an index of mating system. In species with more intense sperm competition, copulations are more frequent and males generally produce more sperm and their ejaculates have a higher concentration of sperm than species with relatively low sperm competition (Møller 1988, 1991b). Whether a greater number of SSTs is expected as a consequence is not clear, since females may also induce sperm competition by reducing the number of available sperm storage sites. The absence of a relationship between the number of SSTs and testis length is also surprising since Birkhead and Møller (1992b) found a positive correlation across 11 species between the number of SSTs and the number of sperm per ejaculate, suggesting that females produce more SSTs when males produce more sperm.

SPERM STORAGE AND SPERM PRECEDENCE

In contrast to variation in the number of SSTs, differences in SST length across species were not correlated with body mass. Instead, SST length was positively correlated with sperm length. Although the potential for sperm layering varied from species to species, a strong relationship between sperm length and SST length was obtained

because females in most species had SSTs that could hold an average of two layers of sperm. In our sample of 20 species, SSTs could hold about two layers of sperm in 15 species, more than three layers of sperm in two species and only a single layer in three species (Fig. 1). Why should the pattern of layering differ from species to species?

One possibility is that sperm long enough to fill an SST might prevent access to SSTs by the sperm of other males. In the featherwing beetles (*Bambaria* spp.), sperm are particularly large and the first male to mate fills the female's spermatheca to capacity, thereby excluding any potential competitors for access to her single large egg (Dybas and Dybas 1981). If a similar process operates in birds, sperm stored by females with SSTs too short to allow sperm layering may result in a paternity pattern biased in favor of first-mating males. However, such a simple mechanism seems unlikely since many SSTs remain empty or capable of storing additional sperm even after several inseminations (Verma and Cherms 1965, Birkhead et al. 1990).

On the other hand, sperm layered within an SST suggests a proximate mechanism of last-male sperm precedence in birds. Because SSTs have only a single opening, it has been hypothesized that the last sperm to enter an SST blocks the exit for sperm already inside (Compton et al. 1978). During ovulation, such last-stored sperm will be the first to exit into the oviductal lumen and make their way to the unfertilized ovum. It is this advantage that presumably favors the last male in subsequent fertilizations (Compton et al. 1978). However, sperm stratification and last male precedence can occur only if SSTs are long enough to accommodate two or more sperm lengths. When sperm are stored in a single layer within short SSTs, all sperm have the opportunity to exit at roughly the same time and the probability of paternity should be determined by the proportion of each male's sperm present, all else being equal. In reality, SST length in most species falls between one and a few sperm lengths and sperm exhibit varying degrees of stratification and mixing within SSTs. Nevertheless, differences between species in the proportion of short and long SSTs suggest that sperm precedence patterns may also vary from species to species.

Differences in the proximate mechanisms of sperm precedence between species could have considerable influence on the evolution of male behavior. When most sperm are stored in stratified layers, as in species with many long SSTs (e.g., American Robin), the last male to copulate before fertilization will be most likely to fertilize the next egg laid. In Zebra Finches, SSTs are about three times as long as sperm (Birkhead 1987) and last male sperm precedence in this species averages about 80% (Birkhead et al. 1988). In this and other species with sperm stratification, males could minimize cuckoldry by insuring they are the last to copulate before each ovulation.

In contrast, the last male to copulate in species with a high proportion of short tubules, and hence no layering (e.g., Red-eyed Vireo), may achieve paternity only in proportion to the number of its sperm present relative to total number of sperm present in all SSTs. Thus it may be necessary for extra-pair males in such species to copulate repeatedly in order to outnumber or displace sperm from previous males. As a result, it is possible that extra-pair copulations do not pose as great a risk to a pair male's paternity in species with no sperm layering. Unfortunately, there are currently no data available on levels of sperm precedence in species with small SSTs to test this hypothesis.

Recently, Lessells and Birkhead (1990) examined several alternative models of sperm competition to determine whether sperm stratification or sperm displacement best explained last-male sperm precedence in domestic chickens. Because last-male precedence did not decline over time, as would be expected if successive layers of sperm were uncovered as sperm was depleted in the SSTs, they concluded that sperm displacement was the most likely mechanism of sperm competition in this species. Our observations of SST anatomy and sperm layering in a variety of wild passerines suggest that sperm stratification may in fact be an important mechanism of sperm precedence. Our results also suggest that the mechanisms of sperm competition thought to occur in chickens may not necessarily apply to other species, particularly if the patterns of sperm storage (i.e., in single vs. multi-layers) differ.

AN ARMS RACE?

In correlational studies it is often difficult to separate cause from effect. Has SST length increased in response to changes in sperm length or has sperm length evolved to accommodate changes

in SST length? From the female's perspective, changing SST length relative to sperm length could give her control over sperm precedence. On the one hand, by having long SSTs, females could control which male fertilizes each egg by making sure he is the last to copulate before ovulation. This strategy might be important if she wishes either to devalue a forced extra-pair copulation or to be fertilized by a male other than her own mate. Alternatively, if greater sperm mixing occurs in short SSTs, this process could induce greater male–male competition and it might be a strategy to insure that a male with the most viable and fastest sperm or highest sperm production fertilizes most of her clutch (Knowlton and Greenwell 1984).

From the male's perspective, sperm stratification resulting in last male precedence allows males to devalue extra-pair inseminations by simply copulating again with his mate before ovulation. Alternatively when sperm are stored in single layers, extra-pair copulations may be swamped by resident male sperm. When males and females are under different selection pressures with respect to extra-pair copulations, the net result may be an "arms race" (Dawkins and Krebs 1979) between sperm length and SST length for control over sperm precedence patterns. Because the ratio between sperm length and SST length varies from species to species, independent of body mass and phylogeny, we suggest that the evolution of both of these structures may be the product of escalating adaptations and counter-adaptations between the sexes to control the proximate mechanism of sperm precedence.

FURTHER STUDY

Our observations on the number and length of SSTs and the arrangement of sperm within SSTs suggest several avenues for further research on aspects of avian reproductive biology not previously considered by behavioral ecologists. For example, we need to know more about the number and length of SSTs in a greater range of species and mating systems before any general conclusions can be drawn. Some taxa, such as the shorebirds (Scolopacidae) or grouse (Phasianidae) might be particularly rewarding for comparative analyses of sperm storage as they comprise diverse mating systems within closely related genera.

More experiments on sperm precedence are also needed to determine if species with relatively shorter tubules are less likely to exhibit a last male mating advantage. Such an experiment might also test the effectiveness of "retaliatory copulations" (Birkhead and Møller 1992a) across a range of species with different SST sizes relative to sperm length. If differences are found, it may be possible to relate these patterns to variation in the mechanisms of sperm storage and vice versa. Perhaps by labelling sperm (e.g., with radioactive tracers) from specific males, it might be possible to determine the degree of mixing and stratification of sperm from both pair and extra-pair copulations within SSTs. Such techniques would then be useful for determining whether sperm displacement is more or less likely in species with different patterns of layering within SSTs.

Finally, we need to determine how stored sperm are used in the fertilization process. For example, some sperm probably never enter the SSTs, but instead travel directly to the infundibulum (Howarth 1974). If inseminations are timed to coincide with ovulation (Birkhead 1988), these non-stored sperm could potentially fertilize eggs and thereby circumvent any selection imposed by SST morphology. Indeed, it is possible that sperm stored in SSTs may seldom be used to fertilize eggs in species that copulate throughout egg-laying (e.g., Smith's Longspur; Briskie 1992), although we could not discern any morphological differences in SSTs among the species in our sample that would suggest such a reduced function.

Although our study gives only a preliminary view of the patterns of sperm storage and precedence across species, it is clear that the proximate mechanisms associated with sperm competition vary widely from species to species and that these differences may play an important role in shaping the evolution of mating behavior. Clearly, to understand how the tremendous variability in avian mating patterns has evolved, it will be necessary to determine how both females and males manipulate the process of sperm competition to their own advantage.

ACKNOWLEDGMENTS

We thank Gilles Seutin, Sarah Kalff, Scott Petrie, Jan Lifjeld, Wally Rendell and Sari Saunders for help collecting oviducts. The Churchill Northern Studies Centre, the University of Manitoba Field Station at Delta Marsh and the Queen's University Biological Station provided logistic sup-

port and accommodation during the course of this study. Funding was provided by Indian and Northern Affairs Canada (Northern Scientific Training Grant), a Natural Sciences and Engineering Research Council (NSERC) Scholarship to J.V.B. and grants from NSERC and the Queen's University Advisory Research Committee to R.M.D. Promislow provided sage advice on interpreting the comparative analyses and T. R. Birkhead and two anonymous reviewers made many useful comments on the manuscript.

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