

# Reproductive biology and sperm competition in Australian fairy-wrens

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## ABSTRACT

In birds, the outcome of sperm competition is determined by the relative timing and order of matings, sperm numbers, and the differential fertilizing capacity of male ejaculates. The intensity of sperm competition varies across species according to mating system, specifically the copulation behavior of females. The Australian Maluridae (fairy-wrens, grasswrens, and emu-wrens) exhibit a diversity of reproductive behaviors and adaptations thought to be associated with sperm competition. Australian malurids live in sedentary social groups and reside on temporally stable territories. The species are socially monogamous, with males and females forming multi-year pair bonds, and exhibit facultative cooperative breeding. In contrast to the relative conservatism of social systems, the malurid genera vary widely in reproductive behaviors. Fairy-wrens (*Malurus*) exhibit some of the highest known rates of reproductive promiscuity for socially monogamous species, and males possess many anatomical and behavioral adaptations associated with sperm competition including relatively large testes and sperm stores. In contrast, male grasswrens (*Amytornis*) and emu-wrens (*Stipiturus*) show relatively small testes and sperm stores. These differences are likely a consequence of relatively lower rates of reproductive promiscuity in the grasswrens and emu-wrens. Fairy-wrens and the other malurids illustrate the complex interactions between social and reproductive behaviors and the intensity of sperm competition and sexual selection in birds.

**Keywords:** Maluridae, fairy-wren, *Malurus*, sperm competition, extra-pair paternity, promiscuity

## INTRODUCTION

Sexual selection is a mechanism generating evolutionary change in traits related to reproductive success, in particular exaggerated traits in males that may reduce survivorship (Darwin, 1871). As a consequence of anisogamy (production of dissimilar sized gametes), variance in reproductive success is asymmetric between the sexes, typically being greater among males than females (Bateman's Principle; Bateman, 1948; Arnold, 1994). In males, reproductive success is primarily related to the number of reproductive partners individuals obtain. Furthermore, in terms of reproductive success, fitness in males is relatively unaffected by indiscriminate matings in that males lose only the time and energy expended in those matings. Indiscriminate matings can, however, significantly reduce reproductive success in females via lost reproductive opportunities

or reduced fitness in offspring. In contrast to males, females generally increase their reproductive success by securing resources (not mates), mating with males that provide such resources, or by mating with quality males (Reynolds, 1996; Jennions and Petrie, 2000).

As a general rule, females invest more in reproduction than males. Consequently, females are a resource over which males actively compete. Such competition among males can be direct (*i.e.* interference competition) and/or indirect (*i.e.* exploitation competition), can occur both pre- and post-copulation, and can involve aspects of morphology, behavior, and physiology. Competition among males can also serve the interests of females by insuring that only the most vigorous males obtain mating opportunities or that the sperm of the 'best' males fertilize the females' eggs. Indeed, females may even incite male-male competition (Cox and Le Boeuf, 1977).

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Commonly, the reproductive interests of males and females differ, leading to conflicting selective pressures for paternity control both between males and between the sexes. The resolution of this reproductive conflict of interest will vary with both the evolutionary history and ecology of a species and will to a large extent influence a species mating system.

One line of evidence suggesting how complex the reproductive conflict of interest can be is the discrepancy between the social mating systems and the reproductive mating systems of organisms. The social mating system describes the social associations among males and females, *i.e.* who lives with whom. In contrast, the reproductive or genetic mating system describes the patterns of copulation and parentage among males and females, *i.e.* who is having offspring with whom. In birds, approximately 90% of species are socially monogamous with males and females forming pair bonds and frequently exhibiting joint parental care. However, reproductive monogamy occurs in only 14% of surveyed passerines with the remaining species exhibiting varying levels of reproductive promiscuity (in this case promiscuity does not necessarily imply indiscriminate mating) (Griffith *et al.*, 2002). It now appears that extra-pair (or extra-group) copulations, and the resulting extra-pair paternity, are common in socially monogamous birds. For males, extra-pair copulations serve as a means of securing additional mates and further benefit the male because the resulting fertilizations do not incur the cost of parental care. For females, extra-pair copulations serve as a means of choosing ‘better’ males to sire, but not raise, their offspring.

When females mate with multiple males during a single reproductive episode, the sperm from these males may simultaneously reside in the female’s reproductive tract and compete for fertilization of ova. This form of post-copulatory sexual selection is referred to as sperm competition (Parker, 1970). Sperm competition is a significant component of sexual selection in birds, involving anatomical, physiological, and behavioral processes.

In this paper we briefly review the mechanisms and consequences of sperm competition in birds and then focus on the reproductive biology of Maluridae, in particular the fairy-wrens, a family of Australasian birds that illustrates the complex interaction between sperm competition, morphology, physiology, and behavior.

## SPERM COMPETITION IN BIRDS

Sperm competition occurs when females mate with two or more males, whose sperm compete for fertilization opportunities (Parker, 1970). The intensity of sperm competition varies with the degree of reproductive promiscuity in females. As the number of males an individual female copulates with increases, or as the mean number of males that an average female in a population copulates with increases, so does the likelihood and intensity of sperm competition.

In birds, paternity success follows a ‘raffle principle’ in which the male with numerical dominance of sperm gains a greater share of paternity (Parker, 1990a,b). More specifically, paternity success is determined by: (1) the timing of inseminations, relative to competitive matings, fertilization, and oviposition; (2) sperm numbers; and (3) differential fertilizing capacity of ejaculates (Birkhead and Pizzari, 2002). Typically it is the last insemination a female receives that fertilizes the majority of subsequent offspring, a phenomenon referred to as last male sperm precedence.

Last male precedence is thought to occur primarily because of passive sperm loss, whereby sperm passively leak from storage sites in the female reproductive tract (Wishart, 1987; Birkhead *et al.*, 1995a; Colegrave *et al.*, 1995; Birkhead, 1998; Birkhead and Biggins, 1998). As a result, the number of sperm available to fertilize successive ova decreases with time since copulation. Consequently, the last male to inseminate a female will have a numerical advantage in terms of sperm numbers and subsequent fertilizations relative to any previous male.

Males can mediate the effects of sperm competition through a variety of strategies, including mate guarding (Birkhead, 1988; Møller and Birkhead, 1991), transferring greater numbers of sperm during copulation (Møller, 1988, 1991), timing their copulations relative to ovulation in females (Birkhead, 1998), or increasing the fertilizing capacity of ejaculates (Birkhead *et al.*, 1995b). Differential fertilizing capacity describes the relative ability of sperm from different males to fertilize ova in a competitive environment (Lanier *et al.*, 1979). Several sperm traits influence fertilizing capacity, including mobility, swimming velocity, viability, length, and morphology (Birkhead and Petrie, 1995; Birkhead *et al.*, 1995b; Birkhead *et al.*, 1999; Donoghue *et al.*, 1999; Blount *et al.*, 2001; Hemberger *et al.*, 2001; Froman *et al.*, 2002; Wishart *et al.*, 2002).

Although sperm competition can occur whenever females mate with multiple males in a single reproductive episode, our specific concern here is with sperm competition in socially monogamous species when extra-pair (or extra-group) fertilizations occur. In socially monogamous species, rates of extra-pair fertilization are typically quantified with respect to the female as rates of extra-pair paternity, expressed as either the proportion of offspring resulting from extra-pair copulation or the proportion of females producing offspring sired by a male other than their social mate. Across species, rates of extra-pair paternity (proportion of offspring) vary from 0% to more than 70% (Griffith *et al.*, 2002). Because extra-pair paternity rates reflect varying rates of multiple mating by females, levels of extra-pair paternity are commonly used as a measure of the intensity of sperm competition (Dunn *et al.*, 2001; Pitcher *et al.*, 2005).

The consequences of sperm competition are diverse, particularly for males. For example, relative testes size is larger in species that experience intense sperm competition (Møller and Briskie, 1995). Larger testes produce more sperm allowing males to inseminate females either with many sperm in few copulations or smaller numbers of sperm over frequent copulations (Brillard and de Reviere, 1981; Møller, 1988). Males of species experiencing sperm competition also exhibit a relatively large cloacal protuberance, the site of sperm storage. The cloacal protuberance projects from the body surface of the male as a result of the enlargement of the paired seminal glomerus (the distal portion of the ductus deferens) (Birkhead, 1988; Birkhead *et al.*, 1993).

Sperm competition is a component of sexual selection and contributes to variance in reproductive success in males. Males possessing traits that increase paternity success will experience a fitness advantage and as such there will be strong selection for these traits. Such traits may include sperm number and morphology, the fertilizing capacity of ejaculates, aspects of seminal fluid, and behavioral traits related to copulation or mate attraction.

## FAIRY-WRENS

Distributed throughout Australia and New Guinea, the Maluridae are a family of passerine birds belonging to the 'old' endemics, groups with Gondwanan origin and subsequent *in situ* diversification. In New Guinea, the malurid genera include fairy-wrens (*Malurus*), tree-

wrens (*Sipodotus*) and russet-wrens (*Clytomias*). In Australia, the malurid genera include fairy-wrens, grasswrens (*Amytornis*), and emu-wrens (*Stipiturus*) (Rowley and Russell, 1997). The phylogeny of Maluridae is reasonably well established (Christidis and Schodde, 1997). The genera *Malurus*, *Amytornis*, and *Stipiturus* represent monophyletic lineages, whereas the phylogenetic positions of *Sipodotus* and *Clytomias* are uncertain (Christidis and Schodde, 1997). Within the fairy-wrens, three distinct clades are recognized: the blue *cyaneus* group, the bi-colored *leucopterus* group, and the chestnut-shouldered *lamberti* group. Phylogenetic topologies suggest the fairy-wrens and emu-wrens are more closely related to each other than either is to the grasswrens (Christidis and Schodde, 1997).

Malurids are relatively small passerines (5 to 40 grams; Rowley and Russell, 1997). All species are known or believed to be sedentary and non-migratory. Individuals reside on permanent and relatively stable territories from one year to the next, although during the winter territorial boundaries break down and individuals exhibit localized wandering. Furthermore, all species are insectivorous, foraging in all layers of vegetation but tending towards the ground. Lastly, all species are known or believed to exhibit cooperative breeding, in which offspring from one generation remain on their natal territory and assist their parents in raising additional offspring (Table 1).

The dynamics of cooperative breeding are best known in the fairy-wrens, in particular the splendid (*M. splendens*) and superb (*M. cyaneus*) fairy-wrens. In these species, groups typically consist of a male and female breeding pair and one or more (up to eight) male offspring from previous breeding efforts. Additional males are alternatively referred to as auxiliary males, subordinates (as the breeding male is behaviorally dominant) or helpers because they generally provide food to the offspring of the breeding female. Family groups may have additional females (offspring from prior breeding efforts) but the presence and number of auxiliary females vary widely across populations, from 3% of splendid fairy-wren groups in drier habitats (Van Bael and Pruett-Jones, 2000) to 24% in the superb fairy-wren (Cockburn *et al.*, 2003). However, in the superb fairy-wren, auxiliary females are not offspring from previous broods, but rather females that have dispersed there from their natal territory. These females either use the new territory as a staging post for a second stage of dispersal or they

**Table 1** Biology and behavior of Maluridae. Data summarized from Rowley and Russell (1997)

Genus	Species	Body mass (g)	Sexual dimorphism	Sexual dichromatism	Cooperative breeding
<i>Malurus</i>	12	7.6–14.0	M > F	Male brighter <sup>a</sup>	Yes <sup>b</sup>
<i>Amytornis</i>	9	17.5–34.1	M > F	Female brighter	Yes <sup>b</sup>
<i>Stipiturus</i>	3	5.4–7.5	M > F	Male brighter	Yes <sup>b</sup>
<i>Sipodotus</i>	1	7.9	M > F	Slight <sup>c</sup>	Likely <sup>e</sup>
<i>Clytomias</i>	1	12.3	F > M	Monomorphic <sup>d</sup>	Likely <sup>e</sup>

<sup>a</sup> In one species, the white-shouldered fairy-wren (*M. Alboscapulatus*), three subspecies are dichromatic and three are monochromatic. In a second species, the broad-billed fairy-wren (*M. grayi*) the dichromatism is slight.

<sup>b</sup> Evidence for cooperative breeding from detailed studies of marked birds in at least one species in the genus (Maguire and Mulder, 2004) and regular observations of large social groups during the breeding season in all species.

<sup>c</sup> In wallace's wren (*Sipodotus wallacii*) males and females are almost inseparable by plumage.

<sup>d</sup> In the orange-crowned wren (*Clytomias insignis*) one subspecies exhibits monochromatism and one exhibits slight dichromatism.

<sup>e</sup> Evidence for cooperative breeding from observations of birds seen in groups during the breeding season.

inherit the territory if the resident female dies (Cockburn *et al.*, 2003).

Auxiliary males assist the group by sharing in territorial defense and nestling provisioning. The offspring that helpers feed are likely, but not necessarily, siblings. The degree of relatedness between helpers and the offspring they are provisioning varies as a result of variation in female promiscuity and turnover in resident breeding females. In the superb fairy-wren, Dunn *et al.* (1995) report that an average of 36% of nestlings were not related to the helpers provisioning them. Generally, the assistance helpers provide does not appear to increase overall reproductive success within a given nest (Tidemann, 1986) or annual reproductive success of the group (Dunn *et al.*, 1995; Green *et al.*, 1995).

In splendid and superb fairy-wrens, cooperative breeding appears to indirectly facilitate extra-pair copulations by the breeding female. In these species, extra-pair paternity is more common when there are helpers in the social group (Mulder *et al.*, 1994). It has been hypothesized (Mulder *et al.*, 1994) that this occurs because the provisioning of offspring by helpers mitigates the cost that females pay for mating outside the social group. This cost is the expected reduction in parental care by the female's social mate as a consequence of her behavior. Helpers also substantially reduce provisioning by dominant males and, at least for superb fairy-wrens, it has been hypothesized that helpers subsidize the cost (reduced paternal care) of seeking extra-pair copulations by dominant males (Green *et al.*, 1995). In contrast, the presence of helpers does not increase the likelihood of extra-pair paternity by the dominant group male in the splendid fairy-wren (Webster *et al.*, 2004).

In fairy-wrens, extra-pair copulation facilitated by the presence of helpers is consistent with comparative studies demonstrating that rates of promiscuity are negatively correlated with the need for paternal care (Birkhead and Møller, 1996; Møller, 2000; Arnold and Owens, 2002; Griffith *et al.*, 2002). When helpers are available to assist in the provisioning of offspring, the amount of required parental care by the breeding male and female is reduced. In such situations, when there is little cost to the females in terms of reduced paternal care, females appear more likely to engage in extra-pair copulations (Griffith *et al.*, 2002).

In contrast to the fairy-wrens, cooperative breeding appears less common in the emu-wrens. Maguire and Mulder (2004) found that just 8% of groups ( $n = 42$ ) of the southern emu-wren (*S. malachurus*) exhibited cooperative breeding at their study sites. Furthermore, cooperative breeding was only observed in two of three study years (Maguire and Mulder, 2004). The population studied by Maguire and Mulder (2004) experienced high adult mortality, presumably resulting in greater turnover rates and more breeding opportunities for one-year old offspring.

As in emu-wrens, the frequency of cooperative breeding appears to be considerably lower in grasswrens than it is in fairy-wrens. In each species of grasswren that has been studied, the striated (*A. striatus*), white-throated (*A. woodwardi*), and thick-billed grasswren (*A. textiles*), groups typically consist of simple breeding pairs, but both groups with helpers and the feeding of offspring by multiple birds are occasionally observed (Noske, 1992; Rowley and Russell, 1997; Karubian, 2001). Additionally, observations of three or more (unmarked) birds at a nest suggest the occurrence of cooperative breeding in the

black (*A. housei*), dusky (*A. purnelli*) and the grey grasswren (*A. barbatus*) (Rowley and Russell, 1997).

In fairy-wrens, limited breeding opportunities may constrain female mate choice at the time of social pairing and females may be forced to accept available males or territories, regardless of quality. In superb fairy-wrens, an experimental study showed that removal of breeding males from existing territories led to immediate dispersal by auxiliary males from nearby territories and that resident females accepted the first dispersing male as a social mate (Pruett-Jones and Lewis, 1990). In that study, both habitat limitation and a male biased sex ratio (common in many fairy-wren species) influenced the occurrence of cooperative breeding. If all suitable territories are occupied when dispersing females search for open areas to settle, females may have limited opportunities for choice when they settle onto a territory. However, in such situations, females can still exert mate choice through selection of extra-pair reproductive partners.

Although extra-pair paternity is now commonly recorded in avian species, the fairy-wrens exhibit some of the highest rates of extra-pair paternity recorded to date. DNA fingerprinting and microsatellite studies have shown that, across species, up to 96% of all broods contain extra-pair young (96% in *M. cyaneus* [Mulder *et al.*, 1994]; 65% in *M. s. splendens* [Brooker *et al.*, 1990]; 55% in *M. s. melanotus* [Webster *et al.*, 2004]; and 75% in *M. melanocephalus* [Karubian, 2002]) and up to 76% of all young are sired by extra-pair males (72–76% in *M. cyaneus* [Dunn and Cockburn, 1998, 1999]; 42% in *M. s. melanotus* [Webster *et al.*, 2004]; and 56% in *M. melanocephalus* [Karubian 2002]). It is expected that similarly high levels of extra-pair paternity will be shown in other species of fairy-wren once they receive detailed study (Rowley and Russell, 1997; Webster *et al.*, 2004). The rates of extra-pair paternity recorded in fairy-wrens are considerably greater than the average levels of extra-pair paternity for socially monogamous species. Across all bird species studied to date, the mean frequency of extra-pair paternity in broods is 18.7% and the average frequency of extra-pair offspring is 11.1% (Griffith *et al.*, 2002).

Although no data are available on extra-pair paternity in emu-wrens, Maguire and Mulder (2004) suggest that the levels are likely to be low in the southern emu-wren for several reasons: (1) males did not exhibit enlarged cloacal protuberances; (2) no extra-group courtship displays were observed, contrasting sharply

with species of fairy-wrens in which males are regularly seen displaying to females outside their social group (Mulder, 1997; Green *et al.*, 2000); and (3) males invested heavily in paternal care of the offspring, even at levels surpassing those of females, a further observation contrasting sharply with data available for fairy-wrens. Similarly, rates of extra-pair paternity are believed to be relatively low in the grasswrens (Karubian, 2001).

Extra-pair paternity constitutes a considerable component of reproductive success in fairy-wrens and males devote considerable time and energy to the courtship of extra-pair females (Mulder, 1997). Extra-group displays by males occur during and up to two months prior to the breeding season but only rarely when the male has nestlings or fledglings present in his own territory (Mulder, 1997; Dunn and Cockburn, 1999; Green *et al.*, 2000). Typically, extra group displays occur in the morning and the displaying males are intruding from neighboring or nearby territories (up to 4 territories away), are socially-dominant within their own group, are two-years of age or older, and target females with multiple helpers (Mulder, 1997; Green *et al.*, 2000). A conspicuous aspect of extra-group displays involves the male carrying and often presenting a flower petal to the female, a behavior rarely directed towards the male's social partner (Mulder, 1997). Petal carrying has been observed in eight of the nine species of fairy-wren (Rowley and Russell, 1997). In superb fairy-wrens, between 7 and 20% of extra-group displays include petal carrying (Mulder, 1997; Green *et al.*, 2000). There is some suggestion that males exhibit preferences for petal colors that maximize contrast with plumage coloration, thereby enhancing their display (Rowley and Russell, 1997; Rathburn and Montgomerie, 2003). Petal carrying appears to have evolved specifically for soliciting future extra-pair copulations and is a unique display in fairy-wrens, indirectly emphasizing the importance of extra-pair paternity in this group (Mulder, 1997).

In the superb fairy-wren, extra group display and mating occur in different contexts. Displays appear to advertise a male as a prospective candidate for future extra-pair copulations as females are generally disinterested, avoiding and evading displaying males. Furthermore, such displays do not lead to copulation, even when females exhibit soliciting behavior (Mulder, 1997). Instead, females conduct pre-dawn forays during their fertile period to copulate with particular

males. Females may make multiple forays over multiple days and even multiple fertile periods, often to the same male. Females appear to select specific males as evidenced by paternity success gained by particular males (Double and Cockburn, 2000; Green *et al.*, 2000).

In fairy-wrens, male reproductive success is highly asymmetrical. In superb fairy-wrens, male within-group success is correlated with male social status and age. Generally, younger males and those with helpers are less successful at siring within-group young (Dunn and Cockburn, 1999). In terms of extra-group success, the majority of males fail to obtain extra-group paternity (68%; Dunn and Cockburn, 1999). Among males that achieve extra-group paternity, success is also highly biased with 5% of the males achieving 46% of the extra-group success. This asymmetrical reproductive success appears to be the result of female choice (Dunn and Cockburn, 1999). Alternatively, these successful males may be individuals possessing ejaculates of higher competitive ability, transferring greater numbers of sperm and/or transferring sperm of higher quality during copulation. However, these two explanations are not necessarily mutually exclusive. For example, females may be choosing males on the basis of a trait, *e.g.* plumage, which signals male fertilizing capacity.

In superb fairy-wrens, the basis of female choice appears to be the timing of nuptial plumage acquisition, with earlier molting males, typically older individuals, achieving greater extra-pair paternity (Dunn and Cockburn, 1999; Green *et al.*, 2000). Upon completion of their molt, males commence displaying to extra-group females suggesting it may be the onset of display, rather than molt date *per se*, that provides females with mate choice cues. Molt also appears to be condition dependent, as males molt later in harsh years and males in better condition tend to molt earlier (Mulder and Magrath, 1994; Dunn and Cockburn, 1999). Early molt incurs costs to males, including the energetic costs of molt and prolonged testosterone-induced immunosuppressive effects. Consequently, it has been suggested that the timing of molt provides an honest indicator of male quality (Mulder and Magrath, 1994; Peters *et al.*, 2000).

## MALE REPRODUCTIVE ANATOMY

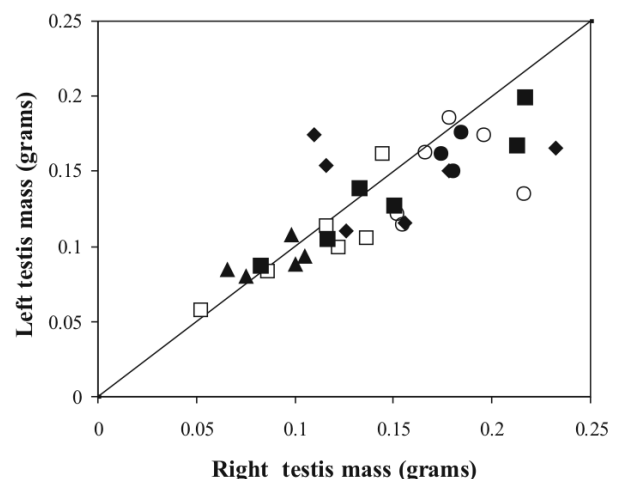
Unless otherwise cited, the data in the following sections come from an on-going study of comparative

reproductive anatomy and sperm competition in the Australian Maluridae (Rowe and Pruett-Jones, in prep.).

## Testes

Male fairy-wrens possessed relatively large testes, comprising up to 6% of male body mass (Table 2). In superb fairy-wrens, Mulder and Cockburn (1993) reported one male with testes comprising 10% of body mass. Testes mass in fairy-wrens was greater than expected based on the regression of Møller (1991) where  $\log(\text{testes mass}) = -1.37 + 0.67 \log(\text{body mass})$ . On average, fairy-wrens had testes 30.45% (range 1.7–55.6%) larger than predicted for their body mass. In contrast, testes mass comprised 1.98% of body mass in the striated grasswren and 0.29% of body mass in the dusky grasswren (Table 2). When compared with the linear model (Møller, 1991) striated grasswrens had testes 12% greater than predicted for body mass, while the dusky grasswren exhibited testes 150% lower than predicted.

Fairy-wrens and grasswrens exhibited asymmetry in the size of their testes, with the right testis larger than the left. Combining data from six species (splendid, superb, variegated, white-winged and red-backed fairy-wrens, and the striated grasswren), the mean mass of the left and right testis was 0.129 g and 0.142 g, respectively (Figure 1;  $t = -2.31$ ,  $P = 0.028$ ).



**Fig. 1** Scatterplot of left testis mass by right testis mass for six species of fairy-wrens (*Malurus*) and grasswrens (*Amytornis*). ◆ splendid fairy-wren, ■ superb fairy-wren, ▲ variegated fairy-wren, □ red-backed fairy-wren, ○ white-winged fairy-wren and ● striated grasswren. Data from Rowe and Pruett-Jones (unpublished). Solid line represents the line of equal left and right testis mass.

**Table 2** Aspects of the reproductive biology of Australian Maluridae, specifically fairy-wrens and grasswrens

Common name	Species	Clade <sup>a</sup>	Mass <sup>b</sup> (male)	Testes mass <sup>c</sup> (% mass)	CP volume <sup>d</sup> (vol index)	Seminal glomera <sup>e</sup> (% mass)	Sperm number <sup>e</sup> ×10 <sup>6</sup>	Sperm conc. <sup>e</sup> ×10 <sup>6</sup> /μl	Stored sperm <sup>e</sup> ×10 <sup>6</sup>	EPP <sup>f</sup> %broods (%/young)
Splendid	<i>M. splendens</i>	B	10.6	0.45 (4.25)	162.6 (16.2)	0.089 (1.03)	58.13 ±6.1	7.46 ±0.4	276.47 ±9.5	(72.5) 55.4 (42)
	<i>M. s. melanotus</i>		8.7	0.30 (3.4)						
Superb	<i>M. cyaneus</i>	B	9.4	0.48 (5.11)	(26.7)	0.095 (1.06)	33.36 ±8.7	7.59 ±0.7	318.69 ±53.3	90.5 (71.1)
	<i>M. c. cyanocephalus</i>	B	8.9	0.29 (3.28)	105.1 (15.1)					
Purple-crowned Variegated	<i>M. coronatus</i>	B	14.5	0.05 (0.35)	73.3 (8.8)	0.055 (0.69)	13.11 ±4.6	10.52 ±1.6	163.19 ±29.8	
	<i>M. lamberti</i>	CS	8.2	0.25 (3.05)						
	<i>M. l. assimilis</i>	CS	8.2	0.21 (2.6)						
Blue-breasted Lovely Red-winged White-winged	<i>M. pulcherrimus</i>	CS	9.27	0.18 (1.94)	117.6	0.087 (1.13)	54.06 ±6.6	4.55 ±1.1	270.58 ±12.4	
	<i>M. amabilis</i>	CS	8.8	0.21 (2.39)						
	<i>M. elegans</i>	CS	9.9	0.46 (4.65)						
	<i>M. leucopterus</i>	BC	7.6	0.33 (4.34)						
	<i>M. l. tuconotus</i>	BC	7.7	0.33 (4.36)						
Red-backed	<i>M. l. edouardi</i>	BC	7.03		118.4	0.047 (0.65)	50.86 ±7.2	8.02 ±0.7	120.66 ±27.9	74.5 (56)
	<i>M. melanocephalus</i>	BC	8.0	0.49 (6.13)	115.7 (21.1)					
White-shouldered Striated grasswren	<i>M. albocapitatus</i>	BC	11.2	0.25 (2.2)	120.3 (6.3)	0.045 (0.24)	6.56 ±2.2	3.71 ±0.9	42.46 ±6.2	
	<i>A. striatus</i>		20.2	0.4 (1.98)						
Dusky grasswren	<i>A. purnelli</i>		21.0	0.06 (0.29)						

<sup>a</sup> Malurus clades. B = Blue group; CS = chestnut-shouldered group; BC = Bi-colored group. These clades represent monophyletic groupings of species within the genus (Christidis and Schodde, 1997).

<sup>b</sup> Mass of males (grams). Data from (Rowley and Russell, 1997), except for *M. s. melanotus*, *M. c. cyanocephalus* and *M. l. tuconotus* (Rowe and Pruett-Jones, unpubl.); *M. l. assimilis* (Tuttle *et al.*, 1996); and *A. striatus* (Karubian, 2001).

<sup>c</sup> Mass of left and right testes combined (grams). Data for *M. splendens*, *M. cyaneus*, *M. coronatus*, *M. lamberti*, *M. l. assimilis*, *M. pulcherrimus*, *M. amabilis*, *M. elegans*, *M. leucopterus*, *M. albocapitatus*, *M. melanocephalus*, *A. striatus* and *A. purnelli* from Dunn *et al.* (2001); *M. s. melanotus*, *M. c. cyanocephalus* and *M. l. tuconotus* (Rowe and Pruett-Jones, unpubl.); and *M. l. edouardi* (Pruett-Jones and Tarvin, 2001).

<sup>d</sup> Volume of the cloacal protuberance in males (mm<sup>3</sup>). Data for *M. s. melanotus*, *M. l. assimilis* and *M. l. tuconotus* from Tuttle *et al.* (1996); *M. l. edouardi* (Pruett-Jones and Tarvin, 2001); *A. striatus* (Karubian, 2001); *M. melanocephalus* (Karubian, 2002); *M. elegans* (Russell and Rowley, 2000); and *M. c. cyanocephalus* (Rowe and Pruett-Jones, unpublished). Cloacal protuberance volume index data for *M. cyaneus* from Mulder and Cockburn (1993); *A. striatus*, *M. melanocephalus*, *M. l. tuconotus*, *M. s. melanotus*, *M. l. assimilis* and *M. c. cyanocephalus* (Rowe and Pruett-Jones, unpublished).

<sup>e</sup> All seminal glomera data (grams) and sperm data from Rowe and Pruett-Jones (unpublished). The sperm sperm data are presented as the mean ± standard error.

<sup>f</sup> Level of extra-pair paternity expressed as percentage of broods containing at least one offspring that resulted from extra-pair fertilization and as the percentage of offspring in the population resulting from extra-pair paternity. Data for *M. splendens* (Brooker *et al.*, 1990); *M. s. melanotus* (Webster *et al.*, 2004); *M. cyaneus* (Mulder *et al.*, 1994; Dunn and Cockburn, 1996; Green *et al.*, 2000); and *M. melanocephalus* (Karubian, 2002).

### Seminal glomera

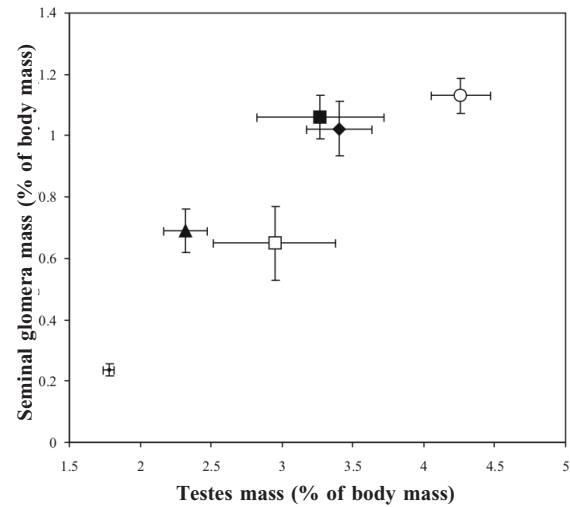
The seminal glomera consists of extensive coiling of the distal portion of the ductus deferens (excurrent ducts) and functions as the site of sperm storage in males. In fairy-wrens, the mean mass of the left and right glomerus ranged from 0.022 g to 0.048 g and 0.025 g to 0.046 g, respectively. The mean combined glomera mass ranged from 0.047 g to 0.094 g, representing approximately 0.69 to 1.13% of male body mass (Table 2). In the striated grasswren, the seminal glomera were relatively smaller, the mean mass of the left and right glomerus weighed 0.022 g and 0.023 g, respectively. The mean combined mass of the seminal glomera was 0.045 g, or 0.24% of body mass (Table 2).

Across species, the seminal glomera mass was positively correlated with testes mass, when both were expressed as percentage of body weight (Figure 2;  $r = 0.910$ ,  $P = 0.008$ ). Across males within species (those species for which we had at least six samples), the relationship between seminal glomera mass and testes mass (expressed as percentage of body mass) was variable. There was a significant and positive correlation in the superb fairy-wren ( $r = 0.878$ ,  $P = 0.018$ ) but non-significant correlations in the splendid fairy-wren ( $r = -0.698$ ,  $P = 0.135$ ), red-backed fairy-wren ( $r = 0.631$ ,  $P = 0.198$ ), and white-winged fairy-wren ( $r = 0.329$ ,  $P = 0.554$ ).

### Cloacal protuberances

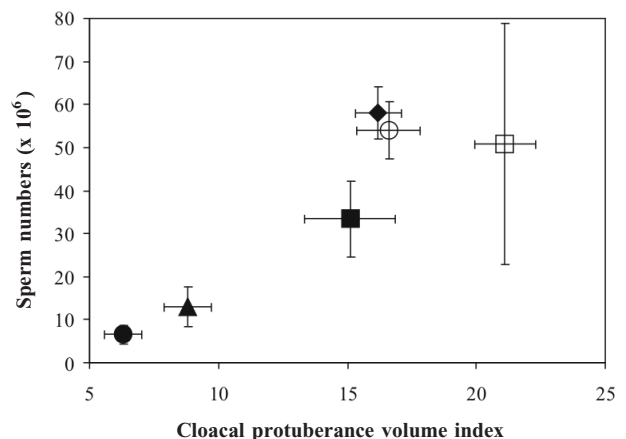
In fairy-wrens and grasswrens, the cloacal protuberance consisted primarily of the paired seminal glomerus. Male fairy-wrens possessed large cloacal protuberances (Table 2) that were greater than expected for their body mass (splendid, superb, white-winged, and red-backed fairy-wrens) based on the regression equation of Briskie (1993). To allow for comparisons with published values for other species, we calculated the cloacal protuberance volume index following Birkhead *et al.* (1991). The mean cloacal protuberance volume index in fairy-wrens ranged from 8.8 to  $26.7 \text{ mm}^3 \text{ g}^{-1}$  (Table 2), some of the largest values for any bird species. In contrast, the striated grasswren had a relatively small cloacal protuberance, with a mean volume of  $120.3 \text{ mm}^3$  and volume index of just  $6.25 \text{ mm}^3 \text{ g}^{-1}$ , lower than expected for their body size (Briskie, 1993).

Across species, cloacal protuberance volume index correlated with the number of sperm in ejaculate



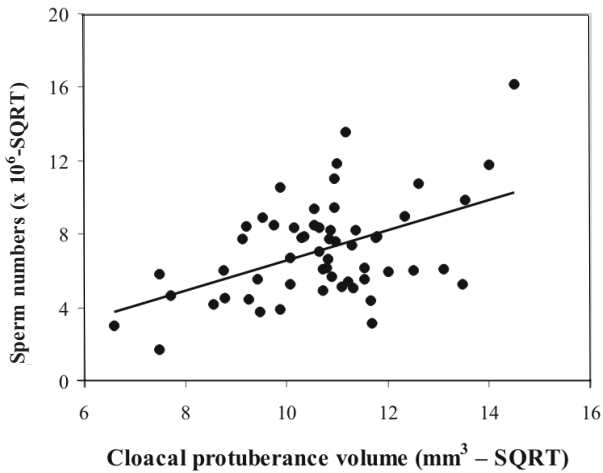
**Fig. 2** Relationship between seminal glomera mass and testes mass (both expressed as % body mass) for six species of fairy-wrens (*Malurus*) and grasswrens (*Amytornis*). ◆ splendid fairy-wren, ■ superb fairy-wren, ▲ variegated fairy-wren, □ red-backed fairy-wren, ○ white-winged fairy-wren, and ● striated grasswren. Shown are the means and standard error for each species. Data from Rowe and Pruett-Jones (unpublished). The regression is statistically significant (see text for details).

samples (*i.e.* the sperm obtained using cloacal massage; Quinn and Burrows, 1936; Tuttle *et al.*, 1996) (Figure 3;  $r = 0.902$ ,  $P = 0.01$ ). Similarly, within the splendid fairy-wren, cloacal protuberance volume was significantly and positively correlated with the number of sperm in ejaculate samples of socially



**Fig. 3** Relationship between sperm numbers in ejaculate samples and cloacal protuberance volume index for six species of fairy-wrens (*Malurus*) and grasswrens (*Amytornis*). ◆ splendid fairy-wren, ■ superb fairy-wren, ▲ variegated fairy-wren, □ red-backed fairy-wren, ○ white-winged fairy-wren, and ● striated grasswren. Shown are the means and standard error for each species. Data from Rowe and Pruett-Jones (unpublished). The regression is statistically significant (see text for details).





**Fig. 4** Regression of cloacal protuberance volume versus sperm numbers in breeding male splendid fairy-wrens. The regression is statistically significant (see text for details).

dominant males (Figure 4;  $R^2 = 0.235$ ,  $F_{1,55} = 16.78$ ,  $P = 0.0001$ ). A similar relationship has also been reported in the white-winged fairy-wren (Tuttle *et al.*, 1996). However, cloacal protuberance size was not related to breeding status. Comparison of cloacal protuberance volume of breeding males versus auxiliary males within the same social group showed no significant difference in either the superb fairy-wren (Mulder and Cockburn, 1993) or the splendid fairy-wrens ( $t$  test,  $t = 0.59$ ,  $P = 0.58$ ). However, this result is likely an effect of the variability in auxiliary male age; auxiliary males can be either one-year old males or males of two years and older. Comparing known one-year old males to breeding males revealed a significant increase in size of the cloacal protuberance in splendid and variegated fairy-wren, but not the white-winged fairy-wren (Tuttle *et al.*, 1996).

## Sperm

Malurid sperm are similar to those of other passerine species, with 'corkscrew' heads (acrosome, nucleus and midpiece), a ribbon-like plasma membrane, and straight tail (splendid, superb, variegated, white-winged and red-backed fairy-wrens and striated grasswren). Our examination of sperm from splendid, variegated and white-winged fairy-wrens revealed an average sperm length of  $84.68 \mu\text{m}$ . Total sperm length did not vary across the species (ANOVA,  $F_{2,38} = 0.268$ ,  $P = 0.766$ ), however significant inter-specific variation existed in the lengths of each of the components of the sperm (acrosome, ANOVA

$F_{2,38} = 5.14$ ,  $P = 0.009$ ; nucleus, ANOVA,  $F_{2,38} = 4.903$ ,  $P = 0.0128$ ; midpiece, ANOVA,  $F_{2,38} = 27.357$ ,  $P < 0.0001$ ; and tail, ANOVA,  $F_{2,38} = 16.532$ ,  $P < 0.0001$ ).

Male fairy-wrens had large numbers of both stored sperm and sperm in ejaculate samples. The total number of sperm in the seminal glomera ranged from  $120.66$  to  $318.69 \times 10^6$  (Table 2). In contrast, the striated grasswren had only  $42.46 \times 10^6$  sperm stored in the seminal glomera (Table 2). The numbers of sperm in ejaculate samples ranged from  $13.1$  to  $58.1 \times 10^6$  in fairy-wrens and equaled  $6.6 \times 10^6$  in the grasswren (Table 2). Across species, the volume of ejaculate samples correlated with sperm numbers ( $r = 0.858$ ,  $P = 0.0259$ ). Across males within species, a similar significant relationship between volume of ejaculate samples and sperm numbers was observed in the splendid fairy-wren ( $R^2 = 0.643$ ,  $F_{1,70} = 126.308$ ,  $P < 0.0001$ ), red-backed fairy-wren ( $R^2 = 0.669$ ,  $F_{1,41} = 82.959$ ,  $P < 0.0001$ ), superb fairy-wren ( $R^2 = 0.933$ ,  $F_{1,4} = 56.103$ ,  $P = 0.0017$ ), and variegated fairy-wren ( $R^2 = 0.802$ ,  $F_{1,6} = 24.233$ ,  $P = 0.0026$ ) but not white-winged fairy-wrens ( $R^2 = 0.096$ ,  $F_{1,5} = 0.532$ ,  $P = 0.4986$ ) although an earlier study of the white-winged fairy-wren did observe such a correlation with larger sample sizes (Tuttle *et al.*, 1996).

The concentration of sperm in ejaculate samples ranged from  $4.55$  to  $10.52 \times 10^6/\mu\text{l}$  in fairy-wrens and equaled  $3.71 \times 10^6/\mu\text{l}$  in the striated grasswren (Table 2). Average sperm concentration differed significantly between species (ANOVA,  $F_{5,133} = 4.268$ ,  $P = 0.0012$ ; splendid, superb, variegated, red-backed, white-winged fairy-wrens and striated grasswren). Sperm concentration and sperm numbers were correlated across males in the splendid ( $R^2 = 0.144$ ,  $F_{1,70} = 11.790$ ,  $P = 0.001$ ) and red-backed ( $R^2 = 0.296$ ,  $F_{1,41} = 17.264$ ,  $P = 0.0002$ ) fairy-wrens.

In the splendid fairy-wren, individual males sampled over two consecutive years showed no significant difference in mean sperm count (number of sperm in ejaculate samples) ( $t_{28} = -0.31$ ,  $P = 0.76$ ) or mean sperm concentration ( $t_{28} = 1.66$ ,  $P = 0.109$ ) across year. In a comparison of sperm sampled from individual males we observed no significant change from one year to the next in sperm count ( $t_{14} = -0.32$ ,  $P = 0.754$ ) or sperm concentration ( $t_{14} = -2.012$ ,  $P = 0.064$ ).

Although no studies have been done on the reproductive anatomy of emu-wrens, initial studies of the

breeding biology revealed the southern emu-wren did not possess a cloacal protuberance (Maguire and Mulder, 2004). The lack of a cloacal protuberance suggests this species may not produce and store the large numbers of sperm observed in fairy-wrens.

## FEMALE REPRODUCTIVE ANATOMY

### Sperm storage tubules

Female birds store sperm for prolonged periods in specialized sperm-storage tubules (Bakst, 1987). Sperm remain viable in sperm-storage tubules for extended periods; for example, the mean duration of sperm storage is 43 days in the turkey *Gallopavo meleagris* (Birkhead and Møller, 1992b). Sperm storage eliminates the need for synchronized copulation and ovulation, provides protection to sperm from contractions associated with the daily ovulation-oviposition cycle, and may provide a reservoir of 'selected' sperm (Bakst, 2001). Sperm storage also appears to enhance the opportunity for sperm competition, as sperm from different males simultaneously residing in sperm-storage tubules are available at the time of fertilization.

In all bird species studied to date, sperm-storage tubules are epithelial invaginations located in the lamina propria of the mucosal folds of the oviductal utero-vaginal junction (Shugart, 1988; Birkhead and Møller, 1992a; King *et al.*, 2002). Sperm-storage tubules are narrow, branched or unbranched, tubular structures with a single opening into the lumen of the oviduct. The sperm-storage tubule epithelium consists of tall, non-ciliated columnar cells with basal nuclei (Bakst, 1987; Birkhead and Møller, 1992b; Briskie, 1996; Bakst, 1998).

Interspecific variation occurs in the number, size, and morphology of sperm-storage tubules (Birkhead and Møller, 1992a,b). Much of this variation appears to be a consequence of allometry (larger birds possess more tubules; Briskie and Montgomerie, 1993). However, after statistically controlling for body size, the number of sperm-storage tubules is positively correlated with the number of sperm in the male ejaculate (Birkhead and Møller, 1992a) and negatively correlated with average tubule length (Briskie and Montgomerie, 1993). In contrast, the length of sperm-storage tubules is not correlated with body mass, but is positively correlated with sperm length (Shugart,

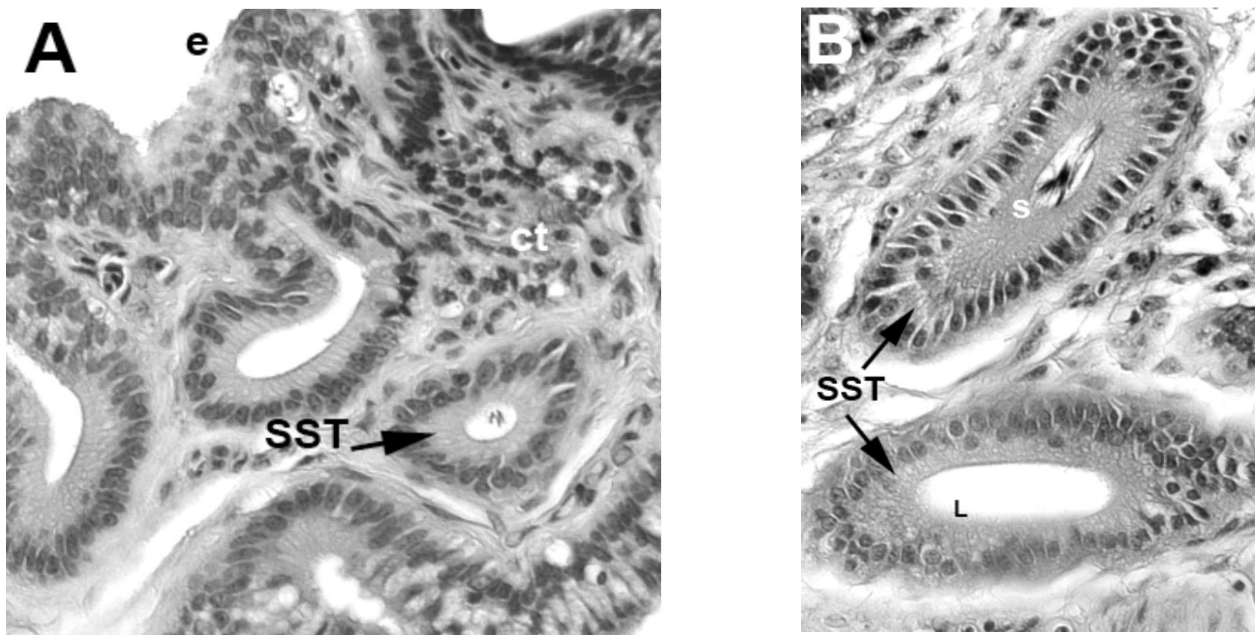
1988; Briskie and Montgomerie, 1992, 1993; Briskie *et al.*, 1997) as well as the rate of extra-pair paternity and intensity of sperm competition (Briskie *et al.*, 1997).

The presence of sperm-storage tubules has been confirmed in females of every species of malurid we have examined to date (superb, splendid, variegated, white-winged and red-backed fairy-wrens and the striated grasswren). In these species, the sperm-storage tubules are found in the utero-vaginal junction of the oviduct and are narrow tubular structures with a single luminal opening. The microscopic structure of the sperm-storage tubules of fairy-wrens (Figure 5) is consistent with that observed in other avian species (*i.e.* alpine accentor: Chiba and Nakamura, 2001; turkey: Bakst, 1987, 1998).

## DISCUSSION

In birds, the social mating system describes the patterns of social pairing among males and females, while the reproductive mating system describes the patterns of copulation and paternity within the population. The Australian Maluridae exhibit relatively conserved social mating systems. All species are known to be socially monogamous and are known or suspected to exhibit cooperative breeding. In contrast, their reproductive mating systems are more diverse, ranging from monogamy or near monogamy in the emu-wrens to what appears to be obligate promiscuity in the fairy-wrens. This variation in reproductive systems, and the associated rates of extra-pair copulation results in varying levels of sperm competition across the species.

Male fairy-wrens exhibit classic adaptations in their reproductive anatomy associated with sperm competition, including relatively large testes and sperm stores, and large numbers of sperm in ejaculate samples. Because sperm competition is a consequence of multiple mating by females, we can predict adaptations in males based on levels of female promiscuity. Conversely, we should be able to predict relative levels of female promiscuity from male adaptations. Thus, we agree with Maguire and Mulder (2004) and predict that emu-wrens will be shown to exhibit the lowest rates of extra-pair paternity in the Australian Maluridae. This prediction is based on the lack of a cloacal protuberance in males, and relatively high rates of paternal care (Maguire and Mulder, 2004). Additionally, we predict that grasswrens will be shown to exhibit low to moderate levels of extra-pair



**Fig. 5** Light microscopic sections of sperm storage tubules in a female white-winged fairy-wren. **A**, Low magnification ( $20\times$ ) of the utero-vaginal junction of the oviduct showing a sperm storage tubule (arrow), epithelial lining (e), and connective tissue (ct). **B**, Higher power view ( $40\times$ ) showing the tubules (arrow), the lumen (L), and sperm within one tubule (s).

paternity. This prediction is based on the size of the cloacal protuberance, relative testes mass, and the numbers of sperm in sperm stores and ejaculate samples observed in male striated grasswrens. If, as we suspect, paternity studies will later support these predictions, the Australian Maluridae will represent an avian family illustrating the entire spectrum of relative sperm competition intensities, from low or non-existent in emu-wrens, to intermediate in grasswrens, and extreme in species of the fairy-wrens.

The sperm counts reported here are based on counts obtained from sperm collected via cloacal massage (manual manipulation of the cloacal protuberance of males; Quinn and Burrows, 1936; Wolfson, 1960; Tuttle *et al.*, 1996; Gee *et al.*, 2004). Although these samples are not natural ejaculates, we refer to them as 'ejaculate samples' in keeping with earlier work (Tuttle *et al.*, 1996). An obvious and important question is whether the counts obtained from these samples are representative of natural ejaculates. While we do not yet have a definitive answer to this question, there is circumstantial evidence that suggests these sperm counts are comparable, if not identical in mean value, to sperm counts of actual ejaculates. First, the volume of ejaculate samples obtained during cloacal massage is comparable to the volumes of natural ejaculates collected from other bird species of similar size

(Tuttle *et al.*, 1996). Second, in an earlier study two males trapped immediately after copulation yielded no sperm with cloacal massage (Tuttle *et al.*, 1996). Lastly, although large cloacal protuberances are typically thought to be required to maintain large sperm reserves for a high copulation rate (Birkhead *et al.*, 1993), fairy-wrens appear to copulate infrequently (Mulder and Cockburn, 1993; Webster *et al.*, 2004), suggesting that stored sperm is available for a single or at the most a few copulations (Tuttle *et al.*, 1996; Webster *et al.*, 2004).

The sperm counts we report here are significantly different (smaller) than the sperm counts reported earlier by Tuttle *et al.* (1996) and Tuttle and Pruett-Jones (2004) for the splendid, variegated, and white-winged fairy-wrens. Following publication of Tuttle and Pruett-Jones (2004), it was discovered that an error had been made in the reported sperm counts, and the counts published in Tuttle *et al.* (1996) and Tuttle and Pruett-Jones (2004) are now known to be much too large (Pruett-Jones and Tuttle, in review). The corrected sperm counts do not differ from the overall mean sperm counts that we report here. Because the error in that earlier work dealt only with the sperm counts, none of the other findings (size of cloacal protuberances, interspecific differences, *etc.*) are affected and thus remain valid. Although fairy-wrens

do not have massive sperm counts as previously reported, they still have large numbers of stored sperm and we believe, as suggested above, that they will be shown to also have large numbers of sperm in ejaculates. The numbers of sperm in ejaculate samples across species of fairy-wren ( $13.1$  to  $58.1 \times 10^6$ ) are substantially larger than known sperm counts for other species of passerines, e.g.  $12.5 \times 10^6$  in red-winged blackbirds *Agelaius phoeniceus* (Westneat *et al.*, 1998),  $0.17 \times 10^6$  to  $5.29 \times 10^6$  in zebra finches *Taeniopygia guttata* (Birkhead *et al.*, 1995b), and  $12.0 \times 10^6$  in japanese quail *Coturnix coturnix* (Clulow and Jones, 1982).

The reasons why females engage in multiple mating remains a debated issue and numerous hypotheses have been proposed to explain this behavior. Potential benefits to females can include direct benefits, such as increased parental care and fertility insurance (Sheldon, 1994; Reynolds, 1996; Li and Brown, 2002). Alternatively, indirect or genetic benefits may accrue to females, such as reduced genetic incompatibility (and inbreeding avoidance), increased genetic diversity, genetic bet-hedging, selection for superior sperm and compensation for low quality social partners (Keller and Reeve, 1995; Zeh and Zeh, 1996; Brown, 1997; Zeh and Zeh, 1997; Jennions and Petrie, 2000; Tarvin *et al.*, 2005). Regardless of the benefits to females of engaging in multiple matings, it is expected that selection will be stronger in males to ensure paternity than it is in females to manipulate paternity (Parker, 1984).

In socially monogamous species, female reproductive promiscuity can involve both the decision not to copulate with the social pair, as well as the choice of an extra-pair reproductive partner. Although these decisions may often overlap, they may also be independent of one another. In the splendid fairy-wren, females appear to engage in extra-pair copulations when they are genetically similar to their social mate, but genetic relatedness *per se* does not explain the choice of copulation partner (Tarvin *et al.*, 2005). In this species, the genetic similarity between females and their extra-pair partners was not significantly different from the genetic similarity between females and an 'average' male from the population, but extra-pair males were significantly less genetically similar to the females than were the social males that were cuckolded (Tarvin *et al.*, 2005).

The likelihood of reproductive promiscuity in fairy-wrens is undoubtedly related in part to the limitation of

suitable territories for females to settle on at the time of their dispersal. Juvenile females of many species of fairy-wrens either disperse voluntarily or are forced out of the group of the resident breeding female whereas juvenile males are tolerated and remain on the natal territory as helpers. For the dispersing juvenile females, most suitable territories are already occupied. Consequently, females may be forced to accept any available territory whether or not the resident male is preferred as a mating partner. In such situations, females may use one set of criteria when deciding where to settle and a separate set of criteria when deciding which male to copulate with.

Regardless of the specific reasons that female fairy-wrens engage in extra-pair copulations, it is clear that reproductive promiscuity in these species is integrated into many aspects of their social behavior. Fairy-wrens are the only group of birds in which males are known to have a ritualized display (petal carrying) associated with seeking extra-pair copulations. Furthermore, at least for superb fairy-wrens, there appears to be a temporal disassociation between extra-pair displays (initiated by males intruding onto a female's territory) and extra-pair copulations (initiated by females traveling to the male's territory). Thus, the dynamics of extra-pair paternity influence patterns of display behavior, space use, and movement patterns in these species.

The likelihood that copulation results in a fertilization may vary if males differentially allocate sperm according to their copulatory partner. Differential allocation may explain the disproportionate success of extra-pair copulations observed in some species. For example, in zebra finches a single extra-pair copulation was found to fertilize 54–84% of the female's eggs (Birkhead *et al.*, 1995b). In this species, the mechanism of sperm allocation is behavioral control of sperm numbers, with the male performing extra-pair copulations when his social mate's fertile period is over and the social pair is no longer copulating. As sperm numbers and quality (sperm velocity) are determined by time since last ejaculation, males transfer larger numbers of higher quality sperm during extra-pair copulations (Birkhead *et al.*, 1995b). Disproportionate success of extra-pair copulations may also occur in fairy-wrens. In the superb fairy-wren, extra-pair copulations represent just 11% of total observed copulations, and yet approximately 75% of all offspring are the result of extra-pair paternity (Mulder *et al.*, 1994; Birkhead and

Møller, 1995). Similarly, in the splendid fairy-wren extra-pair copulations represent just 2.6% of observed copulations and approximately 65% of all offspring result from extra-pair paternity (Brooker *et al.*, 1990; Birkhead and Møller, 1995).

There is considerable variation in birds in the copulation rates of males. Some of this variation is likely due to sperm competition. Although it is the copulation behavior of females that generates sperm competition, such behavior in females can also influence or increase the same behavior in males. Across all bird species, high copulation rates are observed in polyandrous species, in species in which males are not able to guard mates and in species that exhibit forced pair copulations following extra-pair copulations (retaliatory copulations) (Birkhead *et al.*, 1987). Across species, there is also a positive correlation between male copulation frequency and cloacal protuberance size, suggesting that one possible function of a large cloacal protuberance is to store sperm for multiple copulations (Birkhead *et al.*, 1993).

In fairy-wrens, data regarding within-pair and extra-pair copulation rates are limited but it is thought that these rates are relatively low (Brooker *et al.*, 1990; Mulder *et al.*, 1994; Birkhead and Møller, 1995). These limited data may be a result of copulations occurring during pre-dawn hours (Double and Cockburn, 2000), making them difficult to observe or it may be that copulations are simply infrequent. If, as suspected, fairy-wrens exhibit low copulation rates, then they represent an exception to the rule that large cloacal protuberances equate with high copulation rates. Instead, large sperm stores may relate to a strategy of transferring large numbers of sperm in a single or few copulation(s).

In fairy-wrens, large sperm counts may have evolved specifically in relation to extra-pair copulation success or may relate to both the guarding and gaining of paternity. Although mate guarding is a commonly observed behavior in male fairy-wrens, behavioral studies suggest mate guarding behavior ceases after the female has laid the first egg (Pruett-Jones, unpublished) or is generally weak throughout the breeding season (Mulder, 1997). Furthermore, the high rates of extra-pair paternity suggest mate guarding is relatively ineffective in these species. The transfer of large numbers of sperm would enable males to maximize fertilization success during infrequent within-pair and extra-pair copulations, protecting against cuckoldry while cuckolding other males (Webster *et al.*, 2004).

In the splendid and variegated fairy-wren, the size of the cloacal protuberance, and numbers of stored sperm, increases with age when comparing one-year old males with males 2+ years of age (Tuttle *et al.*, 1996). However, this is not the case in the white-winged fairy-wren. In this species, there is no significant difference between one-year old brown-plumed males, two to three year old males in sub-adult plumage, and 3+ year old males in full nuptial plumage in terms of cloacal protuberance size (Tuttle *et al.*, 1996; Rathburn and Montgomerie, 2003) and the numbers of sperm in ejaculate samples (Tuttle *et al.*, 1996). These differences relate to basic differences among the species in sexual and behavioral maturation of males. Splendid and variegated fairy-wrens exhibit the typical pattern of plumage acquisition in *Malurus*, in which one, and sometimes two year-old males, exhibit sub-adult male plumage and are seldom the dominant male within a social group. In contrast, plumage maturation is delayed in the white-winged fairy-wren and males do not obtain full adult plumage until three or four years of age (Rowley and Russell, 1995; Tuttle *et al.*, 1996).

White-winged fairy-wrens also exhibit an unusual social grouping, being organized into a 'clan' system composed of several territories within close proximity to one another. Within these clans, only a single male exhibits full nuptial plumage (Tidemann, 1980; Rowley and Russell, 1995). The social hierarchy between clan males is unclear, but one possibility is that fully plumed males exert dominance over other clan males and may participate or control the activities within other territories. It has also been hypothesized that fully plumed males achieve greater reproductive success, relative to brown and sub-adult plumed males, as a result of extra-pair matings with females within and outside the clan (Tidemann, 1980; Rowley and Russell, 1995). However, both brown-plumed and sub-adult plumed males are capable of holding territories (as the dominant male), have large sperm stores, and appear to obtain some share of paternity (Rowley and Russell, 1995; Pruett-Jones, unpublished).

In white-winged fairy-wrens an obvious potential cost to adult nuptial plumage is increased predation risk. Typically, white-winged fairy-wrens inhabit relatively low vegetation in the most exposed habitats occupied by any species of fairy-wren. Bright males (blue plumage on the mainland and black plumage in the island subspecies) are extremely conspicuous in their habitat. Despite the potential benefit to males of

acquiring adult plumage (in terms of clan control and possible reproductive success), there may also be significant costs in terms of predation. Additionally, plumage maturation may carry a cost of conspecific aggression or be under social constraint. The biology and behavior of white-winged fairy-wrens is obviously complicated and is in need of considerably more research. This species illustrates patterns of population biology and behavior that are not known for any other species of passerine and it may be an example of a species in which acquiring adult plumage in males carries as many costs as it offers benefits.

There has been considerable progress over the last several decades in understanding the reproductive biology and behavior of Australian fairy-wrens. However, there are also many basic questions that remain unanswered. Of greatest importance is additional work on the rates of extra-pair paternity in emu-wrens and grasswrens as well as other species of fairy-wren. Additionally, we need to know how the sperm obtained in ejaculate samples relate to the numbers of sperm that males transfer to female during actual copulations. These data will be critical to understanding whether the large sperm stores in males are for few or many copulations. Data on copulation behavior, and the relative rates of within-pair and extra-pair copulations, will help identify the mechanisms of mixed paternity broods in females. We anticipate that once additional data on extra-pair paternity are available for other species of fairy-wren, as well as emu-wrens and grasswrens, most aspects of the reproductive biology of both males and females of all species of the Maluridae will be shown to be a direct result of the patterns of reproductive promiscuity and the varying intensity of sperm competition.

## CONCLUSIONS

1. Sperm competition is a significant component of sexual selection in Australian fairy-wrens. Furthermore, extra-pair paternity constitutes a considerable source of male reproductive success. The importance of extra-pair paternity is indirectly illustrated by the occurrence of courtship displays (petal carrying) thought to have evolved for the specific purpose of soliciting future extra-pair copulations.
2. Male fairy-wrens exhibit several adaptations in their reproductive anatomy associated with intense sperm competition. We believe that once additional

data on extra-pair paternity are available for other species of fairy-wrens, these adaptations will be shown to vary with the level of reproductive promiscuity in females.

3. We predict emu-wrens will be shown to exhibit low levels of extra-pair paternity based on the lack of male sperm stores and high rates of paternal care. Furthermore, we predict grasswrens will be shown to exhibit low to moderate levels of extra-pair paternity based on the size of male sperm stores, relative testes mass and the numbers of sperm in ejaculate samples.
4. The Australian Maluridae represent a family of birds exhibiting the entire spectrum of relative sperm competition intensities, from low or non-existent in emu-wrens, to intermediate in grasswrens, and extreme in species of the fairy-wrens. These species exemplify the consequences of extreme sperm competition under conditions of female control of copulation and low copulation frequencies.

## ACKNOWLEDGEMENTS

We wish to thank Murray R. Bakst for his invitation to write this review and for other assistance in our research. This paper was improved by comments received from Emma Greig, Susan Longest, Emily Kay, and Jill Mateo. Special thanks go to Michael Webster, Willow Lindsay, Claire Varian, and Jordan Karubian and to field assistants Wen Shen, Veronica Yovovich, Emily Kay and Alex Smith. Our work on fairy-wrens has been supported by the National Science Foundation (SP-J) and the Hinds Fund from the University of Chicago (MR).

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