

SPERM COMPETITION AND THE REPRODUCTIVE ANATOMY OF MALE SUPERB FAIRY-WRENS

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ABSTRACT.—In Superb Fairy-Wrens (*Malurus cyaneus*), groups of males cooperate with a single female to rear young, yet offspring are usually sired by males from outside the group. In this unusual mating system there is potential for intense sperm competition. During the breeding season, males develop a sperm storage structure (cloacal protuberance) and testes that proportionally are among the largest found in passerines. We compared the development pattern and size of cloacal protuberances of males differing in age and social status. Protuberance size increased with body mass. Age, intragroup dominance, and pairing status did not influence the overall size of the protuberance, but old males had a larger tip on their protuberance. This prominent tip has not been reported in other species, and we speculate that it serves as an intromittent organ. Other birds with large testes and cloacal protuberances have high copulation rates, but copulation in Superb Fairy-Wrens is only very rarely observed. We propose that the cloacal protuberance and large testes of Superb Fairy-Wrens provide large sperm reserves primarily for extrapair copulations. These may occur frequently, or involve the transfer of large ejaculates. Received 13 January 1993, accepted 14 March 1993.

FEMALES of many passerine species mate with more than one male during a single fertile period (Birkhead and Møller 1992). Breeding males in these species often respond behaviorally by close following or guarding of their mate. They may also exhibit reproductive anatomical structures that have developed to unusual size in response to sperm competition. For example, male birds commonly develop a swelling around the cloaca known as the cloacal protuberance (Fatio 1864, Salt 1954, Wolfson 1954). The protuberance results from the enlargement of the seminal glomerus (the distal end of the ductus deferens), and is a site for sperm storage and maturation before ejaculation (Lake 1981). Species possessing relatively large protuberances are often polygynandrous and have high copulation frequencies (Nakamura 1990, Birkhead et al. 1991, Briskie 1992). Therefore, it is likely that large protuberances are an anatomical adaptation to sperm competition (Birkhead et al. 1993).

In this paper we describe features of the reproductive anatomy of the Superb Fairy-Wren (*Malurus cyaneus*), a small Australian passerine. In this species extragroup fertilizations are extremely common. In our study population about 80% of all offspring are the result of extragroup matings, and in 95% of clutches at least one of the young is sired by an extragroup male (Mulder et al. unpubl. data).

METHODS

Superb Fairy-Wrens are small (ca. 10 g) passerines common in southeastern Australia. Our study site is the Australian National Botanic Gardens, a 40-ha plantation of Australian native shrubs and trees on the eastern slopes of Black Mountain, Canberra (149°15'E, 36°05'S; elevation 650 m). Fairy-wrens occupy most habitat at this site in 30 to 40 small (ca. 0.6 ha each) contiguous territories. Breeding takes place between September and March. Females are multi-brooded and make numerous nesting attempts (up to seven) per season. Breeding units consist of a female and dominant male, and up to three additional "helper" males, generally philopatric sons from previous nesting attempts. Most males molt from blue nuptial breeding plumage into a cryptic brown eclipse plumage during winter; females remain brown all year. A very small number of old males molt directly from nuptial to nuptial plumage.

Birds were captured in mist nets between dawn and 0900 and weighed to the nearest 0.1 g using a Pesola spring balance. Between August 1991 and May 1992 we measured the dimensions of each male's protuberance (length [including and excluding prominent tip], width, and height) to the nearest 0.05 mm using dial calipers (measured on 124 occasions; $n = 89$ males). All measurements were taken by one of us (R.A.M.) to minimize sampling error. In previous seasons, presence or absence was noted each time birds were captured.

Since the protuberance roughly resembles a barrel shape from its anterior to posterior end, we estimated the volume of the protuberance as

$$\text{Volume} = \text{length} \times \pi r^2, \quad (1)$$

where r is estimated as one-fourth the sum of the width and height. For comparative purposes, we also calculated an alternative index of volume (length \times width \times height/body mass).

We obtained data on testes size from fairy-wren specimens held at the Australian National Wildlife Collection, Canberra. Data were obtained as length \times width of testes, and were recalculated to combined testes mass (g) using Møller's (1991) formula:

$$\text{testes mass} = 2(1.087 \text{ g} \cdot \text{cm}^{-3})1.33\pi a^2 b, \quad (2)$$

where a and b are the smallest and largest radii of each testis (in centimeters). To estimate testes mass at peak breeding, we followed Briskie (in press) by fitting a second-order polynomial for date and testes mass, and used the maximum of this regression as the average testis mass during breeding. We estimated average testis mass in this way for four species of *Malurus* for which more than 15 measures of testes mass were available. We compared actual testes masses (in grams) to those predicted from the allometric relationship with body mass (in grams; Møller 1991), where

$$\log(\text{testes mass}) = -1.37 + 0.67 \log(\text{body mass}), \quad (3)$$

to determine whether testes were relatively large or small for the body mass of the bird. Results are presented as means (\pm SE) in all statistical tests unless stated otherwise.

RESULTS

Cloacal protuberance.—The cloacal protuberance of male Superb Fairy-Wrens appears as a large, bulbous structure surrounding the vent, mildly bilobed at the posterior end and forming a prominent pointed tip at the anterior end (Fig. 1). The shape of the protuberance varied little between males. Protuberances were mostly bare, except for a ring of feathers around the vent. The coiled ducts of the seminal glomera were visible through the skin of the protuberance only in some individuals (e.g. Fig. 1); in others the skin was black.

The average dimensions of the fully developed protuberance were: length, 7.9 ± 0.1 mm ($n = 42$ [excluding tip on anterior end of bulb; 9.4 ± 0.1 mm including tip]); width, 6.3 ± 0.1 mm ($n = 42$); height, 5.3 ± 0.1 mm ($n = 38$). Males averaged 9.8 ± 0.1 g in body mass ($n = 97$). Body mass correlated positively with protuberance width ($r^2 = 0.12$, $P = 0.017$, $n = 45$), height ($r^2 = 0.25$, $P = 0.001$, $n = 45$), and volume ($r^2 = 0.17$, $P = 0.009$, $n = 37$). Cloacal-protuberance size is strongly correlated both with the

mass of the seminal glomera, and the number of sperm contained within them (Birkhead et al. 1993, Briskie in press). Microscopic sections of *Malurus* cloacal protuberances have shown that they contain very large numbers of spermatozoa (Parsons and Cleland 1926, Tidemann 1983).

The volume index (length \times width \times height/body mass) of the protuberance is 26.7. Compared to the same indices for 36 passerines either listed in Birkhead et al. (1993) or calculated using data from Briskie (in press), the cloacal protuberance of the Superb Fairy-Wren is among the five largest.

The earliest date on which we detected swelling of the cloacal area was 26 August. The first sign of the developing protuberance is eversion of the tip, which takes place before any cloacal swelling. Seasonal changes in the length of the protuberance are plotted in Figure 2. Length correlated with width ($r^2 = 0.57$, $P = 0.0001$, $n = 33$) and height ($r^2 = 0.47$, $P = 0.0001$, $n = 32$) and, therefore, provides a good measure of changes in the dimensions of the protuberance. Old individuals molted into nuptial plumage before their protuberances started enlarging (sometimes several months earlier). By contrast, birds approaching their first breeding season often developed full-sized protuberances before acquiring nuptial plumage. No individual maintained a protuberance throughout the year, even though some retained their nuptial plumage. The first signs of decline in volume of the cloacal protuberance were noted on 15 January, and the decline appeared well correlated with the onset of molt into eclipse plumage. The latest date we observed the presence of a (receding) protuberance in a male was 7 April. This male was in his sixth year and had molted from one nuptial plumage into another.

With one exception, males of all ages developed a protuberance during the breeding season, and there appeared to be no difference in the height, width and length of the protuberance across age classes (Table 1; measurements from period of maximum protuberance size only, 1 November–12 January). The length of the tip of the protuberance varied, and was larger in older males (Table 1).

A single male in his second breeding season, measured during peak breeding (24 November), showed no enlargement of the cloacal area (solid circle, Fig. 2). This unusual individual was also the only male not to acquire nuptial

TABLE 1. Cross-sectional comparisons of cloacal protuberance dimensions across male age classes. Values are means \pm SE (*n*). All measurements in mm, except for volume (mm³). Mean values (compared by ANOVA) did not differ significantly for any measure between age classes, except for the tip, which was longer in older males.

Age ^a	Length	Width	Height	Volume	Tip length
1	7.7 \pm 0.2 (9)	5.9 \pm 0.1 (9)	4.9 \pm 0.2 (9)	179 \pm 12 (9)	1.2 \pm 0.1 (9)
2	8.1 \pm 0.1 (14)	6.4 \pm 0.2 (14)	5.4 \pm 0.1 (11)	225 \pm 14 (10)	1.4 \pm 0.1 (12)
3	8.0 \pm 0.4 (5)	6.4 \pm 0.3 (5)	5.4 \pm 0.3 (5)	227 \pm 30 (5)	1.7 \pm 0.1 (5)
4 ^b	7.7 \pm 0.1 (5)	6.2 \pm 0.2 (5)	5.5 \pm 0.2 (4)	204 \pm 7 (4)	1.7 \pm 0.2 (5)
<i>P</i>	0.42	0.27	0.08	0.13	0.05

^a Ranking of known-age males (e.g. 1 = males in their first breeding season).

^b Birds in their fourth or later breeding season.

plumage in this or the previous breeding season.

For 11 breeding groups, we were able to capture all of the males resident in the group on the same morning, allowing us to compare directly the sizes of their protuberances. Seven groups consisted of two males, three of three males, and one of four males. Within these groups, dominant males did not have larger cloacal protuberances than the mean for their helpers (paired *t*-test, *df* = 10, *t* = 0.96, *P* = 0.36). However, in two instances where two males from the same group were captured very early (1 October) or very late (4 February) in the breeding season, the protuberance had receded in the subordinate male but was still present in the dominant individual. Therefore, it is possible that, although sizes of fully developed protuberances do not differ greatly between individuals, protuberances develop later and regress earlier in young subordinate individuals.

Unlike females in some other species (e.g. Alpine Accentor, *Prunella collaris*), female Superb Fairy-Wrens showed no evidence of cloacal swelling or coloration during the breeding season, and we never observed behaviors such as cloacal pecking (Davies and Houston 1983) or ejection of feces by females (Nakamura 1990) before copulation.

Testes mass.—Testes length of Superb Fairy-Wrens measured during the breeding season peaked at 11.0 mm, with a mean mass of 0.48 g (4.9% of male body mass). Mean testes mass is 1.44 times larger than predicted for body mass. For the largest Superb Fairy-Wren testes measured, testes mass (1.03 g, 10% of body mass) was 3.93 times larger than predicted for the body mass of the individual.

Figure 3 compares the relative testes mass of four species of *Malurus* to those of a range of passerines from Møller (1991), correcting for

the body mass of Superb Fairy-Wrens (erroneously reported as 20 g by Møller 1991). The relative testes masses of *Malurus* species are larger than those of almost every other species of comparable body mass.

DISCUSSION

Both the cloacal protuberance and testes of the Superb Fairy-Wren are very large in relation to its body mass. Large testes and sperm-storage structures suggest that male Superb Fairy-Wrens are anatomically adapted for frequent copulation, or for the transfer of large numbers of sperm per copulation. However, unlike all other species with relatively large cloacal protuberances and testes (e.g. Nakamura 1990, Birkhead et al. 1991, Briskie 1992), Superb Fairy-Wrens do not copulate at conspicuously high frequencies. Polygynandrous Dunnocks (*Prunella modularis*), for example, copulate about 14 times a day, female Alpine Accentors up to 32 times per day (Nakamura 1990), and Smith's Longspurs (*Calcarius pictus*) even more frequently (about 350 times per clutch; up to 7 times per hour during the morning; Briskie 1992). By contrast, we observed pair copulation less than 30 times, and extrapair copulation only twice in the course of hundreds of hours of systematic observations of female Superb Fairy-Wrens during mornings from 1987 through 1992. Most females in our study area have never been observed to copulate.

A similarly low rate of observed copulation has been reported for the closely related Splendid Fairy-Wren (*M. splendens*; Rowley and Russell 1990). Paradoxically, these species are known to have the highest recorded levels of extrapair fertilization among birds (Brooker et al. 1990, Mulder et al. unpubl. manuscript). For this reason, large cloacal protuberances and testes in

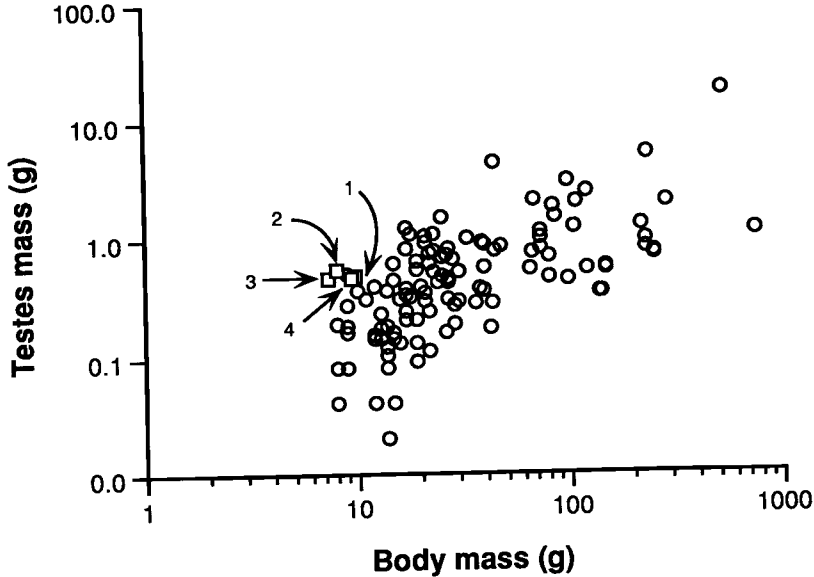


Fig. 3. Testes mass in passerines (adapted from Møller 1991), including four species of *Malurus* shown as squares: (1) *cyaneus*; (2) *leucopterus*; (3) *melanocephalus*; (4) *splendens*.

these species may be more important for frequent extrapair copulations rather than within-pair copulation attempts.

The evolution of large sperm storage organs for the purpose of extrapair copulation is likely because males may attempt frequent inseminations with numerous females. Furthermore, there is some evidence that extrapair fertilization could involve the transfer of larger numbers of sperm than within-pair copulation (Birkhead and Fletcher 1992, Birkhead and Møller 1993). This would also demand larger sperm reserves. The fact that extrapair copulations are typically secretive (Birkhead and Møller 1992) may make them much less likely to be observed. A less plausible alternative possibility is that within-pair copulations are equally common, but also secretive.

Young unpaired helper males also develop large cloacal protuberances. Our evidence from DNA fingerprinting shows that these males only rarely achieve fertilization in the group in which they live. However, they do engage in extragroup courtship. In at least two groups we observed, the subordinate male was observed in extragroup courtship attempts more frequently than the older dominant male from the group.

If protuberances functioned mainly to reduce

the risk of sperm depletion during repeated bouts of within-pair copulation (often employed by males as a paternity guard), then we would predict that the primary male in the group (who stays nearest the female during her fertile period) develops the largest cloacal protuberance. However, our results show that maximum sizes of protuberances during peak breeding vary little between males of differing ages and social status. In contrast, younger, subordinate males may develop their protuberances later, and they may regress earlier. Since development and regression of the testes is controlled by hormones (e.g. testosterone) that also play a role in the development of secondary sexual characters like colorful plumage, earlier regression of the testes in younger males is consistent with their earlier return to eclipse plumage.

The structure of the cloacal protuberance of male Superb Fairy-Wrens, particularly the pointed anterior tip of the protuberance, is unusual and has not been described before. The tip appears to be cartilaginous rather than erectile. Copulation occurs too rapidly (duration about 1 s) for us to observe the mechanics of sperm transfer, so we can only speculate about the function of this tip. Perhaps, like the intro-

mittent organs or phalluses of some other birds, it is inserted into the female's cloaca (Birkhead and Møller 1992). During short copulations, this might facilitate effective sperm transfer.

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