ANATOMICAL ADAPTATIONS TO SPERM COMPETITION IN SMITH'S LONGSPURS AND OTHER POLYGYNANDROUS PASSERINES

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ABSTRACT.—I compared the reproductive anatomy of the polygynandrous Smith's Longspur (*Calcarius pictus*) with two other polygynandrous passerines (Dunnock [*Prunella modularis*] and Alpine Accentor [*P. collaris*]) and with a wide range of socially monogamous species. All three polygynandrous species were found to have enlarged testes and cloacal protuberances (i.e. the site of sperm storage) compared to species with other mating systems. Testes lengths in polygynandrous species averaged 44% longer and cloacal protuberances 213% greater in volume than expected for the body sizes of these species. Testes mass in Smith's Longspurs comprised 4.2% of adult body mass or more than twice (2.0%) that found in the congeneric and monogamous Lapland Longspur (*C. lapponicus*). Smith's Longspurs also had larger cloacal protuberances, larger seminal glomera and higher sperm stores, suggesting a greater overall rate of sperm production than in Lapland Longspurs. In contrast, females of polygynandrous species did not show any consistent differences in the pattern of sperm storage due to increased sperm production by males, although this needs to be evaluated more thoroughly. The enlarged male reproductive organs of Smith's Longspurs and other polygynandrous species appear to have evolved as a consequence of sperm competition, whereby large sperm reserves function to insure paternity through diluting or displacing the ejaculates of rival males. *Received 26 December 1991, accepted 25 November 1992.*

SPERM COMPETITION results whenever females mate with more than one male during the span of a single breeding attempt (Parker 1970). Although multimale mating may be advantageous to females in some situations (e.g. Smith 1988, Møller 1988a), it can also reduce a partner's paternity (e.g. Gibbs et al. 1990). To counter female infidelity and increase the probability of paternity, males have evolved several elaborate counter measures. For example, males in some species continually guard and defend their mates from the advances of other males during the period when eggs are fertilizable (Beecher and Beecher 1979, Birkhead 1979). If extrapair copulations occur, then pair males may reduce their subsequent parental investment to avoid wasting energy or resources on raising another male's offspring (Trivers 1972, Burke et al. 1989). Sometimes female infidelity cannot be prevented because of ecological constraints, but males may nonetheless increase paternity confidence through frequent copulations that dilute or displace rival ejaculates (McKinney et al. 1984, Birkhead et al. 1987). Indeed, inter-

specific comparisons of primates (Harcourt et al. 1981, Harvey and Harcourt 1984), birds (Cartar 1985, Møller 1991) and mammals (Kenagy and Trombulak 1986, Brownell and Ralls 1986) have shown that nonmonogamous animals have relatively larger testes than monogamous species. Since larger testes produce more and larger ejaculates (Møller 1988b), an increase in testes size has been interpreted as an adaptation to sperm competition (Harcourt et al. 1981, Møller 1988b, 1991).

Smith's Longspurs (*Calcarius pictus*) are unusual among passerine birds in that females regularly pair and copulate with more than one male for a single clutch of eggs at the same time that males pair and copulate with several females (Briskie 1992). In a color-banded population near Churchill, Manitoba, 76.2% of 21 females observed mated with two males, 9.5% with three males, and only 14.3% with a single male (Briskie 1992, 1993). Of 27 males observed in the same population, 63.0% mated with two females, 7.4% with three females, and 29.6% with a single female. This combination of polyandry and polygyny is termed polygynandry and has been reported in only two other passerines to date (Dunnock, *Prunella modularis* [Davies 1985]

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and Alpine Accentor, *P. collaris* [Nakamura 1990]). Males in all three species assist females in feeding offspring; in Smith's Longspurs as many as three males may provide food at a single nest (Briskie 1993). Because females copulate with several males during the period when eggs could be fertilized, males risk investing energy in offspring fathered by another male. Possibly to minimize this cost, copulations in these species are extraordinarily frequent and, in the Smith's Longspur, average more than 350 per clutch (Briskie 1992). Observations of such extreme behavior, along with recent findings of high levels of mixed paternity in the Dunnock (Burke et al. 1989) and Smith's Longspur (75% of 12 broods with mixed paternity; R. Montgomerie, J. V. Briskie and T. Poldmaa unpubl. data), strongly suggest that intense sperm competition is common in all polygynandrous species.

In an earlier paper, I described how sperm competition has influenced the mating behavior of Smith's Longspurs (Briskie 1992). Here, I examine the reproductive anatomy of this species to determine what adaptations have arisen to cope with the high levels of sperm competition experienced by males. Recently, Nakamura (1990) and Birkhead et al. (1991) examined Alpine Accentors and Dunnocks, respectively, for the purpose of determining how sperm competition has affected male and female reproductive anatomy in these species. Males in both species were found to have very large testes and cloacal protuberances (i.e. the site of sperm storage) but in neither study was enough information available to make detailed comparisons with socially monogamous species. I collected data on cloacal-protuberance size, testis size, and sperm-storage patterns for a wide variety of North American passerines. Using the data from Alpine Accentors and Dunnocks, I then examine how sperm competition has influenced the reproductive anatomies in all three polygynandrous species.

METHODS

The reproductive system of male birds comprises the two testes and their connections to the cloaca via the ductus deferens (see review in Lake 1981). The distal end of each ductus deferens is known as the seminal glomerus and, during the breeding season, it becomes expanded and highly coiled, pushing out of the body cavity, and forming an externally visible cloacal protuberance around the vent (Wolfson 1952,

1954, Salt 1954). Sperm produced in the testes mature in the glomera and are stored there until ejaculated. Both sperm production and sperm storage capacity are expected to increase as an adaptation to sperm competition (e.g. Smith 1984); thus, males in species exposed to high levels of sperm competition should have such organs enlarged relative to those found in monogamous species.

Cloacal protuberances were measured on 29 passerine species breeding in Ontario and Manitoba, Canada from 1988 to 1991 (see Table 1 for a list of species). Apart from the Smith's Longspur, this sample included 2 socially polygynous species (Yellow-headed Blackbird [*Xanthocephalus xanthocephalus*], Brown-headed Cowbird [*Molothrus ater*]), 2 sequentially polyandrous species (American Goldfinch [*Carduelis tristis*], Common Redpoll [*C. flammea*]) and 24 socially monogamous species (all remaining species listed in Table 1; mating-system data from Ehrlich et al. 1988, Briskie and Montgomerie 1992, and references therein). Using calipers, I measured (to 0.1 mm) the diameter (along left-right axis) and height (on one side) of the cloacal protuberance on each male captured or collected from one week before to one week after the peak clutch-initiation period of that species. Clutch-initiation dates were determined by direct observations and most males were captured or collected at known nest sites or on territories with nesting females. Because cloacal-protuberance size varies over the breeding cycle, for all interspecific comparisons I used only data from those birds measured during the peak period of clutch initiation; this is the period when cloacal protuberance size is at its maximum (Quay 1986, Weatherhead and Robertson 1980, unpubl. data). Using these values, I estimated cloacal-protuberance volume as $h\pi r^2$, where h is the cloacal-protuberance height and r is 0.5 times the protuberance diameter. Sample sizes per species ranged from 1 to 37 (Table 1).

To examine the structure of the seminal glomera and cloaca in greater detail, I collected five male Smith's Longspurs: three during their copulation period, one after the copulation period, and one postbreeding bird. Within an hour of death, the cloacal protuberance was removed and preserved in 10% buffered formalin. Each seminal glomerus was later dissected, measured and weighed. To estimate the number of sperm stored by each male, I finely minced each glomerus and washed the sperm into a known volume of phosphate buffered saline. Five samples were then withdrawn from this solution and the number of sperm counted under a microscope using an Improved Neubauer counting chamber. Estimates for both glomera were summed to obtain an estimate of total number of sperm stored. For comparative purposes, I also estimated the number of sperm in the seminal glomera of one Lapland Longspur (*C. lapponicus*), one American Tree Sparrow (*Spizella arborea*), and three Yellow-headed Blackbirds. All birds were

TABLE 1. Summary of data on cloacal-protuberance dimensions (±SE), testis lengths and sample sizes for species used in study. All data from my study except for the Alpine Accentor (Nakamura 1990) and Dunnock (Birkhead et al. 1991, A. P. Møller pers. comm.).

| Species | Cloacal protuberance | | | Testis length (mm) |
	Diameter (mm)	Height (mm)	n	
1 Least Flycatcher (*Empidonax minimus*)	4.25 ± 0.19	3.34 ± 0.17	8	5.70
2 Red-eyed Vireo (*Vireo olivaceus*)	4.93 ± 0.33	4.15 ± 0.29	4	8.50
3 Warbling Vireo (*V. gilvus*)	5.25 ± 0.25	3.25 ± 0.75	2	6.79
4 American Robin (*Turdus migratorius*)	12.21 ± 0.41	10.51 ± 0.40	7	14.50
5 Veery (*Catharus fuscescens*)	7.00 ± 1.00	5.75 ± 0.75	2	9.50
6 Gray Catbird (*Dumetella carolinensis*)	7.47 ± 0.28	4.09 ± 0.26	11	10.80
7 Black-capped Chickadee (*Parus atricapillus*)	4.40	4.30	1	6.88
8 Barn Swallow (*Hirundo rustica*)	5.57 ± 0.30	3.79 ± 0.24	7	6.90
9 Tree Swallow (*Tachycineta bicolor*)	7.50 ± 0.46	6.28 ± 0.42	4	9.30
10 Horned Lark (*Eremophila alpestris*)	6.80	6.00	1	8.80
11 American Pipit (*Anthus rubescens*)	6.50	6.00	1	9.00
12 Dunnock (*Prunella modularis*)	8.50 ± 0.39	6.50 ± 0.30	6	10.90
13 Alpine Accentor (*P. collaris*)	14.18 ± 0.31	12.78 ± 0.49	13	19.90
14 American Goldfinch (*Carduelis tristis*)	6.00 ± 0.50	5.35 ± 0.35	2	7.00
15 Common Redpoll (*C. flammea*)	7.00 ± 0.58	5.40 ± 0.31	3	6.80
16 Savannah Sparrow (*Passerculus sandwichensis*)	7.65 ± 0.44	6.58 ± 0.35	6	9.61
17 Song Sparrow (*Melospiza melodia*)	7.00	6.30	1	8.62
18 American Tree Sparrow (*Spizella arborea*)	8.80 ± 0.74	7.94 ± 0.60	5	11.06
19 Chipping Sparrow (*S. passerina*)	6.48 ± 0.43	6.00 ± 0.32	4	8.33
20 Clay-colored Sparrow (*S. pallida*)	6.17 ± 0.22	5.78 ± 0.11	6	7.23
21 White-crowned Sparrow (*Zonotrichia leucophrys*)	8.41 ± 0.23	7.14 ± 0.20	10	10.35
22 Smith's Longspur (*Calcarius pictus*)	11.49 ± 0.18	9.89 ± 0.22	15	14.50
23 Lapland Longspur (*C. lapponicus*)	6.70	5.70	1	10.10
24 Tennessee Warbler (*Vermivora peregrina*)	5.40	5.20	1	5.92
25 Blackpoll Warbler (*Dendroica striata*)	6.85 ± 0.05	6.60 ± 0.40	2	7.03
26 Yellow Warbler (*D. petechia*)	5.72 ± 0.14	4.64 ± 0.16	37	5.30
27 Common Yellowthroat (*Geothlypis trichas*)	4.00 ± 0.35	2.90 ± 0.19	5	7.58
28 American Redstart (*Setophaga ruticilla*)	5.20 ± 0.31	5.77 ± 0.27	3	6.70
29 Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*)	11.27 ± 0.43	8.57 ± 0.20	3	13.30
30 Northern Oriole (*Icterus galbula*)	8.62 ± 0.66	6.72 ± 0.52	6	12.22
31 Brown-headed Cowbird (*Molothrus ater*)	6.08 ± 0.37	3.94 ± 0.29	8	7.00

collected (under permit) during the copulation period and had well-developed cloacal protuberances and large testes. These values were then compared to seminal glomera sizes and/or sperm number estimates obtained from: Birkhead et al. (1991) for the Dunnock, Zebra Finch (*Taeniopygia guttata*) and Bengalese Finch (*Lonchura striata*); Wolfson (1954) for the House Sparrow (*Passer domesticus*), American Robin (*Turdus migratorius*) and Dark-eyed Junco (*Junco hymelis*); and T. R. Birkhead (pers. comm.) for the Canary (*Serinus canaria*).

In passerines, the extreme distal end of each ductus deferens forms a conical-shaped papilla that protrudes in the lumen of the cloaca. These structures are thought to function, in conjunction with the proctodeal chamber of the cloaca, as a phallus during sperm transfer (Salt 1954). After the seminal glomera were removed, I cut open the cloaca of the four species I collected and measured the width and height of the papilla of the ductus deferens. The phallus in primates (Dixson 1987) and whales (Brownell and Ralls

1986) is enlarged and elaborated in species subject to sperm competition. Thus, I looked for any similar modifications that may have occurred in the papilla of Smith's Longspurs relative to other passerines.

Data on testis size were obtained from museum skins housed in the Royal Ontario Museum (Toronto), the National Museum of Canada (Ottawa), the Manitoba Museum of Man and Nature (Winnipeg), and from personal observations of fresh material. To estimate testis length at peak breeding for each species, I fitted a second-order polynomial regression between testis length and date, and used the maxima of these regressions as the average maximum testis length during breeding. At least 25 measures of testis length were available for each species included in this study. Testis size in the Alpine Accentor was obtained from Nakamura (1990). Birkhead et al. (1991) did not provide measurements of testis length in the Dunnock, so I obtained these data from Møller (1991, pers. comm.). I also weighed the testes from those birds collected for estimating sperm number and sem-
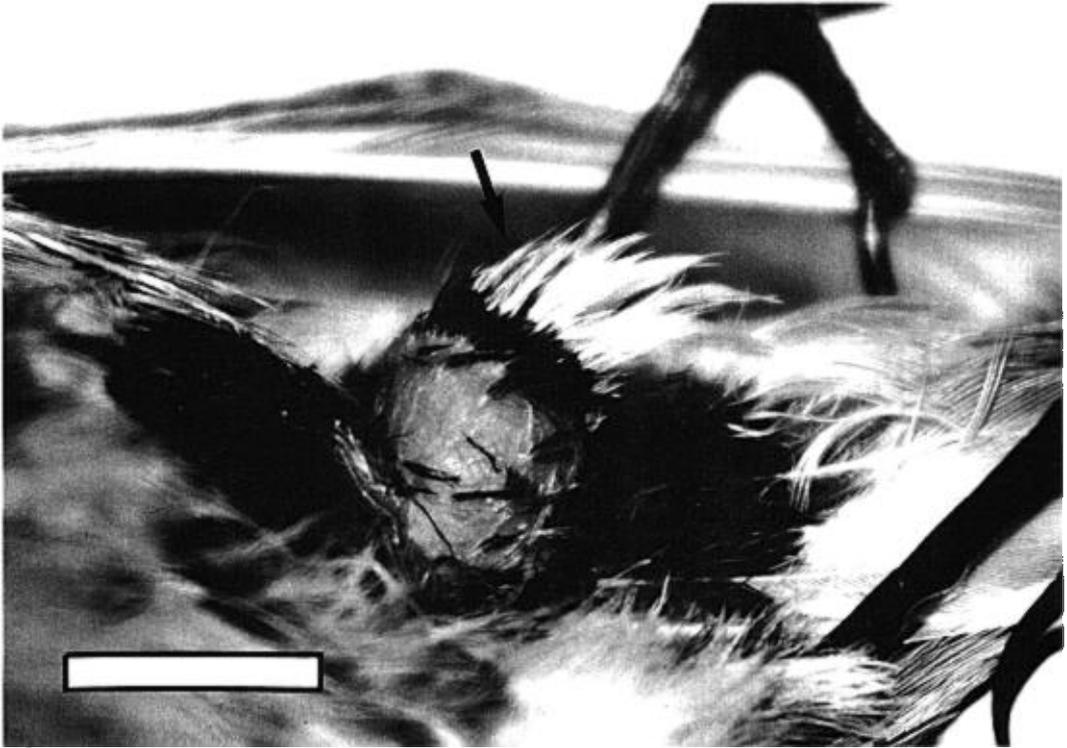
Fig. 1. Lateral view of cloacal protuberance of a male Smith's Longspur. Except for ring of feathers surrounding vent (arrow), protuberance is mostly bare. Abdominal feathers in this individual have been removed to expose base of protuberance; anterior of bird is to left in this view. Scale bar = 10 mm.

inal glomera size. Body masses for North American species were obtained from Dunning (1984).

Females of polygynandrous species also might exhibit certain anatomical features as a consequence of increased sperm competition in males. For example, when males produce more sperm, females may increase the capacity for sperm storage. Sperm storage in female birds occurs in specialized sperm-storage tubules (SSTs) located at the junction of the uterus and vagina (e.g. Howarth 1974, Shugart 1988). To determine if the patterns of sperm storage in female Smith's Longspurs differ from those of other birds, I compared the number and size of SSTs across 20 species of North American passerines. The methods used for estimating SST number and size have been described in detail elsewhere (Birkhead and Hunter 1990, Briskie 1990, Briskie and Montgomerie 1992, Briskie and Birkhead 1993). Some of these data were presented previously with respect to the evolution of sperm size (Briskie and Montgomerie 1992), but are repeated here for comparative purposes along with data on sperm-storage patterns in the Dunnock from the literature (Birkhead et al. 1991).

In all comparative analyses, I used the methods described by Harvey and Pagel (1991) to control for phylogenetic effects. Such methods are required because some traits may be similar in closely related species simply due to common ancestry rather than independent evolution. Failure to control for this artificially weights sample sizes and the influence of some species on the analysis. First, I used Sibley and Ahlquist (1990) to construct a phylogeny for the species studied. Then, using the independent contrast method (Harvey and Pagel 1991), I calculated unique linear contrasts within each taxon with two or more data points. This comparative method calculates comparisons or "contrasts" among daughter taxa for each node in a phylogeny for which there is variation in the test variable. In essence, a phylogeny is first searched for independent evolutionary events of the character in question (e.g. large testes size). These events are then compared with independent evolutionary events in a second or third character (e.g. body mass, mating system). To test for overall comparative relations across taxa, I determined whether linear contrasts for one variable were correlated with those of another. I used Pearson's product-moment coefficient to test for linear relations between two variables and forced the regression through the origin as recommended by Harvey and Pagel (1991). A significant

correlation between two variables suggests that the evolution of one character is associated with that of the second. Sample sizes in these sorts of comparative analyses, thus, are the number of independent contrasts and not the number of species originally used in the analysis. For clarity, I present correlations using both raw data and the contrasts. All variables were log transformed before analyses to help normalize distributions. Standard errors of the mean (SE) were calculated for all results.

RESULTS

Cloacal-protuberance size.—During the breeding season the cloacal protuberance of male Smith's Longspurs appears as a large, barrel-shaped structure surrounding the vent (Fig. 1). It averages 11.49 ± 0.18 mm ($n = 15$) in diameter and 9.89 ± 0.22 mm in height. The protuberance is largely bare except for a ring of 24 to 27 ($\bar{x} = 25.7 \pm 0.88$, $n = 3$) feathers encircling the vent. Cloacal feathers are white with a black base and stand approximately 8.0 mm high posteriorly, but taper to 2.6 mm at the anterior end of the protuberance. Cloacal feathers in Smith's Longspur males did not differ appreciably in number or size from the cloacal feathers examined in one male of the congeneric Lapland Longspur ($n = 30$ feathers, 8.0 mm posteriorly, 2.5 mm anteriorly). Unlike in Alpine Accentors (Nakamura 1990), the ducts of the seminal glomera of Smith's Longspurs were not visible through the skin of the protuberance.

Cloacal-protuberance size in Smith's Longspurs was already large when birds arrived on the breeding grounds in early June (Fig. 2). After copulation behavior ceased in mid-June, cloacal-protuberance volume decreased gradually such that protuberance volume was less than one-half that of copulating birds by the time males were feeding offspring in early July (Fig. 2). Renesting in Smith's Longspurs occurs only rarely (Briskie 1993), but in one renesting male, cloacal-protuberance size during his second copulation period was similar to that found in copulating males during first nesting attempts (Fig. 2).

Protuberance size varied 10-fold across the passerine species that I sampled (Fig. 3A); it was greatest in the Alpine Accentor and smallest in the Common Yellowthroat (*Geothlypis trichas*). Cloacal-protuberance size increased with increasing body mass (Fig. 3A). The three polygynandrous species had protuberances approximately 213% larger than that expected for their
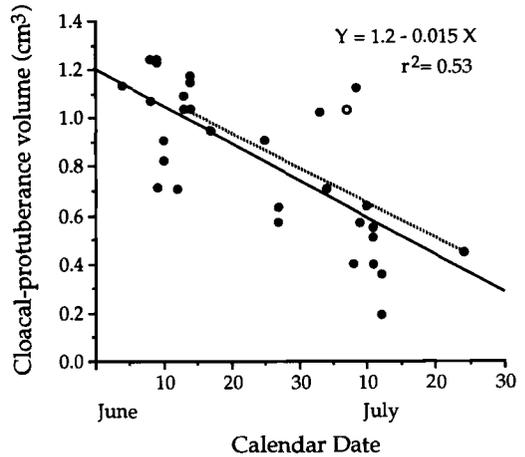

Fig. 2.   Decline in cloacal-protuberance size of male Smith's Longspurs over the breeding season. Males arrive on breeding grounds in early June. Copulation activity begins about 10 days later and ceases by third week in June once all clutches have been laid. Open circle represents cloacal-protuberance size of a single male known to have renested. Sample size is 30 males. Decline in protuberance size in one male measured twice is shown by dotted line.

body size; Smith's Longspur cloacal protuberances alone averaged three times the volume of protuberances in the monogamous Lapland Longspur. Cloacal-protuberance volumes in the two polygynous species (Yellow-headed Blackbird, Brown-headed Cowbird) were 44% less on average than expected for their body size while the two sequentially polyandrous species (Common Redpoll, American Goldfinch) had protuberances about 36% larger than expected (Fig. 3A). Thus, large cloacal-protuberance size was associated with polygynandry (and perhaps with sequential polyandry), but not with any other mating system. Protuberance volume contrasts between polygynandrous species and their closest monogamous relatives or groups of relatives were consistently large and positive but low for body size (Fig. 3B), confirming that large cloacal-protuberance size is associated with polygynandry once phylogenetic effects are removed.

Seminal glomera size and number of sperm.—The left and right seminal glomera from three male Smith's Longspurs collected during the copulation period averaged 0.158 ± 0.017 g and 0.164 ± 0.012 g, respectively (Fig. 4). Combined glomera mass (0.322 g) of copulating-period males was 1.13% of body mass (28.5 g). Glomera mass
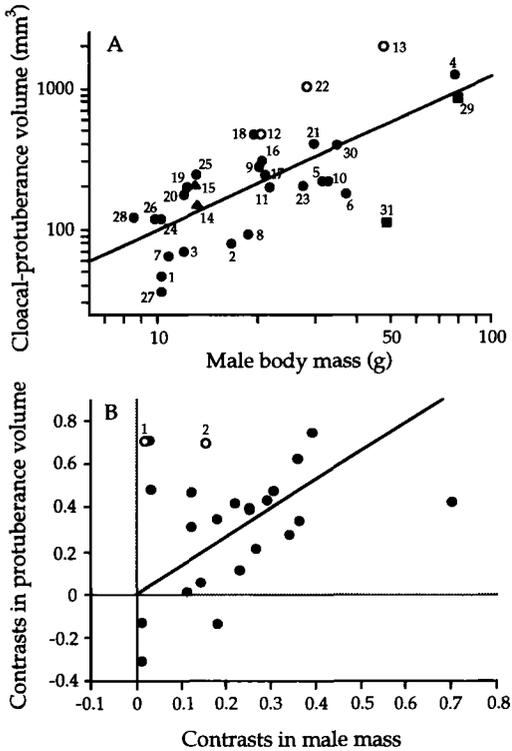
Fig. 3. (A) Relation between male body mass and cloacal-protuberance size for 31 species of passerines. Polygynandrous species represented by open circles, sequentially polyandrous species by closed triangles, polygynous species by closed squares, and monogamous species by closed circles. Numbers refer to species listed in Table 1. (B) Independent contrasts (see Methods) between mass and protuberance size for same group of species. Open circles are contrasts between (1) Smith's and Lapland longspurs, and (2) between the genera *Prunella* (Dunnock and Alpine Accentor) and *Anthus*. Large positive contrasts in protuberance size in these two comparisons suggest that an increase in protuberance size is associated with polygynandry.

from one postcopulation-period male was less than one-half (0.115 g combined) that of copulating males, while the seminal glomera from a single postbreeding male was only 0.030 g or less than 0.10% of male body mass. The dimensions of left and right glomera in copulating males were very similar and averaged 9.59 ± 0.53 mm along the dorsal-ventral axis, 6.93 ± 0.18 mm along the anterior-posterior axis, and 4.34 ± 0.14 mm along the left-right axis.

The average combined seminal glomera mass of Smith's Longspurs (0.322 g) was nearly three

times that of either the Lapland Longspur (0.115 g, $n = 1$) or the American Tree Sparrow (0.102 g, $n = 1$). The average seminal glomera mass of Yellow-headed Blackbirds (0.294 ± 0.014 g, $n = 3$) was similar to that found in Smith's Longspurs even though blackbirds are almost three times the mass of longspurs. When phylogeny is controlled, cloacal-protuberance volume was strongly correlated with seminal glomera mass across species ($Y = 0.93 X$; $r^2 = 0.82$, $P = 0.0003$, $n = 10$ species); thus, species with large cloacal protuberances have proportionately large seminal glomera, as would be expected if the cloacal protuberance forms as an expansion of the glomera.

The combined number of sperm in both glomera of Smith's Longspurs during the copulation period was estimated to be 2.17 ± 0.22 × 10^8 (range 1.76 × 10^8 to 2.50 × 10^8; $n = 3$). This was approximately four times that found in the glomera of the Lapland Longspur (0.53 × 10^8 sperm) or the American Tree Sparrow (0.58 × 10^8 sperm), but only one-half that found in the Yellow-headed Blackbird (4.11 ± 0.24 × 10^8 sperm) and one-fifth that found in Dunnocks (10.6 × 10^8 sperm; Birkhead et al. 1991). However, when controlled for phylogeny, the number of sperm stored in the seminal glomera was strongly correlated with cloacal protuberance height across species ($Y = 4.82 X$; $r^2 = 0.61$, $P = 0.039$, $n = 7$ species), indicating that species with large cloacal protuberances generally have large reserves of sperm.

Cloacal anatomy.—In all four species examined, the papillae of the ductus deferens formed a pair of small conical structures projecting into the lumen of the urodeum of the cloaca (Fig. 5). In Smith's Longspurs collected during the copulation period, each papilla averaged 1.13 ± 0.01 mm at the base and 1.53 ± 0.04 mm in height ($n = 3$). Papillae in the Lapland Longspur (base 1.18 mm, height 1.58 mm, $n = 1$), American Tree Sparrow (base 1.08 mm, height 1.18 mm, $n = 1$), and Yellow-headed Blackbird (base 1.41 ± 0.17 mm, height 1.65 ± 0.15 mm, $n = 3$) were similar in both size and appearance. There was no obvious morphological difference between the papillae of longspurs and the other species examined that would be related to the frequent copulations characteristic of Smith's Longspurs. No information is available on the size of the cloacal papillae in either the Dunnock or Alpine Accentor.

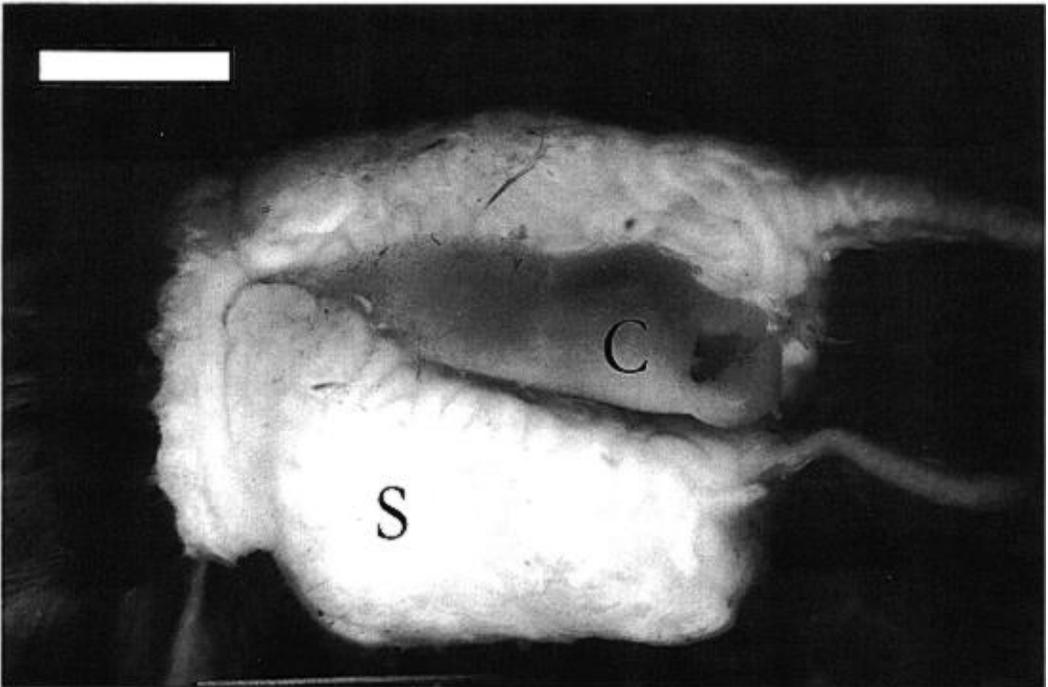*Testis size.*—Testes length in breeding Smith's

Fig. 4. Ventral view of seminal glomera (S) of a male Smith's Longspur. The tortuous coiling of ductus deferens is readily apparent. Cloaca (C) appears between the two glomera. Base of vent feathers are to far left in this view. Scale bar = 3 mm.

Longspurs peaked at 14.5 mm and on average had a combined mass of 1.203 g, or about 4.2% of adult body mass (Fig. 6). This is slightly greater than that of the Dunnock (3.36%; Birkhead et al. 1991), but less than that of the Alpine Accentor (7.7%; Nakamura 1990). Testis length was significantly correlated with body mass across species (Fig. 7). The three polygynandrous species (Smith's Longspurs, Alpine Accentors and Dunnocks) had testis lengths an average of 44% larger than expected for birds with similar body masses. Testis length in Smith's Longspurs was 4.4 mm greater than Lapland Longspur testis length (10.1 mm) and more than twice the mass (Lapland Longspur 0.544 g or 2.0% of male body mass). Testis lengths in the two polygynous species (Yellow-headed Blackbird, Brown-headed Cowbird) were 28% less than expected for their body sizes, while the two sequentially polyandrous species (Common Redpoll, American Goldfinch) had testes lengths about 7% less than expected (Fig. 7A). Thus, large testes size was associated with polygynandry, but not with any other mating system. When phylogeny was controlled, testis-

length contrasts of the polygynandrous species were large and positive, but small for body mass, suggesting that a switch to polygynandry at these nodes resulted in increases in testis size but not body mass (Fig. 7B)

Sperm storage in females.—The mean number of sperm-storage tubules in female Smith's Longspurs collected during egg laying was 1,537 ± 146 (n = 3 females), somewhat less than that found in Lapland Longspurs (2,147 ± 204, n = 6 females), but similar to the Dunnock (1,398; Birkhead et al. 1991). No information is available on sperm storage in the Alpine Accentor, but the number of SSTs in Smith's Longspurs and Dunnocks is close to that expected for birds of their body mass (Fig. 8A). Thus, in these two species females do not appear to provide more sperm-storage sites in response to increased sperm production by males.

The mean length of SSTs in Smith's Longspurs was 237.6 ± 44.9 μm (n = 3 females), only slightly longer than those of the Lapland Longspur (216.2 ± 10.1 μm, n = 6), but less than that found in the Dunnock (370.3 ± 14.4 μm; Birkhead et al. 1991). Since SST length appears to
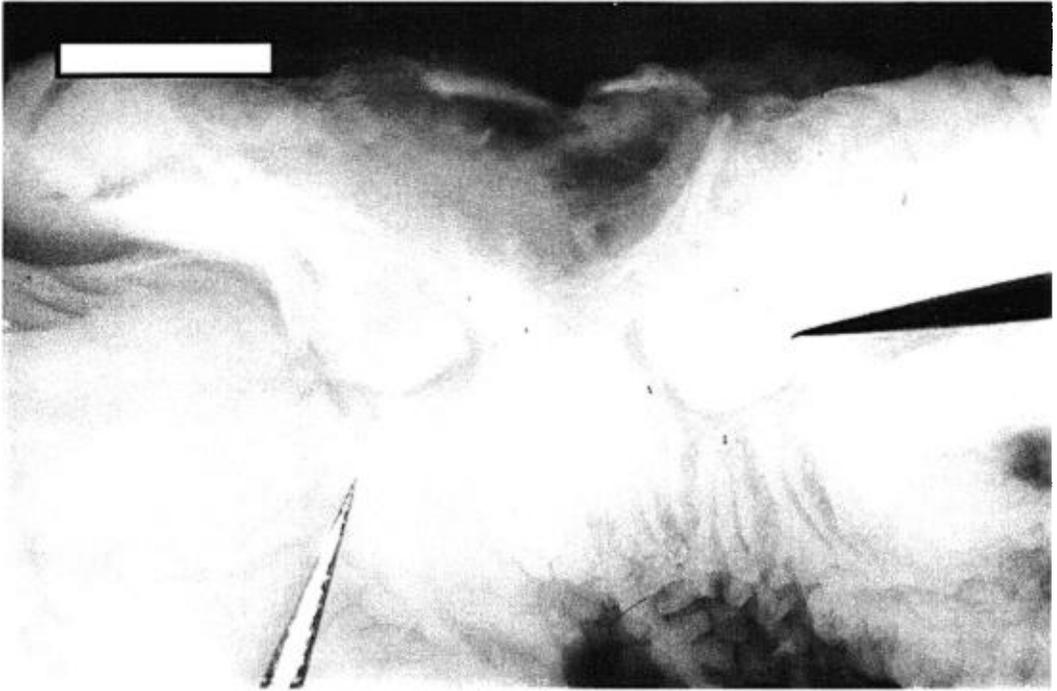
Fig. 5. View of papilla of ductus deferens in urodeum of a male Smith's Longspur. Papillae appear as two conical structures on ventral wall of cloaca (positions indicated by two pins). Vent is at top of photograph. Scale bar = 2 mm.

have coevolved with sperm length (Briskie and Montgomerie 1992, Birkhead and Møller 1992), the appropriate measure for comparing sperm-storage capacity of SSTs among species is the number of layers of sperm that can be accommodated within each SST. When sperm length is compared to SST length (Fig. 8B), it becomes apparent that Smith's Longspurs can accommodate about two layers of sperm per SST, a pattern common to most of the species of passerines examined to date, including the Lapland Longspur. The Dunnock, however, can accommodate up to five layers of sperm, a pattern approached only by the American Robin (Fig. 8B). Thus, Dunnocks appear to have an increased capacity to store sperm within each SST relative to other species, but such an increase does not appear to be related to polygynandry since it was not found in Smith's Longspurs.

The mean number of sperm within each SST was estimated in one Smith's Longspur female to be 25.8 (range 0 to 200, $n = 100$ SSTs censused). Since there were approximately 1,500 SSTs per female, about 39,000 sperm are stored at any given time. Although it is not known

how many sperm are in the ejaculates of Smith's Longspurs, the number of sperm stored by females is less than 0.02% of the sperm present in the seminal glomera of male Smith's Longspurs. This percentage was similar for Lapland Longspur (0.04%) and the Dunnock (<0.01%, calculated from the figures in Birkhead et al. 1991), but information on the amount of sperm stored by females in other species is not available to make a detailed comparison.

DISCUSSION

In common with the other two polygynandrous species studied to date, Smith's Longspur males have very large testes and cloacal protuberances for their body size. Compared with a wide variety of mostly monogamous birds, all three polygynandrous species examined here showed the same pattern. For Smith's Longspurs, testes and cloacal-protuberance size were particularly large and striking compared to those traits in the socially monogamous and congeneric Lapland Longspur.

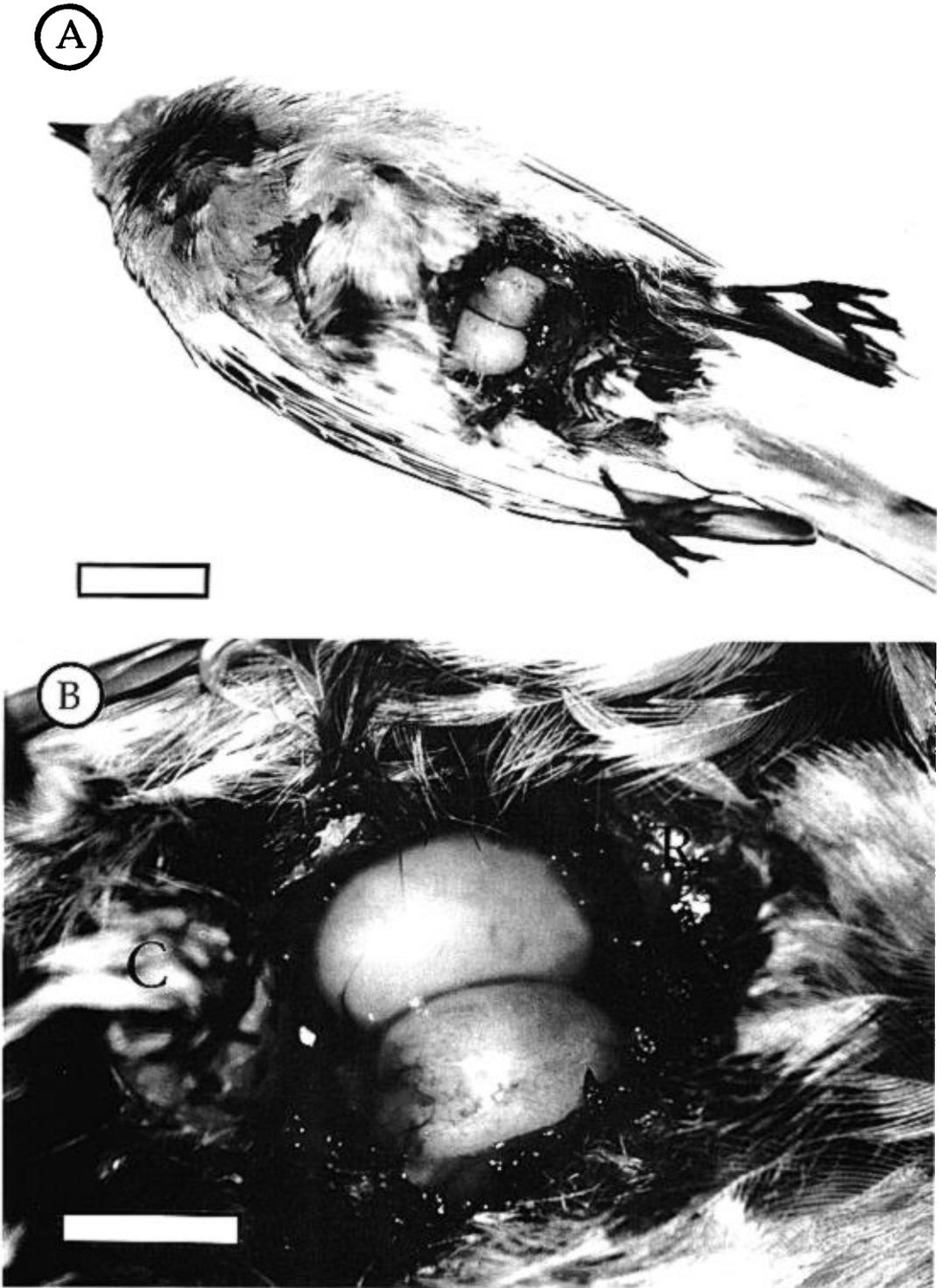Large testes are thought to be an adaptation

Fig. 6. (A) View of testes in a breeding male Smith's Longspur. Scale bar = 20 mm. (B) Close-up view of testes in a second individual. Each testis extends from caudal edge of the rib cage (R) to near anterior edge of cloacal protuberance (C). Scale bar = 10 mm.
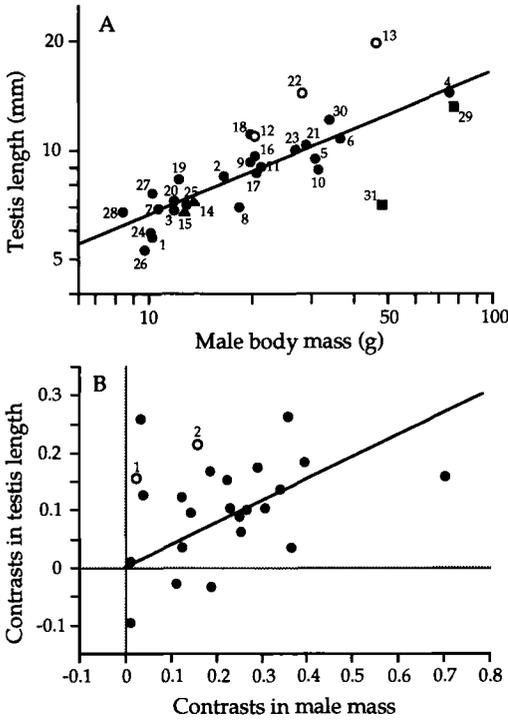
Fig. 7. (A) Relation between testes length and male body mass for 31 species of passerines. Polygynandrous species represented by open circles, sequentially polyandrous species by closed triangles, polygynous species by closed squares, and monogamous species by closed circles. Numbers refer to species listed in Table 1. (B) Independent contrasts between testis length and body mass for same group of species. Open circles are contrasts between (1) Smith's and Lapland longspurs, and (2) between the genera *Prunella* (Dunnock and Alpine Accentor) and *Anthus*. Large positive contrasts in testes size in these two comparisons suggest that an increase in testes size is associated with polygynandry.

to sperm competition because large testes produce larger volumes of more concentrated ejaculates (Møller 1988b). When females copulate with more than one male, such large numbers of sperm may function to dilute or displace ejaculates from rival males. Thus, males that produce and inseminate the largest number of sperm into a given female are likely to father a higher proportion of her offspring and will be favored over males with lower sperm production. This strategy is likely to be most successful when fertilization success is directly proportional to number of sperm per ejaculate and females regularly switch from one male to another over a short time interval. In domestic
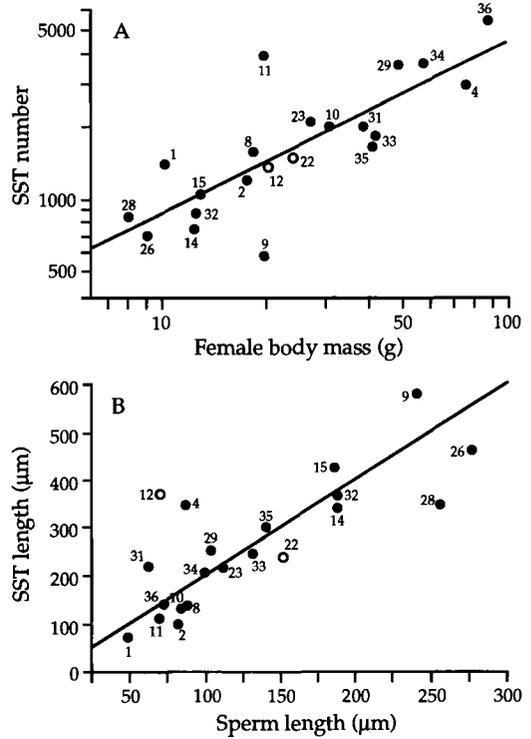
Fig. 8. (A) Relation between female body mass and number of sperm-storage tubules (SST) per female. Smith's Longspurs (22) and Dunnocks (12) are indicated by open circles. Numbers refer to species listed in Table 1 except for the following: (32) *Carduelis hornemanni*, (33) *Plectrophenax nivalis*, (34) *Euphagus cyanocephalus*, (35) *Agelaius phoeniceus*, and (36) *Sturnella neglecta*. Data from Briskie and Montgomerie (1992) and Birkhead et al. (1991). Contrast analyses (see Methods) for most of these data were presented in Briskie and Montgomerie (1992) and show a similar relationship between female body mass and the number of SSTs when controlled for phylogeny. (B) Relation between sperm length and length of SSTs in females. Line indicates SST lengths that can accommodate two layers of sperm. Numbers and symbols as above. Although Smith's Longspur appear to store sperm in a pattern common to most other passerines, Dunnock SSTs are long relative to length of their sperm. Contrast analyses using most of these data were presented in Briskie and Montgomerie (1992) and show the same relationship between sperm length and SST length when phylogeny is controlled.

chickens, when sperm from two males are inseminated within 4 h of each other, paternity is directly proportional to the number of sperm inseminated by each male (Martin et al. 1974). However, after 4 h the last male to copulate generally experiences greater success, a pattern

called last-male sperm precedence (Sims et al. 1987, Birkhead et al. 1988). Last-male precedence is thought to arise from either the covering up of first-male sperm by second-male sperm within the SSTs or by the displacement of first-male sperm from the SSTs by second-male sperm (Lessells and Birkhead 1990).

The pattern of sperm precedence is not known for any of the polygynandrous species studied here, but because each copulates very frequently and at intervals usually less than 4 h (Davies 1985, Nakamura 1990, Briskie 1992), it is possible that paternity is determined by proportional representation. This seems especially likely in Dunnocks and Alpine Accentors, in which females frequently switch or alternate from one mate to another over the copulation period (Davies 1985, Nakamura 1990). However, repeated mate switching is infrequent in Smith's Longspurs (Briskie 1992, unpubl. data). Instead, each female longspur copulates exclusively with one male for a period lasting one to six days, at which point she switches to a second male and copulates exclusively with him for a period lasting one or more days (Briskie 1992). Thus, for male Smith's Longspurs, large testes and the concomitant increase in sperm production may be an adaptation to rapidly dilute and overwhelm sperm at mate switches rather than simply increasing the proportion of sperm inseminated.

The large cloacal protuberances of Smith's Longspurs and other polygynandrous birds seem to function as large sperm reserves for frequent copulations. Cloacal-protuberance size was strongly correlated with both seminal glomera mass and the number of sperm estimated within the glomera. Thus, large protuberances are large simply because they contain huge numbers of sperm. All three polygynandrous species were found to have large cloacal protuberances for their body sizes, a pattern which further suggests that large protuberances are an adaptation to increased sperm competition. Across a wide sample of species, Birkhead et al. (1993) found that protuberance size was correlated positively with the frequency of copulation, a pattern that also supports the hypothesis that variation in cloacal-protuberance size is related to the degree of sperm competition.

An alternative explanation for both large testes and large cloacal protuberances in polygynandrous species is that large sperm stores are required because males must fertilize two or more females, while males in socially monogamous species need only produce enough sperm to fertilize a single female (Cartar 1985, Møller 1991). However, this sperm-depletion hypothesis seems unlikely for the species considered here. Both the Yellow-headed Blackbird and Brown-headed Cowbird are considered polygynous, yet in neither species was testes length or cloacal-protuberance volume larger than expected for their body mass. In fact, the Brown-headed Cowbird had the smallest cloacal-protuberance volume and testes length relative to its body size of any bird in my sample. Thus, sperm production by males with relatively "normal-sized" testes apparently is sufficient to fertilize several females. This suggests that increased testes size with polygynandry more likely evolved to insure paternity under conditions of intense sperm competition rather than through sperm depletion.

Neither female Smith's Longspurs nor female Dunnocks appeared to have any obvious adaptations to increased sperm competition in males. Both the number and size of the SSTs were similar to that expected for their body mass and found in closely related species. Nonetheless, it is difficult to predict exactly how female genital anatomy should respond to sperm competition. One possibility is that females could provide more or larger areas for sperm storage; however, there seems little theoretical reason why this should be favored over any other pattern. Females might even be able to induce greater sperm competition by reducing the number of sperm-storage sites and, thereby, increase the scramble among ejaculates for the few remaining sites. Briskie and Montgomerie (1992) have argued that such a mechanism may account for the wide variation in spermatozoa size found across species. The number of SSTs was slightly less in Smith's Longspurs than in the Lapland Longspur, but the number of SSTs did not differ appreciably between Dunnocks and their nearest monogamous relatives. In contrast, Dunnocks had relatively longer SSTs than most other passerines studied; however, sperm storage needs to be examined in more polygynandrous birds before it can be determined whether long SSTs are an adaptation to high sperm production by males.

Dunnocks appear unusual among birds in that females regularly eject a small volume of ejaculate in response to cloacal pecking by males

(Davies 1983). Cloacal pecking is an obvious advantage to a male if it removes sperm from previous males, but may be advantageous to females as well if it assures males of paternity and increases the likelihood that they will invest in subsequent offspring. Cloacal pecking does not occur in Smith's Longspurs, but it is clear that neither is all sperm later stored by females. Less than 1% of sperm present in the seminal glomera of male Smith's Longspurs is stored in the SSTs at any given time. This value is similar to that of Dunnocks and Lapland Longspurs. Since most sperm is not stored nor ejected, it is likely that most is lost through defecation, although I have not examined female fecal sacs to determine if sperm are present. Alpine Accentors also do not perform cloacal pecking but, prior to mounting by the male, the female defecates while the male watches (Nakamura 1990). Although it was not determined if sperm are voided during this display, it may function in an analogous way to sperm ejection in Dunnocks.

One possible explanation for the large difference in number of sperm stored between males and females is that females may store only a small quantity of sperm simply because that is all they need to maximize their fertility. If storing large quantities of sperm is costly to females (either energetically or through increased probability of contacting parasites or diseases), then females should store only that amount of sperm that insures a maximum rate of fertility. The rest is superfluous and voided. For males, this loss is costly but it should not change the benefits of producing large ejaculates if doing so increases the probability of increasing the number of sperm (however, small in absolute numbers) that eventually find their way into the SSTs.

The number of sperm stored in the seminal glomera of Smith's Longspur males was approximately four times that found in Lapland Longspurs and American Tree Sparrows, but far less than that found in Yellow-headed Blackbirds and Dunnocks. This result was somewhat unexpected because Smith's Longspurs had the largest combined seminal glomera mass of any of the species for which data were available. One possibility is that the Smith's Longspur males I examined were collected in the afternoon ($n = 1$) and evening ($n = 2$), and they may have already used up a large proportion of their sperm reserves by this time. Copulation activity

in longspurs is greatest during the morning and virtually ceases by the afternoon (Briskie 1992). Thus, it is possible that sperm reserves may have been depleted in the birds I collected but this needs to be tested further.

Although my observations provide clear evidence that testis size and cloacal-protuberance size are larger in species in which sperm competition is thought to be most intense, it is less certain how such adaptations function in assuring male paternity. For example, it is not known how the large reserves of sperm in polygynandrous species are allocated to ejaculates or whether there is a trade-off between ejaculation frequency and the number of sperm per ejaculate. Males in polygynandrous species might be expected to have larger ejaculates than monogamous species in order to dilute rival male's sperm, but they also might be forced to "ration" sperm over the copulation period to insure that some sperm is available for future use. Which pattern best insures paternity in each type of mating system may well depend upon the frequency of encountering rival ejaculates and the physiological constraints of sperm production. The advantages and disadvantages of either strategy will further depend upon how females in each species store and use sperm from different ejaculates. Sorting out the interactions between ejaculate size, insemination frequency and sperm storage patterns may prove complex, but the resolution of this problem will provide a basis for understanding the evolution and adaptive modification of the avian reproductive system.

Siva-Jothy let me use his microscope for taking photographs of the seminal glomera.

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