INDIVIDUAL AND TEMPORAL VARIATION IN CLOACAL PROTUBERANCE SIZE OF MALE BEARDED TITS (*PANURUS BIARMICUS*)

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ABSTRACT.—The intensity of sperm competition is one of the factors known to determine interspecific variation in the size of male reproductive organs. However, individual variation in the size of reproductive organs and its relation to male quality have not been considered appropriately. We investigated the annual pattern of variation in the size of the cloacal protuberance (CP) in male Bearded Tits (*Panurus biarmicus*) and correlated CP volume during the fertile period of females with reproductive and morphological features of males. Contrary to other passerines, the cloacal protuberance does not constitute a sperm reservoir but functions as a copulatory organ in Bearded Tits. CP volume differed significantly between unmated and mated males and provided a good indicator of their reproductive status. Within individual mated Bearded Tits, CP size changed in conjunction with their mate's fertile cycle, peaking around the day of clutch initiation. During the fertile period, mated Bearded Tits possess the largest CP (relative to body size) known for a passerine (average volume index = 59.14 mm^3 per g; n = 16). The adaptive value of this feature could be that it improves ejaculate transfer owing to better and longer cloacal contact. Relative to unmated males, mated males have more total sperm stored in their seminal glomera and larger testes, suggesting the occurrence of large disadvantages for unmated males to gain reproductive success. An isolation experiment showed that female presence positively influences the development of CP size in virgin male Bearded Tits. *Received 14 April 1997, accepted 12 March 1998.*

SPERM COMPETITION is widespread among animals (Parker 1970, Birkhead and Møller 1992) and has a profound effect on the behavior and morphology of a variety of species (Le-Boeuf 1974, Clutton-Brock et al. 1980, Birkhead et al. 1987, Davies 1992). Much of the variation in the size of traits related to reproduction can be accounted for by the intensity of sperm competition (McKinney et al. 1984; Cartar 1985; Møller 1988, 1991; Birkhead et al. 1991; Briskie 1993; Schulze-Hagen et al. 1995; Rising 1996). Contrary to many other vertebrates, most male birds (exceptions include ratites and waterfowl) do not possess an intromittent copulatory organ (Lake 1981). The external sexual organ is usually restricted to a swelling of the cloaca, the so-called cloacal protuberance (CP), which is caused by the growth of the seminal glomera (Salt 1954) that function as the site for the storage and maturation of sperm (Wolfson 1954, Lake 1981). Interspecific variation in relative CP size is best explained by the sperm-competition hypothesis, which predicts that when sperm competition is intense, a large protuber-

ance is required for the maintenance of sufficient numbers of sperm (Birkhead et al. 1993). Wolfson (1954, 1960) also suggested that the CP could act as a phallus or at least facilitate copulation. The latter suggestion seems to be particularly relevant for the Bearded Tit (*Panurus biarmicus*), which is unique among passerines in that the CP does not represent the coiled seminal glomera, but consists of a muscular layer and terminates in a small papilla (Birkhead and Hoi 1994). Birkhead and Hoi (1994) thus concluded that in Bearded Tits, the CP forms a copulatory organ.

Because the development and maintenance of morphological features can be costly, the relative size of such characters may function as a cue to the respective male's quality (Sheldon 1994). Studies of morphological features associated with reproduction, such as antlers (Clutton-Brock 1980) and plumage (Petrie et al. 1991), have illustrated the significance of studies at the level of the individual. We examined the morphological and functional differences in the CP in a population of Bearded Tits. Specifically, we: (1) examined seasonal patterns of male CP development over the breeding period, at both the species and the individual level;

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(2) evaluated possible individual variation in CP size; and (3) related such variation to male reproductive parameters and to morphological features important in female choice.

The social system, sexual strategies, and reproductive parameters of Bearded Tits are known in some detail (Hoi 1989, Birkhead and Hoi 1994, Hoi and Hoi-Leitner 1997). Bearded Tits are socially monogamous, nonterritorial passerines of Eurasian "reed belts," where individuals form pair bonds while they are still juveniles (Koenig 1943). Males possess black feathers that form stripes under each eye, the so-called "beard." The beard, along with tail length in males, function as cues in mate choice by females (Hoi unpubl. data). Because the species shows a male-biased sex ratio of 2:1 throughout the breeding season, flocks of unmated males occur regularly (Hoi 1989). From mid-March until July, pairs produce up to four clutches (Bibby 1983). Copulation frequency is high, with up to 200 copulations per clutch (Hoi 1997). Many of the copulations are solicited by females that initiate "chase-flights" in which up to 13 males follow the soliciting female (Koenig 1943). As a consequence, extrapair copulations and extrapair paternity are rather high in Bearded Tits (Hoi 1997, Hoi and Hoi-Leitner 1997).

Sperm competition theory predicts that high copulation frequency should result in relatively large testes (Møller 1991). Indeed, the testes of Bearded Tits are 70% heavier than predicted from Møller's (1991) equation for birds (Birkhead and Hoi 1994). The number of spermatozoa in the seminal glomera is relatively high compared with body mass and the mass of the seminal glomera in Bearded Tits (Birkhead and Hoi 1994). Moreover, the CP is relatively large compared with other passerines examined to date (see Birkhead et al. 1993), and the CP terminates into a papilla (see Birkhead and Hoi 1994) that is everted during sperm transfer (Sax and Hoi pers. obs.) and elongates the CP by approximately 30%.

METHODS

We measured tarsus length, wing length, and tail length of Bearded Tits captured during the breeding seasons of 1994 and 1995 near Lake Neusiedl, Austria (47°56 N, 16°45 E). Length of the beard stripes of males was calculated as the average length (mm) of both stripes measured from the posterior end of the

bill to the tip of the beard. To calculate the size of the CP (which is cylinder-shaped with a circular base), we measured its height from the posterior ventral surface to the fleshy tip of the cloaca and its diameter at the base. In addition, we determined the breeding status of the mate of each of the males that we measured. To avoid pseudoreplication, every individual was only measured once.

Data on changes in CP size during the reproductive period were obtained from birds housed in aviaries at the Konrad Lorenz Institute of Comparative Ethology in Vienna, Austria. Birds were measured every second day at the beginning of the reproductive period; during the incubation and brood-rearing periods, which last for about two weeks each, catches were limited to once per week to avoid desertion of the brood.

Data on variation in CP volume relative to mating status were obtained from birds kept in aviaries in groups consisting of a mated pair accompanied by two single males. Birds were kept in aviaries measuring approximately 5 × 5 × 3 m and fed *ad libitum*. Mated and unmated males were measured on the day the female laid its first egg.

Observations on the development of the CP of isolated males (hand-raised males without previous contact with females) were conducted at the Konrad Lorenz Institute from April to May 1995, which is the time of the year when Bearded Tits start reproducing (Koenig 1952). Six males were kept in aviaries measuring approximately 3 × 3 × 3 m under natural light conditions and fed *ad libitum*. After one month, two females were added, and the development of the CP was observed over the following month. Measurements were undertaken every second day.

Data on reproductive organs were obtained during the reproductive period from captive birds that died by accident; e.g. during capture. These birds were dissected immediately after death or were preserved in 10% formalin. Birds were dissected according to Birkhead et al. (1991). Wet mass of the testes and the seminal glomera were obtained using a Satorius MC1 electronic balance (± 0.0001 g). Next, the seminal glomera were finely minced with a forceps, the spermatozoa were stripped into 5 mL of phosphate buffered saline, and 10 samples were counted under 200× magnification using an improved Neubauer chamber.

Statistical analysis.—CP volume of males was measured in the field on different days before and after clutch initiation (i.e. day 0). Because CP volume peaked around day 0 (see Results), we used the residual CP volume for comparisons involving CP volume. Measurements of the CP (in mm) were inserted into the formula for the volume of a cylinder ($r^2 \times \pi \times$ height), where r is 0.5 times the protuberance diameter. Values for the volume index, which indicate the relationship between body mass and CP size, were calculated following Birkhead et al. (1991).
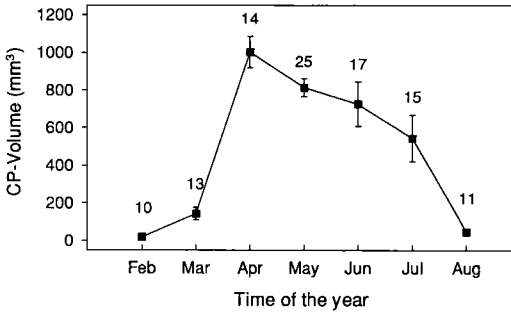
FIG. 1. Development of cloacal protuberance volume of mated male Bearded Tits during the reproductive period (March to July). Vertical bars are ± SE; sample sizes are given for each month.
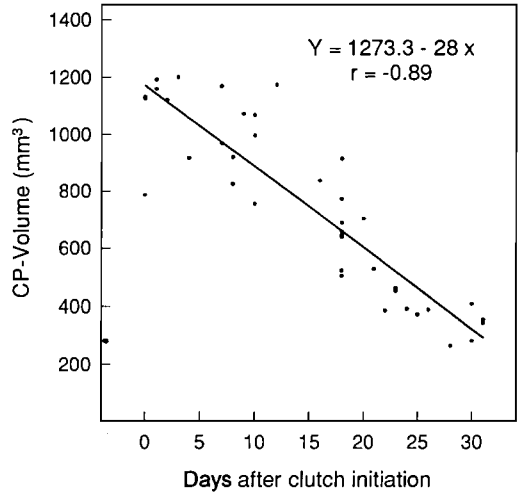
Body mass of paired and unpaired males did not differ (Mann-Whitney U-test, $Z = 1.53$, $P > 0.1$), so we used the average body mass of the species (15 g; Bibby 1983) for this calculation. Because there was no correlation between body mass and volume of the CP for individuals ($r = 0.02$, $n = 10$, $P > 0.9$), we did not correct CP values for body-mass differences. If not mentioned otherwise, values are presented as $\bar{x} \pm$ SE. A Kruskal-Wallis test was used to examine differences in CP volume among mated, unmated, and virgin males, and single post-hoc comparisons were conducted using the method of Conover (1980:231).

RESULTS

Seasonal and breeding-cycle variation in CP size.—The volume of the CP of mated male Bearded Tits showed a pronounced seasonal change. The CP enlarged rapidly at the beginning of the breeding season and reached maximum size in April (Fig. 1). The CP then decreased in size steadily until the end of the reproductive period (July) and was almost completely reduced by August (Fig. 1). Outside of the breeding period, the average volume index of the CP was $7.3 \pm$ SE of 1.3 mm^3 g^{-1} ($n = 11$); at the peak of their mates' fertile period, it was 59.14 ± 13.37 mm^3 g^{-1} ($n = 16$). This latter value is much larger than that of the Alpine Accentor (*Prunella collaris*), which was thought to possess the largest CP of all passerines (i.e. 44.7 mm^3 g^{-1}; Nakamura 1990).

CP volume of mated males also varied within the breeding cycle of their mates, increasing significantly prior to clutch initiation ($r = 0.89$, $n = 9$, $P < 0.01$) and decreasing significantly thereafter ($r = -0.89$, $n = 39$, $P < 0.001$; Fig. 2). The reduction in CP size as indicated by the

FIG. 2. Decrease in cloacal protuberance volume in male Bearded Tits as the nesting cycle progresses. Egg laying occurred from days 0 to 5 (assuming a modal clutch size of six eggs), incubation from days 6 to 18, and brood rearing from days 19 to 31.

slope of the regression equation was 40.3 mm^3 per day.

Bearded Tits produce several clutches per season (Bibby 1983). Repeated measures of individual mated males in the aviary throughout the reproductive period revealed that CP volume increased rapidly a few days before laying, peaked on or near the day that females laid their first eggs (Fig. 3), and decreased significantly after clutch initiation ($r = 0.91$, $n = 8$, P
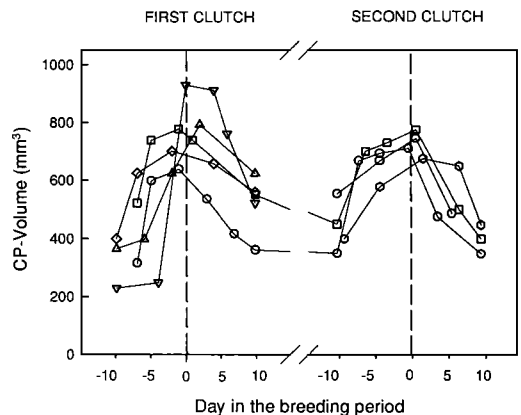

FIG. 3. Changes in cloacal protuberance volume of mated male Bearded Tits relative to the start and end of the female's fertile period. Day 0 is the day the female partner lays the first egg.
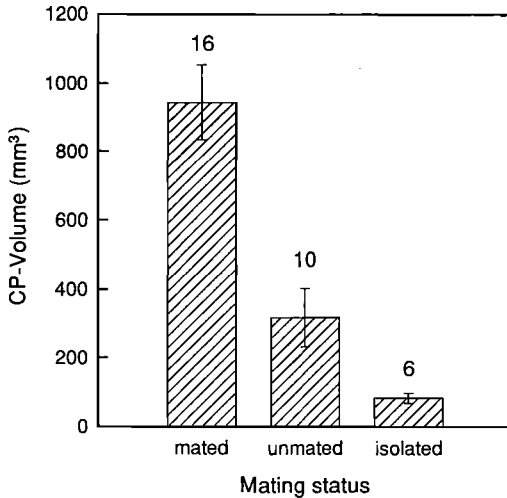
FIG. 4. Cloacal protuberance volume of male Bearded Tits of varying mating status. Volume indices are 59.14 mm³ g⁻¹ for mated males, 19.95 mm³ g⁻¹ for unmated males, and 5.27 mm³ g⁻¹ for isolated males. Numbers above columns are sample sizes.

< 0.02). During the subsequent reproductive period (i.e. for the second clutch), the pattern of increase and decrease in CP size occurred again (Fig. 3).

Variation of CP size relative to mating status.—CP volume differed significantly with mating status (Kruskal-Wallis test, $T = 26.01$, df = 2, $P < 0.001$; Fig. 4). Single post-hoc comparisons revealed that the CP of unmated males was significantly smaller than that of mated males, both within (day 0; $Z = 2.92$, $P < 0.01$) and outside of the fertile period (day +10; $Z = 2.70$, $P < 0.02$); this was also true when comparing mated and virgin males (within and outside of the fertile period; $P < 0.01$ for both periods) and unmated and virgin males ($Z = 2.02$, $P < 0.05$). The CP of isolated virgin males remained at a level typical of males outside the reproductive period (Fig. 4). However, access to females (see Methods) led to a significant increase in the size of the CP when comparing values for 24 April and 25 May (Wilcoxon signed-rank test, $Z = 2.20$, $n = 6$, $P = 0.02$; CP volume one month later = 401.9 ± 51.58 mm³; Fig. 5).

Variation in reproductive organs relative to CP volume.—Two of the six males that we dissected were mated and four were unmated. Of the latter four males, two obviously were not in breeding condition because they possessed
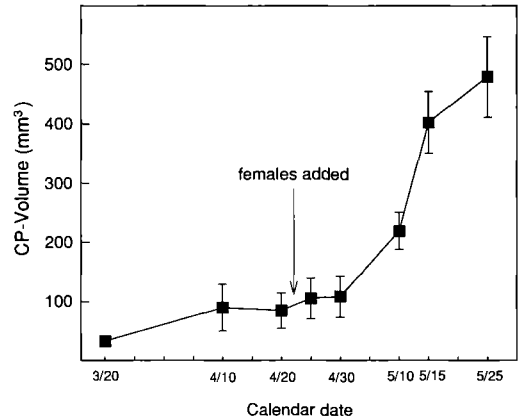

FIG. 5. Course and development of cloacal protuberance volume of isolated male Bearded Tits. The arrow indicates the day males were first allowed access to females.

very small testes and had no detectable seminal glomera. Among the four males in breeding condition, the two mated males stored a much higher total number of sperm in their seminal glomera ($46.47 \pm 19.13 \times 10^6$ vs. $5.81 \pm 0.09 \times 10^6$) and had a much higher combined testes mass than that of the two unmated males (0.53 ± 0.22 g vs. 0.19 ± 0.06 g). The combined mass of the seminal glomera also tended to be higher in the mated males (0.032 ± 0.017 g) than in the unmated males (0.021 ± 0.01 g).

Although each of the three parameters tested (combined testes mass, combined seminal glomera mass, total number of spermatozoa in the seminal glomera) was positively correlated with CP volume, only the correlation with combined testes mass was significant given our small sample sizes ($r = 0.91$, $n = 6$, $P = 0.01$).

Male characteristics and CP size.—Among the morphological parameters taken from mated males (tarsus length, tail length, wing length, beard length, and body mass), only tail length was significantly correlated with CP volume ($r = 0.52$, $n = 16$, $P < 0.031$). However, this P-value became nonsignificant when we applied a Bonferonni correction for multiple comparisons.

DISCUSSION

The cloacal protuberance of male Bearded Tits does not represent the coiled seminal glomera as in other passerines, but is a copu-

latory organ (Birkhead and Hoi 1994). Because the CP is a copulatory organ, its enormous size could be an important factor to ensure sperm transfer. The proportion of copulations that result in sperm transfer ranges from 50 to 70% across species (Birkhead 1991, Birkhead et al. 1993). Low rates of sperm transfer have been attributed to female rejection (Westneat 1987) or to the usually brief duration of copulation (Birkhead and Møller 1992). Therefore, a larger CP should increase the quality and duration of cloacal contact. This idea is supported by the fact that copulation duration is relatively long in Bearded Tits (Birkhead and Hoi 1994). Additionally, the CP of Bearded Tits terminates in a phalloid structure (similar to that found in the Dunnock (*Prunella modularis*; Birkhead et al. 1991) that is everted into the cloaca during copulation (Birkhead and Hoi 1994, Sax pers. obs.). This provides additional support for the idea that males with a large CP (and thus a longer phallus) might be more likely to succeed in sperm transfer and to deliver the ejaculate relatively farther into the female's cloaca. Such an advantage likely would reduce the proportion of sperm the female can reject and also might allow the spermatozoa to enter the foremost sperm-storage tubules, thereby increasing the chances for fertilization (see Briskie and Montgomerie 1993).

The maximum volume of the CP is maintained only during the fertile period of a male's mate. Bearded Tits produce up to four clutches in one season (Bibby 1983), and the size of the CP of mated males changes over the reproductive period, peaking around the day the first egg of each respective clutch is laid. None of the other studies that focused on the CP (e.g. Nakamura 1990, Briskie 1993) have reported similar changes in CP development, although the Alpine Accentor commonly makes two nesting attempts (Nakamura 1990), and Smith's Longspurs occasionally renest (Briskie 1993). This discrepancy could be due to the fact that the other studies did not measured the CP of individual males over the whole breeding season. More likely, however, this is the consequence of the functional and morphological differences in the CP of Bearded Tits and the relatively higher energetic investment associated with the maintenance of this unique muscular feature.

The difference in CP volume observed between mated and unmated males reflects differential development of the reproductive organs. The fact that 50% of the unmated males were not in reproductive condition during the breeding period, and the remaining unmated males had only poorly developed reproductive organs and low numbers of sperm in their seminal glomera, indicates that a considerable proportion of the unmated males is virtually excluded from reproduction, whereas the remaining males face dramatic disadvantages in the pursuit of copulations compared with their mated conspecifics. In our experimental setup, isolated virgin male Bearded Tits showed no signs of fertility, even at the peak of the breeding period, until they were allowed contact with females. This contradicts sperm-competition theory, which predicts that unmated males should have relatively higher numbers of sperm to maximize the success of any extrapair copulations that they may obtain (Parker 1990, Birkhead et al. 1995). However, a previous study suggested that unpaired male Bearded Tits are unlikely to gain extrapair paternity (Hoi and Hoi-Leitner 1997), which might explain why these males invest so little in the development of their reproductive organs.

LITERATURE CITED

BIBBY, C. J. 1983. Studies of West Palearctic birds, 186. Bearded Tit. British Birds 76:549–563.

BIRKHEAD, T. R. 1991. Sperm depletion in the Bengalese Finch *Lonchura striata*. Behavioral Ecology 2:267–275.

BIRKHEAD, T. R., L. ATKIN, AND A. P. MØLLER. 1987. Copulation behaviour of birds. Behaviour 101: 101–138.

BIRKHEAD, T. R., J. V. BRISKIE, AND A. P. MØLLER. 1993. Male sperm reserves and copulation frequency in birds. Behavioral Ecology and Sociobiology 32:85–93.

BIRKHEAD, T. R., F. FLETCHER, E. J. PELLAT, AND A. STAPLES. 1995. Ejaculate quality and the success of extra-pair copulations in the Zebra Finch. Nature 377:422–423.

BIRKHEAD, T. R., B. J. HATCHWELL, AND N. B. DAVIES.

1991. Sperm competition and the reproductive organs of the male and female Dunnock *Prunella modularis.* Ibis 133:306–311.

BIRKHEAD, T. R., AND H. HOI. 1994. Reproductive organs and mating strategies of the Bearded Tit *Panurus biarmicus.* Ibis 136:356–360.

BIRKHEAD, T. R., AND A. P. MØLLER. 1992. Sperm competition in birds. Academic Press, London.

BRISKIE, J. V. 1993. Anatomical adaptations to sperm competition in Smith's Longspurs and other polygynandrous passerines. Auk 110:875–888.

BRISKIE, J. V., AND R. MONTGOMERIE. 1993. Patterns of sperm storage in relation to sperm competition in passerine birds. Condor 95:442–454.

CARTAR, R. V. 1985. Testes size in sandpipers. Naturwissenschaften 72:157–158.

CLUTTON-BROCK, T. H., S. D. ALBON, AND P. H. HARVEY. 1980. Antlers, body size and breeding group size in the Cervidae. Nature 285:565–567.

CONOVER, W. J. 1980. Practical nonparametric statistics, 2nd ed. John Wiley and Sons, New York.

DAVIES, N. B. 1992. Dunnock behaviour and social evolution. Oxford University Press, New York.

HOI, H. 1989. Ökologie und Paarungssystem der Bartmeise (*Panurus biarmicus*). Ph.D. dissertation, University of Vienna, Vienna, Austria.

HOI, H. 1997. Assessment of the quality of copulation partners in the monogamous Bearded Tit. Animal Behaviour 53:277–286.

HOI, H., AND M. HOI-LEITNER. 1997. An alternative route to coloniality in the Bearded Tit: Females pursue extra-pair fertilizations. Behavioral Ecology 8:113–119.

KOENIG, O. 1943. Rallen und Bartmeisen. Niederdonau, Natur und Kultur 25:51–59.

KOENIG, O. 1952. Das Aktionssystem der Bartmeise (*Panurus biarmicus* L.). Österreichische Zoologische Zeitschrift 3:1–82, 247–325.

LAKE, P. E. 1981. Male genital organs. Pages 1–61 *in* Form and function in birds, vol. 2 (A. S. King and J. McLelland Eds.). Academic Press, New York.

LEBOEUF, B. J. 1974. Male-male competition and reproductive success in elephant seals. American Zoologist 14:163–176.

MCKINNEY, F., K. M. CHENG, AND D. BRUGGERS. 1984. Sperm competition in apparently monogamous birds. Pages 523–545 *in* Sperm competition and

the evolution of animal mating systems (R. L. Smith, Ed.). Academic Press, New York.

MØLLER, A. P. 1988. Testes size, ejaculate quality and sperm competition in birds. Biological Journal of the Linnean Society 33:273–283.

MØLLER, A. P. 1991. Sperm competition, sperm depletion, paternal care, and relative testis size in birds. American Naturalist 137:882–906.

NAKAMURA, M. 1990. Cloacal protuberance and copulatory behaviour of the Alpine Accentor (*Prunella collaris*). Auk 107:284–295.

PARKER, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biological Reviews of the Cambridge Philosophical Society 45:525–567.

PARKER, G. A. 1990. Sperm competition games: Sneaks and extra-pair copulations. Proceedings of the Royal Society of London Series B 242:127–133.

PETRIE, M., T. HALLIDAY, AND C. SANDERS. 1991. Peahens prefer Peacocks with elaborate trains. Animal Behaviour 41:323–331.

RISING, J. D. 1996. Relationship between testes size and mating systems in American Sparrows (Emberizinae). Auk 113:224–228.

SALT, W. R. 1954. The structure of the cloacal protuberance of the Vesper Sparrow (*Pooecetes gramineus*) and certain other passerine birds. Auk 71:64–73.

SCHULTZE-HAGEN, K., B. LEISLER, T. R. BIRKHEAD, AND A. DYRCZ. 1995. Prolonged copulation, sperm reserves and sperm competition in the Aquatic Warbler *Acrocephalus paludicola.* Ibis 137:85–91.

SHELDON, B. C. 1994. Male phenotype, fertility and the pursuit of extra-pair copulations by female birds. Proceedings of the Royal Society of London Series B 257:25–30.

WESTNEAT, D. F. 1987. Extra-pair copulations in a predominantly monogamous bird: Observations of behaviour. Animal Behaviour 35:865–876.

WOLFSON, A. 1954. Notes on the cloacal protuberance, seminal vesicles, and a possible copulatory organ in male passerine birds. Bulletin of the Chicago Academy of Sciences 10:1–23.

WOLFSON, A. 1960. The ejaculate and the nature of coition in some passerine birds. Ibis 102:124–125.