

Extra-pair paternity, sperm competition and their evolutionary consequences in the Maluridae

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Abstract. Fairy-wrens (*Malurus*) are socially monogamous and yet exhibit among the highest rates of extra-pair paternity (EPP) known in passerine birds. Here, we review patterns of EPP in fairy-wrens and their allies (Maluridae), and discuss the causes and evolutionary consequences of multiple mating in this group. Rates of EPP vary widely across species, but correlate strongly with relative testes mass suggesting that testes mass can be used as a proxy for EPP in comparative studies. With respect to variation in EPP, there appear to be consistent habitat differences between the subfamilies of Maluridae that may contribute to the observed inter-specific variation in rates of EPP, and specific habitat requirements of some species may influence patterns of paternity between species of *Malurus*. Within species, however, there is equivocal evidence for a role of group size influencing rates of EPP. Regardless of the causes of variation in EPP, available evidence suggests that female fairy-wrens may benefit from extra-pair matings through indirect genetic benefits, such as inbreeding avoidance and choice of high-quality males (i.e. good genes). Using Bayesian modelling, we show that the ancestral state in Australian malurids is one of low sperm competition, and that, in general, increased levels of sperm competition (and hence EPP) are a derived condition in this family. Given the broad range of levels of sperm competition in this family and the relationship between relative testes mass and EPP, we suggest that the Maluridae are a model system for studies of EPP and sperm competition.

Additional keywords: extra-pair mating, fairy-wrens, sexual selection.

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Introduction

In his review of avian breeding systems, Lack (1968) identified monogamy as the predominant breeding system in birds. During the late 20th century, however, the application of molecular techniques to parentage studies revealed that extra-pair paternity (EPP) is a regular phenomenon in birds (reviewed in Petrie and Kempenaers 1998; Wink and Dyrz 1999; Griffith *et al.* 2002; Westneat and Stewart 2003). Indeed, it is now widely accepted that paternity cannot be predicted from observations of social interactions and that sexual monogamy is, in fact, rare in birds, occurring in just 14% of passerine species (Griffith *et al.* 2002). Moreover, EPP is now known in more than 70% of socially monogamous passerines and, on average, over 11% of offspring are the result of copulations outside of the social pair-bond (Griffith *et al.* 2002). Thus, extra-pair fertilisations appear to be the rule, rather than the exception, in socially monogamous birds.

Despite considerable theoretical and empirical attention (Jennions and Petrie 2000; Griffith *et al.* 2002; Westneat and Stewart 2003; Schmoll 2011), the benefits of EPP to females remain unclear. Furthermore, considerable variation in both the percentage of offspring sired by extra-pair males and the frequency of broods containing extra-pair young occurs across species, between populations of the same species and across years within a single population (Petrie and Kempenaers 1998; Griffith *et al.*

2002), yet the factors determining this variation are poorly understood. Thus, ascertaining the adaptive function of EPP and the causes of variation in rates of EPP remains a key challenge in evolutionary biology.

Regardless of the adaptive function of EPP, multiple mating has significant evolutionary consequences for the reproductive biology of individuals. In particular, much attention has been given to the consequences of promiscuity for male reproductive biology. When females copulate with multiple males during a single reproductive episode, sperm from these males compete to fertilise the female's ova (i.e. sperm competition; Parker 1970). Sperm competition influences the evolution of male traits that affect competitive fertilisation success. For example, in birds, sperm competition is associated with increases in testes size (Møller 1991), the proportion of sperm-producing tissue contained within the testes (Lüpold *et al.* 2009a; Rowe and Pruett-Jones 2011) and the efficiency of spermatogenesis (Lüpold *et al.* 2011), as well as changes in sperm morphometry (Kleven *et al.* 2007; Immler *et al.* 2008; Lüpold *et al.* 2009b, 2009c; but see Kliven *et al.* 2009) and performance (Kleven *et al.* 2009; Rowe and Pruett-Jones 2011). Nevertheless, several critical questions remain unexplored (Pizzari and Parker 2009) and further studies of sperm competition will be central to improving our understanding of sexual selection.

Distributed throughout Australia and New Guinea, the Maluridae is represented by 28 species in six genera. In Australia, malurid genera include the fairy-wrens (*Malurus*), emu-wrens (*Stipiturus*) and grasswrens (*Amytornis*) (Rowley and Russell 1997). Fairy-wrens have proven an important system for the study of cooperative breeding and kin selection (Margraf and Cockburn 2013). Additionally, despite initially being referred to as Mormon wrens (owing to early observations leading people to believe a single male lived with a harem of females; Rowley and Russell 1997) it is now known that, despite what appears to be obligate social monogamy, fairy-wrens exhibit some of the highest rates of EPP reported to date. Thus, in perhaps no other group was Lack's assumption of monogamy less appropriate, and clearly extra-pair mating and sperm competition are important in this group.

In this review, we consider the evolutionary causes and consequences of EPP in the Australian Maluridae. We do not aim to review in detail the entire fields of EPP and sperm competition in birds because these have been the subject of several excellent reviews (EPP, Petrie and Kempenaers 1998; Griffith *et al.* 2002; Westneat and Stewart 2003; sperm competition, Birkhead and Møller 1992; Birkhead 1998; Snook 2005; Pizzari and Parker 2009). Instead, we describe the general patterns of EPP observed in the Maluridae and synthesise information relevant to our understanding of the adaptive function and causes of inter- and intra-specific variation in EPP in malurid species. Additionally, we review studies examining the evolutionary consequences of EPP for male reproductive anatomy, ejaculate quality and sperm phenotype, and use evolutionary modelling to investigate the evolution of sperm competition and EPP in this group.

Extra-pair paternity in the Maluridae

General patterns

Rates of EPP are available for six species within the Maluridae: five species of fairy-wren and one species of emu-wren

(Table 1; note that throughout this review we refer to EPP as the paternity gained by any male other than the dominant, pair male, not just 'extra-group' paternity). These data include at least one species from each of the main colour groups of *Malurus* species – (1) the blue group (Superb (*M. cyaneus*) and Splendid (*M. splendens*) Fairy-wrens), (2) the bi-coloured group (Red-backed (*M. melanocephalus*) and White-winged (*M. leucopertus*) Fairy-wrens, and the White-shouldered Fairy-wren (*M. alboscapulatus*) of New Guinea), and (3) the chestnut-shouldered group (Lovely (*M. amabilis*), Variegated (*M. lamberti*), Red-winged (*M. elegans*) and Blue-breasted (*M. pulcherrimus*) Fairy-wrens) – as well as the Purple-crowned Fairy-wren (*M. coronatus*). Importantly, rates of EPP vary widely across species: for example, the proportion of nestlings resulting from extra-pair fertilisations ranges from as low as 4.4% in the Purple-crowned Fairy-wren to >70% in the Superb Fairy-wren (Table 1).

Unfortunately, no species of grasswren or any of the New Guinea malurid species have yet been the focus of paternity studies. However, given the association between rates of EPP and relative testes size (Møller and Briskie 1995; see Box 1), we can predict approximate rates based on relative testes mass (RTM). Although given the low precision surrounding the effect sizes of RTM presented in Box 1 (i.e. wide confidence intervals), we suggest these predictions should be treated as preliminary. Nonetheless, based on RTM, we might expect low levels or possibly an absence of EPP in the Dusky Grasswren (*A. purnelli*; RTM = 0.29%; Rowe and Pruett-Jones 2006) and relatively moderate levels of EPP in the Striated Grasswren (*A. striatus*; RTM = 1.56%; Table 2).

Data on RTM (Table 2) also suggests that the Lovely Fairy-wren may exhibit moderate levels of EPP, whereas the White-winged Fairy-wren may exhibit one of the highest rates. Predictions for the White-winged Fairy-wren are, however, complicated by their unusual biology. Specifically, White-winged Fairy-wrens have a 'clan' mating system (Rowley and

Table 1. Rates of extra-pair paternity in fairy-wrens and emu-wrens

Broods, number of broods sampled; % EPB, proportion of broods containing at least one nestling resulting from an extra-pair fertilisation (with number of broods in parentheses); Nestling, number of nestlings sampled; EPY, nestlings resulting from extra-pair fertilisations (with number of nestlings in parentheses). Under Method: Micro, microsatellite genotyping; DNA, DNA fingerprinting; Alloz, Allozymes. References: 1, Maguire and Mulder (2008); Kingma *et al.* (2009); 3, Mulder *et al.* (1994); 4, Dunn and Cockburn (1998); 5, Dunn and Cockburn (1999); 6, Double and Cockburn (2000); 7, Colombelli-Négrel *et al.* (2009); 8, Brouwer *et al.* (2011); 9, Karubian (2002); 10, Webster *et al.* (2008); 11, Varian-Ramos and Webster (2012); 12, Brooker *et al.* (1990); 13, Webster *et al.* (2004). Under References, an asterisk (*) indicates data used for analysis in Box 1

Species	Species or subspecies	Period	Broods	% EPB (n)	Nestling	% EPY (n)	Method	Reference
Southern Emu-wren	<i>S. malachurus malachurus</i>	2000–02	27	15 (4)	50	12 (6)	Micro	1*
Purple-crowned Fairy-wren	<i>M. coronatus coronatus</i>	2005–08	104	5.8 (6)	227	4.4 (10)	Micro	2*
Superb Fairy-wren	<i>M. cyaneus cyanochlamys</i>	1989–90	40	95 (38)	181	76 (137)	DNA	3
		1989–93, 1986–93	157	92 (144)	618	72 (447)	DNA	4*
	<i>M. cyaneus cyanochlamys</i>	1991–92	–	–	309	67 (207)	DNA	5
	<i>M. cyaneus cyanochlamys</i>	1989–96	253	65.2 (165)	–	–	Micro	6
	<i>M. cyaneus leggei</i>	2006–07	12	83 (10)	27	67 (18)	Micro	7
Red-winged Fairy-wren	<i>M. elegans</i>	2008–09	118	70 (83)	261	57 (149)	Micro	8*
Red-backed Fairy-wren	<i>M. melanocephalus melanocephalus</i>	1998–2000	53	73.6 (39)	144	56 (80)	Micro	9
		1998–2000, 2003–06	185	62.7 (116)	517	51.1 (264)	Micro	10*
	<i>M. melanocephalus melanocephalus</i>	2004–07	110	68 (75)	322	54 (175)	Micro	11
Splendid Fairy-wren	<i>M. splendens splendens</i>	1986–87	40	–	91	72.5 (66)	Alloz	12
	<i>M. splendens melanotus</i>	1992–98	159	55.4 (88)	386	42.2 (163)	Micro	13*

Box 1. Relative testes mass as a proxy for sperm competition

Here we examine whether relative testes mass (RTM, i.e. testes mass controlled for body mass) is correlated with population levels of EPP in Maluridae (i.e. Southern Emu-wren, Purple-crowned Fairy-wren, Red-winged Fairy-wren, Red-backed Fairy-wren, Splendid Fairy-wren, Superb Fairy-wren), given similar findings in birds generally (see Møller and Briskie 1995).

Methods

Data were analysed using phylogenetically controlled generalised least-squares multiple regression (PGLS) (Felsenstein 1985; Pagel 1999; Freckleton *et al.* 2002). We used the molecular phylogeny of Driskell *et al.* (2011), pruning unnecessary taxa from the tree. We used this phylogeny over other available phylogenies (e.g. Gardner *et al.* 2010; Lee *et al.* 2011) because data concerning branch lengths were available. RTM was estimated by including both (ln-transformed) combined testis mass and body mass as independent variables in the statistical models, a more robust approach than the use of residuals (Darlington and Smulders 2001; Garcia-Berthou 2001). Data on testes mass and body mass (Table 2) are from Rowe and Pruett-Jones (2011) and M. Rowe (unpubl. data), with the exception of the Purple-crowned Fairy-wren, for which data are from Dunn *et al.* (2001). EPP data were taken from the literature (Table 1). When multiple sources reported different rates of EPP from the same population, we used the estimates generated from the longest study period (see Table 1). Finally, where possible, we restricted our analysis to data originating from the same subspecies as those sampled for RTM. Analyses were performed using R 2.14.1 (R Core Team 2011), the R package ‘ape’ (Paradis *et al.* 2004) and code written by R. Freckleton.

Results

There was a significant, positive association between RTM and both measures of EPP: proportion of extra-pair young in the population and proportion of broods containing extra-pair young (Table B1; Fig. B1). This suggests that RTM is a valid proxy for rates of EPP. It is worth noting, however, that despite a strong, positive effect (*r*) of RTM the wide 95% confidence intervals, almost certainly resulting from the small number of species included in the analysis, suggest there is considerable uncertainty in these effect sizes. Thus, we recommend additional data from other malurid species be incorporated into future analyses to further validate this relationship. Nonetheless, we suggest that the relative robustness of data concerning RTM, along with the significant relationship between multiple estimates of RTM and available measures of EPP, allow us to draw reasonable inferences regarding predicted levels of EPP in currently unstudied species.

Table B1. Regression analysis controlling for phylogeny (PGLS) demonstrating a positive, significant relationship between extra-pair paternity and relative testes mass in Australian Maluridae

EPY, proportion of extra-pair young in the population; EPB, proportion of broods containing extra-pair young; CL, upper and lower confidence limits. Relative testes mass is estimated by including both (ln-transformed) testes mass and body mass as covariates in the model. The model including the maximum-likelihood value of λ was compared against the models including $\lambda = 1$ and $\lambda = 0$, and superscripts following the λ estimates indicate significance levels of the likelihood-ratio tests (first position: against $\lambda = 1$; second position: against $\lambda = 0$; n.s., not significant)

Predictor	Slope	<i>t</i>	<i>P</i>	λ	<i>r</i>	Effect size	CL
EPY	Testes mass	0.33	4.56	0.02	<0.0001 ^{n.s.,n.s.}	0.80	-0.04 to 2.20
	Body mass	-0.33	-0.89	0.44		-0.25	-1.39 to 0.88
EPB	Testes mass	0.41	4.16	0.025	<0.0001 ^{n.s.,n.s.}	0.77	-0.12 to 2.15
	Body mass	-0.34	-0.67	0.55		-0.19	-1.32 to 0.94

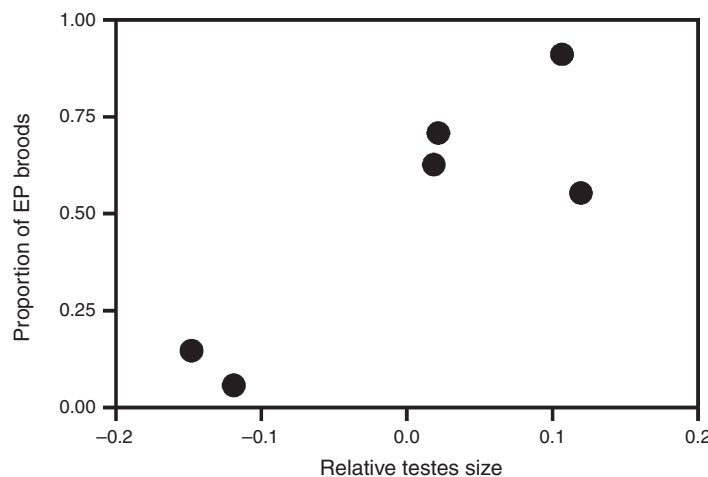


Fig. B1. Relationship between relative testes size and EPP (i.e. proportion of broods containing at least one extra-pair young) in Australian Maluridae. Unlike all analyses, figure does not control for phylogeny. Relative testes size refers to the residual values from a linear regression of testes mass on body mass.

Table 2. Body mass, combined testes mass (CTM), relative testes mass (RTM) and testes dimensions in 11 species of Australian Maluridae

RTM is expressed as gonadosomatic index (GSI): (combined gonad weight ÷ bodyweight) × 100. All values are means ± s.e.; NA, not available. Sample sizes shown in parentheses; sample sizes not available for Purple-crowned Fairy-wren. References (for body mass, CTM and RTM): 1, M. Rowe (unpubl. data); 2, Rowe and Pruett-Jones (2011); 3, Rowley and Russell (1997); 4, Dunn *et al.* (2001). All data on testis dimensions from M. Rowe (unpubl. data)

Species	Species or subspecies	Body mass (g)	CTM (g)	RTM (GSI)	Left testis (length × width; mm)	Right testis (length × width; mm)	Source (body mass, CTM, RTM)
Striated Grasswren	<i>A. striatulus striatulus</i>	19.64 ± 0.24 (10)	0.31 ± 0.02 (6)	1.56 ± 0.13 (6)	8.24 ± 0.39 × 5.69 ± 0.23 (6)	7.48 ± 0.36 × 6.30 ± 0.26 (6)	1
Southern Emu-wren	<i>S. malachurus malachurus</i>	7.32 ± 0.11 (6)	0.045 ± 0.004 (6)	0.62 ± 0.06 (6)	4.30 ± 0.26 × 3.29 ± 0.12 (6)	3.98 ± 0.20 × 3.20 ± 0.07 (6)	2
Lovely Fairy-wren	<i>M. amabilis</i>	8.34 ± 0.10 (6)	0.11 ± 0.01 (4)	1.34 ± 0.16 (4)	5.64 ± 0.26 × 4.48 ± 0.19 (4)	5.03 ± 0.22 × 4.60 ± 0.43 (4)	1
Purple-crowned Fairy-wren	<i>M. coronatus</i> (subspecies unknown)	11.4 (-)	0.05 (-)	0.44 (-)	NA	NA	3 (body mass), 4 (testes mass)
Superb Fairy-wren	<i>M. cyaneus cyanocephalus</i>	8.93 ± 0.22 (6)	0.29 ± 0.04 (6)	3.39 ± 0.48 (6)	7.49 ± 0.34 × 6.35 ± 0.30 (6)	7.81 ± 0.37 × 6.36 ± 0.42 (6)	2
Red-winged Fairy-wren	<i>M. elegans</i>	9.77 ± 0.09 (9)	0.20 ± 0.03 (6)	2.07 ± 0.32 (6)	6.67 ± 0.39 × 5.56 ± 0.49 (6)	6.22 ± 0.39 × 5.45 ± 0.31 (6)	1
Variegated Fairy-wren	<i>M. lamberti assimilis</i>	8.29 ± 0.12 (33)	0.18 ± 0.01 (5)	2.38 ± 0.15 (5)	7.49 ± 0.28 × 5.64 ± 0.28 (5)	8.02 ± 0.74 × 5.15 ± 0.06 (5)	2
White-winged Fairy-wren	<i>M. leucopterus leucopterus</i>	7.70 ± 0.12 (13)	0.33 ± 0.02 (6)	4.45 ± 0.23 (6)	8.41 ± 0.24 × 6.09 ± 0.16 (6)	8.80 ± 0.23 × 6.72 ± 0.34 (6)	2
Red-backed Fairy-wren	<i>M. melanocephalus melanocephalus</i>	7.57 ± 0.05 (76)	0.21 ± 0.01 (17)	2.95 ± 0.18 (17)	7.18 ± 0.15 × 5.77 ± 0.11 (24)	7.29 ± 0.17 × 6.09 ± 0.12 (24)	2
Blue-breasted Fairy-wren	<i>M. pulcherrimus</i>	9.27 ± 0.14 (15)	0.13 ± 0.02 (5)	1.49 ± 0.24 (5)	5.40 ± 0.34 × 4.57 ± 0.50 (5)	5.50 ± 0.34 × 4.49 ± 0.40 (5)	2
Splendid Fairy-wren	<i>M. splendens melanotus</i>	9.45 ± 0.06 (94)	0.30 ± 0.02 (6)	3.56 ± 0.25 (6)	7.93 ± 0.24 × 6.29 ± 0.33 (6)	7.76 ± 0.39 × 6.47 ± 0.23 (6)	2

Russell 1995), in which younger males hold territories and are socially pair-bonded to a female but exhibit female-like plumage (i.e. brown plumage; S. Pruett-Jones, unpubl. data). Superimposed on these individual territories is the territory of a single male in full breeding plumage (i.e. blue-and-white plumage), and this male interacts with the males and females occupying individual territories within this clan (Rowley and Russell 1995). Presumably, the male in full breeding plumage attempts to mate with the females in his clan, and females appear to be paired with both the male on their individual territory as well as the clan male. This makes quantifying mating patterns difficult with respect to pair-bonded groups and suggests that multiple factors might influence selection on testes size in male White-winged Fairy-wrens.

Below (Box 2) we reconstruct ancestral trait values for RTM (as a proxy for levels of sperm competition and thus rates of EPP) and test for directionality in the evolution of sperm competition in the Maluridae. This analysis suggests that there has been a general increase in RTM (presumably driven by EPP) across the entire phylogeny and that this increase has proceeded gradually over time. There are, however, two exceptions to this general pattern. First, in the clade that includes the Purple-crowned Fairy-wren and the chestnut-shouldered group, there was a small decrease in RTM in the ancestor to this clade (i.e. ancestral node 3; Fig. B2a). Next, there is a larger decrease in RTM in the Purple-crowned Fairy-wren (Fig. B2a), presumably as a result of a reduction in rates of EPP.

There is, however, some confusion as to the exact topology of the phylogeny with respect to the Purple-crowned Fairy-wren. Besides the phylogeny of Driskell *et al.* (2011) that we use in our main analysis, there are two additional recently published phylogenies for the group (Gardner *et al.* 2010; Lee *et al.* 2011). These phylogenies generally agree, except with reference to the position of the Purple-crowned Fairy-wren. In the phylogeny of Gardner *et al.* (2010), the Purple-crowned Fairy-wren is in a clade with Superb and Splendid Fairy-wrens (similar to earlier results of Christidis and Schodde 1997). In contrast, in the phylogeny of Lee *et al.* (2011), Purple-crowned Fairy-wrens are sister to all other species in *Malurus* (except the New Guinea species the Broad-billed Fairy-wren (*Chenorhamphus grayi*). Although the specific location of the Purple-crowned Fairy-wren is the only significant disagreement between the alternative phylogenies, the low rates of EPP observed in this species mean its location in the phylogeny is important for understanding EPP and sperm competition in this group (see discussion below and in Box 2).

Determinants of variation in EPP

Variation in EPP has been linked to phylogeny (Owens and Bennett 1997; Arnold and Owens 2002; Griffith *et al.* 2002) and a range of ecological and life-history variables (Petrie and Kempenaers 1998; Griffith *et al.* 2002; Westneat and Stewart 2003). In a comprehensive review, Griffith *et al.* (2002) showed that some factors, such as the need for male parental care and adult survival, are more likely to explain inter-specific variation in EPP, whereas other factors (e.g. breeding density) may be more important in shaping variation across populations of the same species.

With respect to inter-specific variation, survival rates and male provisioning rates are not available for most species of malurid,

Box 2. Evolution of sperm competition in the Maluridae

Here we combine ancestral state reconstruction with Bayesian modelling to assess whether relative testes mass (RTM; as a proxy for levels of sperm competition) shows any dominant direction of evolutionary change. Additionally, we modelled the tempo of evolutionary change in order to understand how selection may have acted over evolutionary time to shape contemporary inter-specific variation in sperm competition levels in species of Australian Maluridae.

Methods

We examined the evolution of sperm competition using BayesContinuous in BayesTraits version 2.0 (Pagel 1999, 2008; Pagel and Meade 2007). Sperm competition was modelled as RTM. In this instance RTM was estimated as the gonadosomatic index ($GSI = (\text{combined gonad weight} \div \text{bodyweight}) \times 100$; Taborsky 1998). We used GSI because these methods model the evolution of a single trait, and thus require a single metric that reflects relative testes size. Importantly, in a similar analysis to those described in Box 1, we found a significant association between the GSI and both measures of EPP (extra-pair young: $t = 5.03$, $P = 0.007$, $\lambda < 0.0001$; extra-pair broods: $t = 4.59$, $P = 0.01$, $\lambda < 0.0001$).

We first evaluated directionality in trait evolution by determining whether a random-walk model (model A) or a directional random-walk model (model B) best described the data. Model A incorporates a single parameter, the instantaneous variance of evolution, and represents a standard constant-variance random-walk model (i.e. Brownian motion model). In contrast, model B is a directional random-walk model and incorporates two parameters: the variance of evolution parameter (as in model A) and a parameter of directional change (β). This latter parameter (β) measures the regression of the trait of interest against total path-length from the root to the tips of the tree and detects trends towards a dominant direction of evolutionary change. For both models, ancestral state reconstructions utilise Markov Chain Monte Carlo methods; thus, we report the estimated ancestral state (α) and the slope (β) of the regression of path-length across the malurid phylogeny (node 1). We also report estimated ancestral states for four additional clades within the malurid tree: ancestor to all *Malurus* (node 2), ancestor to Purple-crowned Fairy-wren + the chestnut-shouldered group (node 3), ancestor to blue group + bi-coloured group (node 4), and ancestor to the chestnut-shouldered group (node 5).

Next, we assessed the tempo (δ) of evolutionary change in sperm traits using the best-fitting evolutionary model (i.e. A v. B). Delta (δ) detects whether the rate of trait evolution has accelerated or slowed over time. Moreover, δ determines if character change is concentrated at the root or towards the tips of a phylogeny: values of $\delta < 1$ indicate change concentrated at the root of the phylogeny (i.e. temporally early evolution of a trait), values of $\delta > 1$ indicate change concentrated towards the tips of the phylogeny (i.e. temporally late evolution of a trait), and values of $\delta = 1$ indicate gradual change over the phylogeny (Pagel 2008). Maximum-likelihood values of δ were estimated using the best-fitting model, which in all instances was model B, and then this model was compared against a null model, in which δ was set at the default value of 1.

When running Markov chains, rate-parameter values were explored to find acceptance rates between 20 and 40% (Pagel and Meade 2006, 2007). Furthermore, Markov chains were run for 1 050 000 generations and sampled every 1000th generation after a burn-in of 50 000 generations. All priors were set as hyperpriors with a uniform distribution, and each run was replicated at least three times in order to ensure the stability of the harmonic mean estimator. Finally, models were compared and selected using Bayes factors (BF) (Kass and Raftery 1995) based on the harmonic mean estimator of the model likelihoods (Nylander *et al.* 2004; Pagel and Meade 2007).

All models were run using the pruned tree from Driskell *et al.* (2011; see also Fig. B2a). As before, we used this phylogeny over other available phylogenies (e.g. Gardner *et al.* 2010; Lee *et al.* 2011) because of the availability of information on branch lengths, as this allows a more robust analysis of change over evolutionary time. Nonetheless, to explore the implications of the alternate topologies (especially with respect to the position of the Purple-crowned Fairy-

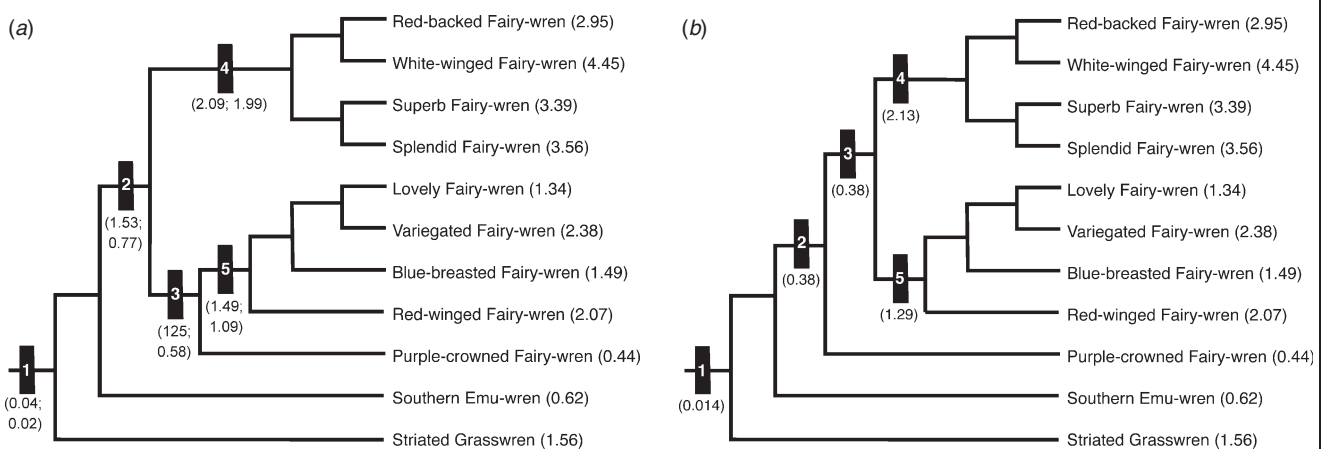


Fig. B2. Evolution of levels of sperm competition in the Australian Maluridae. The figure is a phylogeny showing data for relative testes mass (RTM, using GSI as a proxy for sperm competition level) for 11 species of malurid and reconstructed estimates of GSI for ancestral nodes. (a) Estimates based on phylogeny of Driskell *et al.* (2011). Nodes (numbered black boxes): 1, ancestor to all Australian Maluridae; 2, ancestor to all *Malurus*; 3, ancestor to Purple-crowned Fairy-wren + chestnut-shouldered group; 4, ancestor to blue + bi-coloured groups and 5, ancestor to the chestnut-shouldered group. Values in parentheses at each node are reconstructed ancestral values of GSI (first position for tree with branch lengths; second position for tree with branch lengths set to 1); values in parentheses following species names are GSI values for contemporary terminal taxa. (b) Estimates based on phylogeny of Lee *et al.* (2011). Nodes (numbered black boxes): 1, ancestor to all Australian Maluridae; 2, ancestor to all *Malurus*; 3, ancestor to the chestnut-shouldered + blue + bi-coloured groups; 4, ancestor to blue + bi-coloured groups and 5, ancestor to the chestnut-shouldered group. Values in parentheses at each node represent reconstructed ancestral trait values of GSI; values in parentheses following species names are GSI values for contemporary terminal taxa.

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wren), we performed two further analyses: (1) an analysis using the phylogeny of Lee *et al.* (2011), in this instance branch lengths were not available and all branch lengths were set to 1 and (2) a second analysis using the phylogeny of Driskell *et al.* (2011) but setting the branch lengths equal to 1 (to allow for a direct comparison with the analysis using the phylogeny of Lee *et al.* 2011). We choose to focus on these two recent studies because they provide fully resolved phylogenies, whereas the internal structure of Maluridae remains unresolved in the phylogeny of Gardner *et al.* (2010).

Results

Bayesian modelling revealed that RTM exhibits an evolutionary trajectory that differs significantly from Brownian motion (Table B2a). This suggests that the ancestral state in Australian Maluridae was one of low levels of sperm competition, and that there has been selection for increasing levels of sperm competition

Table B2. Bayesian evolutionary modelling levels of sperm competition in Australian Maluridae

Results are shown for two sets of analyses: (a) analysis using phylogeny with branch lengths (BLs) (Driskell *et al.* 2011) and (b) comparison of phylogenies (i.e. Driskell *et al.* 2011 and Lee *et al.* 2011) where all BLs are assumed to be equal (i.e. BL = 1). Shown are maximum-likelihood estimates (MLEs) of evolutionary tempo (δ), ancestral state (α) and directional trend (β); BF, Bayes factors. Interpretation of Bayes factors follows Kass and Raftery (1995). For BF < 2, no evidence against the null hypothesis (i.e. n.s., not significant); for BF = 2–6, positive evidence against the null hypothesis (*); for BF 6–10, strong support against the null hypothesis (**). Models indicate that relative testes mass has increased over evolutionary time. See main text for full details of evolutionary models

Characters	δ	δ MLE		Random walk (R) v. Directional (D)			
		BF against $\delta = 1$	Interpretation	α	β	BF (R v. D)	Interpretation
(a) Phylogeny of Driskell <i>et al.</i> (2011) with BL							
Relative testes mass	2.01	0.86 ^{n.s.}	Gradual change	0.036	11.40	2.78*	D increasing
(b) Phylogeny of Driskell <i>et al.</i> (2011) v. Lee <i>et al.</i> (2011) (BL = 1)							
Relative testes mass							
Driskell <i>et al.</i> (2011)	0.72	2.71*	Temporally early change	0.015	0.55	6.65**	D increasing
Lee <i>et al.</i> (2011)	0.77	2.93*	Temporally early change	0.014	0.54	7.68**	D increasing

across the phylogeny. Consequently, the model assuming a directional random walk was used for all subsequent analyses. Additional ancestral reconstructions showed a general pattern of increasing sperm competition across all nodes in the phylogeny, with the exception of a minor reduction in ancestral node 3 and a more significant reduction in RTM in the Purple-crowned Fairy-wren; in this species RTM is just 0.44%, whereas RTM for its most recent ancestor is estimated at 1.25% (see Fig. B2a). As previously discussed, however, the phylogenetic position of this species is somewhat uncertain and the quality of data on testes size is not known. Finally, for analysis of evolutionary tempo (δ), the model in which delta was allowed to take its maximum likelihood value did not differ from the null model (Table B2a), suggesting that evolutionary change in levels of sperm competition has occurred gradually over time.

Next, analyses comparing the alternate phylogenetic topologies showed that RTM has increased over evolutionary time irrespective of topology used (Table B2b). Similarly, for the analysis of evolutionary tempo (δ) both topologies returned similar results. Specifically, for both analyses, the model in which delta was allowed to take its maximum likelihood value fitted the data better than the null model of gradual change (Table B2b). Thus the maximum likelihood values of $\delta = 0.72$ (Driskell *et al.* 2011) and $\delta = 0.77$ (Lee *et al.* 2011) were accepted, suggesting that RTM has undergone rapid early evolution followed by slower rates of change among more closely related species (i.e. temporally early change). This result, however, contrasts with the interpretation of gradual changes across the phylogeny found in our main analysis. However, given that the parameter of evolutionary tempo (δ) scales overall path-lengths in the phylogeny, these results are most likely driven by the assumption of equal branch lengths in the phylogenies. Thus we suggest that the interpretation of gradual change in RTM values over time is a more robust interpretation of our findings.

Finally, ancestral state reconstructions using the alternate topologies yielded varying results. Under the assumption of equal branch lengths, analysis of the phylogeny of Driskell *et al.* (2011) returned qualitatively similar results as the analysis performed on the tree incorporating information on branch lengths. Specifically, RTM showed a general trend for increasing trait values with the exception of reductions in ancestral node 3 and the Purple-crowned Fairy-wren (Fig. B2a). In contrast, analysis of the phylogeny of Lee *et al.* (2011) showed a consistent pattern of increasing trait values, and thus levels of sperm competition, across the phylogeny with no evidence of reductions in RTM at any of the reconstructed ancestral nodes or terminal taxa (Fig. B2b).

and thus a direct test of these hypotheses is not currently possible. Nevertheless, these factors could influence EPP through an interaction between habitat productivity, food abundance and the demands on adults during the nestling period, and one recent study of the Southern Emu-wren (*S. malachurus*) provides initial support for this idea. Specifically, the low rates of EPP in that species are suggested to be the result of limited opportunities for female extra-pair copulations owing to a high dependence on paternal care for offspring survival (Maguire and Mulder 2008).

Within *Malurus*, the Purple-crowned Fairy-wren exhibits the lowest rate of EPP (Table 1), a finding that has also been attributed to ecology. Specifically, Kingma *et al.* (2009) suggested that the

linear arrangement of Purple-crowned Fairy-wren territories constrains opportunities for females to seek extra-pair matings. However, although the spatial arrangement of territories may indeed influence paternity in that species, it is unlikely that the occurrence of linear territories provides a general explanation for variation in rates of EPP in malurids generally; in other words, it is hard to see how the absence of linear territories in other species of *Malurus* has led to high rates of EPP.

With respect to intra-specific variation in rates of EPP, the role of breeding density in driving variation remains equivocal (Møller and Ninni 1998). This appears to be the case for the Maluridae as well, for which intra-specific (subspecific) data are available for two species (Superb and Splendid Fairy-wrens).

First, in the Splendid Fairy-wren, the western (*M. splendens splendens*) and eastern (*M. s. melanotus*) subspecies differ in many aspects of their biology and ecology, and also vary with respect to rates of EPP (Table 1) in a manner consistent with the hypothesis that breeding density influences such variation; relative to the eastern subspecies, the western subspecies exhibits a higher population density, larger group sizes and higher rates of EPP. In contrast, despite differences in ecology and behaviour (in particular, the proportion of groups with helpers), two subspecies of Superb Fairy-wren (*M. cyaneus cyanochlamys* in Canberra, ACT, and *M. c. leggei* in South Australia) show almost identical rates of EPP (Table 1; Mulder *et al.* 1994; Colombelli-Négrel *et al.* 2009).

Population density and survival rates would also influence group size, and group size (i.e. number of helpers) influences rates of EPP in both Superb and Splendid Fairy-wrens. In Superb Fairy-wrens, Mulder *et al.* (1994) showed that females in groups with at least one helper are more likely to produce young sired by extra-pair males than those in groups without helpers. Similarly, in Splendid Fairy-wrens, it is the presence of two or more helpers that leads to a dramatic increase in the rate of EPP (Webster *et al.* 2004). Two caveats, however, highlight the variation in these patterns. First, Colombelli-Négrel *et al.* (2009) also studied Superb Fairy-wrens and found high rates of EPP despite the fact that very few groups in their population had helpers. Second, Mulder *et al.* (1994) interpreted the relationship between group size and EPP to indicate that when groups had helpers, females would not pay a cost (in terms of a reduction in paternal care) by engaging in extra-pair matings. However, Varian-Ramos *et al.* (2012) explicitly tested this idea in the Red-backed Fairy-wren and, in that species, females did not suffer any reduction in paternal care regardless of whether they mated within or outside of the pair-bond.

We suggest that the most likely explanation for variation in EPP in the Maluridae is the hierarchical explanation of Griffith *et al.* (2002). In other words, the important variables influencing variation may differ depending on the comparison being examined. Thus, the differences in rates of EPP between emu-wrens, grasswrens and fairy-wrens may be the result of a combination of factors, including phylogeny, survival rates and the interaction between habitat productivity and patterns of parental care. In contrast, differences in rates of EPP between species of *Malurus* may be a result of specific ecological circumstances (as in the Purple-crowned Fairy-wren) or to variation in breeding density and factors such as group size that influence mating opportunities by individuals.

Benefits of EPP

The reasons why females engage in multiple mating remains controversial and numerous hypotheses have been proposed to explain this behaviour. Potential benefits to females can include direct benefits, such as fertility insurance and increased parental care or protection (Sheldon 1994; Reynolds 1996; Li and Brown 2002), as well as indirect or genetic benefits, such as genetic compatibility, inbreeding avoidance, increased genetic diversity, good genes, selection for superior sperm and compensation for low-quality social partners (Keller and Reeve 1995; Zeh and Zeh 1996, 1997; Jennions and Petrie 2000; Tregenza and Wedell 2000; Westneat and Stewart 2003; Slatyer *et al.* 2012).

Before discussing the relevant research on fairy-wrens, it is worth noting that the basic biology of fairy-wrens makes the possibility of most direct benefits unlikely. First, in all malurid species, parental care is provided exclusively by individuals within the social group (Rowley and Russell 1997). Furthermore, as far as is known, extra-pair mates do not provide females or their offspring with resources such as food or protection. In addition, we suggest that it is also unlikely that fertility assurance explains female promiscuity in fairy-wrens because female fairy-wrens store large numbers of sperm (M. Rowe and S. Pruett-Jones, unpubl. data) and, at least in Splendid Fairy-wrens, females cease copulating after their first egg is laid (S. Pruett-Jones, pers. obs.). Although these observations are circumstantial, they suggest that females store sufficient sperm to fertilise their entire clutch of eggs, so sperm are unlikely to be either a limited resource or a direct benefit of female multiple mating.

In contrast, there is evidence that female fairy-wrens may benefit indirectly from multiple matings in several ways. First, for the Superb Fairy-wren, Mulder and Magrath (1994) showed that the timing of pre-breeding moult varied among males according to both the environment and male condition, and suggested that the timing of moult was an honest indicator of male quality. Subsequently, Dunn and Cockburn (1999) showed that females based their choice of mates in part on the timing of the male's moult. Specifically, only males that moulted at least 1 month before the onset of breeding gained any EPP, supporting the idea that females were choosing extra-pair males on the basis of condition-dependent signals. Furthermore, Cockburn *et al.* (2008) showed that this selection regime is also dependent on environmental conditions that vary significantly from one year to the next. Thus, at least in the Superb Fairy-wren, it appears that females may benefit from extra-pair mating through a good genes effect.

Evidence that extra-pair mating may also function to reduce the cost of inbreeding in malurids has been reported in several species. First, Rowley *et al.* (1986) showed that as many as 20% of social pairings in Splendid Fairy-wrens were between females and their sons, fathers and their daughters or full-sibling pairs. Although the designations of relationships were based on social relationships, this work suggested that the risk of inbreeding is indeed high in some species of fairy-wren. Based on this assumption, Brooker *et al.* (1990) interpreted the high rates of EPP observed in the Splendid Fairy-wren as an inbreeding avoidance mechanism.

Next, Cockburn *et al.* (2003) were the first to report, using actual paternity data, that female Superb Fairy-wrens avoided mating with close relatives. In that study, females naturally paired to their sons produced broods with 100% extra-pair young. This finding suggests that female Superb Fairy-wrens may use extra-pair matings as an inbreeding avoidance mechanism, although in this instance females also used divorce to avoid being paired with a close relative or to move to a better territory (Cockburn *et al.* 2003). Similarly, in the Splendid Fairy-wren, Webster *et al.* (2004) found that auxiliary males were more likely to sire offspring within a group if they were unrelated to the breeding female than if they were closely related to her.

Brouwer *et al.* (2011) presented several lines of evidence to support the importance of inbreeding avoidance in determining extra-pair matings in the Red-winged Fairy-wren. First, EPP

was positively associated with the relatedness of the social pair, whereas extra-pair sires were less closely related to the female than her social partner. Next, in the rare cases of within-group paternity by helpers, these cases were confined to males that were unrelated to the breeding female. Finally, females gained paternity from further away when neighbouring territories were occupied by close relatives (Brouwer *et al.* 2011).

In the Red-backed Fairy-wren, evidence for inbreeding avoidance comes from both correlational and experimental work. Specifically, correlational analysis showed that females that produced extra-pair young were more closely related to their social mates relative to females that produced only within-pair young (Varian-Ramos and Webster 2012). Additionally, extra-pair young were more heterozygous than within-pair young in the Red-backed Fairy-wren (Varian-Ramos and Webster 2012), a finding that has also been reported for the Splendid Fairy-wren (Tarvin *et al.* 2005). Using an experimental approach, Varian-Ramos and Webster (2012) also showed that females paired with their sons or other closely related individuals produced a larger proportion of extra-pair young relative to females paired with unrelated males. Similarly, even in Purple-crowned Fairy-wrens, in which rates of EPP are low (Table 1), relatedness among socially paired adults appears to influence extra-pair matings. Specifically, in Purple-crowned Fairy-wrens, the likelihood that a nest contained extra-pair offspring increased significantly with increased relatedness between the socially paired male and female, as well as when the social pair consisted of an incestuous pairing (Anne Peters, pers. comm.).

Finally, using quantitative estimates of relatedness, Tarvin *et al.* (2005) demonstrated that genetic relatedness among females and their social mates influences the likelihood of female multiple matings in the Splendid Fairy-wren: females more genetically similar to their social mates were more likely to produce extra-pair young (Tarvin *et al.* 2005). Interestingly, the authors also found that 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. This suggested that, although females may use relatedness with their social mate as the basis of seeking extra-pair matings, genetic relatedness was not a criterion of extra-pair mate choice *per se* (Tarvin *et al.* 2005). Consequently, these results are consistent with the hypothesis that females benefit from extra-pair mating by inbreeding avoidance and genetic compatibility. In conclusion, although further evidence from experimental manipulations of relatedness in relationship to EPP are clearly warranted, taken together the studies summarised above strongly suggest that female fairy-wrens are sensitive to the relatedness of their social mates and that this influences both the likelihood of their engaging in extra-pair mating and their specific choice of extra-pair mates.

Sperm competition in the Maluridae

Sperm competition is defined as the competition between the sperm from two or more males for the fertilisation of a given set of ova (Parker 1998). In the Maluridae, sperm competition typically appears to be between just two males, the social pair-bonded male and a single extra-pair male. In this review, we refer to the level of

sperm competition faced by males, and we interpret an increase in levels of sperm competition as an increase in the probability that males encounter sperm competition (i.e. a greater proportion of the females in a population engage in extra-pair mating and produce young sired by an extra-pair male).

Male reproductive biology and ejaculate quality

Across a diverse range of taxa, a common evolutionary response to sperm competition is an increase in testes size (Møller 1991; Harcourt *et al.* 1995; Hosken 1997; Stockley *et al.* 1997; Hosken and Ward 2001; Byrne *et al.* 2002; Simmons and García-González 2008) and RTM is often used as a measure of sperm competition in avian studies (e.g. Briskie and Montgomerie 1992; Dunn *et al.* 2001; Pitcher *et al.* 2005; Immler and Birkhead 2007). Relative testes mass varies greatly across species of Australian Maluridae: reported values varying by an order of magnitude, from <0.5% to nearly 5% of male body mass (Rowe and Pruett-Jones 2011, see Table 2). Importantly, selection imposed through sperm competition appears to have selected for increases in testes size within the Australian Maluridae: RTM is larger in species that exhibit higher rates of EPP (see Box 1), validating the use of RTM as a proxy for sperm competition and rates of EPP in this group. Moreover, variation in RTM is associated with variation in many aspects of male reproductive anatomy and physiology, all of which appear to be direct adaptations to sperm competition.

First, however, we must acknowledge that data on testes size in birds are prone to error for several reasons (Calhim and Birkhead 2007), and the estimates presented here are not entirely free from such potential sources of error. In particular, these estimates are based on a rather small number of males (i.e. $n = 4\text{--}17$ v. the recommended sample size of $n \geq 30$; Calhim and Birkhead 2007) and the estimates do not control for possible age-related variation in testes size (e.g. Laskemoen *et al.* 2008). Nevertheless, we consider the data on RTM presented in this review to be of high quality because the data were: (1) based on fresh testes mass; (2) taken from both the left testis and right testis, thus accounting for potential error introduced by testes asymmetry; (3) collected during the breeding season from adult males known to be reproductively active and (4) taken from the same individuals from which body mass was quantified. The one exception to this is the Purple-crowned Fairy-wren, for which data were taken from the literature (Dunn *et al.* 2001), the source of which we do not know.

In addition to increases in RTM, malurid species experiencing strong sperm competition exhibit a greater proportion of spermatogenic tissue in their testes relative to species experiencing little sperm competition (Rowe and Pruett-Jones 2011). Moreover, investment in sperm producing tissue appears to translate into high rates of sperm production (Tuttle and Pruett-Jones 2004; Pruett-Jones and Tuttle 2007). Thus, males experiencing strong sperm competition show increased investment in sperm quantity by adaptations in both testes size and structure that influence sperm production. Finally, as well as selection for sperm quantity, there has been selection for traits related to sperm quality. Across species, RTM correlates with the proportion of motile sperm in ejaculates and the proportion of both viable and morphologically normal sperm in sperm reserves (Rowe and Pruett-Jones 2011).

Table 3. Morphology of the cloacal protuberance (CP) in 11 species of Australian Maluridae

CP volume, volume of the cloacal protuberance; CP volume index, $(\text{CP length} \times \text{CP width} \times \text{CP height}) \div \text{body mass}$; CP tip indicates presence (Y) or absence (N) of a cloacal tip (an asterisk (*) indicates the presence of a non-protruding region of structural modification consistent with the anatomy of the tip observed in other species). Note: data for CP volume for the Purple-crowned Fairy-wren were collected using a different methodology, which is likely to confound direct comparison with other species detailed in the table. Figures are means \pm s.e.; NA, not available; sample sizes are shown in parentheses. References: 1, M. Rowe (unpubl. data); 2, Kingma *et al.* (2009); 3, Rowe and Pruett-Jones (2011); 4, E. Greig (pers. comm.); 5, Rowe *et al.* (2008)

Species	Species or subspecies	CP volume (<i>n</i>) (mm ³)	Reference	CP volume index (<i>n</i>)	Reference	CP tip (Y or N)	Reference
Striated Grasswren	<i>A. striatus striatus</i>	83.61 \pm 7.77 (7)	1	5.50 \pm 0.52 (7)	1	Y	5
Southern Emu-wren	<i>S. malachurus malachurus</i>	0 (13)	1	0 (13)	1	N	5
Lovely Fairy-wren	<i>M. amabilis</i>	29.16 \pm 2.47 (5)	1	4.46 \pm 0.40 (5)	1	Y	1
Purple-crowned Fairy-wren	<i>M. coronatus coronatus</i>	57.4 \pm 3.6 (40)	2	NA	1	N	4
Superb Fairy-wren	<i>M. cyaneus cyanocephalus</i>	106.93 \pm 4.76 (37)	1	14.44 \pm 0.62 (37)	1	Y	5
Red-winged Fairy-wren	<i>M. elegans</i>	55.01 \pm 5.26 (14)	1	7.01 \pm 0.91 (9)	1	Y	1
Variegated Fairy-wren	<i>M. lamberti assimilis</i>	36.44 \pm 3.15 (34)	3	6.01 \pm 0.45 (31)	1	N*	5
White-winged Fairy-wren	<i>M. leucopterus leuconotus</i>	93.73 \pm 9.01 (17)	1	15.82 \pm 1.32 (17)	1	Y	5
Red-backed Fairy-wren	<i>M. melanocephalus melanocephalus</i>	128.24 \pm 3.36 (76)	3	21.53 \pm 0.73 (76)	1	Y	5
Blue-breasted Fairy-wren	<i>M. pulcherrimus</i>	29.02 \pm 2.85 (19)	3	3.98 \pm 0.39 (19)	1	N*	5
Splendid Fairy-wren	<i>M. splendens melanotus</i>	96.99 \pm 3.27 (94)	3	13.10 \pm 0.45 (94)	1	Y	5

Investment in sperm quantity is also reflected in the size of the cloacal protuberance (i.e. the site of sperm storage in passerine birds; Wolfson 1952; see Table 3). Specifically, inter-specific variation in the volume of the cloacal protuberance and the number of sperm stored in the seminal glomera, as well as seminal glomera mass, is positively associated with levels of sperm competition inferred from RTM in malurids (Rowe and Pruett-Jones 2011). Similarly, intra-specific variation in the size of a male's cloacal protuberance correlates with the number of stored sperm (Tuttle *et al.* 1996). Moreover, in the Purple-crowned Fairy-wren, intra-specific variation in the size of the cloacal protuberance appears sensitive to extra-pair mating opportunities; cloacal protuberance size varies in relation to within- and extra-pair mating opportunities faced by males (Kingma *et al.* 2012).

Finally, males of most malurid species possess a unique morphological feature, the cloacal tip, a bulbous structure located on the ventral edge of the cloacal protuberance (Mulder and Cockburn 1993; Rowe *et al.* 2008; see Table 3). Histological examination has revealed that the cloacal tip comprises connective tissue and striated muscle bundles, and that it appears to be a non-erectile muscular structure that is best categorised as a quasi-muscular hydrostatic appendage (Rowe *et al.* 2008). Importantly, inter-specific variation in the presence and length of the cloacal tip is associated with sperm competition: species with relatively larger testes have a more prominent, longer tip. Rowe *et al.* (2008) suggested that the cloacal tip might function to increase a male's likelihood of paternity success through the facilitation of sperm transfer. This could occur through the cloacal tip functioning as an intromittent organ, by mechanical manipulation of the female cloaca or by female stimulation. Female stimulation appears the most likely mechanism, and such stimulation may influence the female to: (a) eject previously deposited sperm, (b) bias sperm use by preferential transport, uptake or storage of sperm or (c) evert the cloacal chamber and thus allow sperm to gain increased access to the vaginal lumen and permit deeper transfer of sperm (Rowe *et al.* 2008). Unfortunately, observations of cloacal contact during copulation in fairy-wrens species are currently lacking. Consequently, discriminating between these functional hypotheses, as

well as the three other proposed hypotheses (i.e. display, structural counterbalance, female choice hypothesis; Rowe *et al.* 2008) is not currently feasible.

Evolution of sperm competition

The results of evolutionary modelling (Box 2) suggest that the ancestral state in the Australian Maluridae is one of low sperm competition, with a RTM of just 0.04% of male body mass. Additionally, our analyses suggest that the general pattern of trait evolution across the phylogeny is one of increasing levels of sperm competition. The major exception to this pattern is observed in the Purple-crowned Fairy-wren, in which RTM is just 0.44%, whereas RTM for its most recent ancestor is 1.25%, suggesting that this species has experienced a relatively large reduction in levels of sperm competition.

However, as discussed above, the Purple-crowned Fairy-wren appears in different locations in each of the three published phylogenies (Gardner *et al.* 2010; Driskell *et al.* 2011; Lee *et al.* 2011), and whether the Purple-crowned Fairy-wren has indeed experienced a reduction in sperm competition is completely dependent upon its phylogenetic position. More specifically, if the Purple-crowned Fairy-wren is sister to the chestnut-shouldered group, as suggested by the phylogeny of Driskell *et al.* (2011), then there has been a reduction in RTM in that specific lineage. In contrast, if the Purple-crowned Fairy-wren is placed as sister to all other *Malurus* species (except the Broad-billed Fairy-wren), as suggested by the phylogeny of Lee *et al.* (2011), then our analysis provides no evidence of reductions in RTM at any of the ancestral nodes or terminal taxa. Consequently it remains uncertain as to whether Purple-crowned Fairy-wren has experienced a loss of EPP or if their low rates of EPP simply reflect the phylogenetic relationships among malurid species.

Importantly, understanding the evolutionary trajectories of levels of sperm competition in the Australian Maluridae can begin to offer insight into the evolution of sperm competition and EPP in avian taxa generally. For example, under the assumption that the Purple-crowned Fairy-wren has experienced a loss of EPP, intra-

familial comparisons of life history and ecology may help elucidate factors driving variation in rates of EPP. Kingma *et al.* (2009) presented qualitative results for such an analysis among four congeneric species of *Malurus* but concluded that the loss of EPP in the Purple-crowned Fairy-wren was not associated with marked differences in life-history parameters. However, future tests of correlated evolution between RTM and a range of life-history and ecological variables, incorporating a wider range of malurid species, may yield information concerning the evolution of EPP in this family. However, we suggest an important initial step in this process is to resolve the phylogenetic placement of the Purple-crowned Fairy-wren.

Sperm morphometry

Sperm cells exhibit considerable variability in size and shape (Pitnick *et al.* 2009) and sperm competition is thought to be an important driver of evolutionary change in sperm traits. For example, sperm competition is thought to favour the evolution of longer sperm (Gomendio and Roldan 1991; Pizzari and Parker 2009), a hypothesis that is generally supported in birds: sperm length is typically greater in species experiencing higher levels of sperm competition (Kleven *et al.* 2009; Lüpold *et al.* 2009b, 2009c; but see Immler and Birkhead 2007). Total sperm lengths have been reported for just four species of fairy-wren, and, on average, vary between 81.02 and 86.3 µm (Tuttle *et al.* 1996; Immler *et al.* 2011; Table 4). Thus, compared to other passerines, in which sperm length ranges from 40 to 280 µm (Jan Lifjeld, pers. comm.), fairy-wrens appear to have relatively short sperm. Given the high levels of sperm competition in some species of *Malurus* and selection for longer sperm under conditions of sperm competition, the immediate question to arise is why do fairy-wrens have such short sperm?

In passerine birds, selection for increases in sperm length is thought to be weak relative to selection for increases in sperm density (Immler *et al.* 2011). In many malurids, males have large numbers of sperm stored in their cloacal protuberances (Rowe and Pruett-Jones 2011) and it is thought that males transfer large numbers of sperm in ejaculates in order to maximise paternity success under conditions of infrequent copulation by males but multiple matings by females (Rowe and Pruett-Jones 2011). Thus strong selection for sperm density may explain the relatively short sperm observed in species of *Malurus*.

Alternatively (or additionally), selection may favour the evolution of shorter sperm. Calhim *et al.* (2011) reported an

association between male cuckoldry success and sperm morphometry in the Superb Fairy-wren: lifetime EPP success was associated with sperm with a shorter flagellum and relatively longer head. Thus, Calhim *et al.* (2011) suggested that the selective advantage of shorter sperm might explain the relatively small size of sperm of the Superb Fairy-wren and the Maluridae as a whole. Finally, Calhim *et al.* (2011) also suggested that the short sperm of Maluridae might be a consequence of space-constrained sperm production in the testes related to selection for a higher proportion of sperm producing tissue (Rowe and Pruett-Jones 2011). However, phylogeny is also thought to explain much of the inter-specific variation in sperm morphology in birds (Jamieson *et al.* 1995; Immler and Birkhead 2007; Kleven *et al.* 2009). Thus inference that sperm are short in malurid species requires information from closely related taxa or estimates of the size of ancestral sperm. Such data, along with knowledge of sperm size in additional malurid species, would provide a deeper understanding of how selection has acted over time to shape contemporary variation in sperm phenotype in this family.

Sperm morphology in fairy-wrens is also characterised by low inter- and intra-male variation in sperm size (Kleven *et al.* 2007; Immler *et al.* 2008; Calhim *et al.* 2011; see also Table 4). Low variation in sperm morphology fits with expectations based on rates of EPP in birds generally (Lifjeld *et al.* 2010), and suggests that males are selected to produce sperm matching an optimal morphology. Interestingly, however, in the Superb Fairy-wren, it appears that, despite extremely high levels of EPP, variance in sperm morphology is maintained as a result of opposing, selection forces specific to the copulation role of males (Calhim *et al.* 2011). Whether such copulation-role-specific variation in sperm morphometry occurs in other malurid species experiencing high levels of sperm competition remains to be explored.

Future directions

We see several areas where additional work is needed in order to understand the causes and evolutionary consequences of EPP in this family. First, quantifying rates of EPP in additional malurid species is important for comparative purposes, and we suggest that studies of grasswrens should be a priority as there are no data currently available for any member of this genus. Perhaps the most likely candidate for paternity studies is the Striated Grasswren as this species is fairly widely distributed (Rowley and Russell 1997). Moreover, at least at some locations (e.g. Cooltong

Table 4. Sperm length and inter-male variation in sperm length in fairy-wrens

Figures are means, ± standard error where available and with sample sizes in parentheses where available. Inter-male variation in sperm length calculated from total sperm length as the between-male coefficient of variation ($CV_{bm} = s.d. \div \text{mean} \times 100$), which was then adjusted for sample size according to the formula: adjusted $CV_{bm} = (1 + (1/4n)) \times CV_{bm}$ (Sokal and Rohlf 1995). References: 1, Immler *et al.* (2011); 2, Tuttle *et al.* (1996); 3, Rathburn and Montgomerie (2003); 4, Calhim *et al.* (2011); 5, M. Rowe (unpubl. data)

Species	Subspecies	Total sperm length (µm)	Inter-male variation in sperm length	Reference	
				Total sperm length	Variation in length
Superb Fairy-wren	<i>M. cyaneus cyanochlamys</i>	81.02 (46)	2.1 (46)	1	4
Variagated Fairy-wren	<i>M. lamberti assimilis</i>	83.0 ± 0.4 (17)	2.02 (17)	2	5
White-winged Fairy-wren	<i>M. leucopterus leuconotus</i>	86.3 ± 0.3 (19)	1.54 (19)	2	5
	<i>M. leucopterus leuconotus</i>	81.9 ± 0.5 (16)	2.48 (16)	3	5
Splendid Fairy-wren	<i>M. splendens melanotus</i>	83.1 ± 0.2 (52)	1.74 (52)	2	5

National Park, Gluepot Reserve, South Australia) this species can be found in fairly high numbers during periods of good rainfall and low incidence of fire, and both males and females are responsive to vocal playback (M. Rowe, pers. obs.). Paternity data will also be especially interesting for the Lovely Fairy-wren, as this species has one of the lowest RTM values for any *Malurus* species studied to date. Similarly, paternity data for the White-winged Fairy-wren will be of interest because of their clan mating system, and for the White-shouldered Fairy-wren of New Guinea, because of the extreme variation the subspecies show in patterns of plumage dimorphism.

In addition, we suggest that studies of different populations of the same species will be critical in understanding the interaction of habitat productivity, group size, relatedness and paternity. Ideally, such studies will be conducted along a habitat gradient, but at present any inter-population comparisons would be useful given the paucity of these kinds of data. Data on relatedness among individuals across groups in relationship to population density and paternity will be particularly interesting. Relatedness across groups in permanently resident species like fairy-wrens almost certainly varies with population density, and coupled with a general trend by females to avoid mating with close kin, cross population studies in fairy-wrens could elucidate the mechanism underlying a correlation between density and extra-pair mating. Experimental approaches will ultimately yield the most informative and conclusive data, and we suggest that experimental studies of paternity in which group composition (e.g. relatedness of the social pair, *sensu* Varian-Ramos and Webster 2012) or number of helpers is manipulated will significantly contribute to our understanding of variation in rates of EPP.

Next, owing to the range of levels of sperm competition observed throughout the Maluridae, we suggest this group also offers several unique opportunities to study the selective advantage of a range of ejaculate and sperm phenotypic traits. Data on sperm morphometry in a larger set of species, as well as the relationship between sperm morphology and fitness (*sensu* Calhim *et al.* 2011) will be particularly important. Similarly, the group is ideal for studies of the kinetics of spermatogenesis. In birds, sperm competition selects for increases in the proportion of sperm-producing tissue contained within the testes (Lüpold *et al.* 2009a; Rowe and Pruett-Jones 2011) and the efficiency of spermatogenesis (Lüpold *et al.* 2011). However, it is not known whether the rate of spermatogenesis has evolved in response to sperm competition in birds (see Ramm and Stockley 2010 for evidence in mammals). Thus, we suggest that research examining the duration of the cycle of the seminiferous epithelium in malurids would provide a more comprehensive view of how selection shapes male reproductive anatomy in order to maximise sperm density.

Studies on adaptive allocation of sperm resources (*sensu* Parker 1998) would also be informative. Such studies, however, require being able to collect natural ejaculates, which is normally done with the use of model females fitted with a false cloaca (Pellatt and Birkhead 1994), and if extra-pair copulations are female-initiated in malurid species, as they are in the Superb Fairy-wren (Double and Cockburn 2000), such experiments may prove challenging. Indeed we attempted such a study with the Splendid Fairy-wren without success. Nevertheless, we encour-

age researchers to test male acceptance of female mounts in other species in order to test hypotheses of ejaculate allocation in this group.

The unique nature of the cloacal tip of male malurids also warrants further research. Artificial stimulation of the tip musculature could be used to elucidate how the tip moves, and histological examination of the tissue surrounding the female cloaca (e.g. silver staining of nerve tissue) could be used to search for a potential site of female stimulation. Finally, correlational and experimental studies could be used to investigate the relationship between the size and movement of the cloacal tip and male reproductive success.

Finally, as we discuss above, the specific phylogenetic position of the Purple-crowned Fairy-wren needs to be established as this affects interpretations of the evolution of EPP and sperm competition in the Maluridae. For example, Kingma *et al.* (2009) conclude that there has been a radical loss of EPP in the Purple-crowned Fairy-wren. Although this conclusion is correct when the phylogenetic topology matches either that used by Kingma and colleagues (i.e. Christidis and Schodde 1997) or the topology of Driskell *et al.* (2011), the alternate topology of Lee *et al.* (2011) implies a completely different evolutionary scenario. Thus resolving the placement of this enigmatic species should be considered a high priority.

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