# REPRODUCTIVE STRATEGIES OF FEMALES IN THE RED-BACKED FAIRY WREN (*MALURUS MELANOCEPHALUS*)

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of CLAIRE WILLOW VARIAN RAMOS find it satisfactory and recommend that it be accepted.

Chair

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# REPRODUCTIVE STRATEGIES OF FEMALES IN THE RED-BACKED FAIRY WREN (*MALURUS MELANOCEPHALUS*)

## Abstract

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When females are limited in the quantity of offspring they can produce, one way they can improve their reproductive success is by increasing offspring quality. Here we examine two ways in which females red-backed fairy-wrens may produce higher quality offspring, first through sex ratio manipulation, and second through choice of extra-pair mates. If one sex of offspring has a greater chance of surviving and reproducing then females benefit if they preferentially produce that sex. Depending on the benefit provided by auxiliary helpers, females in cooperatively breeding species may either prefer to produce the helping sex, in order to gain their assistance with future reproduction, or the dispersing sex, in order to avoid local competition between relatives. In the red-backed fairy-wren we found that females gain little or no fitness benefit from auxiliary males and accordingly females with auxiliaries tend to produce broods biased towards the dispersing sex. Further, the population wide primary sex ratio is biased towards the dispersing sex. Extra-pair mating is another way that females may increase the quality of their offspring. However, because the benefits of extra-pair mating are indirect, if large direct costs are associated with this behavior, it cannot evolve as an adaptive female strategy. In the red-backed fairy-wren we found little or no cost associated with extra-pair mating. This suggests that indirect benefits to females may partially drive the evolution of extra-pair mating. One benefit that females may gain is through extra-pair mating is increased offspring heterozygosity. This may be particularly important when dispersal is limited and inbreeding is common. We found that female red-backed fairy-wrens who produce extra-pair offspring are more related to their social mates than those who do not and that extra-pair offspring are more heterozygous than within-pair offspring. Further, an experimental test shows that females paired to closely related males produce a greater proportion of extra-pair young than those paired to unrelated males. Thus there appear to be several behaviors female red-backed fairy-wrens use to increase offspring quality.

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# GENERAL INTRODUCTION

Natural selection drives adaptations that maximize an individual's reproductive success. A male's reproductive success is generally limited by the number of mates he can obtain, with a few exceptions. However, because they usually invest more in offspring, females are limited in the quantity of offspring they can produce by physiological or ecological factors. When the quantity of offspring produced is limited, one way females can increase their reproductive success is by maximizing offspring quality. Females can increase offspring quality through increased maternal care and other behaviors that increase offspring survival and recruitment. However, recent developments in molecular techniques have revealed that females may also employ behaviors that improve genetic quality in offspring. For example, females may manipulate the sex of offspring in order to produce the most beneficial sex or choose mates that provide better or more compatible genes for their offspring. In this way, females can produce offspring that have a better chance of surviving and reproducing.

In my dissertation I examined two ways that females can influence the quality of their offspring, first through sex ratio manipulation and second through extra-pair mating. Investigations of these behaviors can be complicated by the social structure of the population. It is very important to consider these influences and take them into account when investigating these types of questions. I studied a population of red-backed fairy-wrens in northeastern Queensland, Australia. These birds are cooperative breeders and young males often remain on the natal territory and assist in raising subsequent broods. They also have extremely limited dispersal, particularly among males who typically settle only a territory or two from where they were born. These factors can potentially have a strong effect on female reproductive decisions.

In my first chapter, I investigate how sex ratio manipulation may be affected by the cooperative breeding system in this species. In cooperative breeders it is often thought that females should produce the helping sex in order to gain auxiliary helpers that will increase their future reproductive success. However, these helpers tend to remain philopatric and can compete with each other for breeding opportunities. Thus it may be better for females to produce the dispersing sex if helpers provide little direct benefit to females. In this chapter I take the unusual, but preferable, approach of testing the assumption that helpers provide a benefit in terms of increased female fitness before testing for a sex ratio bias.

In my second and third chapters, I turn the focus to extra-pair mating. While extra-pair mating is quite common among birds, the reasons why females engage in extra-pair mating behaviors remain unclear. In fact, some argue that extra-pair mating cannot evolve as an adaptive female strategy because direct costs of these behaviors are likely to outweigh any indirect genetic benefits gained. In chapter 2, I look at the costs associated with extra-pair mating and whether some females may be able to mitigate these costs through the presence of auxiliary helpers. In chapter 3, I move on to the potential benefits of extra-pair mating. Because of the limited dispersal observed in this species, extra-pair mating may serve as an important mechanism to avoid close inbreeding. The social structure of this species allowed me to use both correlative and experimental tests of this hypothesis. This is the first experimental test of the use of extra-pair mating as an inbreeding avoidance mechanism in any free-living vertebrate.

All research questions, data analyses, and interpretation were entirely my own, as well as the majority of field and laboratory work. Mike Webster provided valuable advise and feedback throughout all stages of the research. Collaborators Jordan Karbian and Willow Lindsay contributed significantly to the field research. In particular, I utilized Jordan's field data from 1998-2000, which are included in chapters 1 and 2, and Willow's field data from 2007-2008 which are included in all three chapters. Sex of offspring were determined genetically in the lab by undergraduates Irma Tapia and Vanessa Talbot. Additional microsatellites for chapter 3 were optimized by Molly McIntosh and half of the samples were run by Sarah Crilly. In addition, Scott Sillett assisted me with the survival analyses in MARK in chapters 1 and 2.

All research was performed in accordance with the Institutional Animal Care and Use Committee (IACUC, permit # 03653). In addition we received a Scientific Purposes Permit from the Queensland Environmental Protection Agency, Collection Permits from the Queensland State Department of Forestry, and approval of our research by the James Cook University Animal Ethics Committee.

# CHAPTER 1

## The effects of repayment and competition on offspring sex ratios in a cooperatively breeding bird

# INTRODUCTION

Sex ratio theory has led to a number of clear predictions about how and when individuals should bias the sex of their offspring (Seger & Stubblefield 2002, West et al 2002), many of which have proven remarkably accurate in studies of invertebrates (reviewed in Godfray & Werren 1996). In contrast, applying classical sex ratio theory to birds and mammals has proven more difficult, and actual sex ratios often deviate from simple predictions based on theory (West et al. 2002; Griffin et al. 2005). One possible explanation for this is that chromosomal sex determination may constrain the evolution of adaptive sex ratio biasing (Williams 1979; West et al. 2005). Alternatively, it may be that behaviors affecting sex ratios, such as helping behavior, are flexible and dependent on environmental conditions in birds and mammals (Cockburn et al. 2002). This could be particularly relevant for social species in which related individuals interact over multiple generations (Wild 2006).

Among vertebrates, many recent sex ratio studies have focused on cooperative species in which offspring of one sex remain on their natal territory and help their parents to raise younger siblings (e.g. Allaine et al. 2000; Clarke et al. 2002; Berg 2004; Doutrelant et al. 2004; Rubenstein 2007). Most of these studies have focused on testing the repayment hypothesis (Emlen et al. 1986), which posits that, in systems where one sex helps and the other does not, the helping sex is ultimately less costly to produce because that sex “repays” some of the cost of being raised. Because parents should invest equally in sons and daughters (Fisher 1958), the repayment hypothesis predicts a primary sex ratio bias toward the helping sex (Emlen et al

1986). This hypothesis also suggests that parents will be particularly likely to produce more of the helping sex when there are few or no auxiliary adults in the group in order to gain the assistance of those auxiliaries during subsequent breeding attempts.

Empirical tests of the repayment hypothesis have yielded mixed results (e.g. Koenig & Dickinson 1996; Berg 2004; Rathburn & Montgomerie 2004; Rubenstein 2007). Several theoretical treatments have attempted to explain these inconsistencies by incorporating additional factors that might counter the effects of repayment (Lessels & Avery 1987; Koenig & Walters 1999; Pen & Weissing 2000). In the most recent and general of these, Wild (2006) developed a model that incorporates the effects of auxiliaries on parental fitness. When auxiliaries increase parental fitness, they partially repay their own cost and we expect the primary sex ratio to be biased towards the helping sex, as predicted by the repayment hypothesis. However, if the presence of an auxiliary provides little or no increase in parental fitness, then other factors may have a more important influence on the primary sex ratio. As suggested by others (Koenig & Walters 1999; Pen & Weissing 2000), Wild's (2006) model shows that one key factor likely to shape primary sex ratios in such systems is local competition between relatives.

The local competition model suggests that competition between relatives (e.g., for breeding territories or mates) can cause offspring sex ratios biased towards the dispersing sex, because adults should produce more of the dispersing sex when resources are limited to avoid competition among their offspring (Hamilton 1967). This hypothesis may be particularly applicable to long-lived, socially monogamous species with related auxiliaries who remain on or near the natal territory to breed (e.g. Komdeur et al. 1997; Dickinson 2004). Indeed, the sex-limited dispersal that is thought to lead to cooperative breeding (reviewed in Hatchwell & Komdeur 2000) is also likely to lead to local competition for the non-dispersive (helping) sex (Dickinson 2004). Accordingly, local competition and offspring repayment exert opposing selective pressures on offspring sex ratios. The optimal offspring sex ratio will depend on the extent to which auxiliary offspring actually enhance parental fitness. Sex ratios biased toward the dispersing sex can be expected even if auxiliaries provide slight fitness benefits to the parents (Wild 2006).

Because of this relationship between repayment and competition, it is important to assess the benefits that parents gain from the presence of auxiliaries before making predictions about the overall population sex ratios of cooperative species (Griffin et al. 2005). In species where auxiliaries provide a large fitness benefit to adults, we expect a sex ratio bias towards the helping sex, but if auxiliaries provide little or no benefit, then a bias towards the dispersing sex is expected (Wild 2006). As predicted, Griffin et al. (2005) showed a correlation between the amount of benefit provided by auxiliaries and brood sex ratio. However, the analysis of Griffin et al. (2005) was based on a small number of studies, as to date few species have had both the contribution of auxiliaries to parental fitness and the degree of sex ratio biasing examined.

Here we examine the benefits of the presence of an auxiliary to parental fitness as well as primary sex ratio using a large multi-year dataset from the red-backed fairy-wren (*Malurus melanocephalus*). These birds are socially monogamous and form multi-year pair bonds (though levels of extra-pair paternity are high; Webster et al. 2008). Females typically disperse to breed in their first year, while young males are philopatric, often remaining on their natal territory for one or more years and assisting in the raising of subsequent broods (Rowley & Russell 1997) before breeding on a territory usually adjacent to their natal territory (unpubl. data). Thus, like many other cooperatively breeding species, in this system both repayment and local competition may be occurring.

## METHODS

### Study Species and Field Methods

Red-backed fairy-wrens are a small Australian passerine. They are socially monogamous and usually pair for life, but like other species in their genus they have very high rates of extra-pair paternity (Webster et al. 2008). Red-back fairy-wrens breed cooperatively, with young males sometimes remaining on the natal territory as auxiliary helpers. In contrast, females disperse from their natal territory and rarely, if ever, assist their parents. Male auxiliaries are nearly always the son of the breeding female, however, because of high extra-pair paternity rates (Webster et al. 2008) they may not be related to the breeding male. There is a plumage dimorphism among males where most breeding males display bright red and black plumage, whereas auxiliaries and some young breeding males, nearly always without auxiliaries, display a dull brown female-like plumage (Karubian 2002; Webster et al. 2008). Females build domed nests low in the grassy under story and lay 2-4 (usually 3) eggs per clutch. Females are solely responsible for building the nest and incubation, but all members of the group participate in feeding nestlings and fledglings. Eggs usually hatch after 12 days of incubation and chicks fledge after 12 days in the nest. Fledglings remain dependent for approximately 40 days post fledging and often remain on the territory through the winter with females and breeding males dispersing in the early spring prior to breeding. Natal dispersal is highly sex biased; males nearly always settle within a territory or two of where they were born (<500m) whereas females disperse further, often off of our field site (unpub. data). Therefore local competition for breeding opportunities is much higher for young males than for young females.

We studied a population of red-backed fairy-wrens breeding in the forest surrounding the Herberton Shire Reservoirs on the Atherton Tablelands in Queensland, Australia (145º25'E, 17º22'S) during the breeding seasons (October - February) of 1998-2000 and 2003-2007 (breeding seasons are designated by the calendar year in which they ended). In each of these seasons we captured most adults on the study site; on average we monitored 49 groups on our study site per year and generally failed to capture at most 3-4 adults per year. We banded each captured adult with a unique combination of plastic color bands to aid identification. We also collected blood samples (ca. 30-50 μl) from the brachial vein for genetic analyses.

We observed banded individuals to determine group composition and monitored all nesting attempts (a small number of attempts were likely missed in each year, but virtually all of these failed prior to hatching).Groups consist of a breeding male and female as well as one or more auxiliary males and fledglings from the current breeding season. In our population approximately 20% of groups have at least one auxiliary male. These auxiliaries feed nestlings and fledglings and although presence of an auxiliary male reduces both maternal and paternal provisioning rates, the overall provisioning rate does not differ between nests with and without auxiliaries (unpubl. data). On average, 27% of females without an auxiliary male in a given season gained an auxiliary in the subsequent season, and 60% of females with an auxiliary male in one season would have no auxiliary in the next (most auxiliaries remain on their natal territory for only a single breeding season). Females produced 1.8 ± 0.9 (Mean ± SD) nests per year, with a maximum of five and no more than two that successfully fledged. Nests were classified as having an auxiliary if an adult (at least one year old) male other than the dominant male was observed consistently with the group and was observed feeding at the nest. Females who had an auxiliary for at least one reproductive attempt were classified as having an auxiliary for the season for the purpose of season wide analyses.

We found and monitored 662 nests over the 8 years of this study; 390 of these persisted long enough for us to sample the offspring for genetic analyses, resulting in samples from 1004 offspring. On average we sampled 1.3 ± 0.5 nests per female per year and we sampled each female in an average of 1.6 ± 0.9 (mean ± SD) years. In 2003-2007, nestlings were banded on day 6 after hatching whenever possible. In other years, nestlings were banded between day 5 and 11. At the time of banding we measured nestling weight, and in later years (2004-2007), tarsus length and fat stores. Fat stores were scored on a scale of 0 - 5, with 0 indicating no fat stores, and 5 indicating that the furcular hollow was bulging with fat. We also collected blood samples (approx. 25 µl) from the tarsal vein of nestlings and stored them in lysis buffer (White &

Densmore 1992) at 4º C. In 2004-2007 all unhatched eggs were collected and examined (around 18% of eggs failed to hatch), and any partially developed embryos were removed for genetic analysis (N = 76). We extracted DNA from samples using a standard phenol-chloroform extraction (Westneat 1990).

## Female Fitness Measures

In birds, females are the heterogametic sex and should therefore have more influence on the sex of offspring than males. It is likely that males are only able to influence offspring sex ratios through differential feeding or other forms of brood reduction. Since we used only complete broods in which all eggs laid were analyzed to measure sex ratios, in effect we examined only pre-laying influences on brood sex ratios by females. Accordingly, we examined only the effects of auxiliaries on adult female fitness and not on adult male fitness.

We assessed several components of female reproductive success, including the number of nests per year (including nests in which eggs were never laid), number of successful nests per year, clutch size (number of eggs laid), nest fledging success (probability of a nest fledging at least 1 offspring), number of chicks fledged per successful nest and per year, and measures of offspring condition and survival. To estimate offspring condition, we used body mass controlled for offspring size (see statistical methods) and fat measurements (above). We only used measurements taken on 6-day-old chicks for these analyses to control for chick age. We used observation of yearling birds recruiting to the study site as a measure of offspring survival;

failure to observe an individual could either be due to death or dispersal off the site.

We also estimated adult female survival probabilities to determine whether females with auxiliaries had higher survival rates than those without (e.g., Russell et al. 2007). Our survivorship data were based on annual surveys conducted at the beginning of each field season to re-sight color-banded females, who are highly sedentary after natal dispersal and rarely change territories between years (unpubl. data).

## Sexing Methods

We determined the sex of all offspring sampled between 1998-2000 and 2003-2005 by amplifying an intron within the CHD gene using primers 1237L and 1272H (Kahn et al. 1998). We ran 10 µl PCR reactions containing 0.15 mM dNTPs (each), 0.50 µM primers (each), 2.0 mM $MgCl_2$, 2.5 units *Taq* polymerase, and 1 µl DNA suspended in sterile water (approximately 50 ng genomic DNA). These reactions were run on an Applied Biosystems GeneAmp PCR System 9700 (Applied Biosystems) with an initial 3 min denaturation at 94° C followed by 30 cycles of 94° C for 60 sec, 57° C for 60 sec, and 72° C for 45 sec. PCR products were visualized through electrophoresis using a 2% agarose minigel and stained with ethidium bromide. Gels were scored by eye; individuals with a single band were scored as male and individuals with two visible bands were scored as female (see Kahn et al. 1998). To check the accuracy of this method, we assayed the sex of 54 adult birds of known sex; of these, only 1 (1.9%) was mis-sexed using our genetic method.

## Statistical Methods

We analyzed all of our measures of female fitness, except for female survival, using generalized linear mixed models (GLMM) run in PROC GLIMMIX in SAS 9.1. For all of our count measures (# eggs, # fledglings per nest, # nests per year, # fledglings per year, and nestling fat score) we used a Poisson distribution and a log link. For measures with categorical responses (nest success and fledgling recruitment) we used a binomial distribution and a logit link. Finally, for the continuous measure (nestling mass) we used a normal distribution and an identity link. For all analyses, female age and presence of an auxiliary were included as fixed effects and female identity and year were included as random effects. For measures from individual nests (# eggs, # fledglings per nest, nest success, and all measures of chick condition and survival) nest initiation date also was included as a fixed effect since timing of breeding can have an effect both on the probability of having an auxiliary (auxiliaries are more common early in the breeding season) and various measures of nesting success (food is more abundant later in the breeding season but predation rates are higher; unpubl. data). When nests were found after the eggs had been laid, we estimated nest initiation date by back-counting from the hatch date. For all measures of chick condition and survival, we used the nest of the chick as an additional random factor since chicks from the same nest are not independent from each other. We added tarsus as an additional fixed effect in our analyses of nestling mass to control for chick size. However, similar results were obtained when tarsus was excluded from the analyses. In our analysis of fledgling recruitment we also included nestling sex as a fixed effect since there is strong sex biased dispersal in this species (unpub. data). Because females are the dispersing sex, the probability that they will disperse off the study site and fail to be detected is much higher. Therefore observed recruitment rate of females is much lower than that of males and likely lower than the true recruitment rate of females.

We examined first order interactions between presence of an auxiliary and all other fixed effects. The only significant interaction was between offspring sex and presence of an auxiliary in the analysis of offspring recruitment, so we included that interaction in our final model of offspring recruitment. No other interaction terms were significant in any model and all others were excluded from the final analyses. We also tried including year as a fixed rather than random effect and including the interaction between presence of an auxiliary and year to see if auxiliaries provided benefits in some years but not others, as might be expected if having an auxiliary is particularly beneficial in poor years. However, this interaction term was never significant in any analysis, so we excluded it from the final analyses and left year as a random effect.

For our analysis of female survival, we used multi-state capture-recapture models (Hestbeck et al. 1991, Brownie et al. 1993, Nichols and Kendall 1995) and Program MARK (White and Burnham 1999) to compare survival probabilities between females with and without auxiliaries. This method simultaneously estimates three probabilities: probability of survival ($S$), probability of re-sighting ($p$), and probability of state change ($\psi$, i.e., the probability of changing from a female with auxiliaries to one without or vice versa). This gives much more accurate measures of survival because it is able to control for the detection probability which is often lumped into the survival probability in other methods. Each of these three probabilities can be modeled as a function of year, presence or absence of a helper, both, or neither, giving a total of 64 possible models. In order to narrow down the number of models we made some *a priori* assumptions based on the biology of the birds and our study methods. We assumed that $p$ varied both with year (because of possible differences in field observers or bird behavior across years), and presence of an auxiliary (because, being less common, groups with auxiliaries sometimes received more observer attention than those without). We held $S$ constant over time in all candidate models to obtain a single estimate for survival; $S$ was modeled as either dependent or independent of presence of an auxiliary. We modeled $\psi$ as dependent on presence of an auxiliary, on year, on a factorial combination of year*auxiliary, and as constant. These assumptions gave us 8 candidate models (Table 1). The relative likelihood of each model was estimated with second-order Akaike's information criterion values, or $\mathrm{AIC}_c$ (Burnham and Anderson 2002). We calculated a weighted model average of survival rates of females with and without auxiliaries using $\mathrm{AIC}_c$ weights.

In all of our analyses of sex ratio we used only complete broods (i.e., all eggs that were laid were sampled) to control for any possible sex-biased mortality prior to sampling. Therefore we excluded 79 of 268 sampled nests either because one or more eggs failed to hatch and they contained no visible embryo (N = 34), mortality of one or more nestlings prior to sampling (N = 2), failure to capture one or more chicks due to late sampling (N = 7), doubt as to whether all eggs laid were sampled due to finding the nest late (N = 25), or failure of the sex-linked fragments to amplify in one or more samples (N = 11). The sampled chicks from these excluded broods tend to be more likely to be male than complete broods (52% male vs. 45% male) however, it is impossible to determine with the information available to us whether this is because these broods are more male biased or because females are less likely to be sampled and sexed. We used Neuhauser's (2004) test for sex ratio bias with clustered data to test for biases in the population-wide primary sex ratio. This test takes into account that the sex of chicks within a brood may not be independent of each other and is a more powerful test for population-wide sex ratio biases in birds (Neuhauser 2004). For our analyses of the effects of auxiliaries on individual broods, we again used SAS 9.1 and PROC GLIMMIX to create a GLMM. In these analyses we used a binomial distribution and a logit link because we are using a proportion as our measure of sex ratio. We included presence of an auxiliary, nest initiation date, and female age as fixed effects and female identity and year as random effects. We tested for first order interactions between presence of an auxiliary and our other fixed effects, but because we found no significant interactions, none of these are included in our final model. In order to show a facultative adjustment of sex ratio in response to the presence of an auxiliary, we compared the sex ratios produced by females when they had an auxiliary to their sex ratio produced when they did not using a two-tailed paired t-test. Due to the small number of females for whom we had sampled complete broods both with and without an auxiliary (N = 20) we were unable to control for age in this analysis.

## RESULTS

### Female Reproductive Success and Survival

Between 1998-2007 we followed a total of 604 nests from 216 females to completion (fledging or failure). Of these, 121 (20%) were produced by groups with at least one auxiliary (groups with more than one auxiliary were rare, accounting for only 2% of nests). Timing within the breeding season had a significant effect on several measures of female reproductive success (Table 2). Nests initiated early in the season tended to be more likely to fledge young than nests late in the season, but nests initiated later in the season contained more eggs. Similarly, nestlings from nests later in the season were heavier and tended to be more likely to survive and recruit to the field site in the subsequent year.

The presence of auxiliaries, in contrast, had little if any effect on measures of female reproductive success (Table 2). With respect to individual nests, females with auxiliaries did not differ significantly from females without auxiliaries in number of eggs laid, probability of fledging, or number of young fledged. Similarly, offspring raised by groups with auxiliaries did not differ from offspring raised without auxiliaries in mass controlled for tarsus length or amount of fat stores. There was also no difference in the probability of offspring recruitment to the site as adults. However, there was a significant interaction between offspring sex and presence of an auxiliary on offspring recruitment. A post-hoc comparison of all levels using Tukey's adjustment revealed that this was largely driven by the difference in recruitment rates between males and females likely due to sex biased dispersal, but females raised by groups without auxiliaries had a non-significant tendency to recruit more frequently than females raised by groups with auxiliaries ($t = 2.25$, $P = 0.11$) where as there was no effect of the presence of an auxiliary on male recruitment rates (t = -0.53, P = 0.95). Females with auxiliaries did have a non-significant trend towards more nesting attempts per season, but they did not fledge more nests or produce more fledglings per year than did females without auxiliaries.

Annual adult female survival was relatively high, with 59.0 ± 2.8% (Mean ± s.e.) of females surviving between years. However, the presence of an auxiliary did not have a large effect on female survival (Table 1) with 58.7% of females without auxiliaries surviving (95% C.I. = 52.8% - 64.4%) and 59.9% of females with auxiliaries surviving (95% C.I. = 51.4% - 67.8%).

## Sex Ratios

Because the presence of an auxiliary had little or no positive effect on female fitness (above, and see Discussion), we predicted that the sex ratio would be biased towards the dispersing sex rather than toward the philopatric helping sex in our study population. We sexed 558 offspring from 189 complete broods (Table 3). Of these, 250 (44.8%) were male, which represents a significant (Z = 2.43, P = 0.016) bias in the population primary sex ratio toward females (the dispersing sex). Our GLMM of brood sex ratio revealed that while female age had no effect on brood sex ratios ($F_{1,64}$ = 0.20, P = 0.65), broods produced earlier in the season contained more males than broods produced later in the season ($F_{1,64}$ = 7.30, P = 0.01). In addition females with auxiliaries produced significantly fewer sons than females without auxiliaries ($F_{1,64}$ = 12.72, P < 0.001). Indeed, on average across years, females with auxiliaries produced brood sex ratios that differed significantly from parity, whereas females without auxiliaries did not (Fig. 1). In order to determine if this is a facultative response by females, we compared the brood sex ratios of individual females when they had and lacked an auxiliary. There was a trend for females to produce fewer sons when they had an auxiliary than when they did not (with auxiliaries 34.1% male, without auxiliaries 47.6% male, $t = 1.83$, $P = 0.084$). There were a relatively small number of females for which we had complete broods produced with and without helpers ($N = 20$) so it is possible that with a larger sample size, this trend would become significant.

There was considerable year-to-year variation in the primary sex ratio, ranging from 32% to 57% males. To determine whether this variation might be explained by variation in local competition among males, we regressed the yearly primary sex ratio against proportion of groups with auxiliaries in that year. The logic of this analysis is that a high number of groups with male auxiliaries should be indicative of very limited breeding opportunities for males, and hence particularly high costs of producing sons. Despite having only 6 years of data, we found a significant negative relationship ($R^2 = 0.78$, $F_{1,4} = 13.82$, $P = 0.021$) between the proportion of groups with auxiliaries and primary sex ratio (Fig. 2a). Since this effect could be due to females with auxiliaries producing more daughters, we repeated the regression using only offspring from females without auxiliaries. The second analysis was marginally non-significant ($R^2 = 0.61$, $F_{1,4} = 6.23$, $P = 0.067$) but still showed a strong trend towards a negative association between proportion of groups with auxiliaries and primary sex ratio (Fig. 2b).

# DISCUSSION

## Benefits of Auxiliaries

In this study we found that the presence of an auxiliary had little if any effect on female fitness. Presence of an auxiliary had no significant effect on our measures of female reproductive output per nest, and there was no evidence that nestling condition or offspring survival were improved by auxiliaries. Similarly, although females with auxiliaries tended to produce more nests in a season than females without auxiliaries, this did not lead to a significant difference in the number of successful nests produced or the total number of young fledged per year. Auxiliaries do make many feeding visits to the nest (Chap 2), suggesting that they might enhance the reproductive success of the adults they are assisting, but this does not seem to be the case. This may be because females with auxiliaries take advantage of the additional care by feeding less (e.g., Brown et al. 1978; Crick 1992; Dickinson et al. 1996; unpub. data) or otherwise investing less in their offspring (Russell et al. 2007) relative to females without auxiliaries. However, if such compensation does occur, it does not result in a pronounced difference in survival between females with and without auxiliaries. It is also possible that auxiliaries bring food to the nest but do not deliver all of it to the nestlings (Boland et al. 1997; Canestrari et al. 2004; Clutton-Brock et al. 2005). In either case, our data indicate that in red-backed fairy-wrens, the direct fitness benefits of having an auxiliary are weak or absent. In contrast, in the superb fairy-wren (*Malurus cyaneus*) auxiliary males have been shown to increase female survival from year to year (Cockburn et al. 2008) perhaps because females are able to reduce their investment in reproduction (Russell et al 2007). It is unclear why auxiliaries have such differing effects on female fitness in these two species despite their similar biology.

The apparent lack of benefit provided by auxiliaries raises the question of why helping behavior exists at all in the red-backed fairy-wren. Although indirect fitness benefits to auxiliaries have long been thought to be key to the evolution of cooperative breeding (Hamilton 1964; Maynard Smith 1964), our results suggest that such benefits from auxiliaries are low or absent in the red-backed fairy-wren. Natal philopatry and helping by auxiliaries might be favored, even in the face of negligible indirect fitness benefits to auxiliaries, if breeding opportunities are highly limited (e.g., Pruett-Jones and Lewis 1990). In our study population, experimental removals of breeding males have shown that auxiliary males quickly disperse from their natal territories to assume vacant breeding positions, often within a matter of hours (Chap. 3), indicating that breeding opportunities are indeed limiting in the red-backed fairy-wren. Moreover, adults might be selected to tolerate the presence of auxiliaries on the natal territory if auxiliaries enhance the ability of adults to produce extra-pair offspring (Mulder et al. 1994, Green et al. 1995, Webster et al. 2004).

Alternatively, helping behavior could be maintained through direct fitness benefits to the auxiliary, such as increased survival or increased future reproductive success, resulting in fitness benefits for the parents (Clutton-Brock 2002; Ekman et al. 2004). These alternative benefits of helping behavior could explain the presence of auxiliaries in the absence of improved female reproduction and survival. However, it is important to note that in nearly all cases when the reproductive benefit of auxiliaries are low, it will be more advantageous to the parent to produce a reproductive offspring than an auxiliary offspring.

Studies demonstrating clear improvements in parental fitness caused by helping are less common than might be expected; although many studies that have controlled for potentially confounding factors have found a positive effect of helping on parental fitness, about a third have found no significant fitness benefit of having an auxiliary helper (Cockburn 1998). Moreover, many of the classic studies showing a correlation between auxiliaries and reproductive success have failed to control for territory quality or individual reproductive potential, either of which could simultaneously increase the incidence of auxiliaries and adult reproductive success (Brown 1987; Mumme 1992). Although we did not control for territory quality or reproductive potential in this study, these confounding factors are unlikely to cause the non-significant results that we found since generally these factors cause a correlation between reproductive success and presence of an auxiliary which is caused by high territory quality rather than an effect of the auxiliary. However it is also possible that helpers provide a benefit only on the poorest of territories (e.g. Blackmore & Heinsohn 2007) and that we were unable to detect this effect because we were unable to control for territory quality. Alternatively, a problem may arise if auxiliaries are only accepted into a group when they are needed, therefore equalizing the reproductive success of individuals on territories of different quality (Cockburn 1998). This does not seem to be a likely scenario in our study population, as we have never seen young unmated males unaffiliated with any group (unpubl. data), as would be expected if some males are not allowed to join as auxiliaries.Young males tend to remain as auxiliaries until they leave, apparently voluntarily, either to pair with a new female who has dispersed onto the site and established a new territory or to assume the breeding position vacated by the death of another breeding male. Therefore, it appears that auxiliaries in red-backed fairy-wrens repay, at best, very little of the cost of their production.

While auxiliaries did not affect measures of reproductive success, timing within the breeding season did. Nests laid earlier in the season contained fewer eggs and chicks from these nests had less mass than those laid later in the season, but later nests were less likely to fledge than earlier nests. These results are likely due to changes in food and predator abundance throughout the season. Our study site is relatively dry early in the season and there are few insects available prior to the onset of the rainy season (unpub. data). After the onset of the rains, the predation rates increase resulting in fewer successful nests later in the season (unpub. data). Thus timing of breeding appears to be more important for female reproductive success than the presence of an auxiliary.

## Offspring Sex Ratios

The lack of benefit provided by auxiliaries in the red-backed fairy-wren, combined with the relatively limited dispersal observed in this species, should lead to a situation in which local competition between relatives has a larger effect on offspring sex ratios than does repayment (Wild 2006). This expectation holds even if auxiliaries provide a small fitness benefit that we were unable to detect statistically (e.g., in female survival), as local competition will be outweighed by repayment effects only when the latter are strong (Wild 2006). This is particularly true when natal dispersal is highly limited (as in fairy-wrens). As predicted, we found a significant bias in the primary population sex ratio toward females, the dispersing sex. Population-wide sex ratio biases are relatively rare in birds, and in cooperatively breeding species, only biases toward the helping sex have been reported prior to this study (e.g. Ligon & Ligon 1990;  Allaine et al. 2000). Local competition seems a likely explanation for this sex ratio bias due to the extreme philopatry displayed by males in our population. An alternate explanation could be that females require fewer resources to produce because they are smaller than males. However, in our population there is no difference in size between male and female chicks at day 6 as measured by tarsus length ($R^2 = 0.10$, $F_{1,120} = 1.20$, $P = 0.276$), and mean adult male tarsus length is only about 2% longer than mean adult female tarsus length ($R^2 = 0.09$, $F_{1,466} = 46.90$, $P < 0.001$). This small degree of sexual size dimorphism is unlikely to explain the sex ratio bias seen in this population. In contrast to the red-backed fairy-wren, the closely related superb fairy-wren's population-wide primary sex ratio is biased towards males (Cockburn & Double 2008). However, this makes sense in light of the fact that auxiliaries in the superb fairy-wren improve female survival (Russell et al. 2007; Cockburn et al. 2008) which may cause repayment to play a larger role in this system than does local competition.

We found evidence suggesting facultative adjustment of brood sex ratios by females with auxiliaries; specifically, females with auxiliaries were more likely to produce daughters than were females without auxiliaries. Additionally, individual females tended to produce more female biased broods when they had an auxiliary versus when they did not, although this result was not statistically significant. These results might arise if having multiple auxiliaries reduces the fitness of breeders (e.g., Komdeur 1994). However, since nearly all auxiliaries in the red-backed fairy-wren help only for one year (unpubl. data), females with auxiliaries could produce additional sons without risking a surplus of auxiliaries. Facultative adjustment of offspring sex ratios in cooperatively breeding species might also occur if females in good condition, including those with auxiliaries, produce more of the sex with greater variance in reproductive success, as suggested by the Trivers and Willard (1973) model (see Rubenstein 2007). However, because extra-pair paternity is prevalent in the red-backed fairy-wren (Webster et al. 2008), male reproductive success is much more variable than that of females (unpubl. data). Consequently, we might expect females with auxiliaries to be more likely to produce sons, yet we found the opposite pattern. Thus, the best explanation for the possible facultative adjustment of sex ratio in the red-backed fairy-wren is that a female with auxiliaries reduces the number of males produced as a means to avoid direct competition for breeding opportunities between her sons (i.e., the local competition hypothesis).

Additionally, we found that nests laid earlier in the breeding season tend to be more male biased than nests laid later in the season. It is possible that this pattern is a result of local competition as well if males hatched earlier in the season have a greater chance of achieving a breeding position in their first year than males hatched later in the season. Alternatively, females in areas of higher competition could lay later than females in areas of lower competition. This pattern could also reflect females in good condition who nest early and also produce more of the sex with higher variance in reproductive success (Rubenstein et al 2007). In order to tease apart these possibilities, more information about which females nest early in the season would be needed.

Many other studies of cooperative species have suggested facultative adjustment of offspring sex ratios but found no bias in the population-wide primary sex ratio (e.g. Koenig & Dickinson 1996; Komdeur al. 1997; Legge al. 2001; Doutrelant al. 2004). Local competition may be an important determinant of facultative sex biasing in these species, as some studies have shown that individuals bias their broods towards the dispersing sex in situations where additional auxiliaries would not be beneficial (e.g., Komdeur al. 1997, Legge al. 2001). A comparative study by Griffin et al. (2005) found a significant correlation between the benefits provided by auxiliaries and the sex ratios of broods with auxiliaries, however none of the studies used reported a population-wide offspring sex ratio biased towards the dispersing sex. Although several studies have shown population-wide biases towards the helping sex (e.g. Ligon & Ligon 1990; Allaine et al. 2000; Cockburn & Double 2008), to our knowledge ours is the first study to demonstrate a population-wide bias towards the dispersing sex in the primary sex ratio for any cooperatively breeding bird or mammal.

The importance of local competition is also supported by the correlation between annual population sex ratio and proportion of groups with auxiliaries. In years when there were many groups with auxiliaries, the primary population sex ratio was skewed more towards the dispersing sex than in years with relatively few groups with auxiliaries. Although this correlation could be caused by the sex ratio bias in the nests of females with auxiliaries, we found a similar trend when only broods of females without auxiliaries were considered. This suggests that females have some way of assessing the availability of local breeding opportunities, either through direct assessment of the presence of auxiliaries in surrounding groups or through some other indicator associated with local competition, such as territory density. A similar result was found in western bluebirds (*Sialia mexicana*) by Dickinson (2004), who suggested that the presence of an auxiliary indicates a lack of suitable nest cavities and thus high competition between males for breeding opportunities, leading females to produce fewer sons. However, in western bluebirds there was no population-wide primary sex ratio bias (Koenig & Dickinson 1996). Unlike the red-backed fairy-wren, in the western bluebird auxiliary males increase the fledging success of the nests at which they help (Dickinson et al. 1996), so perhaps repayment and competition balance out, resulting in no population-wide bias. Since auxiliaries provide no detectable fitness benefit to their parents in red-backed fairy-wrens, competition among sons should have a larger effect on offspring sex ratios than repayment.

The results reported in this study support the idea that competition between offspring may be a better explanation than repayment for offspring sex ratio biases in at least some cooperatively breeding vertebrates, and also suggest the possibility that female red-backed fairy-wrens may be able to facultatively adjust brood sex ratios in response to local conditions. Although it can be somewhat counter-intuitive that auxiliaries provide little or no fitness benefit to the females they help, once this has been established it is not surprising that local competition plays a role in shaping offspring sex ratios. This study underscores the importance of testing the underlying assumptions of sex ratio hypotheses before applying them to any given species.

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**Table 1.** AIC$_c$ values and weights for candidate models of female survival

| | | | Survival (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Model | AIC$_c$ | weight | Aux. | Up. CL | Low CL | No aux. | Up. CL | Low CL |
| $S(.)p(\mathrm{ya})\psi(\mathrm{a})$ | 706.98 | 0.654 | 59.0 | 64.4 | 53.4 | 59.0 | 64.4 | 53.4 |
| $S(\mathrm{a})p(\mathrm{ya})\psi(\mathrm{a})$ | 708.79 | 0.264 | 62.1 | 71.9 | 50.5 | 58.1 | 64.2 | 51.7 |
| $S(.)p(\mathrm{ya})\psi(\mathrm{ya})$ | 712.63 | 0.039 | 58.2 | 63.6 | 52.6 | 58.2 | 63.6 | 52.6 |
| $S(\mathrm{a})p(\mathrm{ya})\psi(\mathrm{ya})$ | 714.41 | 0.016 | 61.3 | 72.1 | 49.3 | 57.2 | 63.4 | 50.9 |
| $S(.)p(\mathrm{ya})\psi(.)$ | 714.47 | 0.016 | 60.1 | 65.4 | 54.6 | 60.1 | 65.4 | 54.6 |
| $S(\mathrm{a})p(\mathrm{ya})\psi(.)$ | 716.37 | 0.006 | 62.5 | 73.2 | 50.5 | 59.2 | 65.5 | 52.6 |
| $S(.)p(\mathrm{ya})\psi(\mathrm{y})$ | 716.96 | 0.004 | 60.1 | 65.3 | 54.6 | 60.1 | 65.3 | 54.6 |
| $S(\mathrm{a})p(\mathrm{ya})\psi(\mathrm{y})$ | 719.11 | 0.001 | 61.1 | 72.0 | 48.8 | 59.7 | 66.1 | 53.0 |
| Model average | | | 59.9 | 67.8 | 51.4 | 58.7 | 64.4 | 52.8 |

Candidate models and model average from the analysis of female survival using MARK. The 3 estimated probabilities, survival ($S$), detection ($p$), and state change ($\psi$), are modeled as being dependent on presence of an auxiliary (a), year (y), neither (.) or both (ya). Survival estimates for each model are given with upper and lower 95% confidence limits.

**Table 2.** Effect of auxiliaries on measures of female reproductive output

| Analysis | Fixed effect | N | Estimate | Auxiliary (mean ± S.E.) | No Auxiliary (mean ± S.E.) | Test Stat. | P |
|---|---|---|---|---|---|---|---|
| # of eggs per nest* | presence of auxiliary | 419 | -0.046 | 2.94 ± 0.20 | 3.07 ± 0.10 | $F_{1,242} = 0.37$ | 0.544 |
| | female age | | -0.039 | | | $F_{1,242} = 0.45$ | 0.502 |
| | nest initiation date | | 0.002 | | | $F_{1,242} = 4.53$ | 0.034 |
| # of fledglings per nest* | presence of auxiliary | 203 | -0.002 | 2.55 ± 0.23 | 2.55 ± 0.13 | $F_{1,29} < 0.01$ | 0.987 |
| | female age | | -0.150 | | | $F_{1,29} = 2.82$ | 0.097 |
| | nest initiation date | | 0.001 | | | $F_{1,29} = 1.29$ | 0.259 |
| % Nests fledging young* | presence of auxiliary | 574 | 0.006 | 42.8 ± 7.1% | 42.9 ± 5.6% | $F_{1,360} < 0.01$ | 0.978 |
| | female age | | 0.046 | | | $F_{1,360} = 0.06$ | 0.802 |
| | nest initiation date | | -0.012 | | | $F_{1,360} = 19.62$ | <0.001 |
| Chick weight in g.** | presence of auxiliary | 228 | 0.118 | 5.66 ± 0.13 | 5.54 ± 0.06 | $F_{1,134} = 0.76$ | 0.385 |
| | female age | | -0.045 | | | $F_{1,134} = 0.17$ | 0.684 |
| | nest initiation date | | 0.003 | | | $F_{1,134} = 4.49$ | 0.036 |
| | tarsus | | 0.375 | | | $F_{1,134} = 166.28$ | <0.001 |
| Chick fat score** | presence of auxiliary | 218 | 0.036 | 2.01 ± 0.27 | 1.95 ± 0.11 | $F_{1,129} = 0.07$ | 0.793 |
| | female age | | 0.065 | | | $F_{1,129} = 0.35$ | 0.554 |
| | nest initiation date | | 0.103 | | | $F_{1,129} = 3.10$ | 0.081 |
| Probability of recruitment** | presence of auxiliary | 445 | 0.233 | 18.1 ± 9.5% | 31.6 ± 10.7% | $F_{1,267} = 2.71$ | 0.101 |
| | female age | | -0.179 | | | $F_{1,267} = 0.45$ | 0.501 |
| | nest initiation date | | 0.008 | | | $F_{1,267} = 4.92$ | 0.027 |
| | sex | | -3.039 | | | $F_{1,267} = 22.77$ | <0.001 |
| | help*sex | | 1.948 | 4.6±3.9 / 50.2±16.9 (♀/♂) | 21.1±8.9 / 44.3±12.7 (♀/♂) | $F_{1,267} = 5.11$ | 0.025 |
| # of nests per year* | presence of auxiliary | 322 | 0.187 | 2.11 ± 0.22 | 1.75 ± 0.14 | $F_{1,118} = 3.75$ | 0.055 |
| | female age | | -0.041 | | | $F_{1,118} = 0.25$ | 0.618 |
| # of successful nests per year* | presence of auxiliary | 332 | 0.160 | 0.81 ± 0.13 | 0.69 ± 0.08 | $F_{1,118} = 1.09$ | 0.298 |
| | female age | | -0.001 | | | $F_{1,118} < 0.01$ | 0.996 |
| # of fledglings per year* | presence of auxiliary | 332 | 0.086 | 1.81 ± 0.26 | 1.66 ± 0.20 | $F_{1,118} = 0.62$ | 0.432 |
| | female age | | -0.057 | | | $F_{1,118} = 0.34$ | 0.559 |

(Table 2 cont.)Results for measures of female fitness from GLMM's. Values for means are back transformed to the original scale using the inverse link function. * Models included year and female identity as random effects. ** Models included year, female identity, and nest as random effects.

**Table 3.** Sex ratios across years

| Year | # Broods | # broods ♂ biased (%) | # Chicks | # ♂'s (%) |
|---|---|---|---|---|
| 1998 | 37 | 13 (35.1) | 101 | 45 (44.6) |
| 1999 | 43 | 11 (25.6) | 128 | 41 (32.0) |
| 2000 | 40 | 16 (40.0) | 122 | 64 (52.5) |
| 2003 | 9 | 4 (44.4) | 23 | 13 (57.5) |
| 2004 | 25 | 7 (28.0) | 74 | 34 (45.9) |
| 2005 | 35 | 15 (42.9) | 110 | 53 (48.2) |
| Total | 189 | 65 (34.4) | 558 | 250 (44.8) |

This data includes multiple broods from females within and between years.

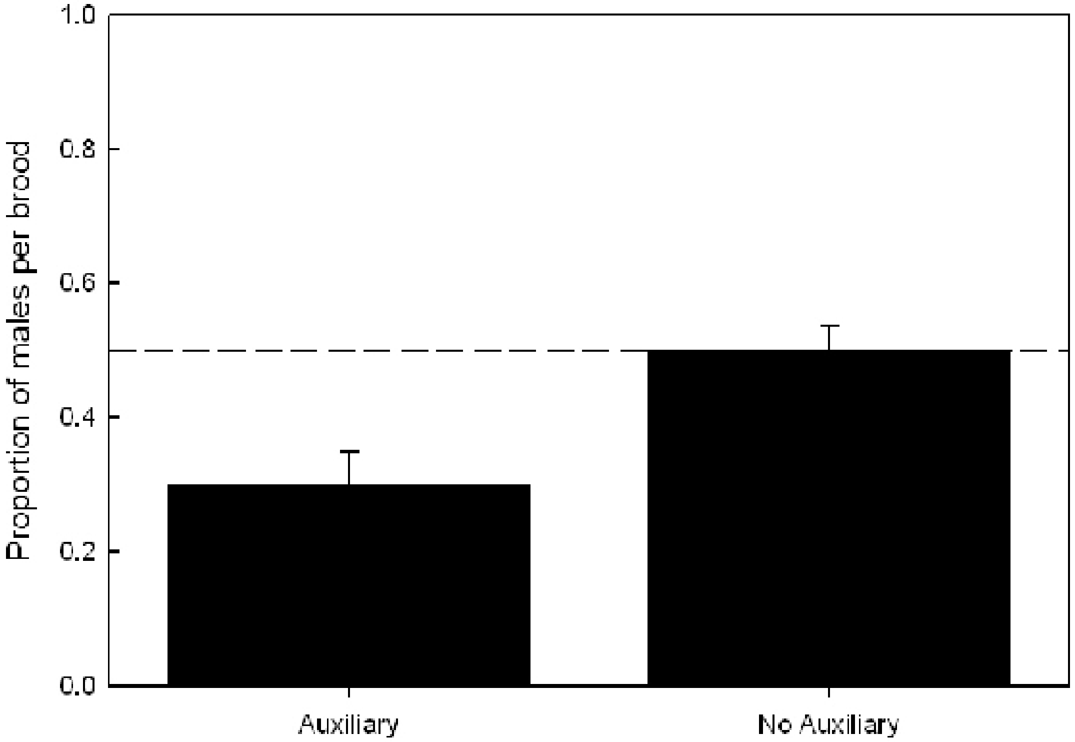

**Fig 1**: Effect of presence of an auxiliary on brood sex ratios. Column values are least squared means from the GLMM. Error bars represent one standard error. The horizontal dashed line shows an equal 50:50 sex ratio.

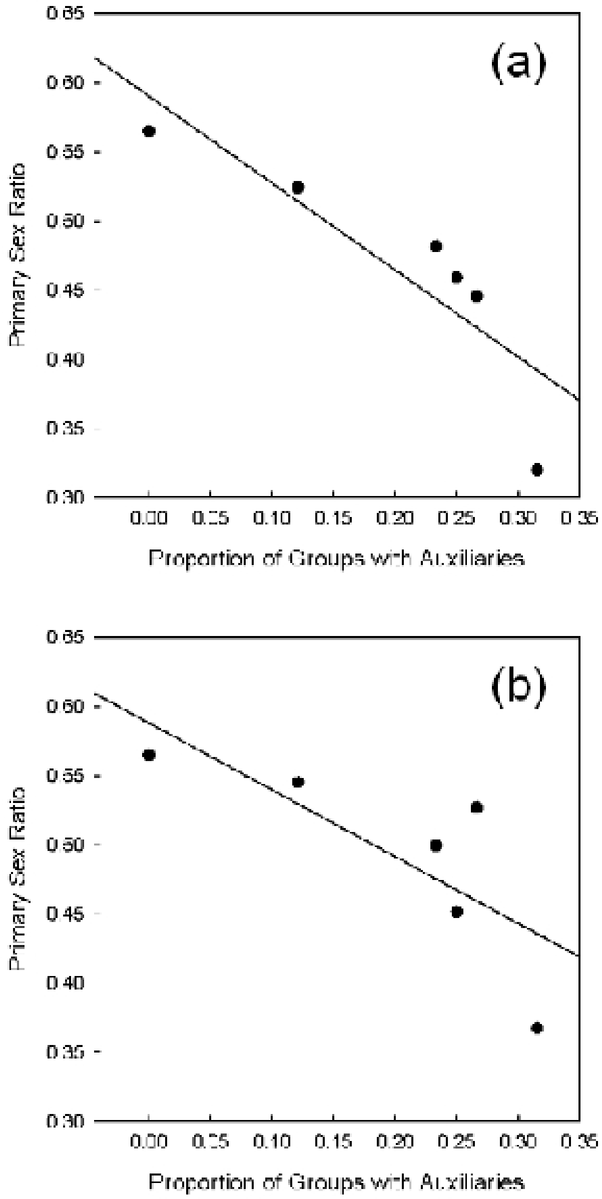

**Fig. 2**: Relationship between primary sex ratio and proportion of groups with auxiliaries between years, for (a) all complete broods, and (b) broods without auxiliaries.

# Chapter 2

# Is extra-pair paternity costly to female red-backed fairy-wrens (*Malurus melanocephalus*)?

## INTRODUCTION

With the advent of molecular techniques in behavioral studies, many species formerly thought to be monogamous have been discovered to engage in extra-pair mating behaviors. These behaviors are particularly common among socially monogamous birds, with over 75% of those studied showing some level of extra-pair paternity (Griffith et al. 2002). However, the frequency of extra-pair paternity (EPP) varies widely across species and even between populations of the same species (Petrie and Kempenaers 1998), and accordingly there is considerable interest in determining the factors that affect levels of EPP within and between populations. Much of the research thus far has focused on ecological factors, such as density and breeding synchrony, which may affect the opportunity for extra-pair mating (Slagsvold and Lifjeld 1997; Griffith et al. 2002; Neudorf 2004). However, an alternate approach to explaining variation in extra-pair mating behavior is to examine the costs of extra-pair mating to females, as these are likely to shape female reproductive strategies (Gowaty 1996; Westneat & Stewart 2003).

The benefits of EPP to females are thought to be indirect in most cases (e.g. good or compatible genes for the offspring; Jennions & Petrie 2000), and therefore will likely be weak (Kirkpatrick and Barton 1997). In contrast, the costs of EPP to females are likely to be direct (see below) and could potentially outweigh the weak indirect benefits (Cameron et al. 2003; Arnqvist and Kirkpatrick 2005). High direct costs of EPP could preclude the evolution of extra-pair mating as an adaptive female strategy. Under these circumstances, EPP rates should be low and those females who do mate with multiple males may be making the "best of a bad deal" by mating with extra-pair suitors rather than putting up a potentially costly defense against them (Westneat and Stewart 2003). This conflict between males and females may maintain a low rate of extra-pair copulations in species where the costs to the female are relatively high (Arnqvist and Kirkpatrick 2005). In contrast, we would expect relatively high rates of EPP in species where costs to females are low, either because females have little reason to resist the extra-pair copulation attempts of males or because the indirect benefits of EPP to females outweigh the weak costs (Albrecht et al. 2006). Because direct costs of EPP can be a strong selective force, it is important to consider these costs in explanations of the evolution of mating behavior in birds (Cameron et al. 2003).

Similarly, if the costs of EPP vary across females within a population, then all else being equal we would expect females able to bear or otherwise mitigate these costs to have higher rates of EPP than those who cannot (Gowaty 1996). In some cooperatively breeding fairy-wrens (Maluridae), for example, females with auxiliary helpers have been found to produce more extra-pair young than females without auxiliaries (Mulder et al. 1994; Webster et al. 2004). One possible reason for this is that females with auxiliaries are less dependent on male assistance in raising the young, and therefore are “emancipated” from retaliation by their mates in the form of reduced parental care (Mulder et al. 1994). However, few studies to date have examined the relationships between EPP and offspring feeding by adult males in cooperative species (but see Dunn & Cockburn 1996; Williams & Hale 2008), and so the effects of EPP on offspring care in these species are unclear.

Recent comparative meta-analyses have strongly implicated costs to females influencing the evolution of EPP rates in birds. First, Arnqvist and Kirkpatrick (2005) examined the relative strength of direct and indirect selection on extra-pair mating behavior in female birds and found that the direct costs to females greatly outweighed the indirect benefits gained from increased fitness of their offspring. Additionally, Albrecht et al. (2006) found that rates of EPP were correlated with the cost of EPP to females across species. However, sample sizes for these studies were relatively small, and in most cases the investigators were forced to use estimates of costs and rates of EPP from different populations of the same species. More importantly, these studies considered only one potential cost of EPP to females – reduced male parental care – and in many cases it is unclear how reduced male parental care affects long term female fitness. Finally, these meta-analyses necessarily focus on interspecific variation and therefore did not consider the possibility that the costs of EPP may vary across females within a population.

There are, in fact, several potential costs of EPP to females. The most commonly examined cost in biparental species is loss of paternal care from the cuckolded social mate (e.g. Dixon et al. 1994; Chuang-Dobbs et al. 2001; Bouwman et al. 2005). If males are able to assess their paternity in broods, it often will benefit them to invest less in broods containing fewer of their own offspring (Fishman & Stone 2004). This reduction in paternal care can result in reduced reproductive success for the female (e.g. Weatherhead et al. 1994), but may not always (Dunn & Cockburn 1997). In cases where females can compensate for the loss of paternal care by increasing their own investment, females may have reduced survival due to a trade-off between investment in reproduction and self-maintenance. Another potential cost of EPP to females is the energetic investment of finding a suitable extra-pair mate (Dunn and Whittingham 2007). There may also be costs associated with the actual act of mating, such as the danger of contracting a sexually transmitted infection (Sheldon 1993; Thrall et al. 2000; Kokko et al. 2002). Costs of copulation are common in insects (Morrow et al. 2003) but have only rarely been demonstrated in wild vertebrates (e.g. Westneat and Rambo 2000). Any of these costs – reduced male parental care, energetic search costs, or costs of mating – will be evolutionarily important only if they affect either female fecundity or survival.

We examined the costs of EPP in the red-backed fairy-wren, *Malurus melanocephalus*. These small Australian passerines are socially monogamous and usually pair for life. They also are cooperative breeders, with young males often remaining on the natal territory as auxiliaries who assist in raising subsequent broods (Schodde 1982; Rowley & Russell 1997). The female is solely responsible for constructing the nest and incubating, but both sexes and auxiliaries feed nestlings and fledglings (Schodde 1982; Rowley & Russell 1997). Like other members of their genus, red-backed fairy-wrens have very high EPP rates; about 50% of offspring are the result of extra-pair mating (Webster et al. 2008). There is also wide variation in EPP rates between females, with 37% of broods containing entirely extra-pair young and an equal number consisting of entirely within-pair young (unpub. data). Because of the variation in EPP across females, this is an excellent species in which to investigate the costs of extra-pair mating.

Because loss of paternal care is the most commonly studied cost of extra-pair mating in birds, we examined male feeding rates relative to their level of cuckoldry (i.e., whether or not extra-pair young were present in the nest). If males are able to assess their paternity within a nest, we would expect them to reduce paternal care to nests that contain fewer of their own offspring (Dixon et al. 1994). Reduced paternal care may not impose much of a cost on females if they are able to compensate for the reduction in some other way. In particular, for other cooperatively breeding species of fairy-wren it has been suggested that care provided by auxiliary male helpers may compensate for lost care from social males, allowing those females with auxiliaries to produce more extra-pair young (Mulder et al. 1994; Webster et al. 2004). Accordingly, we also examined the EPP rates of females with and without auxiliaries as well as feeding rates by all group members. Females may also be able to compensate for loss of care by increasing their own feeding rates, which could lead to reduced reproductive success or survival. Because of this we examined the effect of EPP on measures of female reproductive success and survival. These measures will also detect significant but cryptic costs of extra-pair mating, such as energetic costs or sexually transmitted infections. Because EPP rates are high in this species (Webster et al. 2008), our general predictions were that the costs of extra-pair mating to females should be low, and also that females who produce extra-pair young should be those who are able to mitigate the costs (e.g., through the presence of helpers).

## METHODS

### Field Methods:

We conducted our research on a population of red-backed fairy-wrens in the forest surrounding the reservoirs of the Herberton Shire on the Atherton Tablelands in Queensland, Australia (145º25'E, 17º22'S). Research was conducted during the breeding seasons (Oct. - Feb.) of 1998-2000 and 2003-2007 (Breeding seasons are designated by the year in which they ended). In each year we attempted to capture all adults on the study site. Each captured individual was measured and a small (ca. 30 μL) blood sample was taken from the brachial vein for later genetic analysis. Each individual was also banded with a numbered aluminum band (Australian Bird and Bat Banding Scheme) and a unique combination of three colored leg bands to allow for identification in the field. Group compositions were determined through many observations of the birds in the field. We monitored all breeding attempts by groups on the field site and found nearly all nests that received eggs. We monitored nests by visiting them every three days. On the 6th day after hatching, nestlings were banded with a numbered aluminum band and, in 2005-2007, a single color band for post-fledging identification. A small (30 μL) blood sample was also taken from the tarsal vein of each nestling for later genetic analysis (below).

Nest observations were conducted 7-9 days after hatching. We chose days later in the nestling cycle because larger chicks should require more food investment from their parents and thus the male's contribution should be more important at this time (see Chuang-Dobbs et al. 2001). Most nest observations were conducted by an observer sitting in a blind 5-10 m from the nest, however, in 2006 some observations were conducted with a video camera. In these cases, the videos were later transcribed using the same protocols as used by the live observers. After set up of the blind, observers waited 5-10 minutes before beginning the observation to allow the birds to acclimate to their presence. During the observation, all visits to the nest were recorded, as well as the identity of the visitor, length of the visit, and, in 2004-2007, size of food (if any) brought to the nest. The size of the food items were scored relative to the length of the bird's bill such that food smaller than 1/3 of the bill was given a score of 1, food 1/3 of the bill to the size of the bill was given a score of 2, food 1 to 2 times the size of the bill was given a score of 3, food 2 to 4 times the size of the bill was given a score of 4, and food greater than 4 times the size of the bill was given a score of 5. All observations were conducted for at least 1 hour and the average length of observation was 3.07 ± 1.46 hours (mean ± SD). Observations were conducted in the morning hours and completed before 10 AM. We calculated feeding rates per hour and percentage of feeds provided by each adult feeding at the nest.

### Genetic methods:

Blood samples were stored in lysis buffer (White & Densmore 1992) and kept at 4° C until they were returned to the lab and processed. We extracted the DNA using a standard phenol chloroform procedure (Westneat 1990). Once DNA was extracted, we genotyped all individuals using a panel of 10 highly polymorphic microsatellite loci (detailed in Webster et al. 2008). Briefly, we amplified each loci using fluorescently labeled primers and standard PCR procedures and visualized the alleles using an automated sequencer. These microsatellites gave us a probability of 0.9998 of excluding a random male in the population as the sire an offspring. We used the program CERVUS (Marshall et al. 1998) to assign fathers to all sampled offspring. We evaluated all paternity calls from CERVUS using other available data including possible presence of null alleles, identity of sires, sires of nest mates. In most cases we accepted the paternity assignments, but in some cases, the other sources of data led us to assign paternity to another male (see Webster et al. 2008 for details).

### Statistical methods:

We used a generalized linear mixed model (GLMM) to look at correlates of female EPP rates. In these models we included only nests in which paternity was determined for every egg laid. This insured that our measure of female EPP was as accurate as possible, as incompletely sampled broods are often the result of failure of eggs to hatch or partial mortality prior to sampling. Since it is possible that there may be different mortality rates between extra-pair and within-pair offspring, it is important to exclude these incomplete broods. Because our response variable was the proportion of offspring that were the result of extra-pair copulations, we used a binomial distribution and a logit link. We included female identity, male identity and year as random effects in our model to control for multiple sampling of females and males both within and between years. Our initial model included female age, male age, male plumage color, presence of an auxiliary in the group, and nest initiation date as fixed effects. We then sequentially removed fixed effects with P values > 0.1 starting with the effects with the highest P value. After sequentially removing non-significant effects, our final model included female age, male age, presence of an auxiliary, and nest initiation date.

We again used GLMMs to model parental investment of both males and females. We used three measures to estimate parental investment, number of visits to the nest per hour, proportion of total visits, and average size of food items brought to the nest. For the models of the number of visits per hour we used a normal distribution and an identity link and for models of the proportion visits provided we used a binomial distribution and a logit link. For each analysis, we included individual identity and year as random effects. For our initial models we included proportion of extra-pair offspring, individual age, presence of an auxiliary, number of nestlings, nest initiation date, and, for the analyses of males, plumage color (see Karubian 2002). We also included first order interactions between proportion of extra-pair offspring and all other fixed effects. We then sequentially removed non-significant terms starting with interaction terms and then other terms with P values > 0.1 with the exception of proportion of extra-pair offspring and presence of an auxiliary, which were left in all models regardless of P value as they are the effects of interest in these analyses. Our final models for number of visits for both males and females included proportion of extra-pair offspring, presence of an auxiliary, number of nestlings and nest initiation date. We included only presence of an auxiliary and proportion of extra-pair young as fixed effects in our final model of proportion of care provided by both males and females.

Similarly, we constructed a GLMM to test if there were more subtle effects of extra-pair paternity in terms of the size of food items brought to the nest by males. Because we only recorded size of food items in later years of the study, we had many fewer repeated measure of males. In fact we only had two males for which we had multiple measure of food size, so rather than including male identity as a random factor, we randomly chose one observation per male. For our initial model we included year as a random effect and extra-pair paternity rate, presence of an auxiliary, male age, male plumage color, and nest initiation date as fixed effects. Again we sequentially removed non-significant (P > 0.1) fixed effects from the model with the exception of paternity rate and presence of an auxiliary which were left in regardless. Our final model included paternity rate, presence of an auxiliary, and nest initiation date.

Generalized linear mixed models were also constructed for our analyses of female reproductive success. Our measures of reproductive success were number of eggs per clutch, number of offspring fledged per successful nest, number of successful nests per year and number of fledglings produced per year. Becaues all of these are counts, we used a Poisson distribution and a log link for all models of reproductive success. For all analyses we included female identity and year as random factors and a measure of extra-pair mating rate (see below), female age, and presence of an auxiliary as fixed effects. In addition, for measures from individual nests (# of eggs per nest, # of fledglings per nest) we included nest initiation date as an additional fixed effect. We also included all first order interactions between EPP rate and all other fixed effects. Again, we removed non-significant ($P > 0.1$) terms from the model sequentially starting with interaction terms and then other fixed effect terms with the exception of EPP rate which was included in all cases as it was the fixed effect of greatest interest. Our final models for number of eggs per nest, number of fledglings per nest, and number of nests per year included only EPP rate as a fixed effect since no other effect was significant. Our final model of number of fledglings per year included EPP rate and female age.

For our measure of extra-pair mating we used the proportion of extra-pair young produced by a female in a clutch or in a year. A female with a large proportion of extra-pair young was assumed to have engaged in more extra-pair mating than a female with a small proportion of extra-pair young. However, since proportion of extra-pair young is somewhat related to female reproductive success (i.e., females who have many offspring are more likely to produce both extra-pair and within-pair chicks than females who have few offspring; Parker & Tang-Martinez 2005), I categorized extra-pair mating behavior so that the number of offspring would not be expected to be different between the categories. Specifically, we compared females who produced entirely extra-pair young to females who produced entirely within-pair young. The amount of extra-pair mating was almost certainly different between these two categories, but we excluded females who produce mixed broods, which accounted for around 30% of our samples. Moreover, using a similar but expanded categorization of females – i.e., females who produced more than 50% extra-pair young versus females who produced less than 50% extra-pair young – produced nearly identical results (analyses not shown).

We also estimated adult female survival probabilities to determine whether females who engage in extra-pair matings have lower survival than those who do not. Our survivorship data were based on annual surveys of color-banded females, who are highly sedentary after natal dispersal and rarely change territories between years (unpubl. data). We used multi-state capture-recapture models (Hestbeck et al. 1991, Brownie et al. 1993, Nichols and Kendall 1995) and Program MARK (White and Burnham 1999) to compare survival probabilities, while controlling for probability of re-sighting, between females who produced extra-pair offspring and those who did not. We categorized females into three classes; females who produced at least one extra-pair offspring in a given year, females who produced offspring none of which were extra-pair, and females who produced no sampled offspring. This last category consists both of females whose nests did not survive to be sampled and females seen on the edges of the study site whose nests were never found. Program MARK simultaneously estimates three probabilities: probability of survival ($S$), probability of re-sighting ($p$), and probability of state change ($\psi$, i.e., the probability of changing from a female who produces extra-pair young to one who does not or vice versa), and thereby gives much more accurate measures of survival. Each of these three probabilities can be modeled as a function of year, extra-pair paternity, both, or neither, giving a total of 64 possible models. In order to narrow down the number of models we made some *a priori* assumptions based on the biology of the birds and our study methods. We assumed that $p$ varied with year (because of possible differences in field observers or bird behavior across years), but not extra-pair paternity (because there is no reason to expect that a female's mating behavior should affect our ability to detect her). We held *S* constant over time in all candidate models to obtain a single estimate for survival; *S* was modeled as either dependent or independent of extra-pair paternity. We modeled $\psi$ as dependent on extra-pair paternity, on year, on a factorial combination of year*paternity, and as constant. These assumptions gave us 8 candidate models (Table 1). The relative likelihood of each model was estimated with second-order Akaike’s information criterion values, or $AIC_c$ (Burnham and Anderson 2002). We calculated a weighted model average of survival rates of females with and without auxiliaries using $AIC_c$ weights.

## RESULTS

### Paternity Results:

We determined paternity of 673 young from 231 complete broods. Of these, 54% (362) were extra-pair young and 68% (223) of broods included at least one extra-pair offspring. Many females have multiple breeding attempts in a single season and 67% of females produced at least one extra-pair chick in a given breeding season. Extra-pair paternity rates varied between years with the lowest being 39% in 2004 and the highest being 68% in 2006 (Table 2).

Our GLMM revealed several factors that were correlated with the proportion of extra-pair offspring (Table 3). One-year-old females produced a smaller proportion of extra-pair young (47.4 ± 6.3 %) than did older females (72.1 ± 5.1%, N = 87, $F_{1,50} = 12.78$, $P < 0.001$), whereas females paired to one-year-old males produced more extra-pair young (69.7 ± 6.0%) than those paired to older males (50.3 ± 5.7 %, $F_{1,50} = 7.11$, $P = 0.01$). In addition, nests initiated later in the season contained a greater proportion of extra-pair young than did nests initiated earlier in the season ($F_{1,50} = 10.57$, $P = 0.002$). Finally, as in other species of fairy-wren that have been studied to date (Mulder et al. 1994; Webster et al. 2004), females with auxiliary helpers produced a greater proportion of extra-pair offspring (68.4 ± 6.9%) than females without auxiliaries (Fig. 1; 51.8 ± 4.7%, $F_{1,50} = 4.7$, $P = 0.04$).

**Parental care and paternity:**

We completed 95 feeding observations for a total of 291 hours of observations. Males on average made 2.32 ± 0.27 (mean ± SE) feeding trips to the nest per hour resulting in 22% of visits to the nest, whereas females made an average of 6.79 ± 0.39 feeding trips to the nest per hour resulting in 72% of visits to the nest. The remaining 6% of nest visits were made by auxiliary helpers. We had 87 observation periods with complete information with which to examine the factors that might affect parental care. We found that males with auxiliaries made fewer visits to the nest per hour than did males without auxiliaries (Fig. 2, $F_{1,7} = 6.68$, $P = 0.04$), and that males nesting later in the season made fewer visits to the nest per hour ($F_{1,7} = 9.10$, $P = 0.02$). Males also tended to feed at a higher rate when they had more chicks ($F_{1,7} = 4.03$, $P = 0.08$). However, the proportion of extra-pair young in the nest had no effect on male feeding rates (Table 4; $F_{1,7} = 0.25$, $P = 0.63$) or on the proportion of feeds provided by the male ($F_{1,9} = 0.19$, $P = 0.67$). We also found no effect of the presence of an auxiliary on proportion of feeds provided by the male (Table 4; $F_{1,9} = 1.12$, $P = 0.32$). Indeed, we found no difference in feeding rates even between males raising only within-pair young (no extra-pair young in the brood) and those raising only extra-pair young (no within-pair young in the brood; Fig 3). Similarly, we found no effect of extra-pair paternity (Table 4; $F_{1,25} = 0.11$, $P = 0.74$) or presence of an auxiliary ($F_{1,25} = 1.71$, $P = 0.20$) on the size of the food that males brought to the nest. However, males brought larger food items later in the season ($F_{1,25} = 10.84$, $P < 0.01$).

We found similar effects on female parental care patterns. Females with auxiliaries made fewer feeding visits to the nest per hour than females without auxiliaries (Fig 2, $F_{1,10} = 6.52$, $P = 0.03$), females nesting later in the season visited then nest less frequently ($F_{1,10} = 7.39$, $P = 0.02$), and females with more nestlings visited the nest more often ($F_{1,10} = 8.07$, $P = 0.02$). Again, the proportion of extra-pair young in the nest did not affect the rate at which females visited the nest (Table 5; $F_{1,10} = 0.14$, $P = 0.72$). Finally, as for males, neither presence of an auxiliary ($F_{1,11} = 1.11$, $P = 0.31$) nor proportion of extra-pair young ($F_{1,11} = 0.06$, $P = 0.86$) had any significant effect on the proportion of visits provided by the female (Table 5).

**Female reproductive success and EPP:**

For our measures of female reproductive success we used number of eggs per nest and number of fledged young per nest as well as number of successful nests per year and number of fledgling produced per year. We found no difference in the number of eggs laid by females who produced only extra-pair offspring and females who produced only within pair offspring (Fig 4a, $N = 157$, $F_{1,39} < 0.01$, $P = 0.97$). Neither did we find an effect on the number of chicks fledged from successful nests (Fig 4b, $N = 75$, $F_{1,13} = 0.20$, $P = 0.66$). Females who produced only within-pair offspring did not have more successful nests per year than female who produced only extra-pair offspring (Fig 4c, N = 160, $F_{1,30} = 0.05$, P = 0.82), nor did they produce more fledglings per year (Fig 4d, N = 160, $F_{1,29} = 0.36$, P = 0.55). However, one year old females did tend to produce more fledglings per year (1.98 ± 0.26) than did older females (1.55 ± 0.21), though the difference was not significant (N = 160, $F_{1,29} = 3.24$, P = 0.08).

### Female survival and EPM behavior:

Annual adult female survival was relatively high, with 57.5 ± 2.6% (Mean ± s.e.) of females surviving between years. There was no significant effect of EPP on female survival, with 58.0% of females who produced at least one extra-pair offspring surviving (95% C.I. = 52.1% - 63.7%) and 57.7% of females who produced only within-pair offspring surviving to the following year (95% C.I. = 51.6% - 63.4%; Fig. 5). Females who produced no sampled offspring had an annual survival rate of 57.1% (95% C.I. = 50.6% - 63.3%).

## DISCUSSION

### Costs and overall EPP rates:

Our measures indicate that there is little or no cost to females associated with extra-pair mating in the red-backed fairy-wren. We found that extra-pair paternity rate had no effect on rates of paternal feeding of nestlings. There are other types of paternal care besides feeding of offspring (e.g. incubation, defense from predators) but we have no evidence to suggest that these types of care exist in the red-backed fairy-wren. If providing parental care is costly, it is intuitive to expect males to avoid providing care to unrelated chicks (Sheldon 2002), and while some studies have found evidence of adjustment of paternal care in response to paternity ( e.g. Weatherhead et al. 1994; Lifgeld et al. 1998; Dixon et al. 1994; Chuang-Dobbs 2001, Neff 2003) others have found no effect (e.g. Kempernaers et al. 1998; Peterson et al. 2001; Bouwman et al. 2005, Rytkönen et al. 2007; Williams & Hale 2008). It is important to note that our study is correlative and experimental studies may provide better tests of male responses to EPP as they control for possible confounding effects (Kempenaers & Sheldon 1997). Nonetheless, this type of descriptive study showing no correlation between paternity and paternal care can reveal important patterns (Lifjeld et al. 1998).

There are several possible explanations why males may not adjust their level of paternal care based on paternity (Westneat & Sherman 1993). First, males may not be able to determine their relatedness to individual offspring, so if they reduce their care to a brood they run the risk of harming any offspring they may have sired in that brood (Bouwman et al. 2005; Rytkönen et al. 2007). This would not be a problem if they sire no offspring in the brood as occurs in a third of our nests, but males may have no means to determine that they have been completely cuckolded. Second, there may be other benefits in providing paternal care, for example, level of paternal care could demonstrate male quality and thus ensure more fertilizations in subsequent reproductive attempts with the same female (Freeman-Gallant 1997). Alternatively, the rate of care that males provide to their offspring may not impose much of a cost (Bouwman et al. 2005). Males in this species only provide 23 ± 18% (mean ± SD) of feeding trips to the nest and this lower rate of feeding may be a result of general uncertainty of paternity. If the benefit of providing care to mixed broods is greater than the cost of providing that care, then males would be expected to feed in all cases (Whittingham et al. 1992; Westneat & Sherman 1993).

Most studies of the costs of extra-pair mating have focused on the loss of paternal care and studies providing evidence of other costs are generally lacking (Arnqvist & Kirkpatrick 2005). A few have examined the energetic costs of seeking extra-pair mates (Bouwman & Komdeur 2006; Dunn & Whittingham 2007) and another has suggested a mechanism behind potential costs associated with multiple copulations (Westneat & Rambo 2000), but few studies have examined long term effects of extra-pair mating on overall female fitness. In this study we tested for correlations between extra-pair paternity and female fecundity and survival. It appears that extra-pair mating does not have either a direct cost or a direct benefit in terms of female fitness. Although we were unable to measure all possible costs of EPP, such as energetic costs or costs associated with copulation, one benefit of this study is that, if such costs exist in the red-backed fairy-wren, we would detect them in our measures of female reproductive success and survival. Thus it appears that direct fitness costs (or benefits) to females associated with extra-pair mating are generally weak in the red-backed fairy-wren.

These low costs may help to explain the overall high rates of extra-pair paternity observed in this species. High rates of extra-pair paternity are associated with low costs in studies of other species (Arnold & Owens 2002, Albrecht et al. 2006). When costs are low, females have no reason to resist extra-pair copulation attempts from males and higher rates of extra-pair paternity can evolve through direct benefits to extra-pair males. Additionally, if indirect benefits to females exist, females may seek out extra-pair copulations and the benefits to the female may contribute to high rates of EPP. However, when costs are high females are likely to resist extra-pair mating attempts because direct costs are likely to outweigh indirect benefits, if present (Arnqvist & Kirkpatrick 2005). This conflict between females and extra-pair males is likely to reduce extra-pair paternity rates. The relationship between costs to females and rates of EPP has been revealed in a meta-analysis by Albrecht et al. (2006) that found an inverse correlation between reduction in paternal care of cuckolded males and average EPP rates. This suggests that it is very important to consider costs to females when investigating the evolution of extra-pair paternity rates.

### Costs and individual EPP rates:

Like other species of fairy-wrens (Mulder et al. 1994; Webster et al. 2004), we found that females with auxiliaries produced a greater proportion of extra-pair offspring than did females without auxiliaries. This pattern suggests that the care provided by auxiliaries can compensate for reduced parental care from the social mate, and thereby “emancipates” females to seek extra-pair copulations (Mulder et al. 1994). However, in this study we found that male red-backed fairy-wrens do not adjust their feeding rates based on the paternity of the brood. Thus, females who produce extra-pair young do not appear to suffer reduced paternal care, and some other explanation must underlie the increased EPP in the broods of females with auxiliaries. One possibility is that there is another factor (e.g. territory quality or local density) that increases the chances of a female both engaging in extra-pair copulations and maintaining an auxiliary (Gowaty 1996). For example, if local density of territories is high, females may have more opportunities to engage in extra-pair copulations, and, simultaneously, young males may have fewer opportunities to acquire a breeding territory and would be more likely to remain as an auxiliary on their natal territory. Similarly, a female who tends to engage in more extra-pair copulations, possibly with particularly attractive males, may produce more male biased broods (Kempenaers et al. 1997) and thus have a greater chance of having an auxiliary. In any case, these results suggest that it is important to confirm that males impose costs on unfaithful females before concluding that auxiliaries “liberate” females from those costs.

Although we found no effect of paternity on male feeding rates in the red-backed fairy-wren, Dunn & Cockburn (1996) found that male superb fairy-wrens reduced their care in response to loss of paternity when they had multiple auxiliaries, but not when they had no or only one auxiliary. (we did not find a significant interaction between presence of an auxiliary and paternity on male feeding rates in this study). The authors suggested that this provides support for the constrained female hypothesis and that females produce more EPP when they have auxiliaries because the auxiliaries compensate for the reduction in paternal care (Dunn & Cockburn 1997). We disagree with this interpretation; because males without auxiliaries did not adjust their feeding rates according to paternity, there does not seem to be a cost of EPP to females without auxiliaries. We suggest that a better interpretation of the pattern seen in superb fairy-wrens is that auxiliaries “liberate” males and allow them to reduce their investment in paternal care and instead focus on extra-pair mating (Green et al. 1995). This interpretation suggests that the potential costs to males of reducing care to a few genetic offspring may outweigh the benefits gained by reducing care (Whittingham et al. 2002). In short, there is little evidence that females are constrained by their mate's parental behavior in any of the species of fairy-wrens studied to date.

Although it appears that females are not constrained in their extra-pair mating decisions by reductions in male feeding rates, there is still a large amount of variation in EPP among females in our study population and this variation remains unexplained. It may be that there are costs other than male feeding rate that constrain a females ability to seek extra-pair paternity, however, we found no effect of EPP on any of our measures of female reproductive success or on female survival. In fact, most of the non-significant effects were in the opposite direction than would be predicted if EPP was costly, suggesting that there is not even a slight net cost to mating with extra-pair males.

One possible explanation of these results is that only females who can withstand the costs engage in extra-pair matings (Gowaty 1996; Møller 2000). For example, females who are in particularly good condition or have high quality territories may be more able to engage in extra-pair matings (Gowaty 1996), and these females might also maintain high reproductive success and survival even if costs exist. Therefore the reproductive success and survival would be equalized between females who engage in extra-pair matings and those that do not. Without experimental tests, it is impossible to tell whether some females are better able to withstand costs of extra-pair mating, but correlational evidence can suggest some connections. In this study we found that older females, who are more experienced and may be in better condition, produce a greater proportion of extra-pair young, suggesting that perhaps older females are less susceptible to the costs of extra-pair paternity. Additionally, extra-pair paternity rates are lower early in the season than later in the season. This could reflect that females are limited in their ability to seek extra-pair copulations early in the season, perhaps because food resources are more limited then (unpub. data). However, since EPP rates are relatively high, compared to other species, at all points in the season and in all female age classes, it seems that few females are limited in their extra-pair mating choice. Therefore, it appears that if costs of extra-pair mating exist in this system, few females are limited by them.

Alternatively, the variation in EPP between females could be caused by differences in the benefits that females gain from engaging in extra-pair copulations (Dietrich et al. 2004). A female socially paired to a high quality or genetically compatible male will stand to gain little from seeking extra-pair copulations because the genes provided to her offspring by her extra-pair mate would only be marginally better, or possibly worse, than the genes she would gain from her social mate. In contrast, a female paired to a low quality or genetically incompatible male would likely benefit substantially, in terms of offspring quality, from engaging in extra-pair copulations. Interestingly, in this study we found that young males were cuckolded at a higher rate than were older males, and it has been suggested that male age may be an indication of good genes (Brooks & Kemp 2001; Proulx et al. 2002). There are other possible indirect benefits that females may gain from mating with extra-pair males, such as sexy sons or genetic compatibility (Jennions & Petrie 2000), and further study is needed to determine whether females increase offspring quality by mating with extra-pair males (e.g. Johnsen et al. 2000; Foerster et al 2003; Forsman et al. 2008).

This study provides further support for the idea that the rate of EPP observed across populations is related to the costs that extra-pair mating imposes on the female (Møller 2000; Arnold & Owens 2002; Albrecht et al. 2006). Therefore, it is important to consider the existence of these costs as they may preclude the evolution of extra-pair mating as an adaptive female strategy (Arnqvist and Kirkpatrick 2005). However, although costs seem important in the overall EPP rates across species/populations, in this study the costs of extra-pair mating did not appear to be the driving force explaining variation in EPP across females within a population. Rather, we suggest that it may be differences in the benefits of EPP that may explain the variation in EPP rates in this species, an idea that requires further investigation.

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**Table 1:** $AIC_c$ values and weights for candidate models of female survival

| | | | Survival (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Model | $AIC_c$ | weight | EPP | Up. CL | Low CL | No EPP | Up. CL | Low CL |
| $S(.)p(y)\psi(ye)$ | 875.79 | 0.6392 | 57.5 | 62.3 | 52.5 | 57.5 | 62.3 | 52.5 |
| $S(.)p(y)\psi(e)$ | 877.62 | 0.2570 | 57.9 | 63.2 | 52.5 | 57.9 | 63.2 | 52.5 |
| $S(e)p(y)\psi(e)$ | 879.59 | 0.0957 | 61.5 | 68.7 | 53.8 | 58.0 | 68.6 | 46.6 |
| $S(.)p(y)\psi(.)$ | 885.68 | 0.0046 | 57.9 | 63.2 | 52.5 | 57.9 | 63.2 | 52.5 |
| $S(e)p(y)\psi(.)$ | 887.55 | 0.0018 | 61.5 | 68.8 | 53.8 | 58.0 | 68.5 | 46.7 |
| $S(.)p(y)\psi(y)$ | 888.65 | 0.0010 | 57.9 | 63.2 | 52.5 | 57.9 | 63.2 | 52.5 |
| $S(e)p(y)\psi(y)$ | 890.50 | 0.0004 | 61.6 | 68.8 | 53.8 | 58.3 | 68.8 | 47.0 |
| $S(e)p(y)\psi(ye)$ | 891.22 | 0.0003 | 61.3 | 68.3 | 53.8 | 57.3 | 67.6 | 46.5 |
| Model average | | | 58.0 | 63.7 | 52.1 | 57.7 | 63.5 | 51.6 |

Candidate models and model average from the analysis of female survival using MARK. The three estimated probabilities, survival ($S$), detection ($p$), and state change ($\psi$), are modeled as being dependent on extra-pair paternity (e), year (y), neither (.) or both (ye). Survival estimates for each model are given with upper and lower 95% confidence limits.

**Table 2.** EPP rates over various years of the study.

| Year | # Young | # Nests | # EPY (%) | # Nests w/ EPY (%) |
|---|---|---|---|---|
| 1998 | 93 | 34 | 63 (68%) | 26 (76%) |
| 1999 | 131 | 44 | 64 (49%) | 30 (68%) |
| 2000 | 104 | 34 | 47 (45%) | 21 (62%) |
| 2003 | 23 | 9 | 13 (57%) | 5 (56%) |
| 2004 | 62 | 22 | 24 (39%) | 10 (45%) |
| 2005 | 116 | 38 | 72 (62%) | 29 (76%) |
| 2006 | 53 | 19 | 36 (68%) | 16 (84%) |
| 2007 | 91 | 31 | 43 (47%) | 20 (65%) |

**Table 3.** Results from GLMM of EPP

| Factor | Estimate | LS Means (% EPY ± SE) | | Test stat. | P value |
|---|---|---|---|---|---|
| Presence of auxiliary | 0.701 | Aux.: 68.4 ± 6.9 | No aux.: 51.8 ± 4.6 | $F_{1,50} = 4.70$ | 0.035 |
| Female age | -1.055 | 1 year old: 47.4 ± 6.3 | 2+ years old: 72.1 ± 5.1 | $F_{1,50} = 12.78$ | > 0.001 |
| Male Age | 0.818 | 1 year old: 69.7 ± 6.0 | 2+ years old: 50.3 ± 5.7 | $F_{1,50} = 7.11$ | 0.010 |
| Nest initiation date | 0.012 | N/A | N/A | $F_{1,50} = 10.57$ | 0.002 |

**Table 4:** Results from GLMMs of male parental care measures

| | N | Estimate | LS Means | | Test Stat. | P value |
|---|---|---|---|---|---|---|
| Male feeds per hour | 87 | | | | | |
| Proportion of EPY | | 0.321 | | | $F_{1,7} = 0.25$ | 0.631 |
| Presence of an auxiliary | | -1.762 | Aux.: 0.94 ± 0.36 | No Aux.: 2.69 ± 0.68 | $F_{1,7} = 6.68$ | 0.036 |
| # of offspring | | 0.779 | | | $F_{1,7} = 4.03$ | 0.085 |
| Nest initiation date | | -0.028 | | | $F_{1,7} = 9.10$ | 0.020 |
| Percentage of total feeds by male | 87 | | | | | |
| Proportion of EPY | | 0.274 | | | $F_{1,9} = 0.19$ | 0.670 |
| Presence of an auxiliary | | -0.929 | Aux.: 11.6 ± 8.5% | No Aux.: 24.9 ± 5.1% | $F_{1,9} = 1.12$ | 0.318 |
| Size of food brought by male | 32 | | | | | |
| Proportion of EPY | | 0.097 | | | $F_{1,25} = 0.11$ | 0.739 |
| Presence of an auxiliary | | -0.437 | Aux.: 2.77 ± 0.33 | No Aux.: 3.21 ± 0.18 | $F_{1,25} = 1.71$ | 0.203 |
| Nest initiation date | | 0.020 | | | $F_{1,25} = 10.84$ | 0.003 |

**Table 5:** Results from GLMMs of female parental care measures

| | N | Estimate | LS Means | | Test Stat. | P value |
|---|---|---|---|---|---|---|
| Female feeds per hour | 87 | | | | | |
| Proportion of EPY | | 0.328 | | | $F_{1,10} = 0.14$ | 0.719 |
| Presence of an auxiliary | | -2.526 | Aux.: 4.58 ± 1.11 | No Aux.: 7.11 ± 0.6874 | $F_{1,10} = 6.52$ | 0.029 |
| # of offspring | | 1.560 | | | $F_{1,10} = 8.07$ | 0.018 |
| Nest initiation date | | -0.037 | | | $F_{1,10} = 7.39$ | 0.022 |
| Percentage of total feeds by female | 87 | | | | | |
| Proportion of EPY | | -0.139 | | | $F_{1,9} = 0.06$ | 0.815 |
| Presence of an auxiliary | | -0.640 | Aux.: 59.6 ± 13.1% | No Aux.: 73.7 ± 5.2% | $F_{1,9} = 1.11$ | 0.315 |

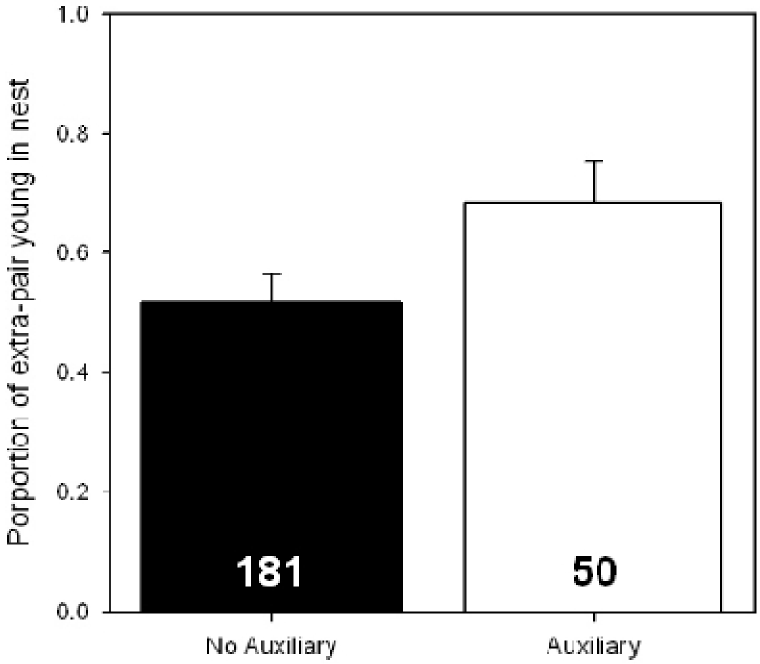

**Fig 1:** Proportion of extra-pair young produced by females with and without auxiliary male helpers. Columns are least squared means and bars are standard errors both from a GLMM including presence of an auxiliary, nest initiation date, female age, and social male age as fixed effects and female identity and year as random effects. Numbers in the columns are sample sizes.

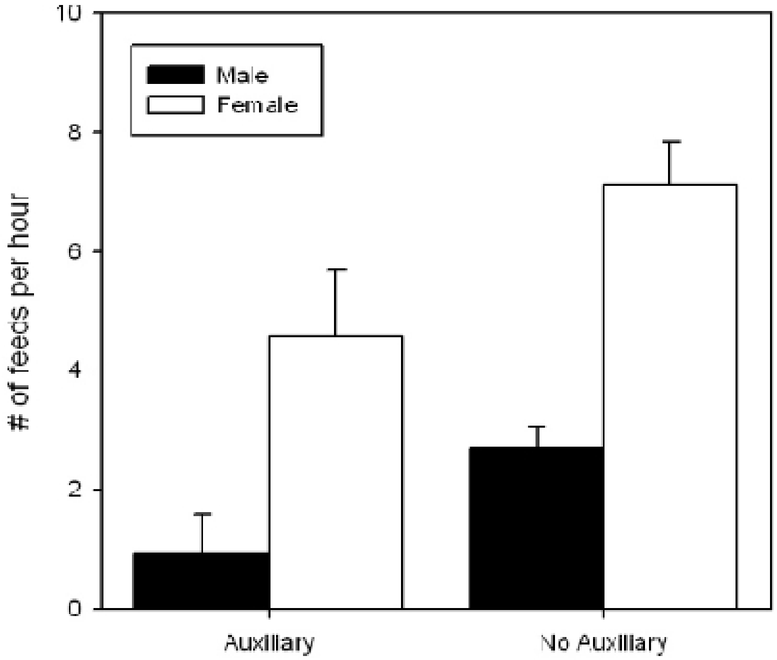

**Fig. 2:** Effect of presence of an auxiliary on parental feeding rates. Columns are least squared means and bars are standard errors are from GLMMs of male and female feeding rates modeled separately including extra-pair mating rate, nest initiation date, presence of an auxiliary, and number of chicks as fixed effects and parent identity and year as random effects.

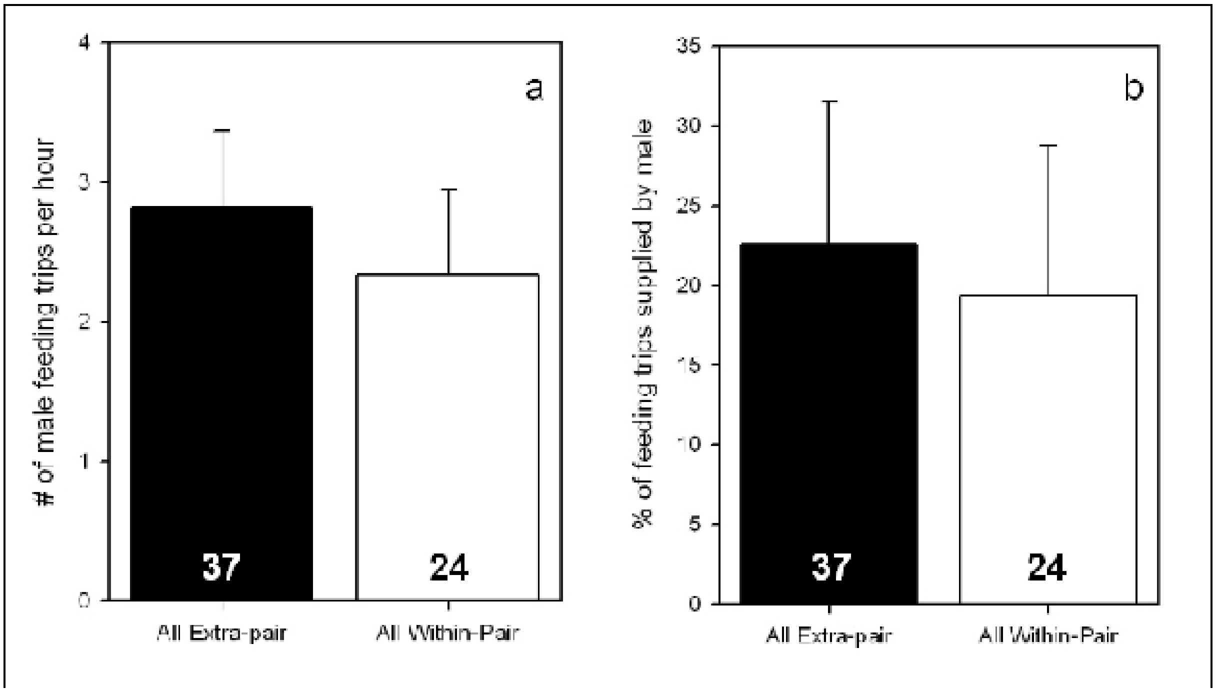

**Fig 3:** Difference in paternal care to nests containing entirely extra-pair and entirely within pair chicks. EPP rate has no effect either on number of male visits to the nest (P = 0.52) or the proportion of feeds provided by males (P = 0.77). a) Number of male visits to the nest per hour. Columns are least squared means and bars are standard errors from a GLMM including extra-pair mating rate, year, nest initiation date, presence of an auxiliary, and number of chicks as fixed effects, and male identity and year as random effects. b) Proportion of visits provided by the male. Columns are least squared means and bars are standard errors from a GLMM including extra-pair mating rate and presence of an auxiliary as fixed effects and male identity and year as a random effects.

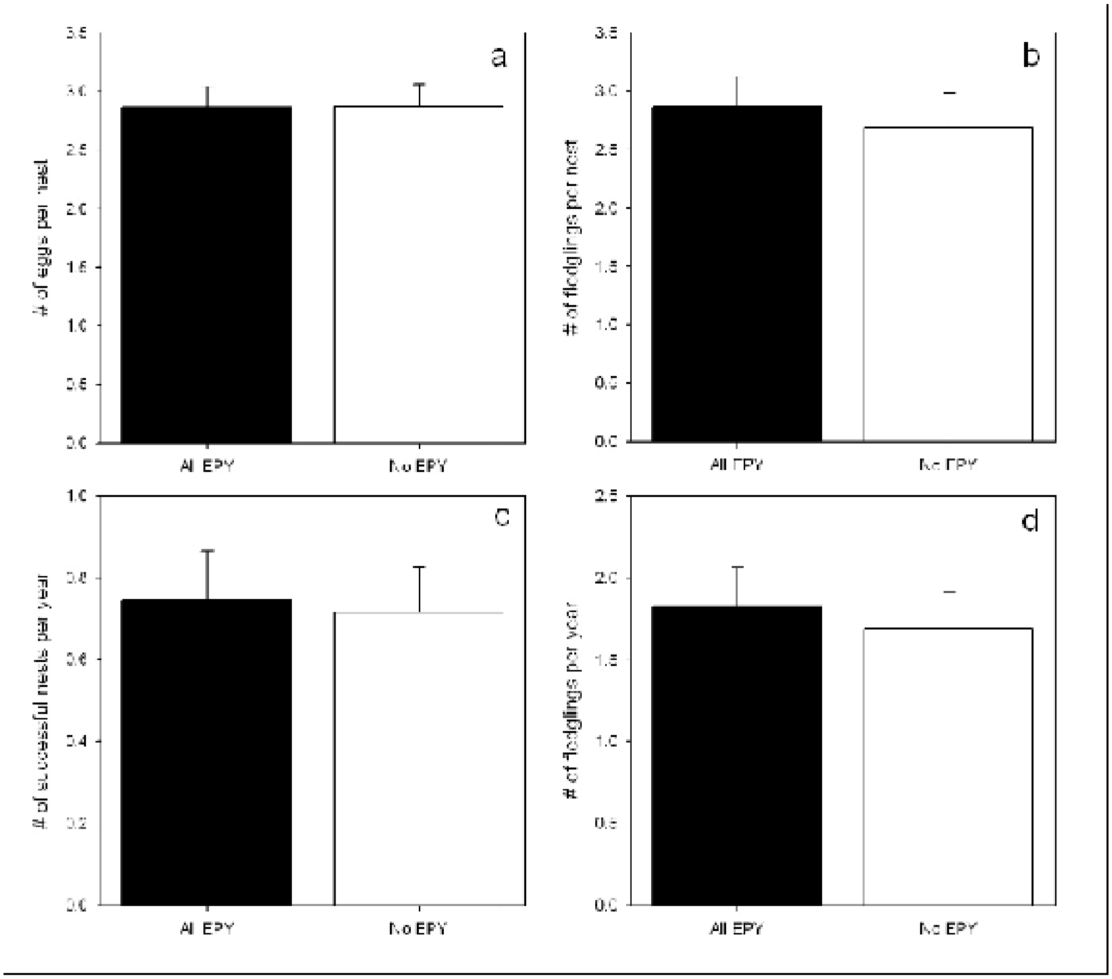

**Fig 4:** Effects of extra-pair mating on reproductive success. Columns are least squared means and bars are standard errors from GLMMs. All models include EPP rate as a fixed effect and female identity and year as random effects. The number of fledglings produced per year also includes female age as a fixed effect.

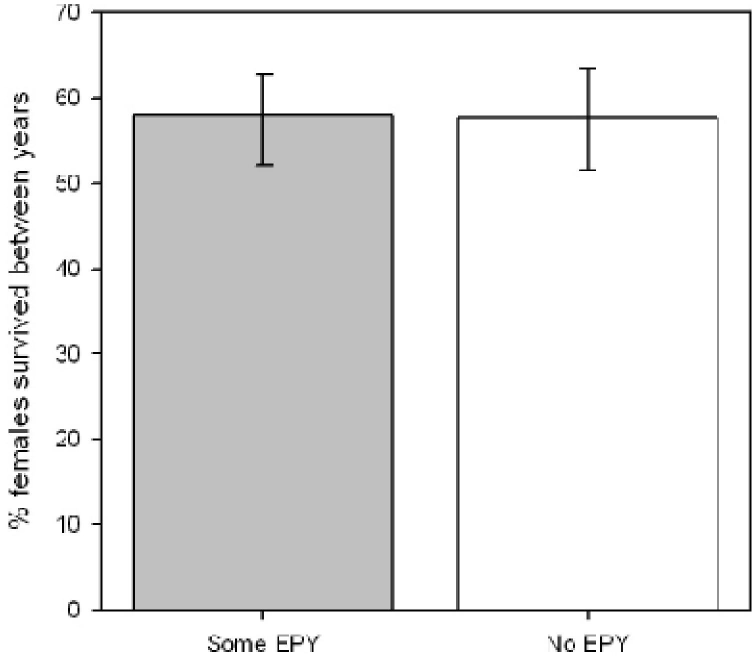

**Fig 5:** Effect of extra-pair mating on female survival. Columns are the weighted model average and bars are the 95% confidence interval.

# Chapter 3

# Female fairy-wrens avoid inbreeding through extra-pair paternity

## INTRODUCTION

It is now clear that females of many socially monogamous species copulate with males other than their social mates (Griffith et al. 2002). It is generally thought that females benefit from production of young sired by these extra-pair males, yet despite nearly two decades of work, the adaptive benefits of extra-pair paternity to females remain unclear and controversial (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005). In some systems females gain direct benefits from their extra-pair mates (e.g. Gray 1997; Kempenaers et al. 1999; Li & Brown 2002), but these seem to be the exception rather than the rule. In most cases it is thought that the only benefits females might gain are indirect benefits in the form of increased offspring quality (reviewed in Jennions & Petrie 2000; Zeh & Zeh 2001; Griffith et al. 2002). One possibility is that females choose extra-pair mates who possess a particular gene or set of genes that impart some benefit to the offspring (Hamilton 1990; Westneat et al. 1990). However, studies of the "good genes" hypothesis have produced mixed results (e.g. Schmoll et al. 2003; Gustafsson & Qvarnström 2006). More recently, the focus has turned to the idea that it is the interaction between the male and female's genes that can increase offspring fitness, and thus females should choose extra-pair mates with whom they are genetically compatible (Tregenza & Wedell 2000; Mays & Hill 2004).

The term "genetic compatibility" has been used to describe several subtly different types of genetic interactions. In some cases it is used to describe a particular gene that has beneficial combinations of alleles (Brown 1999), such as the major histocompatibility complex (MHC) which plays a role in immune response regulation in vertebrates (Hughes & Yeager 1998). It is thought that being heterozygous at MHC genes (e.g. Doherty & Zinkernagel 1975; Arkush et al. 2002) or having particular combinations of MHC genes (e.g. Lohm et al. 2002) can improve individual fitness, and females in many species, including humans, have been shown to prefer mates with compatible MHC genotypes (e.g. Wedekind et al. 1995; Freeman-Gallant et al. 2003; Richardson et al. 2005; reviewed in Penn & Potts 1999; Tregenza & Wedell 2000). In other cases "genetic compatibility" has been used in the context of intragenomic conflict to refer to compatibility between different genetic elements, for example cytoplasmic versus nuclear (Zeh & Zeh 1996; Zeh & Zeh 1997).

Although these forms of "genetic compatibility" may have important evolutionary consequences, more general genome-wide compatibility is likely to have important fitness consequences in most populations. For example, pairings between genetically dissimilar individuals produce highly heterozygous offspring, and overall heterozygosity has been linked to individual fitness in many species (reviewed in Hansson & Westerberg 2002). Therefore females should choose maximally dissimilar males in order to maximize offspring heterozygosity (Mays & Hill 2004). Under this *genetic dissimilarity hypothesis* we would expect that females socially paired to closely related males will be most likely to produce extra-pair young (relative to other females), and that the males they choose as extra-pair mates will be less related than an average male in the population (Griffith et al. 2002). Females paired with closely related males might also mate with extra-pair males to avoid the costs of inbreeding, such as homozygosity at deleterious recessive alleles or decreased heterozygosity (Tregenza & Wedell 2000). Costs of inbreeding have been well established in many species (Keller & Waller 2002; Kruuk et al. 2002; Hansson 2004; Spottiswoode & Møller 2004). Under this *inbreeding avoidance hypothesis*, we would again expect females paired to closely related males to produce more extra-pair young, but the extra-pair mates chosen may not be more distantly related to the female than an average male in the population. In this case, females benefit by avoiding the production of low-quality, inbred, offspring rather than by maximizing offspring heterozygosity. In this paper we will focus on these two sub-hypotheses, the dissimilarity and inbreeding avoidance hypotheses, within the genetic compatibility hypothesis.

Genetic relatedness has been shown to affect mate choice in species that do not form long-term pair bonds (e.g. Bull & Cooper 1999; Stow & Sunnucks 2004; Thuman & Griffith 2005), and also social mate choice in some species that do form pair bonds (Cockburn et al. 2003; Cohen & Dearborn 2004). However, in some systems social mate choice may be limited or constrained, and in these cases extra-pair mating can act as a second chance for females to choose a genetically compatible mate (Brooker et al. 1990; Freeman-Gallant et al. 2006). Genetic compatibility may be a strong force driving the evolution of extra-pair mating behavior in some populations (Tregenza & Wedell 2002), particularly those with limited dispersal, such as cooperative breeders and island populations (Frankham 1998), because in these systems the chance of becoming socially paired with a closely related individual is much higher than in populations with high natal dispersal (Richardson et al. 2004).

Studies of the effect of genetic compatibility on extra-pair mating behavior have become more common in recent years with the increasing prevalence and ease of genetic tools to determine relatedness and heterozygosity. However there are a couple of major weaknesses in most, if not all, of these studies. First, many studies use only a small number of loci to look at questions of relatedness and heterozygosity (e.g. Charmantier et al. 2004; Schmoll et al. 2005; Smith et al. 2005; Foerster et al. 2006; Edly-Wright et al. 2007). Models suggest that studies using only a few markers may lack sufficient power to detect biologically significant differences in relatedness or provide accurate measure of overall heterozygosity (Smith et al. 2005). Second, all previous studies of the effects of relatedness on extra-pair mating have been correlative. Ideally, these hypotheses should be tested experimentally in order to control for possible confounding factors such as male age and attractiveness, pair bond length, and other unknown factors (Griffith et al. 2002). For example, in a species with female biased dispersal, a female may be more likely to be related to younger males as they could be her philopatric descendants, and simultaneously, younger males may have a greater chance of being cuckolded. An experimental test would allow us to separate these factors. However, experimental tests in wild populations are extremely challenging as it is difficult to manipulate the relatedness of pairs.

In this study we used both correlational and experimental approaches to study the inbreeding avoidance and dissimilarity hypotheses in the red-backed fairy-wren, *Malurus melanocephalus*, a small Australian passerine. Red-backed fairy-wrens are socially monogamous and typically pair for life, but have very high rates of extra-pair paternity, with over 50% of offspring being the result of extra-pair copulations (Webster et al. 2008). This species is also a cooperative breeder, with some young males remaining on their natal territory to assist their parents in raising subsequent broods. Like many other cooperative breeders (Hatchwell & Komdeur 2000), natal dispersal is extremely limited in our study population; young females often disperse only a kilometer or less from their natal territory and young males typically establish their own breeding territory within a territory or two of where they were born (unpub.

data). Because of this, clusters of related individuals tend to form and a female can find herself surrounded by the territories of male relatives (Fig 1). Thus, there is a high potential for close inbreeding within this species. Additionally, there are little or no costs associated with extra-pair mating in the red-backed fairy-wren (Chap. 2), so indirect benefits to females can influence the evolution of extra-pair mating (Albrecht et al. 2006). Because of these factors, it is likely that genetic compatibility may play a role in extra-pair mating decisions in this species.

We examined the effects of social pair relatedness on extra-pair mating rates of females. If females use extra-pair mating to avoid inbreeding, we predict that females who are more closely related to their social mates will produce more extra-pair offspring, that extra-pair mates would be less related to the female than the social mates who are cuckolded, and that extra-pair offspring would be more heterozygous than their within-pair half-siblings. In addition to the previous predictions, if females are using extra-pair mating to maximize offspring heterozygosity, then we predict a female will be less related to her extra-pair mate(s) than to an average male in the population. In addition to these correlative analyses, we also conducted an experimental test of the inbreeding avoidance hypothesis. In our population, “widowed” females quickly obtain a new social mate and re-pair, either with an auxiliary male from the same social group (typically the son of the female) or an auxiliary male from a neighboring group (see also Pruett-Jones and Lewis 1990). Thus, by removing breeding males from groups with and without auxiliaries, we were able to create new pairs consisting of females paired with their own son or with an “unrelated” male from a neighboring groups (see below). This experimental approach allowed us to control for the effects of social male attractiveness, length of pair bond, and other unknown factors. The inbreeding avoidance hypothesis predicts that experimental females socially paired to their own sons would produce more extra-pair offspring than control females paired to unrelated males.

## METHODS

### Field Methods:

We conducted our research on a population of red-backed fairy-wrens at a long-term study site in the forest surrounding the reservoirs of the Herberton Shire on the Atherton Tablelands in Queensland, Australia (145º25'E, 17º22'S). Research was conducted during the breeding seasons (Oct. - Feb.) of 2004-2007 (breeding seasons are designated by the year in which they ended). In each year we attempted to capture all adults on the study site. Each captured individual was measured and a small (ca. 30 μL) blood sample was taken from the brachial vein for later genetic analysis. Each individual was also banded with a numbered aluminum band (Australian Bird and Bat Banding Scheme) and a unique combination of three colored leg bands to allow for identification in the field. Group compositions were determined through many observations of the birds in the field. We monitored all breeding attempts by groups on the field site and found nearly all nests that received eggs. We monitored nests by visiting them every three days. On the sixth day after hatching, nestlings were banded and a small (30 μL) blood sample was taken from the tarsal vein of each nestling for later genetic analysis.

### Experimental Methods:

Removal experiments were conducted in 2006 (N = 7) and 2007 (N = 25) at a second study site approximately 15 km SW of our long-term study site. For our experimental treatment, we captured and removed breeding males from groups with a male auxiliary (Fig. 2). Auxiliary helpers usually have a dull brown female-like plumage rather than the bright red and black plumage of most males. However, they are reproductively capable and occasionally sire young (Webster et al. 2008). The removed breeding males were driven about 10 km away and released at another site where fairy-wrens were present. No males returned within the same breeding season, although one male removed in 2006 did return to the study site in the subsequent year. Within hours of each breeding male's removal, the group's auxiliary assumed the breeding position, socially pairing with his own mother. Our observations confirmed that these former auxiliaries interacted with the breeding females and showed behaviors typical of dominant breeding males (see below). For our control groups, we removed breeding males from groups without auxiliaries (Fig. 2). Again, within a few hours a new male, usually an auxiliary male from a neighboring group, moved in and assumed the breeding position. Thus, new breeding males in all manipulated groups were one-year-old former auxiliaries with dull brown plumage – the new breeding males differed between treatments only in terms of whether they were closely related to the breeding female (experimentals) or not (controls).

To control for female breeding status, all removals were conducted when the female was in the latter stages of nest building and/or had begun to lay or incubate eggs. In both experimental and control treatments we destroyed any existing nest and collected the eggs if present in order to force the females to re-nest with their new mate. Females usually re-nested within a few weeks of the removal (18.2 ± 1.3 days). Because of high predation rates, in 2007 we collected the eggs from the first nest of each group, after approximately six days of incubation, to ensure sampling of offspring from as many removals as possible. Embryos were removed and frozen for later genetic analysis. Any subsequent clutches were allowed to hatch normally and blood samples were taken from the nestlings when they were three days old.

**Genetic Methods:**

Blood samples were stored in lysis buffer (White & Densmore 1992) and kept at 4° C until they were returned to the lab and processed. We extracted the DNA using a standard phenol chloroform procedure (Westneat 1990). Embryos were kept frozen until processing and extracted using DNEasy blood and tissue kit from Qiagen. Once DNA was extracted, we genotyped all individuals using a panel of 12 highly polymorphic microsatellite loci developed for other passerine species (Table 1) following the method outline in Webster et al. 2008. Briefly, we amplified each loci using fluorescently labeled primers and standard PCR procedures and visualized the alleles using an automated sequencer. We used 10 microsatellites for the paternity analysis which gave us a probability of 0.9998 of excluding a random male in the population as the sire of an offspring.

We used the program CERVUS 2.0 (Marshall et al. 1998) to assign fathers to all sampled offspring. We evaluated all paternity calls from CERVUS using other available data including possible presence of null alleles, identity of sires, sires of nest mates. In most cases we accepted the paternity assignments, but in some cases, the other sources of data led us to assign paternity to another male (see Webster et al. 2008 for details). To increase the power for our estimates of relatedness between individuals we used all 12 microsatellites. We used the program SPAGeDi (Hardy & Vekemans 2002) to calculate Queller & Goodnight's (1989) R, which estimates relatedness based on gene sharing where uncommon genes are given a higher weight than more common genes. In this estimate, a score of -1 represents two maximally dissimilar individuals, a score of 1 indicates clones, and a score of 0 represents the average relatedness of two randomly chosen individuals in the population (Queller & Goodnight 1989). For our measurement of heterozygosity we excluded one microsatellite with a high frequency of null alleles, as such loci can lead to an artificially decreased estimate of heterozygosity. We used the remaining 11 microsatellites to calculate the standardized heterozygosity (SH), which is an estimate of heterozygosity where each locus is weighted by the average heterozygosity at that locus such that loci with lower average heterozygosity are weighted more heavily than loci with higher average heterozygosity (Coltman et al. 1999). This measure of heterozygosity appears to be well correlated with other measures of heterozygosity and offspring performance (Amos et al. 2001).

### Statistical Methods:

We compared the relatedness of females to their social mate for females who produced at least one extra-pair offspring in a brood and for females who produced no extra-pair offspring in a brood using a generalized linear mixed model (GLMM) assuming a normal distribution of relatedness scores and an identity link. This is a valid assumption because although relatedness values are limited to between -1 and 1, their distribution does not differ significantly from a normal distribution (Goodness of fit test; W = 0.99, P = 0.20). We used presence of extra-pair young in the brood as our fixed effect and female identity as a random effect to control for multiple sampling of some females. We also used only one randomly selected brood per female/social male dyad (to eliminate pseudoreplication) and all broods were complete, i.e. we determined paternity for every egg laid, to ensure that we had accurate measurements of extra-pair paternity. To compare the relatedness of the female to the social mate and the extra-pair mate , we created a GLMM of the difference in relatedness score between social and extra-pair mates ($R_{(\text{female} \rightarrow \text{social male})} - R_{(\text{female} \rightarrow \text{extra-pair male})}$) with female as a random factor to control for multiple sampling of some females. Again we assumed a normal distribution of relatedness scores and used an identity link. The intercept of this model represents the average difference between the two relatedness values, and if it is significantly different from zero then there is a significant difference in relatedness of social and extra-pair mates. We used each female/social mate/extra-pair mate triad only once to control for psuedoreplication. Similarly, we used a GLMM with a normal distribution and an identity link to model the difference in heterozygosity between extra-pair chicks and their within-pair half siblings ($SH_{WPY} - SH_{EPY}$), except in this case we used brood as our random effect to control for multiple comparisons within broods (e.g. if there is one within-pair chick and three extra-pair chicks, then there are three comparisons in the brood). For our experiment we compared the proportion of extra-pair young produced by experimental and control females with generalized linear model (GLM) with a binomial distribution and a logit link since our response variable was a proportion.

## RESULTS

### Paternity Results:

We completed paternity analysis on 322 chicks from 110 complete broods from 58 females. Of these, 175 (54%) were the result of extra-pair copulations, and 75 (68%) of nests contained at least one extra-pair chick. Extra-pair paternity varied between years with 39% extra-pair young in 2004 and 68% in 2006 (Table 2).

**Correlative Results:**

We found that females who produce extra-pair young were more closely related to their social mate than were females who produce only within-pair young (N = 64; $F_{1,5} = 6.79$; P = 0.048; Fig. 3). While we did not find a difference in relatedness of females to their social mate and extra-pair mate (N = 65; $t_{44} = 1.74$; P = 0.089), the borderline non-significant effect was in the predicted direction (Fig 4). The relatedness of females to their extra-pair mates did not differ from the relatedness of females to the average male in the population (N = 65; $t_{44} < -0.40$; P = 0.69), suggesting that females do not choose maximally dissimilar males for extra-pair mates. We also found that extra-pair young were more heterozygous than their within pair half-siblings (N = 85; $t_{43} = 2.10$; P = 0.041; Fig 5).

**Experimental Results:**

We conducted a total of 31 removals across the two years of the experiment, but some were discarded from the analysis: four females repaired with an older (bright plumaged) neighboring male, three females disappeared after the removal, six females either failed to produce a clutch prior to the end of our field season or the clutch was depredated prior to sampling, and in one case we had no genetic sample from the replacement male. Of the 17 successful removals from which we obtained genetic samples from the offspring, 9 were experimental removals and 8 were control removals. Our removals were successful at manipulating pair relatedness, as experimental pairs were more closely related ($R = 0.44 \pm 0.05$) than were control pairs ($R = -0.05 \pm 0.05$; $t_{15} = 6.52$; $P < 0.0001$). After the removal, both related and unrelated replacement males displayed typical breeding male behaviors, including duetting with the female, defending the territory, and mate guarding the female (i.e., aggressively chasing away intruding males); non-breeding auxiliaries do not typically display these behaviors. We found that experimental females paired to a closely related male produced a larger proportion of extra-pair young than did control females paired to an unrelated male ($F_{1,15} = 5.37$; $P = 0.035$). In fact, of the 27 offspring produced by experimental pairs, only one was an incestuous within-pair offspring, whereas 8 of the 24 offspring produced by control pairs were within-pair (Fig. 6). Furthermore, none of the offspring were sired by the removed male through sperm storage, suggesting that all of the extra-pair young represent new extra-pair copulations occurring after the removal of the original male.

We also examined our long-term data set to see if there were any “natural experiments” where a female was naturally widowed and ended up paired with her son. In the eight years of our long term monitoring of this population (1998-2007), there have only been two cases where a son paired with his mother under natural conditions. In one case (1999) the pair produced two broods, both of which were entirely extra-pair young. In second case (2007), no successful broods were produced. These data fit with the patterns seen in our experiment.

## DISCUSSION

In our correlational analysis we found that females who produced extra-pair young are more related to their social mate than were females who produced only within-pair young, and that extra-pair offspring were more heterozygous than their within-pair half siblings. This suggests that females avoid breeding with more closely related social males by engaging in extra-pair copulations and that these extra-pair copulations result in potential benefits for her offspring. However, it has not yet been demonstrated that increased heterozygosity leads to increased fitness in this species and further study would be needed to confirm that extra-pair paternity leads to increased offspring fitness. Breeding between related individuals has been shown to be costly in a wide variety of species (Amos et al 2001), and thus the increase in heterozygosity of extra-pair young is likely to reflect an increase in offspring fitness. This study joins a number of others providing correlational support of the use of extra-pair mating as an inbreeding avoidance strategy, including studies that show differences in cuckoldry rates based on relatedness (Blomqvist et al. 2002; Eimes et al. 2005; Tarvin et al. 2005; Freeman-Gallant et al. 2006; Jouventin et al. 2007; Blackmore & Heinsohn 2008; Cohas et al. 2008), differences in the relatedness of within-pair and extra-pair mates (Tarvin et al. 2005; Lindstedt et al. 2007; Fossøy et al. 2008), and differences in offspring heterozygosity (Foerster et al. 2003; Tarvin et al. 2005; Rubenstein 2007; Stapleton et al. 2007 ; Fossøy et al. 2008).

However, this is the first study to experimentally test the inbreeding avoidance hypothesis, and our experimental results support the findings of the correlational analyses;

females experimentally paired to very closely related males had higher rates of extra-pair paternity than did those experimentally paired to unrelated males. These types of experimental tests are important to understanding the evolution of behaviors in natural systems because they can control for confounding factors that may confuse results in correlative studies (Griffith et al. 2002). In this experiment all replacement males were one year old, had dull brown plumage, were former auxiliaries, and had been socially paired to the breeding female in that season. Thus, we controlled for a number of potentially confounding factors, and the only known difference between experimental and control males was their relatedness to the female. However, there are a few caveats to consider. First, it is possible that the mother-son pairs may not have been actual breeding pairs, but rather an unpaired female and her auxiliary. However, our observations of pair behavior suggest otherwise, as the mother-son pairs, as well as control pairs, showed all of the behaviors associated with social mates in this species, including duetting and aggressive mate guarding behavior by the son. Second, even if they were a breeding pair, mother-son pairs may have shown some subtle breeding inhibition (e.g., if young males are reluctant to copulate with their mother or have reduced sperm counts). However, one incestuous offspring was produced from a pairing between a mother and a son, so at least some copulations must have occurred. Moreover, even if some sort of breeding inhibition does occur in these experimental pairs, this represents another cost of inbreeding – females paired with close relatives would suffer reduced fertility that can be avoided through extra-pair matings. It is important to note that no offspring in either our experimental or our control treatments were sired by the removed male, suggesting that removing the prior nest in order to force the females to re-nest provided enough time for any stored sperm to be removed from the system. Thus, all extra-pair young sired are likely to represent new (post-removal) extra-pair copulations by the female.

It is unknown how birds are able to assess their relatedness to other individuals. For our experiment, it is possible that social association between the breeding female and her philopatric son allows her to identify him as a relative (e.g. Komdeur et al. 2004). However, it is unlikely that this mechanism can explain our correlational results because natural mother son pairings have only occurred twice in the eight years of our long-term study. Since pairings where the female would have social cues to detect relatedness are rare, it seems that females must have some other mechanism(s) by which to assess relatedness to their social mate and then adjust their extra-pair mating behavior accordingly. In mammals it is thought that individuals can determine relatedness through smell (Penn 2002) specifically by detecting differences in MHC genes (e.g. Penn & Potts 1998).Until recently there is some doubt as to whether this could occur in birds (Mays & Hill 2004) since they are commonly thought to have a less acute sense of smell than mammals (Jones & Roper 1997). More recent studies suggest that sense of smell may be more developed in birds than previously thought (Balthazart 2001) and some birds have been shown to choose extra-pair mates with dissimilar MHC genes (Freeman-Gallant et al 2003; Richardson et al 2005), so this remains a possible mechanism through which birds can assess their relatedness to potential mates. Alternatively, females may “recognize” sperm from closely related males via some post-copulatory mechanism that results in fewer embryos being sired by closely related males (Tregenza & Wedell 2002). In either case, extra-pair paternity can serve as a mechanism whereby females avoid the costs of inbreeding with closely related social mates.

Although female fairy-wrens appear to be avoiding close inbreeding via extra-pair paternity it does not appear that females choose maximally dissimilar males for extra-pair mates (e.g. Amos et al. 2001) as extra-pair mates were not less related to the female than the average male in the population. This suggests that females are avoiding close inbreeding rather than maximizing offspring heterozygosity. It is also possible that females are only able to assess the relatedness of their social mate and not in potential extra-pair mates and thus choose extra-pair mates at random from the population (Tarvin et al. 2005) or based on some other factor, such as plumage color (Webster et al. 2008). Alternatively, females may be limited in their ability to seek out maximally dissimilar males and instead choose nearby extra-pair males who may not be especially dissimilar to them (Freeman-Gallant et al. 2006).

Studies of the effects of relatedness on extra-pair mating have produced somewhat mixed results; support for the genetic compatibility hypothesis has been found in 73% of the species studied, either in terms of patterns of mate relatedness or offspring heterozygosity (Table 3). One possible reason for these mixed results is that some studies may be unable to detect an effect either because of limited sample size or limited power in the measures of genetic similarity or heterozygosity (Smith et al 2005). In particular negative results from studies using relatively few genetic markers (e.g. Charmantier et al. 2004; Schmoll et al. 2005; Smith et al. 2005; Foerster et al. 2006; Edly-Wright et al. 2007) should be interpreted very carefully.

Another explanation of these mixed results is that there are some systems where extra-pair mating may be less likely to be an important inbreeding avoidance mechanism. In particular, species with high natal dispersal, such as long distance migrants, are likely to be at very low risk of inbreeding (e.g. Kleven & Lifjeld 2005; Fossøy et al. 2008). In fact, natal dispersal has been suggested to have evolved as a means avoid inbreeding (Greenwood & Harvey 1982). Because individuals are likely to unrelated to all other breeders in the local population, choosing an extra-pair mate would result in a only a small improvement in offspring heterozygosity. Unless individuals have very accurate mechanisms to determine relatedness and the ability to compare many extra-pair mates, the selection for this behavior would be much weaker than in a population where close inbreeding was more likely. Thus inbreeding avoidance through extra-pair mating is most likely to occur in populations where the risks of inbreeding are high. In these species it may be the case that inbreeding avoidance through mate choice is less costly than inbreeding avoidance through dispersal. Populations with very low natal dispersal distances, such as seen in many cooperatively breeding animals and/or island populations, should be at relatively high risk of inbreeding. In this regard, it is noteworthy that many of the species showing evidence for inbreeding avoidance through extra-pair paternity are populations with relatively low dispersal distances, whereas those showing a lack of support for the hypothesis are species with high natal dispersal (Table 3), although exceptions exist (e.g., Richardson et al. 2004). This pattern could be further elucidated through a study of populations of the same species at different risks of inbreeding, for example comparing island and mainland populations. One study included data from island and mainland populations of blue tits (Charmantier et al. 2004), and while they did not find any effect of relatedness of females and their mates on extra-pair paternity either on islands or on the mainland, they only used a very few genetic markers (4-7 microsatellites) and so their lack of significant effects may be suspect. Further studies of this nature with more robust genetic tools could help explain the difference in extra-pair paternity rates in different populations.

The very low rates of dispersal observed in the red-backed fairy-wren leads to greater risk of inbreeding between relatives. Because of this, there is likely to be strong selection favoring inbreeding avoidance mechanisms if costs of inbreeding exist. Although individuals can potentially avoid inbreeding through social mate choice, when this fails, as may happen often when social mates are limited, then extra-pair mating is an alternative method of inbreeding avoidance (Freeman-Gallant 2006). Social pairing occurs very rapidly in this species, as was evidenced by our removal experiments and this suggests that female have little opportunity to assess potential social mates, possibly resulting in a higher incidence of pairing between related individuals. Interestingly, in other species of fairy-wrens, incestuous social pairings are more common than in the red-backed fairy-wren (Brooker et al 1990; Cockburn et al 2003) and these species have been shown to have some of the highest extra-pair paternity rates of any animal (Brooker et al 1990; Mulder et al 1994). In addition, Tarvin et al. (2005) found a relationship between pair relatedness and extra-pair paternity in the splendid fairy-wren (*Malurus splendens*) similar to the pattern reported here. The extremely high rates of extra-pair paternity observed in this genus may be, in part, caused by the need to avoid close inbreeding (Brooker 1990). This study provides compelling correlative evidence for the use of extra-pair mating as an inbreeding avoidance mechanism, as well as the first experimental test of this hypothesis in a free living population, and provides further evidence that it may be genetic compatibility rather than "good genes" that drives the evolution of extra-pair mating in monogamous species.

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**Table 1:** Microsatellites used in various analyses

| Locus | # Alleles | $H_O$ | P(excl) | Freq. Null |
|---|---|---|---|---|
| Mcy1*†[1] | 6 | 0.73 | 0.51 | 0.01 |
| Mcy2*†[1] | 6 | 0.36 | 0.20 | 0.00 |
| Mcy3*†[1] | 19 | 0.69 | 0.81 | 0.14 |
| Mcy4*†[1] | 11 | 0.84 | 0.71 | 0.01 |
| Mcy7*†[1] | 13 | 0.70 | 0.56 | 0.03 |
| Msp4*†[2] | 21 | 0.83 | 0.77 | 0.03 |
| Msp6*†[2] | 11 | 0.74 | 0.56 | 0.02 |
| Ase9*†[3] | 10 | 0.80 | 0.63 | 0.00 |
| Cuu28*†[4] | 20 | 0.43 | 0.46 | 0.20 |
| Phtr3*[5] | 6 | 0.23 | 0.44 | 0.42 |
| Smm1†[6] | 9 | 0.41 | 0.22 | 0.02 |
| Smm7†[6] | 20 | 0.80 | 0.78 | 0.05 |

All microsatellites were used for measures of relatedness. * Used for paternity analysis. † Used for measure of heterozygosity. [1] Developed for the superb fairy-wren (*Malurus cyaneus*) (Double et al. 1997). [2] Developed for the splendid fairy-wren (*Malurus splendens*) (Webster et al. 2004). [3] Developed for the Seychelles warbler (*Acrocephalus sechellensis*) (Richardson et al. 2000). [4] Developed for the Swainson's thrush (*Catharus ustulatus*) (Gibbs et al. 1999). [5] Developed for the willow warbler (*Phylloscopus trochilus*) (Fridolfsson et al. 1997). [6] Developed from the southern emu-wren (*Stipiturus malachurus*) (Maguire et al. 2006).

**Table 2.** Extra-pair paternity rates over various years of the study.

| Year | # Young | # Nests | # EPY (%) | # Nests w/ EPY (%) |
|---|---|---|---|---|
| 2004 | 62 | 22 | 24 (39%) | 10 (45%) |
| 2005 | 116 | 38 | 72 (62%) | 29 (76%) |
| 2006 | 53 | 19 | 36 (68%) | 16 (84%) |
| 2007 | 91 | 31 | 43 (47%) | 20 (65%) |
| Total | 322 | 110 | 175 (54%) | 75 (68%) |

**Table 3:** Summary of studies regarding inbreeding avoidance and dissimilarity

| Species | Diff. in mate relat. | Diff. in offspring het. | Natal Dispersal | Genetics | References |
|---|---|---|---|---|---|
| Kentish Plover (*Charadrius alexandrinus*) | yes | N/A | moderate dispersal | minisat. | Blomqvist et al. 2002, Foppen et al. 2006. |
| Common Sandpiper (*Actitis hypoleuca*) | yes | N/A | moderate dispersal | minisat | Blomqvist et al. 2002, Dougall et al. 2005. |
| Western Sandpiper (*Calidris mauri*) | yes | N/A | low dispersal | minisat | Holmes, R.T. 1971, Blomqvist et al. 2002 |
| Splendid Fairy-Wren (*Malurus splendens*) | yes | yes | very low dispersal | 6 microsat. | Russell & Rowley 1993; Tarvin et al. 2005 |
| Grey-crowned Babbler (*Pomatostomus temporalis*) | yes | N/A | very low dispersal | 7-8 microsat. | Edwards 1993; Blackmore & Heinsohn 2008 |
| Mexican Jay (*Aphelocoma ultramarina*) | yes | N/A | very low dispersal | minisat. | Eimes et al. 2004; McCormack & Brown 2008 |
| House Wren (*Troglodytes aedon*) | yes? | N/A | high dispersal | 5-7 microsat. | Drilling & Thompson 1988; Masters et al. 2003. |
| Bluethroat (*Luscinia svecica*) | yes | yes | high dispersal? | 11 microsat. | Fossøy et al. 2008. |
| House Finch (*Carpodacus mexicanus*) | yes | N/A | high dispersal | 16 microsat. | Hill 1993; Lindstedt et al. 2007. |
| Savannah Sparrows (*Passerculus sandwichensis*) | yes | N/A | very low dispersal | 5 microsat. | Wheelwright & Mauck 1998; Freeman-Gallant et al. 2006. |

| Species | Diff. in mate relat. | Diff. in offspring het. | Natal Dispersal | Genetics | References |
|---|---|---|---|---|---|
| Common Mole-Rat (*Cryptomys hottentotus*) | yes | yes | low dispersal | 7 microsat. | Bishop et al. 2007. |
| Wandering Albatross (*Diomedea exulans*) | yes | no | low dispersal | 10 microsat. | Bried et al. 2007; Jouventin et al. 2007. |
| Blue Tit (*Parus caeruleus*) | no | yes | low dispersal | 5-7 microsat.; minisat. | Kempenaers et al. 1996; Foerster et al. 2003; Charmantier et al. 2004; Foerster et al. 2006 |
| Tree Swallow (*Tachycineta bicolor*) | no | yes | moderate dispersal | minisat.<br>11 microsat. | Barber et al. 2005; Stapleton et al. 2007. |
| Superb Starling (*Lamprotornis superbus*) | no | yes | very low dispersal | 17 microsat. | Rubenstein 2007. |
| Alpine Marmot (*Marmota marmota*) | yes | no | low dispersal | 5-12 microsat. | Grimm et al. 2003; Cohas et al. 2006; Cohas et al. 2007; Cohas et al. 2008. |
| Coal Tit (*Parus ater*) | no | N/A | very low dispersal | minisat | Dietrich et al. 2003; Schmoll et al. 2005 |
| Seychelles Warbler (*Acrocephalus sechellensis*) | no | no | low dispersal | 14 microsat. | Richardson et al. 2004. |
| Great Reed Warbler (*Acrocephalus arundinaceus*) | no | N/A | low dispersal | 21 microsat. | Hannson et al. 2002; Hansson et al. 2004. |
| House Sparrow (*Passer domesticus*) | no | N/A | low dispersal | 10 microsat.<br>minisat. | Fleischer et al. 1984; Stewart et al. 2006; Edly-Wright et al. 2007. |

| Species | Diff. in mate relat. | Diff. in offspring het. | Natal Dispersal | Genetics | References |
|---|---|---|---|---|---|
| Black-throated Blue Warbler (*Dendroica caerulescens*) | no | no | high dispersal | 5 microsat. | Holmes et al. 2005; Smith et al. 2005. |
| Reed Bunting (*Emberiza schoeniclus*) | no | no | high dispersal? | 6-9 microsat. | Kleven & Lifjeld 2005; Bouwman et al. 2006; Bouwman et al. 2007. |

Review of studies of the inbreeding avoidance and dissimilarity hypotheses for extra-pair mating. Measures of dispersal were usually obtained from different studies and may be from different populations. The two species labeled with “higher dispersal?” are species for which no information on dispersal could be found, but they are both continental populations of migratory species and are thus likely to have high rates of dispersal.

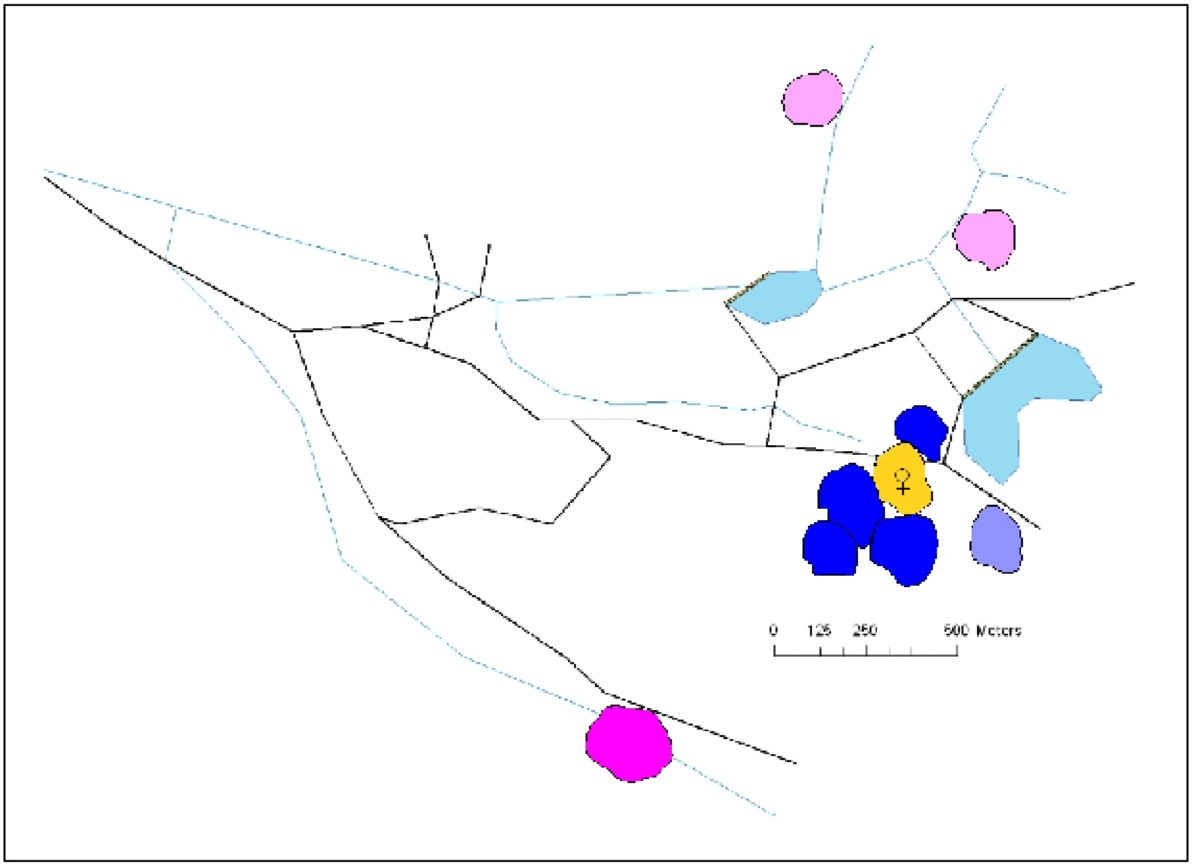

**Fig. 1:** Map of study site showing dispersal of recruiting offspring from their natal territory. The yellow shape represents the territory of a banded female (QYY) present on the site between 2003 and 2008. The blue and pink shapes are the territories of her offspring and grand-offspring. Darker blue shapes are the territories of her sons, lighter blue shapes are the territories of her grandsons. Similarly, the darker pink shape is the territory of her daughter, whereas the lighter pink shapes are the territories of her granddaughters. Territory sizes are approximate. This demonstrates the extremely limited natal dispersal in this species.

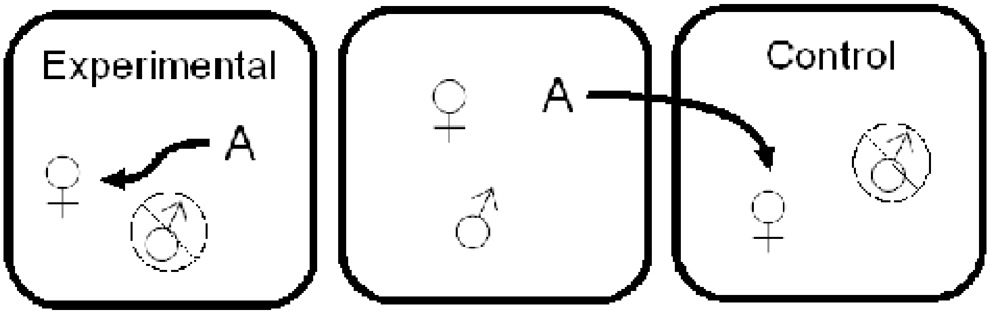

**Fig. 2:** Schematic of the experimental procedure. The boxes represent territories of free living fairy-wrens. Male and female signs are the dominant pair in each group. “A” represents auxiliary males on a territory. When the dominant male on a territory is removed, auxiliary males from the same (experimental) or neighboring (control) territories form a social bond with the dominant female.

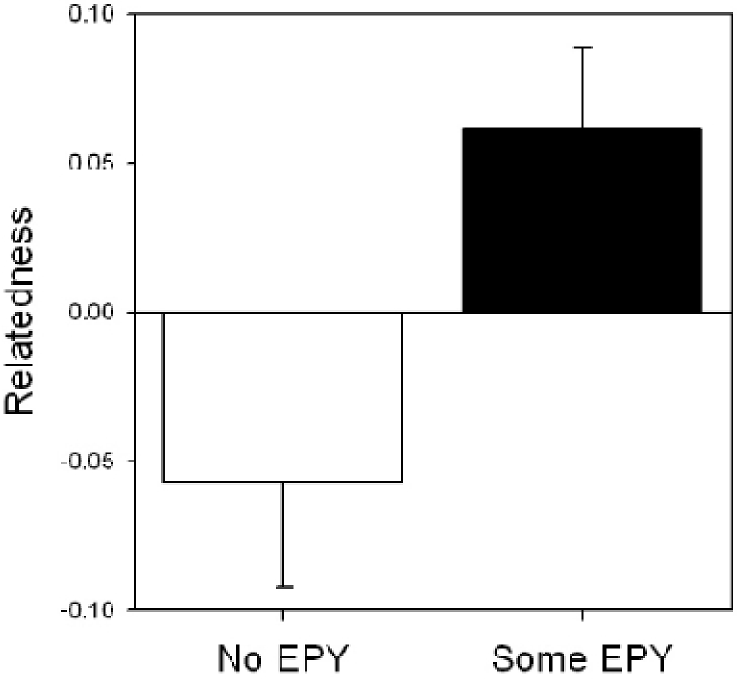

**Fig. 3:** Relatedness (R from Queller and Goodnight) of social males to females who produce no extra-pair young versus females who produce at least one extra-pair young. Values are least squared means and bars are standard errors from the GLMM controlling for multiple measures for females. Females who produce extra-pair young are significantly more related to their social mate than those who do not ($P < 0.05$)

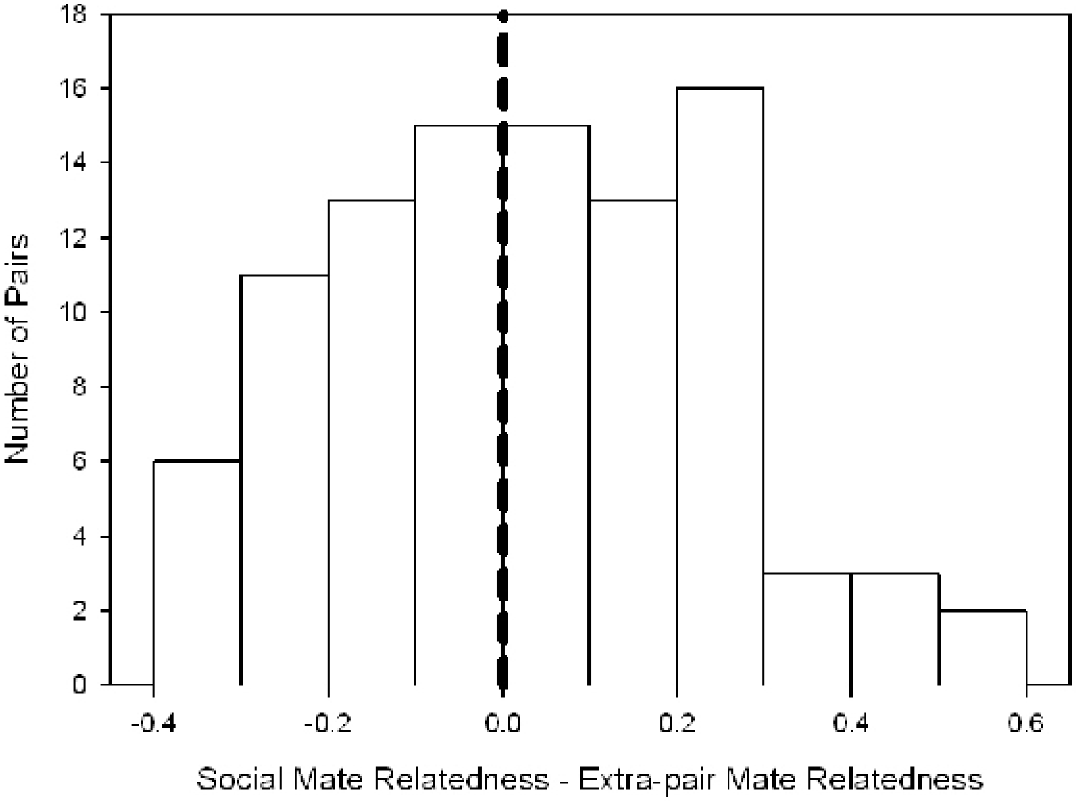

**Fig. 4:** Distribution of differences in relatedness (R from Queller and Goodnight) of females to the social mate they cuckold and the extra-pair mate they choose. Bars to the left of the dashed line represent extra-pair mates who are more related to the female than was the social mate. Bars to the right of the dashed line represent the opposite.

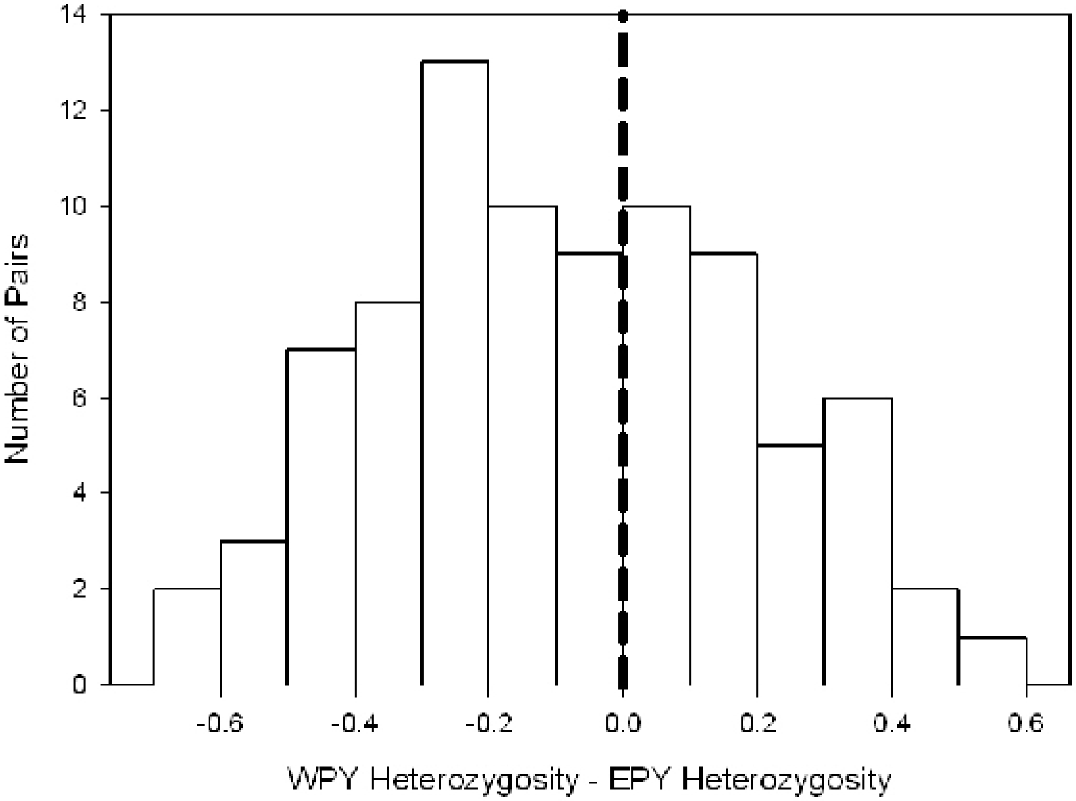

**Fig 5.** Distribution of differences in heterozygosity (standardized heterozygosity) between within-pair and extra-pair half siblings. Bars to the left of the dashed line represent half sibling pairs where the extra-pair chick was more heterozygous than the within-pair chick. Bars to the right of the dashed line represent the opposite.

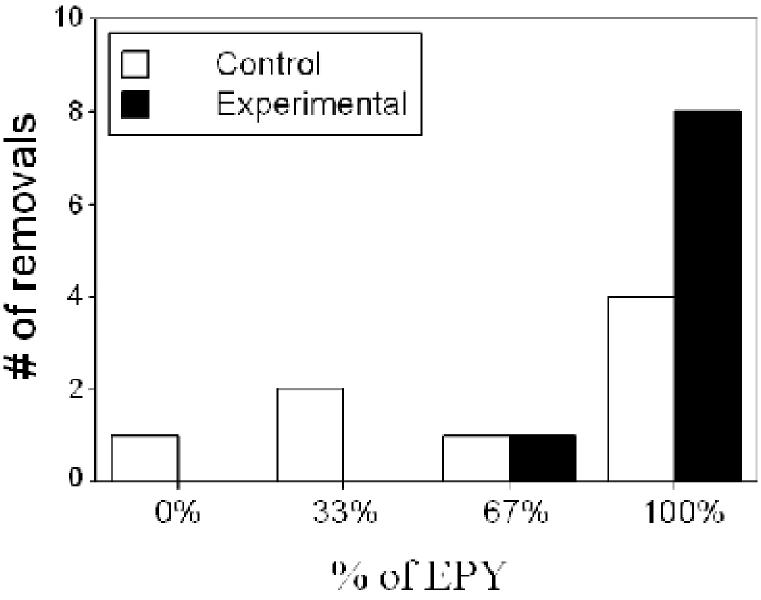

**Fig. 6:** Distribution of extra-pair paternity rates in groups from the experiment. Black bars are experimental groups and white bars are control groups. Females from experimental groups produce significantly more extra-pair offspring than females from control groups ($P < 0.05$).