

Reproductive anatomy of male Southern Emu-wrens (*Stipiturus malachurus*) and Striated Grasswrens (*Amytornis striatus*)

Melissah Rowe^{A,B} and Stephen Pruett-Jones^A

^ADepartment of Ecology and Evolution, University of Chicago, 1101 East 57th Street, Chicago, Illinois 60637, USA.

^BCorresponding author. Email: melissah@uchicago.edu

Abstract. In birds, several features of male reproductive anatomy vary with the degree of promiscuity and the intensity of sperm competition. We describe the male reproductive anatomy of the Southern Emu-wren (*Stipiturus malachurus*) and the Striated Grasswren (*Amytornis striatus*) in the context of sperm competition. Male Southern Emu-wrens exhibited a very low relative testes size of just 0.6% of male body mass, lacked a cloacal protuberance and possessed a relatively small seminal glomera containing an average of just 12.6×10^6 sperm. Male Striated Grasswrens exhibited relatively small testes, comprising 1.78% of male body mass, and small cloacal protuberances. The relative size of the seminal glomera was also small and seminal glomera contained an average of 42.5×10^6 sperm. When compared with the well-studied fairy-wrens (*Malurus*) and based on male reproductive anatomy, it appears that emu-wrens experience a relatively low level, and grasswrens an intermediate level, of sperm competition.

Introduction

The reproductive biology and anatomy of a species often reflects competition, both within and between the sexes, for mating opportunities, fertilisation success and control of paternity. For example, in birds, colourful male plumage is often associated with mate attraction (Møller 1988a), whereas large testes and frequent copulation are associated with fertilisation success and sperm competition, which itself is influenced by the copulation behaviour of females (Møller 1991; Birkhead *et al.* 1993). Sperm competition refers to competition between the sperm from two or more males for fertilisation of a given set of ova (Parker 1970). Extra-pair paternity rates are commonly used as a measure of the intensity of sperm competition (Dunn *et al.* 2001; Pitcher *et al.* 2005) and several features of male avian reproductive anatomy vary with the rate of extra-pair paternity and the intensity of sperm competition. For example, species experiencing more intense sperm competition typically have relatively larger testes, cloacal protuberances, seminal glomera, and numbers of stored sperm than those species experiencing lower levels of competition (Møller 1988b, 1991; Birkhead *et al.* 1993; Møller and Briskie 1995; Tuttle *et al.* 1996).

Emu-wrens (*Stipiturus*) and grasswrens (*Amytornis*) are small, insectivorous passerines belonging to the family Maluridae. In Australia, the Maluridae also include the closely related fairy-wrens (*Malurus*), species known to exhibit high rates of extra-pair paternity and several anatomical and behavioural adaptations associated with intense sperm competition (Brooker *et al.* 1990; Mulder and Cockburn 1993; Tuttle *et al.* 1996; Dunn and Cockburn 1998, 1999; Karubian 2002; Webster *et al.* 2004; Rowe *et al.* In press). Although fairy-wrens, emu-wrens and grasswrens are similar in several aspects of their breeding biology, including social monogamy and facultative

cooperative breeding (Rowley and Russell 1997; Maguire and Mulder 2004), rather little is known of the reproductive anatomy of emu-wrens and grasswrens compared to that of the fairy-wrens (Rowe and Pruett-Jones 2006).

Three species of emu-wren are currently recognised, all of which exhibit a high degree of habitat specificity (Rowley and Russell 1997). Emu-wrens are the smallest of the malurids (5.4–7.5 g) and all species are sexually dichromatic, with adult males having a pale-blue chin and throat and females a tawny chin and throat (Rowley and Russell 1997). In contrast to other malurids, sexual dichromatism in throat colour may be apparent in nestlings as young as 5 days old and is maintained year-round during the breeding and non-breeding seasons (Fletcher 1915; Rowley and Russell 1997; Maguire and Mulder 2004). In the Southern Emu-wren (*Stipiturus malachurus*), the one species for which detailed studies have been conducted (Maguire and Mulder 2004), the frequency of cooperative breeding is low in comparison with other malurids, with only 8% of groups ($n = 48$) having helpers at the nest. Helpers were always male and were shown to contribute to provisioning of nestlings and fledglings. Maguire and Mulder (2004) also reported the absence of extra-pair courtship displays, low annual survival rates, and the lack of a cloacal protuberance in this species, and suggested that extra-pair fertilisations, and hence sperm competition, might be uncommon. Subsequent microsatellite analysis has shown that extra-pair paternity is indeed low, with just 12% of offspring ($n = 50$) being sired by extra-pair males, and 15% of nests containing extra-pair young (Maguire 2005).

Grasswrens are the largest of the malurids (17–35 g) (Rowley and Russell 1997). Currently, nine species are recognised (Christidis 1999) and, in contrast to the bright plumage

exhibited by male fairy-wrens and emu-wrens, all species of grasswren are relatively drab and cryptically plumaged. Additionally, females are somewhat brighter than males, having stronger rufous plumage on the flanks and belly (Rowley and Russell 1997). As in emu-wrens, the frequency of cooperative breeding in grasswrens appears to be low (Noske 1992; Johnstone and Kolichis 1999; Karubian 2001). Striated Grasswrens (*Amytornis striatus*) have fairly small cloacal protuberances and low volumes and concentrations of sperm, which suggest low levels of sexual promiscuity and little sperm competition among males (Karubian 2001). To date, however, there has been no analysis of paternity in the Striated Grasswren or any other species of grasswren.

The aim of this paper is to describe the features of male reproductive anatomy in Southern Emu-wrens and Striated Grasswrens and to discuss these features in the context of sperm competition. Additionally, we discuss the reproductive anatomy of these species with reference to the extreme levels of sperm competition experienced by the closely related fairy-wrens.

Materials and methods

Three male Striated Grasswrens and six male Southern Emu-wrens were used this study. Striated Grasswrens were collected from Pooginook and Cooltong Conservation Parks, near Berri, South Australia (34°16'S, 140°35'E), during October and November 2005, and Southern Emu-wrens were collected from the Smiths Lake region, New South Wales (32°22'S, 152°28'E), during October 2006. We trapped birds by herding focal individuals into mist-nets with the aid of playback of song. All individuals were confirmed to be in breeding condition, based upon their behaviour, anatomy and physiology. Upon capture, individuals were weighed using a Pesola spring balance (to nearest 0.1 g), and morphological measurements taken. We measured the length (L) (distance between the cloacal vent and posterior edge of the protuberance, excluding the cloacal tip), width (W) and depth (D) of the cloacal protuberance, and the length and width (at base) of the cloacal tip (if present). Volume of the cloacal protuberance was estimated as (following Tuttle *et al.* 1996):

$$\text{volume} = \pi (D/2 \times W/2) \times L,$$

and cloacal protuberance volume index as (following Birkhead *et al.* 1991):

$$\text{volume index} = (L \times W \times D) / \text{body mass}.$$

Ejaculate semen samples were collected from Striated Grasswrens using standard cloacal massage techniques (Quinn and Burrows 1936; Tuttle *et al.* 1996; Gee *et al.* 2004). We refer to the samples collected as ejaculate samples. Although we do not know how ejaculate samples relate to natural ejaculates, these data provide a comparison to the other closely related species for which similar ejaculate samples have been collected via cloacal massage. Exuded semen was collected in micro-capillary tubes, transferred to micro-centrifuge tubes containing a known volume of Lago Formulation Avian Semen Extender (Hygieia Biological Laboratories, Woodland, CA, USA) and mixed thoroughly. We were unable to collect ejaculate samples from Southern Emu-wrens as they did not have a cloacal protuberance. Following collection of ejaculate samples and mor-

phological measurements, individuals were euthanased via injection of an overdose of pentobarbitone sodium (5 mL per 10 kg) in accordance with animal ethics approval (see below) and under licence to possess a Schedule 4 drug for administration (see below). Use of museum specimens was considered, but suitable specimens of Striated Grasswrens and Southern Emu-wrens were limited, and the use of preserved tissue to measure testis weight suffers from several sources of error (Calhim and Birkhead 2007). In addition, museum specimens cannot provide information regarding numbers or morphology of sperm in seminal glomera and ejaculates.

We dissected out the testes, seminal glomera, and ductus deferens from all birds. We measured length, width and fresh weight of the left and right testis. Relative testes size was calculated as the combined mass of the left and right testis expressed as a percentage of male body mass. For each individual, we also measured length, width and fresh weight of the left and right seminal glomerus. Relative seminal glomera mass was calculated as the combined mass of the left and right seminal glomerus expressed as a percentage of male body mass. To determine the concentration of sperm in the seminal glomera and the total number of stored sperm, we flushed the sperm from each glomerus with a known volume of avian semen extender and stored diluted semen samples in micro-centrifuge tubes. All measurements were made with digital calipers (to nearest 0.1 mm) and all weights determined using an electronic balance (Ohaus Navigator, to nearest 0.01 g).

The concentration of sperm in ejaculate samples and within the seminal glomera was determined using a calibrated Makler counting chamber (Irvine Scientific, Santa Ana, CA, USA). All samples were mixed thoroughly before counting. Ten microlitres of diluted semen (sperm and semen extender) were placed in the counting chamber and the density of sperm cells in each of 10 grid-squares was counted, representing the concentration of sperm ($\times 10^6$) per millilitre. We replicated the counts using separate aliquots of the diluted semen sample, and calculated an average sperm concentration based on the two replicates. To calculate the total sperm count in an ejaculate sample (Striated Grasswrens only) we corrected the sperm concentration values by the dilution factor, converted this value into a concentration per microlitre, and multiplied the concentration by the volume of the ejaculate. To calculate the total number of sperm stored in the seminal glomera (following ejaculate sample collection) we corrected the sperm concentration values by the dilution factor, converted this value into a concentration per microlitre, and combined the total counts for the left and right seminal glomerus. For the Striated Grasswren, we estimated the total numbers of sperm in the seminal glomera before collection of the ejaculate sample by summing the number of sperm in the seminal glomera and ejaculate sample.

Finally, we measured the total length of ten haphazardly chosen and morphologically normal sperm from each individual using phase contrast microscopy at 100 \times magnification and an ocular micrometer. Length of sperm was measured using sperm from ejaculate samples in grasswrens and sperm collected from the seminal glomera sperm stores in emu-wrens. We calculated the average length of sperm (to the nearest 0.5 μ m) per individual and an average length of sperm for both the Southern Emu-wren and Striated Grasswren.

All methodologies were approved by The University of Chicago Animal Care and Use Committee (ACUP# 71453), as well as the Department of Environment and Heritage (South Australia) Wildlife Ethics Committee (Project No. 13/2004), and the Director-General of New South Wales Department of Primary Industries Animal Care and Ethics Committee (Trim File No. 06/3846). Study specimens of the Southern Emu-wren were accessioned in the collection of the Australian Museum (Sydney), and specimens of Striated Grasswren were accessioned in the collection of the South Australian Museum (Adelaide). All statistics were performed using the R (2.4.1) software package (R Development Core Team 2006). Standard errors of the mean (\pm s.e.) were calculated for all results.

Results

Striated Grasswren

Striated Grasswrens possessed a bulbous-type cloacal protuberance with a prominent cloacal tip at the ventral edge. Cloacal protuberances were bare, except for a circle of feathers surrounding the cloacal vent (cloacal circlet). As shown by dissection, the cloacal protuberance consisted primarily of the paired seminal glomera. The mean volume of the cloacal protuberance was $94.15 \pm 9.5 \text{ mm}^3$ (range 80.4–112.3 mm^3) and the mean volume index was $6.25 \pm 0.7 \text{ mm}^3 \text{ g}^{-1}$. The mean length of the cloacal tip was $3.4 \pm 0.4 \text{ mm}$ and mean area of the tip was $4.89 \pm 1.0 \text{ mm}^2$. There was a significant negative correlation between male body mass and the volume of the cloacal protuberance ($r = -0.99$, $n = 3$, $P = 0.047$).

The sperm of Striated Grasswrens were typical of passerine sperm, with a total length of $80.1 \pm 0.4 \mu\text{m}$. The mean number of sperm present in ejaculate samples was $6.6 \pm 2.2 \times 10^6$ and the mean concentration of sperm was $3.7 \pm 0.9 \times 10^6$ sperm per μL^{-1} . We found no relationship between the concentration of sperm and total sperm counts in ejaculate samples, or between male body mass and concentration of sperm or total sperm counts in ejaculate samples (all $P > 0.1$). Similarly, there was no relationship between volume of the cloacal protuberance and concentration of sperm, total sperm count in ejaculate samples, or total numbers of stored sperm (all $P > 0.1$).

The mean mass of the left and right seminal glomera was $0.022 \pm 0.004 \text{ g}$ and $0.023 \pm 0.003 \text{ g}$ respectively. The mean mass of both seminal glomera combined was $0.045 \pm 0.005 \text{ g}$, representing just 0.24% of male body mass. The average number of sperm remaining in the seminal glomera after collection of ejaculate samples was $35.9 \pm 8.2 \times 10^6$ and the average total number of stored sperm was $42.5 \pm 6.1 \times 10^6$ (seminal glomera sperm count plus ejaculate sperm count). Therefore, an ejaculate sample represented 15.5% of the total sperm available in the seminal glomera. For the left and right testis respectively, the mean length of the testis was $8.4 \pm 0.3 \text{ mm}$ and $7.9 \pm 0.2 \text{ mm}$ and the mean width $6.1 \pm 0.2 \text{ mm}$ and $6.6 \pm 0.1 \text{ mm}$. The average mass of left and right testis was $0.16 \pm 0.007 \text{ g}$ and $0.18 \pm 0.002 \text{ g}$, respectively, and the average combined testes mass $0.34 \pm 0.01 \text{ g}$, representing 1.78% of male body mass.

Southern Emu-wren

In contrast to the Striated Grasswren, the Southern Emu-wren lacked a cloacal protuberance and cloacal tip. The total length of

sperm of Southern Emu-wrens was $75.2 \pm 1.1 \mu\text{m}$ and their morphology was also typical of passerine sperm. The average mass of the left and right seminal glomera was $0.005 \pm 0.001 \text{ g}$ and $0.006 \pm 0.001 \text{ g}$ respectively. The mean mass of both seminal glomera combined was $0.01 \pm 0.002 \text{ g}$, representing just 0.14% of male body mass. The average number of sperm in the seminal glomera, and thus the total available stored sperm, was $12.6 \pm 4.6 \times 10^6$. For the left and right testis respectively, the mean length of the testis was $4.3 \pm 0.3 \text{ mm}$ and $4.0 \pm 0.2 \text{ mm}$ and the mean width was $3.3 \pm 0.1 \text{ mm}$ and $3.2 \pm 0.07 \text{ mm}$. The average mass of left and right testis was $0.024 \pm 0.003 \text{ g}$ and $0.021 \pm 0.002 \text{ g}$, respectively, and the average combined testes mass $0.045 \pm 0.004 \text{ g}$, representing just 0.6% of male body mass.

Discussion

The sperm of passerines have a helical form and a midpiece comprising a mitochondrial helix twisted around the tail (McFarlane 1963, 1971). This basic sperm phenotype appears to be highly conserved in passerines and the sperm of both the Striated Grasswren and the Southern Emu-wren were consistent with this phenotype. In the Striated Grasswren the values of cloacal protuberance volume, sperm concentration and sperm counts of ejaculate samples found in this study generally agree with those reported previously for a different population (Karubian 2001). In contrast to previous work, however, we found a significant negative correlation between male body mass and volume of the cloacal protuberance, with heavier males having smaller protuberances. The volume of the cloacal protuberance has been shown to correlate positively with body mass in Superb Fairy-wrens (*Malurus cyaneus*) (Mulder and Cockburn 1993), but is uncorrelated with body mass in Splendid (*M. splendens*), Variegated (*M. lamberti*) and White-winged (*M. leucopterus*) Fairy-wrens (Tuttle *et al.* 1996). While the results of our study should be viewed with caution owing to the small sample size, it appears that the relationship between the volume of the cloacal protuberance and body size may vary across species.

Both the cloacal protuberance and seminal glomera of the Striated Grasswren were small in relation to body mass when compared with other malurid species (Table 1). The presence of a cloacal protuberance has also been reported in the Thick-billed Grasswren (*A. textiles*) (Rowley and Russell 1997). In that species, the volume of the cloacal protuberance is relatively small, with a mean volume index of $6.39 \pm 0.9 \text{ mm}^3 \text{ g}^{-1}$ ($n = 4$; B. Cale, unpubl. data). The testes of the Striated Grasswren were also relatively small at just 1.78% of male body mass (Table 1). Additionally, when compared with the fairy-wrens, the mean number of sperm stored within the seminal glomera and the total number of sperm in ejaculate samples of Striated Grasswrens were also relatively low (Table 1).

The mean number of sperm within the seminal glomera of Striated Grasswrens was, however, comparable to the intermediate values observed in some non-malurid species, including those species exhibiting a larger cloacal protuberance (e.g. Chaffinch (*Fringilla coelebs*); Sheldon and Birkhead 1994). The Striated Grasswren, however, had shorter sperm than the Chaffinch (sperm length 252.5 μm ; Sheldon and Birkhead 1994) (Table 1). The spermatozoa size hypothesis predicts that species with shorter sperm have relatively smaller cloacal pro-

Table 1. Aspects of male reproductive anatomy in the Southern Emu-wren and Striated Grasswren in comparison with similar data for representative species of fairy-wrens (*Malurus*)
 Samples sizes from this study are presented in Materials and methods (above); sample sizes for other data vary, and are found in the literature sources. EPP, extra-pair paternity

Species	Cloacal protruberance volume index (mm ³ g ⁻¹)	Relative glomera mass (% of male body mass)	Number of stored sperm	Relative testis size (% of male body mass)	Numbers of ejaculate sperm	Length of sperm (µm)	EPP % young broods (% broods)	Reference
Southern Emu-wren	0	0.14	12.6 × 10 ⁶	0.62	na	75.2	12 (15)	This study, except for rates of EPP from Maguire (2005)
Striated Grasswren	6.25	0.24	42.5 × 10 ⁶	1.78	6.6 × 10 ⁶	80.1		This study
Superb Fairy-wren	26.7			4.9			76 (95)	Mulder and Cockburn (1993), Mulder <i>et al.</i> (1994)
Superb Fairy-wren (<i>M. c. cyanocephalus</i>)	15.1	1.06	318.69 × 10 ⁶	3.27	33.36 × 10 ⁶			Rowe and Pruett-Jones (2006), Rowe <i>et al.</i> (in press)
Splendid Fairy-wren	16.2	1.03	276.47 × 10 ⁶	3.4	58.13 × 10 ⁶	83.1	55.4 (42)	Tuttle <i>et al.</i> (1996), Webster <i>et al.</i> (2004), Rowe and Pruett-Jones (2006)
Red-backed Fairy-wren	21.1	0.65	120.66 × 10 ⁶	2.95	50.86 × 10 ⁶		74.5 (56)	Karubian (2002), Rowe and Pruett-Jones (2006), Rowe <i>et al.</i> (in press)

tuberances (Birkhead *et al.* 1993). Comparative studies provide some support for this hypothesis and suggest that sperm length may account for a portion of the interspecific variation in cloacal protuberance size (Birkhead *et al.* 1993). Additionally, across species, numbers and length of sperm are often negatively correlated (Parker 1982; Tuttle *et al.* 1996). Therefore, the numbers of sperm stored in the seminal glomera of Striated Grasswren may be comparable to values in the Chaffinch because the Grasswren has shorter sperm.

The lack of a cloacal protuberance in male Southern Emu-wrens has been reported previously (Maguire and Mulder 2004), and we found the same result in a different population. The Mallee Emu-wren (*S. mallee*) and the Rufous-crowned Emu-wren (*S. ruficeps*) also appear to lack a cloacal protuberance (S. Brown and P. Horton, respectively, pers. comm.). In contrast to the other malurids, the relative size of the seminal glomera and the mean number of sperm stored within the seminal glomera in the Southern Emu-wren was very low (Table 1). Similarly, at just 0.6% of male body mass, the testes of the Southern Emu-wren were also very small (Table 1). In fact, the relative testes size of the Southern Emu-wren is among the lowest recorded, with only the Eurasian Bullfinch (*Pyrrhula pyrrhula*) reported to have a smaller relative testes size (0.29%; Birkhead *et al.* 2006).

The fairy-wrens are the closest taxonomic relatives of the emu-wrens and grasswrens. Some species of fairy-wrens have extremely high rates of extra-pair paternity, with up to 95% of all broods containing at least one extra-pair offspring and up to 76% of all offspring sired by extra-pair males (Brooker *et al.* 1990; Mulder *et al.* 1994; Dunn and Cockburn 1998, 1999; Karubian 2002; Webster *et al.* 2004). Fairy-wrens appear to exhibit intense sperm competition and consequently, males have several morphological and anatomical adaptations that reflect this intense level of sperm competition (Mulder and Cockburn 1993; Tuttle *et al.* 1996; Rowe and Pruett-Jones 2006; Rowe *et al.* In press). In fairy-wrens the cloacal protuberance, seminal glomera and the numbers of sperm stored within the seminal glomera, the testes, and the total number of sperm in ejaculate samples are all very large in relation to male body mass (Table 1).

This study provides the first comprehensive description of the reproductive anatomy of male emu-wrens and grasswrens. Two lines of evidence suggest that the intensity of sperm competition in emu-wrens is low. First, the rates of extra-pair paternity reported in the Southern Emu-wren are low, with just 12% of offspring sired by extra-pair males (Maguire 2005). Second, the relatively small testes, seminal glomera and sperm stores of Southern Emu-wrens and the lack of a cloacal protuberance in each of the three species of emu-wren suggest that male emu-wrens are anatomically adapted to low levels of sperm competition. Similarly, the reproductive anatomy of male grasswrens suggests adaptation to intermediate levels of sperm competition (i.e. higher than in the emu-wren but considerably lower than in the fairy-wren species). Consequently, the species of Maluridae appear to experience varying intensities of sperm competition: from low levels in the emu-wrens, low to intermediate in the grasswrens and some fairy-wrens, to extreme levels of sperm competition in other species of fairy-wren. Integrating these data into knowledge of the exact mating systems of species of

grasswrens and emu-wrens must await additional detailed paternity analyses and behavioural studies, but we anticipate that such data will confirm our interpretation that emu-wrens and grasswrens represent species at low and intermediate positions on a spectrum of reproductive promiscuity levels, at least in comparison with fairy-wrens.

Acknowledgements

We are grateful for the assistance and advice from Christina Rockwell, Wen Shen, Lionel Filewood, Jody Gates, Philippa Horton, David Hair, and David Turner. We thank Sarah Brown, Philippa Horton, and Jaynia Tarnawski for helping us with information regarding cloacal protuberances in emu-wrens and grasswrens. We are especially grateful to Belinda Cale for the cloacal protuberance data for the Thick-billed Grasswren. We also thank Trevor Price, Jill Mateo, and one anonymous reviewer for helpful comments on this manuscript. This research was supported by a National Science Foundation grant (to SP-J) and a Hinds Fund grant from the University of Chicago (to MR). All procedures were undertaken with approval from The University of Chicago Animal Care and Use Committee (ACUP# 71453). All work on the Striated Grasswren was approved by the Department of Environment and Heritage (South Australia) Wildlife Ethics Committee (Project No. 13/2004) and was conducted under a scientific permit (Q24832) and AW licence (No. 142). All work undertaken on the Southern Emu-wren was approved by the Director-General of New South Wales Department of Primary Industries Animal Care and Ethics Committee (Trim File No. 06/3846) and was conducted under a NSW NPWS scientific licence (S12048). Animals were administered pentobarbitone sodium under a permit to possess an S4 drug for administration (2006-60012). Finally, export of samples from Australia was approved by the Australian Government Department of Environment and Heritage (WT2005-10120 and WT2006-10958).

References

- Birkhead, T. R., Hatchwell, B. J., and Davies, N. B. (1991). Sperm competition and the reproductive organs of the male and female Dunnock *Prunella Modularis*. *Ibis* **133**, 306–311.
- Birkhead, T. R., Briskie, J. V., and Møller, A. P. (1993). Male sperm reserves and copulation frequency in birds. *Behavioral Ecology and Sociobiology* **32**, 85–93. doi:10.1007/BF00164040
- Birkhead, T. R., Immler, S., Pellatt, E. J., and Freckleton, R. (2006). Unusual sperm morphology in the Eurasian Bullfinch (*Pyrrhula pyrrhula*). *Auk* **123**, 383–392. doi:10.1642/0004-8038(2006)123[383:USMITE]2.0.CO;2
- Brooker, M. G., Rowley, I., Adams, M., and Baverstock, P. R. (1990). Promiscuity: an inbreeding avoidance mechanism in a socially monogamous species. *Behavioral Ecology and Sociobiology* **26**, 191–200.
- Calhim, S., and Birkhead, T. R. (2007). Testes size in birds: quality versus quantity – assumptions, errors, and estimates. *Behavioral Ecology* **18**, 271–275.
- Christidis, L. (1999). Evolution and biogeography of the Australian grasswrens, *Amytornis* (Aves: Maluridae): biochemical perspectives. *Australian Journal of Zoology* **47**, 113–124. doi:10.1071/ZO98054
- Dunn, P. O., and Cockburn, A. (1998). Costs and benefits of extra-group paternity in Superb Fairy-wrens. In 'Avian Reproductive Tactics: Female and Male Perspectives'. (Eds P. G. Parker and N. T. Burley.) pp. 147–161. (American Ornithologists Union: Washington, D.C.)
- Dunn, P. O., and Cockburn, A. (1999). Extrapair mate choice and honest signaling in cooperatively breeding superb fairy-wrens. *Evolution* **53**, 938–946. doi:10.2307/2640733
- Dunn, P. O., Whittingham, L. A., and Pitcher, T. E. (2001). Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution* **55**, 161–175.
- Fletcher, J. A. (1915). Further field notes on the emu-wren (*Stipiturus malachurus*). *Emu* **14**, 213–217.

- Gee, G. F., Bertschinger, H., Donoghue, A. M., Blanco, J., and Solet, J. (2004). Reproduction in nondomestic birds: physiology, semen collection, artificial insemination and cryopreservation. *Avian and Poultry Biology Reviews* **15**, 47–101.
- Johnstone, R. E., and Kolichis, N. (1999). First description of the nest and eggs of the Black Grasswren *Amytornis housei* (Milligan) with notes on breeding. *Records of the Western Australian Museum* **19**, 259–265.
- Karubian, J. (2001). The social organization and mating system of the Striated Grasswren. *Condor* **103**, 412–417. doi:10.1650/0010-5422(2001)103[0412:TOSAMS]2.0.CO;2
- Karubian, J. (2002). Costs and benefits of variable breeding plumage in the red-backed fairy-wren. *Evolution* **56**, 1673–1682.
- McFarlane, R. W. (1963). The taxonomic significance of avian sperm. *Proceedings of the International Ornithological Congress* **13**, 91–102.
- McFarlane, R. W. (1971). The ultrastructure and phylogenetic significance of avian spermatozoa. Ph.D. Thesis, The University of Florida, Gainesville, FL.
- Maguire, G. S. (2005). Behavioural ecology of the Southern Emu-wren (*Stipiturus malachurus*). Ph.D. Thesis, The University of Melbourne, Melbourne.
- Maguire, G. S., and Mulder, R. A. (2004). Breeding biology and demography of the southern emu-wren (*Stipiturus malachurus*). *Australian Journal of Zoology* **52**, 583–604. doi:10.1071/ZO04043
- Møller, A. P. (1988a). Female choice selects for male sexual tail ornaments in the monogamous swallow. *Nature* **332**, 640–642. doi:10.1038/332640a0
- Møller, A. P. (1988b). Testes size, ejaculate quality and sperm competition in birds. *Biological Journal of the Linnean Society* **33**, 273–283. doi:10.1111/j.1095-8312.1988.tb00812.x
- Møller, A. P. (1991). Sperm competition, sperm depletion, paternal care, and relative testis size in birds. *American Naturalist* **137**, 882–906. doi:10.1086/285199
- Møller, A. P., and Briskie, J. V. (1995). Extra-pair paternity, sperm competition and the evolution of testis size in birds. *Behavioral Ecology and Sociobiology* **36**, 357–365.
- Mulder, R. A., and Cockburn, A. (1993). Sperm competition and the reproductive anatomy of male superb fairy-wrens. *Auk* **110**, 588–593.
- Mulder, R. A., Dunn, P. O., Cockburn, A., Lazenby-Cohen, K. A., and Howell, M. J. (1994). Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **255**, 223–229. doi:10.1098/rspb.1994.0032
- Noske, R. A. (1992). The status and ecology of the white-throated grasswren *Amytornis woodwardi*. *Emu* **92**, 39–51.
- Parker, G. A. (1970). Sperm competition and its evolutionary consequences in the insects. *Biological Reviews* **45**, 525–567. doi:10.1111/j.1469-185X.1970.tb01176.x
- Parker, G. A. (1982). Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *Journal of Theoretical Biology* **96**, 281–294. doi:10.1016/0022-5193(82)90225-9
- Pitcher, T. E., Dunn, P. O., and Whittingham, L. A. (2005). Sperm competition and the evolution of testes size in birds. *Journal of Evolutionary Biology* **18**, 557–567. doi:10.1111/j.1420-9101.2004.00874.x
- Quinn, J. P., and Burrows, W. H. (1936). Artificial insemination of fowls. *Journal of Heredity* **27**, 31–37.
- R Development Core Team (2006). 'R: A Language and Environment for Statistical Computing.' (R Foundation for Statistical Computing: Vienna, Austria.) ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Rowe, M., and Pruett-Jones, S. (2006). Reproductive biology and sperm competition in Australian fairy-wrens. *Avian and Poultry Biology Reviews* **17**, 21–37.
- Rowe, M., Bakst, M. R., and Pruett-Jones, S. (in press). Good vibrations? Structure and function of the cloacal tip of male Australian Maluridae. *Journal of Avian Biology*, in press.
- Rowley, I., and Russell, E. (1997). 'Fairy-wrens and Grasswrens: Maluridae.' (Oxford University Press: Oxford, UK.)
- Sheldon, B. C., and Birkhead, T. R. (1994). Reproductive anatomy of the Chaffinch in relation to sperm competition. *Condor* **96**, 1099–1103. doi:10.2307/1369121
- Tuttle, E. M., Pruett-Jones, S., and Webster, M. S. (1996). Cloacal protuberances and extreme sperm production in Australian fairy-wrens. *Proceedings of the Royal Society of London. Series B.* **263**, 1359–1364. doi:10.1098/rspb.1996.0199
- Webster, M. S., Tarvin, A., Tuttle, E. M., and Pruett-Jones, S. (2004). Reproductive promiscuity in the splendid fairy-wren: effects of group size and auxiliary reproduction. *Behavioral Ecology* **15**, 907–915. doi:10.1093/beheco/arih093

Manuscript received 28 September 2007, accepted 12 February 2008