MOLECULAR ECOLOGY

Molecular Ecology (2011) **20**, 4796–4807 doi: 10.1111/j.1365-294X.2011.05325.x

Strategic promiscuity helps avoid inbreeding at multiple levels in a cooperative breeder where both sexes are philopatric

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Abstract

In cooperative breeders, the tension between the opposing forces of kin selection and kin competition is at its most severe. Although philopatry facilitates kin selection, it also increases the risk of inbreeding. When dispersal is limited, extra-pair paternity might be an important mechanism to avoid inbreeding, but evidence for this is equivocal. The red-winged fairy-wren is part of a genus of cooperative breeders with extreme levels of promiscuity and male philopatry, but is unique in that females are also strongly philopatric. Here, we test the hypothesis that promiscuity is an important inbreeding avoidance mechanism when both sexes are philopatric. Levels of extra-pair paternity were substantial (70% of broods), but did not arise through females mating with their helpers, but via extra-group mating. Offspring were more likely to be sired by extra-pair males when the social pair was closely related, and these extra-pair males were genetically less similar to the female than the social male and thus, inbreeding is avoided through extra-pair mating. Females were consistent in their choice of the extra-pair sire over time and preferred early moulting males. Despite neighbouring males often being close kin, they sired 37% of extra-pair offspring. However, females that gained paternity from neighbours were typically less related to them than females that gained paternity further away. Our study is the first to suggest that mating with both closely related social partners and neighbours is avoided. Such sophistication in inbreeding avoidance strategies is remarkable, as the extreme levels of promiscuity imply that social context may provide little cue to relatedness.

Keywords: cooperative breeding, dispersal, extra-pair paternity, kin competition, kin selection, *Malurus*

Received 28 June 2011; revision received 30 August 2011; accepted 7 September 2011

Introduction

In cooperatively breeding species, individuals—often previous offspring of the breeding pair—assist others in raising offspring (Brown 1987; Stacey & Koenig 1990; Cockburn 1998). Evolutionary theory suggests that helping can be adaptive because of the indirect fitness benefits helpers gain by the production of kin (kin selection, Hamilton 1964). Limited movement of individuals (viscosity) results in a high degree of relatedness among group members and facilitates kin selection (Hamilton 1964). However, living with closely related individuals also results in kin competition and increases the risk of inbreeding depression.

Sex-biased natal dispersal, where one sex disperses with a higher probability, earlier and/or further, has been hypothesized to have evolved to avoid inbreeding by preventing siblings and other close relatives from settling in close proximity to each other (Greenwood 1980; Pusey 1987). In many cooperatively breeding birds, one sex typically disperses while the other stays

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and helps (Koenig *et al.* 1992; Clarke *et al.* 1997; Cockburn 1998). Where this does not happen or is not sufficient, cooperative breeders often have additional mechanisms to avoid inbreeding, such as dispersal that is initiated or enforced following the death of a same-sex breeder (Balcombe 1989; Koenig *et al.* 1998), and abandonment of breeder positions when all group members of the opposite sex are close relatives (Koenig *et al.* 1998; Daniels & Walters 2000; Cockburn *et al.* 2003; Koenig & Haydock 2004). While these dispersal mechanisms may prevent inbreeding within groups, population viscosity is likely to lead to clusters of relatives on adjacent territories (Double *et al.* 2005). Inbreeding avoidance under these circumstances is much more poorly understood (Dobson *et al.* 1997; Koenig & Haydock 2004).

The potential for inbreeding is particularly high in cooperative breeders, as a shortage of breeding vacancies means that individuals have limited opportunities to choose their social partner. Close relatives can end up forming a social pair, especially when both sexes are philopatric. In such cases, extra-pair paternity (EPP), whereby offspring are sired by a male other than the social male, has been suggested as an alternative strategy to avoid inbreeding (Brooker *et al.* 1990; Pusey & Wolf 1996). Notwithstanding, there are several other nonexclusive hypotheses that can explain why females seek EPP. For example, females could gain EPP to insure against infertility, obtain direct benefits, gain 'good genes' or increase genetic compatibility (for reviews see: Petrie & Kempenaers 1998; Jennions & Petrie 2000; Tregenza & Wedell 2000; Westneat & Stewart 2003; Slatyer *et al.* 2011).

A constraint on extra-pair mate choice is retaliation by the male, leading to reduced paternal care when the male loses confidence in paternity (Arnold & Owens 2002). The presence of helpers, however, may liberate females from this constraint on EPP, because helpers may decrease the need for care from the social male (Mulder *et al.* 1994). Alternatively, EPP might undermine the likelihood of cooperative breeding as it reduces the relatedness between offspring and helpers, which is expected to negatively affect helping effort, as has been shown in several species accounts (Richardson *et al.* 2003; Wright *et al.* 2010), and been suggested to be of general importance in comparative studies (Griffin & West 2003; Cornwallis *et al.* 2010).

Overall, empirical evidence that EPP has a crucial function in inbreeding avoidance is ambiguous in both noncooperative and cooperative species (Griffith *et al.* 2002; Akçay & Roughgarden 2007). Despite the fact that reproductive promiscuity is common in cooperative breeders (e.g. Brooker *et al.* 1990; Mulder *et al.* 1994; Richardson *et al.* 2001; Williams 2004; Cockburn 2004; Berg 2005), direct associations with inbreeding avoidance are weak and have only been shown in a few species. Male superb fairy-wrens (*Malurus cyaneus*) paired to their mother never mate with her, but the female continues to produce broods via extra-group fertilization (Cockburn *et al.* 2003), and extra-group mating is most likely when the dominant pair have high genetic relatedness in splendid fairy-wrens (*Malurus splendens*, Tarvin *et al.* 2005): grey-crowned babblers (*Pomatostomus temporalis*, Blackmore & Heinsohn 2008) and Mexican jays (*Aphelocoma ultramarina*, Eimes *et al.* 2005). Furthermore, although these studies showed that inbreeding was avoided within the group, they did not show whether closely related neighbours are also avoided in extra-pair mate choice.

The nine species of Australian fairy-wrens (genus *Malurus*) are all cooperative breeders with extreme levels of male philopatry, the highest known from year-round territorial birds (Cockburn *et al.* 2008b). Some of the highest levels of EPP observed in birds were reported by studies on the splendid (85% of broods, Brooker *et al.* 1990; 55%, Webster *et al.* 2004), superb (95%, Mulder *et al.* 1994) and red-backed fairy-wren (*M. melanocephalus,* 75%, Karubian 2002), suggesting that EPP has a function in inbreeding avoidance. In all these species, the dominant male is most likely to be cuckolded by a male from outside the social group. However, in *M. cyaneus* and *M. Melanocephalus,* incestuous pairing is rare because of female dispersal (Rowley & Russell 1997; Cockburn *et al.* 2003), so most EPP must have an explanation other than incest avoidance. Although superb fairy-wren females avoid mating with a son if he inherits the territory, such relationships are rare and short-lived (Cockburn *et al.* 2003). Behavioural evidence suggests that 100% of superb fairy-wren females seek extra-group fertilizations with a specific preferred male, regardless of their relatedness to their mate (Double & Cockburn 2000), but also always copulate with their social partner unless he is their son (A. Cockburn, unpublished data).

A role of EPP as an important inbreeding avoidance mechanism is also equivocal in fairy-wrens that do have some female philopatry: female splendid fairy-wrens whose entire broods were sired by extra-pair males were genetically more similar to their social than their extra-pair male; however, this was not the case for mixed-paternity broods (Tarvin *et al.* 2005), which is consistent with the avoidance of within-pair fertilizations when sons are paired to their mother. Finally, it has recently been shown that EPP is virtually absent in the purple-crowned fairy-wren (*M. coronatus*; 4.5% of broods, Kingma *et al.* 2009), suggesting that EPP cannot be the primary measure preventing inbreeding avoidance in all fairy-wrens.

In this study, we will investigate the role of EPP as an inbreeding avoidance mechanism in the cooperatively breeding red-winged fairy-wren (*Malurus elegans*). This species is the most interesting with respect to the costs of viscosity, as it has by far the highest incidence of female philopatry (Rowley & Russell 1997). In addition to complete male philopatry, all female red-winged fairy-wrens stay and help in their natal territory for at least 1 year (Russell & Rowley 2000). Consequently, social pairings between closely related individuals are common, with 21% of males being paired with their mother or sister, and females breeding in their natal neighbourhood also likely to be surrounded by close male relatives (Russell & Rowley 2000). The consequent high risk of inbreeding in red-winged fairy-wrens suggests that EPP might be used as a strategy to avoid inbreeding, but parentage data have thus far been unavailable.

Here, we report an examination of the genetic mating system of the red-winged fairy-wren using microsatellite markers to test the hypothesis that EPP has an important role in inbreeding avoidance when both sexes are philopatric. We first determine the level of EPP and identify whether both male and female helpers share in reproduction. Subsequently, we investigate whether EPP has a function in inbreeding avoidance within the group by testing whether more related social pairs have a higher probability of having EP offspring and whether females are less related to EP males than to the social male. In viscous populations, the relatedness of a female with EP males is expected to decrease slowly with distance, and therefore, we next investigate whether there is evidence for inbreeding avoidance outside the group (i.e. within the local neighbourhood of the subpopulation). Finally, we investigate whether females are consistent in their EPP between broods and whether male EP success depends on the timing of moult into nuptial plumage, a sexual signal that has been shown to be a predictor of mate choice in other fairy-wrens (Dunn & Cockburn 1999; Karubian 2002; Cockburn *et al.* 2008a).

Methods

Study area and data collection

Data were collected in Smithbrook Nature Reserve 10/(116°′E, 34°20′S) in southwest Western Australia in 2008 and 2009. This site was used for a long-term study of demography in this species from 1980 to 1995 (Russell & Rowley 2000). The 95-ha reserve consist of eucalypt wet forest (Karri *Eucalyptus diversicolor* and Jarrah *E. marginata*) with a dense understorey (see for more details: Rowley *et al.* 1988). Eighty-eight percent of the border of the study area is bounded by unsuitable habitat (farmland), but three narrow corridors lead away from the reserve afford potential for emigration from and immigration to the reserve. The main study area comprised 70 territories and completely includes the smaller site where Russell & Rowley (2000) studied demography. More than 99% of the adult birds were individually colour-banded and blood sampled during the 2008 breeding season (October 2008–January 2009); during the 2009 breeding season, we colour-banded and sampled the remaining unringed adults ($n = 6$) in the main study area and an additional 80% of the birds in 31 territories surrounding the main study area.

In the main area, each territory was checked at least fortnightly for group composition and breeding activity throughout the breeding season. Each group comprised a 'dominant' pair-bonded male and female and anywhere from zero up to seven helpers (mean group size: 3.9 ± 1.4 SD). Social status was based on behavioural observations and plumage variation (Russell & Rowley 2000). Eighty-three percent of the territories had one or more helpers, and on average, 45% of helpers were female. Plural breeding, where more than one female in the group builds a nest (Rowley *et al.* 1989), was observed in 7% of the territories (Russell & Rowley 2000). Territory borders were mapped (using a Garmin 76 GPS) based on observations of individual fairy-wrens and the outcome of disputes between groups. We tried to follow the fate of each nest and sample all the offspring (clutch size 1–3), although it is likely that some unsuccessful attempts during the 2008 breeding season were not detected. Nestlings were blood sampled when at least 2 days old and colour-banded when 8 days old. Abandoned clutches and unhatched eggs were collected for genotyping. Each group produced on average 1.1 (range 0–3) broods per season that survived to be sampled. In total, we sampled 345 adults and 320 offspring from 158 broods ($n = 267$ nestlings, 16 unhatched eggs and 37 fledglings); 118 broods with 261 offspring from 73 females (average per female = 1.6 brood) were completely sampled before any mortality took place. All tissue and blood samples (*c.* 15 μL) were stored in 1 mL of 100% ethanol and stored at room temperature.

Microsatellite genotyping and parentage analyses

To assign parents to the offspring, eight hypervariable microsatellite markers developed for other bird species were selected: *Mcy3*, *Mcy6*, *Mcy7*, *Mcy8* (Double *et al.* 1997), *Tmm6* (Adcock & Mulder 2002), *Msp4* (Webster *et al.* 2004), *Smm2* and *Smm7* (Maguire *et al.* 2006). Genomic DNA was extracted using a salt extraction method as described in Bruford *et al.* (1998). The genomic DNA was amplified in standard 20 μL PCRs containing 2.5 μL 10 × PCR buffer (concentrations of Tris–HCl, KCl and $(NH4)_2SO_4$ are proprietary; Qiagen), 0.2 mM of each nucleotide, 0.15 μM of each primer (forward primer being labelled with M13 HEX, FAM, NED or VIC; Applied Biosystems, Foster City, CA, USA), 1 U of Taq-polymerase and approximately 40 ng genomic DNA. Following an initial 5-min denaturation at 94 °C, the reaction mix went through the following touch-down thermal cycling programme: 30 s at 94 °C, 30 s at 66 °C and 1 min at 72 °C—dropping the annealing temperature by 4 °C every four cycles until it reached 50 °C, at which it was kept for another 20 cycles. The last cycle was followed by a 10-min extension at 72 °C. After PCR, products from all eight loci were combined and visualized on an ABI PRISM 3100 Genetic Analyzer using Genescan-500 LIZ size standard (Applied Biosystems). Genotypes were then assigned using GeneMapper 3.7 software (Applied Biosystems).

All eight microsatellite loci were hypervariable with on average 29 alleles (range: 11–56 alleles) and observed heterozygosities from 0.71 to 0.97. The exclusion probabilities per locus ranged from 0.49 to 0.88 for the first parent and from 0.66 to 0.93 for the second parent, with a combined exclusion probability for all loci of >0.99. There was no evidence of sex linkage, but loci *Mcy*3 and *Msp*4 deviated significantly from Hardy–Weinberg expectations, with a low null allele frequency estimate of 0.05 for *Mcy*3 and a higher estimate of 0.16 for *Msp*4. However, as the presence of null alleles results in underestimation rather than overestimation of the exclusion probability (Dakin & Avise 2004) and the exclusion probabilities for both loci were high (*Mcy*3: 0.85; *Msp*4: 0.98), these loci were included in the parentage analyses but treated with care.

Parentage was assigned using a parent-pair analysis in program Cervus 3.0 (Kalinowski *et al.* 2007). Through a simulation module, Cervus incorporates the occurrence of null alleles and mutations as well as incomplete sampling into likelihood calculations to decrease the probability of false exclusions. The simulation parameters (98% of candidate parents sampled; error rate of 1% to allow for mistyping; assignment at a 95% confidence level) were estimated from the field data with all adult males and females in the population included as possible parents for each offspring. In four cases, two sires had similar likelihood. We assigned paternity to a male if he was unambiguously assigned parentage to another young in the brood or when one of the males was more likely due to the presence of a null allele.

Relatedness

Relatedness measures the proportion of alleles shared between individuals that are identical by descent. We estimated relatedness by calculating the pairwise *r* according to Wang (2002) in program KINGROUP v2 (Konovalov *et al.* 2004). We used Wang's estimator here as calculating pairwise *r* for mother–offspring pairs (predicted pairwise *r* = 0.5) showed that this measure performed best compared with three other commonly used measures (pairwise *r*: 0.47 ± 0.09 SD, Queller & Goodnight 1989; 0.47 ± 0.12 SD, Lynch & Ritland 1999; 0.48 ± 0.06 SD, Wang 2002; 0.45 ± 0.10 SD, Konovalov & Heg 2008). The presence of null alleles in locus *Msp4* resulted in lower pairwise *r* values for mother–offspring and was therefore omitted from the relatedness calculations. The mean and SD pairwise *r*-values of mother–offspring pairs was used to group pairwise *r* for graphical purposes as follows: *r* = 0.5: 0.48 ± 0.06; *r* = 0.375: 0.36 ± 0.06; *r* = 0.25: 0.24 ± 0.06; *r* = 0.125: 0.12 ± 0.06.

Timing of nuptial moult

Male red-winged fairy-wrens can moult from a brown eclipse plumage to a bright blue and chestnut nuptial plumage up to 6 months before the breeding season, whereas females retain their grey-brown plumage throughout the year (Russell *et al.* 1991). Early acquisition of male nuptial plumage has been shown to be the main trait influencing female choice of extra-group sires in other fairy-wren species (Dunn & Cockburn 1999; Karubian 2002). In superb fairy-wrens, such early acquisition is highly condition-dependent, being more likely among older birds and in favourable (wet) conditions (Cockburn *et al.* 2008a; van de Pol & Cockburn 2011). In red-winged fairy-wrens, only 20% of males moult into full nuptial plumage in the first breeding season after hatching, and birds acquire nuptial plumage earlier as they get older, regardless of the dominance status of the male (Russell *et al.* 1991).

We recorded the moulting stage for 109 males in the winter (July) and breeding season of 2009. Males were divided into three classes: (i) early moult (in full nuptial plumage before 1 August), (ii) mid moult (moulted between 1 August and the onset of the breeding season, 15 October) and (iii) late moult (moulted during the breeding season, not fully completed nuptial moult or not moulted at all before 15 January).

Statistical analyses

To assess whether EPP is used as an inbreeding avoidance strategy, we determined whether it was predicted by the pairwise *r* of the social pair. The proportion of EPP per brood was fitted as a binomial response variable with brood size as denominator and analysed using a logit link function in a generalized linear mixed model (GLMM). We included whether or not helpers were present, the year, and their interaction, as the presence of the helpers is the main predictor of cuckoldry rates in superb fairy-wrens (Mulder *et al.* 1994; Dunn & Cockburn 1999), and sexual selection is less intense in dry years in this species (Cockburn *et al.* 2008a). As broods of the same female may not be statistically independent, female identity was included as a random effect.

To determine whether neighbouring males are less likely to sire extra-pair offspring, we quantified the distance (minimum number of territory borders crossed) between each offspring to their sire and to all other adult males in the study area. Subsequently, we calculated the proportion of neighbouring males with high relatedness for females that gained paternity with immediate neighbours and compared this to the neighbour relatedness of females that gained paternity in more distant territories. Behavioural inbreeding avoidance mechanisms are likely to be based on learning and association, such as nest sharing (Bateson 1978; Komdeur & Hatchwell 1999), therefore, we defined highly related as half-sib (pairwise $r = 0.25$) or higher order. Although there could potentially be misclassification of individuals with respect to pairwise r, this is not expected to vary between the two groups (paternity with neighbouring males vs. paternity with males in more distant territories). We coded the distance of EPP as a binary response variable (EPP gained by neighbour or more distant male; logit link function) and included the proportion of neighbouring males with high relatedness and number of neighbouring males as continuous linear predictors and female identity as a random factor in the GLMM.

The repeatability (ρ: the intraclass correlation coefficient) of females in their level and distance of EPP between broods was calculated as the ratio of the between-female variance over the sum of the between- and within-female variance (Rasbash *et al.* 2005), with standard errors calculated following Fisher (1925).

To investigate whether a male's EPP success is associated with the timing of nuptial moult (early, mid or late moulting), we analysed whether or not a male sired EP offspring using a logistic regression. Similarly, for dominant males, we analysed whether the probability of siring within-pair offspring depended on the time when they acquired nuptial plumage.

Statistical analyses were performed in MLwiN V2.20 (Rasbash *et al.* 2005). Model selection was based on stepwise backward elimination of nonsignificant fixed terms in order of their significance. Significance of fixed terms was assessed by the Wald test and of random terms by a likelihood ratio test, which were both compared with a chi-square distribution. Effect sizes were based on the final model that contained all significant terms.

Results

Maternity

Parentage analysis suggested that egg dumping by helper or extra-group females does not occur. For >99% of the sampled offspring ($n = 317$), the most likely mother was the female attending the nest, which could either be the dominant female (96%) or a plural breeder (4%) on the territory (Fig. 1). For three offspring, both the dominant and a helper female had similar likelihood of being the genetic mother. It seems unlikely that these cases involved egg dumping as there was no other evidence for brood parasitism, such as clutches with more than three eggs or two eggs appearing in a nest within 24 h. Consequently, we assigned these offspring to the dominant female on the territory.

Paternity

Paternity was assigned to 97% of the offspring with a combined parent-pair–offspring confidence level of 95%. For 1.2% of all offspring ($n = 4$), two candidate males had a very similar likelihood; for three of these offspring, a sire could be assigned after considering the presence of null alleles and whether or not one of these males had sired other offspring in the brood. For 1.8% of the offspring ($n = 6$), paternity could not be assigned, these offspring were probably sired by an unsampled extra-group male.

Considering just the completely sampled and assigned broods, the majority (70%) of the broods contained at least one EP offspring and 57% of the

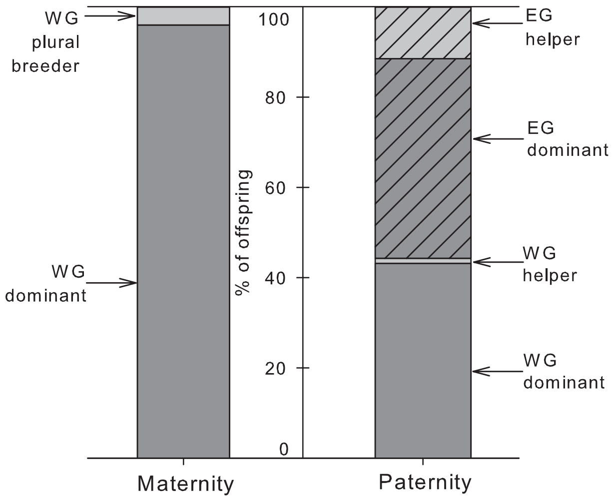

Fig. 1 Within-group and extra-group maternity and paternity of red-winged fairy-wren offspring of fully sampled broods ($n = 118$ broods, 261 offspring).

offspring were sired by a male other than the social male (Fig. 1). The majority of this EPP was gained by males from outside the group (extra-group paternity), with only 1.9% of the EP offspring ($n = 3$) being sired by within-group (WG) helper males (Fig. 1). Consequently, EPP is virtually synonymous to extra-group paternity in this population. Helper males did gain a substantial proportion of the extra-group paternity, though, siring 21% of the extra-group offspring (12% of all offspring; Fig. 1).

Pairwise relatedness and extra-pair paternity

There was a strong positive association between the proportion of EPP in a brood and the pairwise r of the social pair (Table 1), with first order relatives nearly always producing EP offspring (22 out of 25 offspring; Fig. 2). A pairwise comparison showed that females were significantly less related to their extra-pair mates than to their social male (pairwise r ± SE extra-pair vs. social male: −0.003 ± 0.01 vs. 0.07 ± 0.03; paired $t = -2.6$, $n = 58$, $P = 0.01$). These results suggest that EPP is more likely when mating with the social male is likely to be incestuous.

Distance of extra-pair paternity

The mean distance between extra-group offspring and their sire was 2.2 ± 1.4 SD territories ($n = 180$), with 90% of all EPP within three territories, even though females can mate with males as much as 12 territories from their own (Fig. 3). Comparing the distances between offspring and the sampled males in the study area showed that our study design allowed for distances up to 20 territory widths to be detected (Fig. 3).

Table 1 Result from a generalized linear mixed model of the proportion of extra-pair paternity in a brood in relation to the pairwise relatedness of the social pair and to possible confounding factors ($n = 118$ broods). Estimates of effect sizes are on logit scale

Parameter	Estimate ± SE	$\chi^2_{\text{d.f.}=1}$	P
Final model			
Intercept	0.14 ± 0.18		
Pairwise r	3.07 ± 1.00	9.4	0.002
Female random effect (σ^2)	0.96 ± 0.36	7.0	0.008
Rejected terms			
Year	−0.35 ± 0.28	1.62	0.20
Helper presence	0.61 ± 0.40	2.27	0.13
Helper presence × pairwise r	3.36 ± 2.67	1.58	0.21

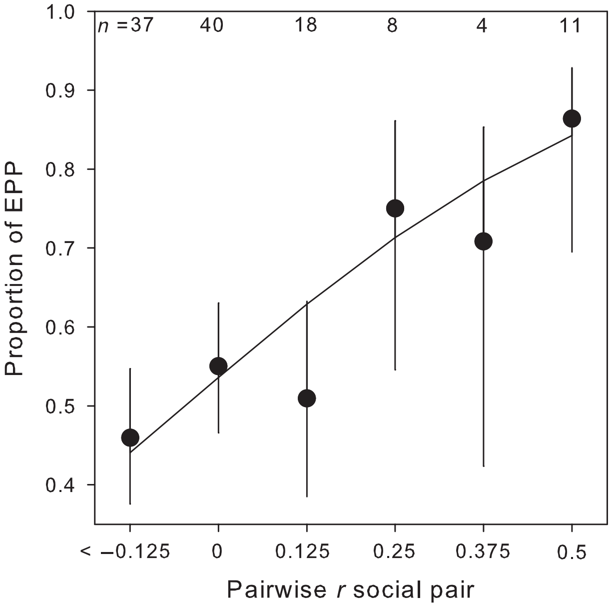

Fig. 2 The relationship between the pairwise relatedness (r) of the social pair and the proportion of extra-pair paternity in a brood. Pairwise r values were grouped for graphical purposes on the x-axis, but analyses were performed on original values. Numbers on top indicate sample size (no. of broods).

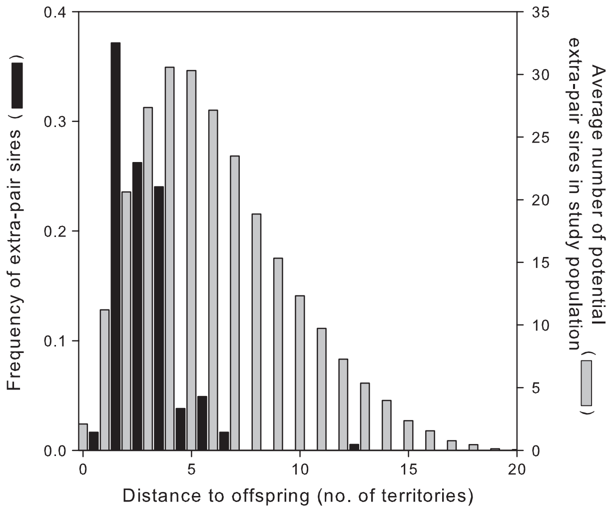

Fig. 3 Frequency distribution of distances between extra-pair offspring and their sires (left y-axis; $n = 183$). A distance of zero means that the extra-pair sire was a within-group helper male. Also shown is the frequency distribution of the average number of potential extra-pair sires (adult males) in the study population in relation to distance to each offspring (right y-axis), which describes our study's power to detect extra-pair paternity at long distance (up to 20 territories). Note that the number of potential extra-pair sires in the study area declines at high distances because of the confined spatial configuration of the study population.

The pairwise r between dominant males and dominant females within the same territory was often high, with 21% of the social pairs having a pairwise $r = 0.25$

or higher and 8% of the social pairs being first order relatives ($r \approx 0.5$, distance to female of zero, Fig. 4a). The probability of close relatedness between a dominant female and dominant males from other territories declines with increasing distance, with the largest decline between one and two territories away, after which the proportion of closely related dominant males remains at the background population level (Fig. 4a). Thus, an average female is expected to gain little additional reduction in the risk of inbreeding by seeking EPP from further than two territories away.

Given the extreme philopatry of males, it is unsurprising that most of the within-group helper males (57%) are in some way related to the dominant female, with 41% of the helper males being first order relatives (probably sons, Fig. 4b). Helper males from neighbouring territories, however, are substantially less related to the dominant female than the within-group helpers, but still 11% have a pairwise $r \approx 0.25$ or higher (Fig. 4b).

The large proportion of highly related males in neighbouring territories suggests that gaining EPP from males in neighbouring territories—which accounts for 37% of all EPP (Fig. 3)—typically results in a higher risk of inbreeding than gaining EPP with males further away. However, the EPP distance varied as a function of the frequency of high relatedness among neighbouring males (GLMM, $\chi^2 = 11.2$, d.f. = 1, $P < 0.001$), with females that gained EPP from neighbours having less closely related neighbouring males than females that gained EPP from more distant males (Fig. 4c, d). This result suggests that in situations of spatial kin clustering, the choice/distance of extra-group sire is also used as an inbreeding avoidance strategy in the local neighbourhood (i.e. outside the group). The number of

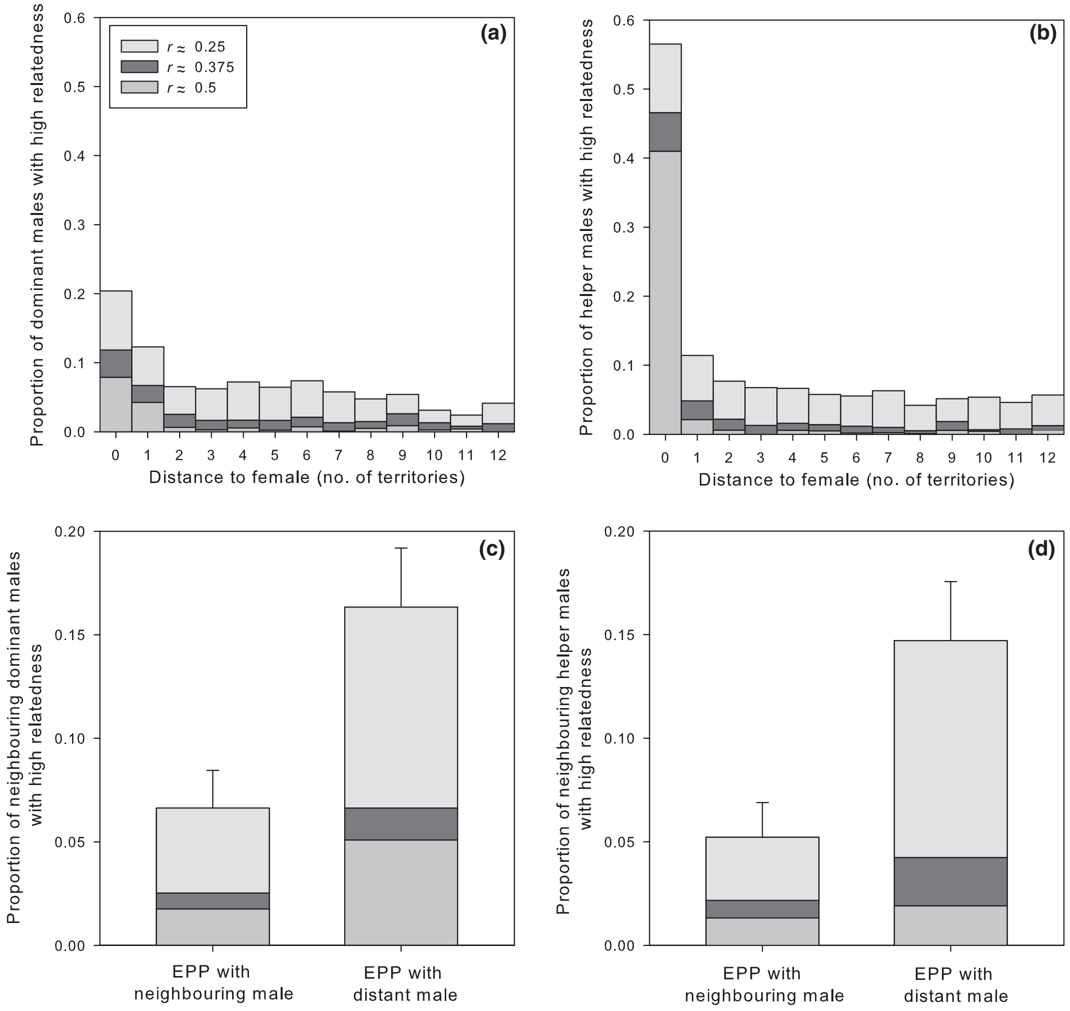

Fig. 4 (a,b) The average proportion of (a) dominant males and (b) helper males with high pairwise *r* to a dominant female as a function of their distance in territory widths (distance 0 is female's own territory). (c, d) The average proportion of neighbouring (c) dominant and (d) helper males with high pairwise *r* to a female for females that gained extra-pair paternity with immediate neighbours or with males from more distant territories ($n = 66$ females, 180 extra-group offspring).

neighbouring males was not associated with the distance of EPP (GLMM, $\chi^2 = 0.62$, d.f. = 1, $P = 0.43$).

Female consistency in extra-pair behaviour

Females were consistent in their level of EPP between broods ($\rho = 0.42 \pm 0.04$). The intraclass correlation coefficient ρ remained high after controlling for pairwise r of the social pair ($\rho = 0.31 \pm 0.04$), suggesting that pairwise r only explained part of the repeatability of EPP levels of females. Furthermore, females were extremely consistent in the distance at which they gained EPP ($\rho = 0.86 \pm 0.02$), suggesting that females repeatedly mated in the same territory. Indeed, in all broods with more than one extra-pair offspring, the same EP male sired multiple offspring in 88% of the cases ($n = 49$ broods). Moreover, EP males had a 52% probability of siring another EP offspring with the same female in subsequent broods (both within and across seasons; conditional on the males being alive at both occasions; $n = 31$ males).

Timing of nuptial moult and extra-pair paternity

Males that moulted early into nuptial plumage were more likely to sire EP offspring than males that moulted later in the year (logistic regression, $\chi^2 = 6.3$, d.f. = 1, $P = 0.01$; Fig. 5). There was no indication that timing of moult of the social male affected his risk of being cuckolded (logistic regression, $\chi^2 = 0.57$, d.f. = 1, $P = 0.45$).

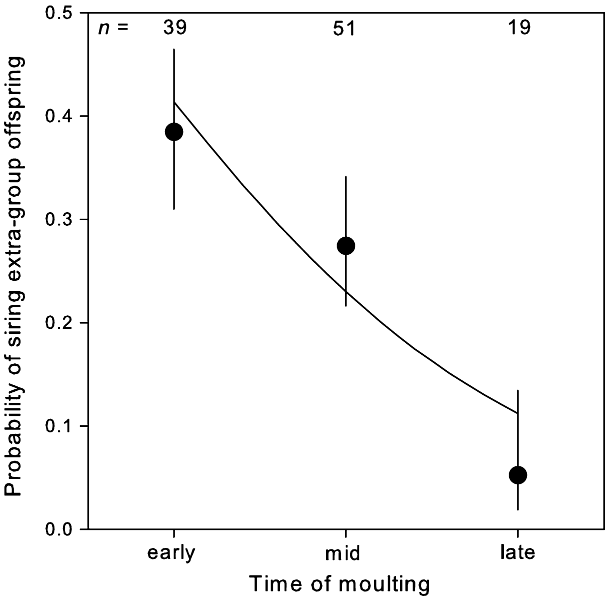

Fig. 5 The relationship between the time of year adult males moult into nuptial plumage and the probability (±SE) that they sired extra-group offspring (early: before 1 August, mid: 1 August–15 October, late: after 15 October). Numbers on top indicate sample size (no. of males).

Discussion

Both male and female cooperatively breeding red-winged fairy-wrens are highly philopatric, leading to frequent incestuous pairing and clustering of relatives in local neighbourhoods (Russell & Rowley 2000). Here, we show that like most other fairy-wrens, this species has a very high rate of extra-group fertilization (Fig. 1). While such extra-group mating is universal when nuclear family members are paired, indicative of strong incest avoidance, extra-group fertilizations remain common when females are unrelated to their partner (Fig. 2). Females prefer to mate with older males that are capable of sustaining nuptial plumage before the mating season commences (Fig. 5). While these data are consistent with reports from other fairy-wren species, this study suggests that females also avoid mating with related neighbours (Fig. 4), indicating that incest avoidance through extra-pair mating occurs at multiple levels.

Cooperative breeding and inbreeding avoidance

The potential for inbreeding is particularly high in cooperative breeders based on natal philopatry, both within the group, because of the presence of offspring that delay dispersal and within the local neighbourhood, where most of the dispersers end up. A recent meta-analysis failed to find an association between genetic similarity of the social pair and EPP in birds (Akçay & Roughgarden 2007), indicating that there is not much evidence for a role of EPP in inbreeding avoidance in general. However, of the studies in the meta-analysis, an association between genetic similarity and EPP was found in two of the three cooperatively breeding species versus only three of the 15 noncooperatively breeding species. A role of EPP in inbreeding avoidance might therefore be more likely in cooperatively breeding species, especially in species such as the red-winged fairy-wren, where both sexes are philopatric and incestuous pairings are common (Russell & Rowley 2000).

EPP and inbreeding avoidance within the group

Our molecular dissection of relatedness confirms previous suggestions that philopatry among both females and males means that social mates in the red-winged fairy-wrens are frequently close relatives and are often likely to be nuclear family relatives (Russell & Rowley 2000). However, our results also suggest that the frequency of nuclear family pairings may have been overestimated (10% of pairs have pairwise $r \approx 0.5$ vs. 21%

as estimated by Russell & Rowley 2000). Such overestimation is likely to come about because while mothers who pair to their sons will be predictably related to them, extra-group mating means that the same will not be true for daughters who pair to their social father or for putative siblings.

The inbreeding avoidance hypothesis for extra-pair mating predicts that extra-pair mating should increase with the relatedness of the social pair (Brooker *et al.* 1990). This prediction was supported in our study, where we found that levels of EPP increased with increasing relatedness of the social pair and that extra-pair sires were less closely related to the female than her social partner. In addition, within-group paternity by helpers was very rare and confined to males unrelated to the dominant female. Studies on the superb and splendid fairy-wren similarly showed that helper males were more likely to sire within-group offspring after the dominant female was replaced (Cockburn *et al.* 2003; Webster *et al.* 2004).

However, although infidelity helps to avoid inbreeding, we are unconvinced that the inbreeding avoidance hypothesis alone adequately accounts for patterns of extra-pair mating in fairy-wrens. First, contrary to the predictions of the hypothesis, the levels of EPP in red-winged fairy-wrens are not as high as in other fairy-wren species where incestuous pairing is rare because females always undergo natal dispersal, and mothers initiate divorce when their son inherits the territory (Rowley & Russell 1997; Cockburn *et al.* 2003). Second, even in red-winged fairy-wrens, extra-group mating is the dominant contributor to fertilization even when pairs were not related. An alternative view of the fairy-wren system is that there are other benefits of EPP (e.g. gaining 'good genes') that makes incestuous pairing possible, because extra-group mating is so frequent that the female can continue to produce offspring even when paired to a close relative (Cockburn 2004). Future research comparing the fitness of half-sibs within the same brood or with the same genetic sire might give new insights into this (Griffith *et al.* 2002), although genetic benefits of EPP can also be hidden and act through more complex mechanisms (Brouwer *et al.* 2010).

Alternatively, the observed patterns between EPP and relatedness could also be the result of 'cryptic' female mate choice through genetic incompatibility, whereby fertilization success decreases with increasing relatedness (Zeh & Zeh 1996), the so called 'genetically loaded raffle' (Ball & Parker 2003; Griffith & Immler 2009). Evidence for the genetic loaded raffle hypothesis comes, for example, from a recent experimental study on Gouldian finches (*Erythrura gouldiae*), which showed that genetically incompatible males were less likely to sire offspring as a result of sperm competition (Pryke *et al.* 2010). When most females seek EPP—as appears to be the case in red-winged fairy-wrens—cryptic female choice could also result in a positive association between relatedness and EPP. However, a role for genetic incompatibility is undermined by evidence that females actively choose their mates rather than engaging in polyandry to acquire a compatible mate. The high repeatability of both the proportion of EPP and distance of the EP sire suggests that females are consistent in their EP mating behaviour and repeatedly mated in the same territory, as also found in the superb fairy-wren, where radio-telemetry revealed that females initiate fertilizations before dawn by flying directly to the male's singing post on his territory (Double & Cockburn 2000). Like superb fairy-wrens, female red-winged fairy-wrens preferred males that moult into nuptial plumage well before the breeding season. Such early moulting is likely to be costly (Peters *et al.* 2000) and condition-dependent (Cockburn *et al.* 2008a) and hence a reliable cue of male quality. Neighbouring helpers did obtain substantial amounts of our paternity in our study, but in the superb fairy-wren, most such success is acquired parasitically by singing near high-quality males during the dawn chorus (Double & Cockburn 2003; Cockburn *et al.* 2009), and the same may well be true for red-winged fairy-wrens. Nevertheless, we cannot rule out that the results are a combination of precopulatory (female choice and mating behaviour) and postcopulatory mechanisms (cryptic female choice).

Interestingly, our results reveal a paradox to the hypothesis that extra-group mating can be driven by incest avoidance: the same EPP behaviour that would allow inbreeding avoidance also makes social context an unreliable cue for relatedness. For example, 57% of females will be completely unrelated to their social father, yet many potential extra-pair sires raised in different nests or territories might still be close kin because they share a sire with the female. Indeed, females are at risk of mating with their own father if they leave their territory to seek fertilizations.

EPP and inbreeding avoidance in local neighbourhood

Our data suggest that females are sensitive to the risk of mating with relatives in their extended neighbourhood. A completely novel result of our study is that females gained paternity further away when neighbouring territories are occupied by their close relatives. Red-winged fairy-wrens are long-lived, and it is premature to assess these results against a full pedigree, but the aversion to mate with closely related neighbours appears to be more complex than avoidance of mating with sons that had dispersed into neighbouring territories, as females also gained EPP further when there was a large proportion of males with $r \approx 0.25$ (Fig. 4c, d).

We know of no other study of birds that has shown evidence for inbreeding avoidance in an extended subpopulation (Foerster *et al.* 2006). Avoidance of inbreeding in the subpopulation would require sophisticated kin recognition or behavioural mechanisms that might be costly to acquire. Inbreeding avoidance could even restrict the ability to mate (Koenig *et al.* 1998; Cooney & Bennett 2000; O'Riain *et al.* 2000). Thus, in many species, such mechanisms to avoid inbreeding in the local neighbourhood might be more costly than inbreeding depression.

Choice of extra-group sire

At this stage, we cannot know the mechanism by which females avoid mating with related males in their extended neighbourhood. Because of the high level of EPP, social context will be an unreliable cue to recognise kin. Kin recognition based on MHC provides an alternative when social context is unreliable (Zelano & Edwards 2002). Females could also use behavioural mechanisms to avoid inbreeding outside the group that do not require kin recognition. For example, females that gain paternity with males in more distant territories might be females that inherited the territory rather than those that dispersed into it from somewhere else. Future research on known age and origin breeders might shed light on the importance of such behavioural mechanisms. Finally, if our findings are (partly) the result of a 'genetically loaded raffle' (Ball & Parker 2003; Griffith & Immler 2009), fertilization is the result of postcopulatory processes (i.e. sperm competition), which do not require any kin recognition mechanism.

Conclusion

Red-winged fairy-wrens differ from their congeners in having high levels of natal philopatry among both male and female offspring, so that social partners are often closely related. Extra-pair mating in this species may permit such social pairing, but expose females to the risk of incestuous mating in their immediate neighbourhood. Although the mechanisms remain unknown, our data indicate that in response to this pressure, females surrounded by related males travel further to mate outside the group, suggesting that this species represents an ideal model for examining sophisticated kin recognition among birds.

Acknowledgements

The Department of Environment and Conservation gave permission for fieldwork and sampling. We would like to thank staff of the Science division of DEC in Manjimup for their support and hospitality and John Angus for logistical support. Nicolas Margraf provided invaluable advice in the laboratory. This work was supported by a Rubicon fellowship of the Netherlands Organisation for Scientific Research (Grant no. 825.08.003) awarded to LB and by the Australian Research Council (Grant no. DP0451018) awarded to AC.

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Data accessibility

Sample locations and microsatellite data deposited at DRYAD: doi:10.5061/dryad.b3t0g38s.