

Offspring sex ratios reflect lack of repayment by auxiliary males in a cooperatively breeding passerine

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Abstract The repayment hypothesis posits that primary sex ratios in cooperative species should be biased towards the helping sex because these offspring “repay” a portion of their cost through helping behavior and therefore are less expensive to produce. However, many cooperatively breeding birds and mammals do not show the predicted bias in the primary sex ratio. Recent theoretical work has suggested that the repayment hypothesis should only hold when females gain a large fitness advantage from the presence of auxiliary adults in the group. When auxiliaries provide little or no fitness advantage, competition between relatives should lead to sex ratios biased towards the dispersing (non-helping) sex. We examined the benefits auxiliaries provide to females and corresponding offspring sex ratios in the red-backed fairy-wren (*Malurus melanocephalus*), a cooperatively breeding Australian bird with male auxiliary helpers. We found that auxiliaries provide little or no benefit to female reproductive success or survival. As predicted, the population primary sex ratio was biased towards daughters, the dispersing sex, and females with auxiliaries

produced female-biased broods whereas females without auxiliaries produced unbiased broods. Moreover, offspring sex ratios were more strongly biased toward females in years when auxiliaries were more common in the population. These results suggest that offspring sex ratios are associated with competition among the non-dispersing sex in this species, and also that females may use cues to assess local breeding opportunities for their offspring.

Keywords Cooperative breeding · *Malurus melanocephalus* · Local competition · Offspring sex ratios · Red-backed fairy-wren · Repayment hypothesis

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 and Stubblefield 2002; West et al. 2002), many of which have proven highly accurate in studies of invertebrates (reviewed in Godfrey and Werren 1996). In contrast, applying classical sex ratio theory to birds and mammals has proven more difficult, and actual sex ratios often vary 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 more so than haplodiploid determination seen in many insects (Williams 1979; West et al. 2005). Alternatively, it may be that behaviors affecting sex ratios in birds and mammals, such as helping behavior, are flexible and dependent on environmental conditions (Cockburn et al. 2002). This could be particularly relevant for social species in which related individuals interact over multiple generations because of potential cooperation or competition between relatives (Wild 2006).

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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; Clark 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 might 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 (Table 1).

Empirical tests of the repayment hypothesis have yielded mixed results (e.g., Koenig and Dickinson 1996; Berg 2004; Rathburn and Montgomerie 2004; Rubenstein 2007). Several theoretical treatments have attempted to explain these inconsistent results by incorporating additional factors, such as relationship between breeders and helpers and the amount of benefit an additional helper may provide, that might counter the effects of repayment (Lessells and Avery 1987; Koenig and Walters 1999; Pen and 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 a primary sex ratio bias 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 and Walters 1999; Pen and Weissing 2000), Wild (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., local resource competition, Clark 1978, or local mate competition, Hamilton 1967) 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; Table 1). 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 and Komdeur 2000) is also likely to lead to local competition for the non-dispersive (helping) sex (Dickinson 2004). Accordingly, local competition and offspring repayment effects 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, and sex ratios biased toward the dispersing sex are expected even if auxiliaries provide small fitness benefits to the parents (Wild 2006).

Because of this dichotomy 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, repayment occurs and we expect a sex ratio bias towards the helping sex, but if auxiliaries provide little or no benefit, then competition may be more important and a bias towards the dispersing sex is expected, particularly when natal dispersal is limited (Wild 2006; Table 1). 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 studies have examined both the contribution of auxiliaries to parental fitness and the degree of sex ratio biasing.

Here we examine the benefits of the presence of an auxiliary to maternal fitness using a large multi-year dataset from the red-backed fairy-wren (*Malurus melanocephalus*). Using this information, we then test for biases in offspring sex ratios predicted by the repayments and local competition hypotheses. Because young male red-backed fairy-wrens can either remain on the natal territory and assist their parents or settle to breed on a neighboring territory,

Table 1 Sex ratio hypotheses for cooperatively breeding vertebrates

	More important when...	Population primary sex ratio	Brood sex ratios
Repayment	Auxiliary helpers increase parental fitness	Primary bias toward helping sex.	Females without auxiliaries produce the helping sex
Local Competition	Natal dispersal is low/competition between relatives is high	Primary bias towards the dispersing sex.	Females with auxiliaries produce the dispersing sex.

Predictions from the repayment and local competition hypotheses

both repayment and local competition may occur in this system. Here we attempt to ascertain whether repayment or competition plays a larger role in determining offspring sex ratios in this system.

Methods

Study species and field methods

Red-backed fairy-wrens are a 6–7-g Australian passerine. They are socially monogamous and maintain social pairs throughout the year, remaining with the same mate for multiple breeding seasons. However, like other species in their genus, they have very high rates of extra-pair paternity (51%; Webster et al. 2008). Red-backed fairy-wrens breed cooperatively, with 34% of 1-year-old males remaining on the natal territory as auxiliary helpers (unpublished data). Male auxiliaries are nearly always the son of the breeding female; however, because of high extra-pair mating rates, they may not be related to the breeding male. Nearly all (96%) auxiliary males help only for a single year and become breeders by their second breeding season. Males who breed in their first year nearly always settle very close to where they were born (<500 m). In contrast, females disperse further (>500 m), making it likely that most settle off of our 2 km×3 km field site (unpublished data) and have never been observed to help at the nest. Because of this, males are more likely to compete with philopatric male relatives than females who are likely to compete with unrelated individuals after dispersal.

There is a plumage dimorphism among males where most (77%) breeding males display bright red and black plumage, whereas auxiliaries and 79% of 1-year-old breeding males display a dull brown female-like plumage (Karubian 2002; Webster et al. 2008). Females build domed nests low in the grassy understorey and lay two to four (usually three) 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 (unpublished data). 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.

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 (145°25'E, 17°22'S) during the breeding seasons (October–February) of 1998–2000 and 2003–2007 (breeding seasons are designated by the year in which they ended). In each of these seasons we captured most adults on

the study site; on average we monitored 49 groups per year and failed to capture at most three to four adults per year. We banded each captured adult with an Australian Bird and Bat Banding Scheme numbered aluminum band and a unique combination of plastic color bands to aid identification. We also collected blood samples (ca. 30–50 μ l) from the brachial vein for genetic analyses.

We observed banded individuals at least twice per week 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 fledging). In our population approximately 20% of groups have at least one auxiliary male and only around 2% of groups have two or more auxiliaries. 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 (unpublished data). On average, 27% of females without an auxiliary male in a 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. Females produced 1.8 ± 0.9 (mean \pm 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 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 1,004 offspring. Of the 390 nests, 264 were completely sampled (we obtained a genetic sample from every egg laid). On average we sampled 1.3 ± 0.5 nests per female per year and we measured the reproductive success of each female in an average of 1.6 ± 0.9 (mean \pm 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 store and 5 indicating that the furcular hollow was bulging with fat. We also collected blood samples (approximately 25 μ l) from the tarsal vein of nestlings and stored them in lysis buffer (White and Densmore 1992) at 4 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 control over the sex of offspring than males. It is likely that males are only able to control 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 manipulation of brood sex ratios by females. Accordingly, we focused only on the effects of auxiliaries on adult female fitness and not on adult male fitness.

We assessed several components of female reproductive success, including number of nests per year (in which eggs were laid), number of successful nests per year, clutch size (number of eggs laid), nest fledging success (probability of a nest fledging at least one offspring), number of chicks fledged per successful nest and per year, and measures of offspring condition. Offspring condition has been associated with survival in the fledgling period in other Passerine species (e.g., Monrós et al. 2002). To estimate offspring condition, we used weight controlled for offspring size (see “Statistical methods” section) and fat measurements (above). We only used measurements taken on 6-day-old chicks for these analyses to control for chick age.

We also estimated adult female survival probabilities to determine whether females with auxiliaries had better survival 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-ringed females, which are highly sedentary after natal dispersal and rarely change territories between years (unpublished 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 μ l PCR reactions containing 0.15 mM dNTPs (each), 0.50 μ M primers (each), 2.0 mM $MgCl_2$, 2.5 units *Taq* polymerase, and 1 μ l DNA suspended in sterile water (approximately 50 ng genomic DNA). These reactions were run on an Applied Biosystems GeneAmp PCR System 9700 with an initial 3 min denaturation at 94 C followed by 30 cycles of 94 C for 60 s, 57 C for 60 s, and 72 C for 45 s. 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 one (1.9%) was mis-sexed.

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 (number of eggs, number of fledglings per nest, number of nests per year, number of 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 weight) 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 (number of eggs, number of 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; unpublished 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 weight to control for chick size. We also examined first order interactions between presence of an auxiliary and all other fixed effects. Only significant interaction terms were retained in the final analyses.

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 (ψ , 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 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 ψ as dependent on presence of an auxiliary, on year, on a factorial combination of year \times auxiliary, and as constant. These assumptions gave us eight candidate models (Table 2). 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.

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. Because of this, 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 ($N=2$), we failed to capture one or more chicks due to late sampling ($N=7$), we were unsure whether all eggs laid were sampled due to finding the nest late ($N=25$), or the sex-linked fragments failed to amplify in one or more samples ($N=11$). We used Neuhauser (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 more powerful than a Wilcoxon signed rank test for population-wide sex ratio biases in birds (Neuhauser 2004). We also 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.

For our analyses of the effects of auxiliaries on individual broods, we again used SAS 9.1 and proc GLIMMIX to create a GLMM with a binomial distribution and a logit link where the response was the number of males in a brood and the binomial was the total brood size. 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 of all offspring produced by individual females when they had an auxiliary vs. the sex ratio of all offspring produced when they did not, using a two-tailed paired t test. The sex ratios are calculated from multiple broods within and between years. 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 or year in this analysis.

Results

Female reproductive success and survival

Between 1998 and 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 3). Nests initiated early in the

Table 2 AIC_c values and weights for candidate models of female survival with and without auxiliary males

Model	AIC_c	Weight	Survival (%)		No aux.	Up. CL	Low CL	
			Aux.	Up. CL				
$S(\cdot)p(ya)\psi(a)$	706.98	0.654	59.0	64.4	53.4	59.0	64.4	53.4
$S(a)p(ya)\psi(a)$	708.79	0.264	62.1	71.9	50.5	58.1	64.2	51.7
$S(\cdot)p(ya)\psi(ya)$	712.63	0.039	58.2	63.6	52.6	58.2	63.6	52.6
$S(a)p(ya)\psi(ya)$	714.41	0.016	61.3	72.1	49.3	57.2	63.4	50.9
$S(\cdot)p(ya)\psi(\cdot)$	714.47	0.016	60.1	65.4	54.6	60.1	65.4	54.6
$S(a)p(ya)\psi(\cdot)$	716.37	0.006	62.5	73.2	50.5	59.2	65.5	52.6
$S(\cdot)p(ya)\psi(y)$	716.96	0.004	60.1	65.3	54.6	60.1	65.3	54.6
$S(a)p(ya)\psi(y)$	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 (ψ), are modeled as being dependent on presence of an auxiliary (a), year (y), neither (\cdot), or both (ya). Survival estimates for females with and without auxiliaries from each model are given with upper and lower 95% confidence limits

Table 3 Effect of auxiliaries on measures of female reproductive output

Analysis	Fixed effect	<i>N</i>	Estimate	Auxiliary (mean±S.E.)	No auxiliary (mean±SE)	Test Stat.	<i>P</i>
No. of eggs per nest ^a	Presence of auxiliary	419	-0.046	2.94±0.20	3.08±0.10	$F_{1,1}=0.37$	0.652
	Female age		-0.039			$F_{1,1}=0.45$	0.623
	Nest initiation date		0.002			$F_{1,1}=4.53$	0.280
No. of fledglings per nest ^a	Presence of auxiliary	203	-0.002	2.55±0.23	2.55±0.13	$F_{1,1}<0.01$	0.989
	Female age		-0.150			$F_{1,1}=2.82$	0.342
	Nest initiation date		0.001			$F_{1,1}=1.29$	0.459
Nests fledging young ^a (%)	Presence of auxiliary	574	0.006	42.8±7.1%	42.9±5.6%	$F_{1,529}<0.01$	0.978
	Female age		0.046			$F_{1,374.5}=0.06$	0.802
	Nest initiation date		-0.012			$F_{1,570}=19.62$	<0.001
Chick weight ^b (g)	Presence of auxiliary	228	0.118	5.66±0.13	5.54±0.06	$F_{1,67.4}=0.76$	0.387
	Female age		-0.045			$F_{1,78.3}=0.17$	0.685
	Nest initiation date		0.003			$F_{1,61.6}=4.49$	0.038
	Tarsus		0.375			$F_{1,161}=166.28$	<0.001
Chick fat score ^b	Presence of auxiliary	218	0.036	2.01±0.27	1.95±0.11	$F_{1,1}=0.07$	0.837
	Female age		0.065			$F_{1,1}=0.35$	0.659
	Nest initiation date		0.103			$F_{1,1}=3.10$	0.329
No. of nests per year ^a	Presence of auxiliary	321	0.21	2.07±0.23	1.69±0.14	$F_{1,318}=4.33$	0.038
	Female age		-0.05			$F_{1,318}=0.37$	0.542
No. of successful nests per year ^a	Presence of auxiliary	332	0.160	0.81±0.13	0.69±0.08	$F_{1,329}=1.09$	0.297
	Female age		-0.001			$F_{1,329}<0.01$	0.996
No. of fledglings per year ^a	Presence of auxiliary	332	0.086	1.81±0.26	1.66±0.20	$F_{1,329}=0.62$	0.431
	Female age		-0.057			$F_{1,329}=0.34$	0.559

Results for measures of female fitness from GLMMs. Values for means are back transformed to the original scale using the inverse link function

^a Models included year and female identity as random effects

^b Models included year, female identity, and nest as random effects

season tended to be more likely to fledge than nests late in the season, but nests initiated later in the season contained more eggs (Table 3). 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 (Table 3).

In contrast to the effect of time in the breeding season, the presence of auxiliaries had little if any effect on measures of female reproductive success (Table 3). 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 weight controlled for tarsus length or amount of fat stores. Females with auxiliaries did have more nesting attempts per season, but they did not fledge more nests or produce more fledglings per year than did females without auxiliaries (Table 3). There were no significant interaction terms in any of these analyses.

From our analysis of survival of 226 females, on average, 59.0±2.8% (mean±SE) of females survived between

years. However, the presence of an auxiliary did not have an effect on female survival (Table 2) with 58.7% of females without auxiliaries surviving (95% CI=52.8–64.4%) and 59.9% of females with auxiliaries surviving (95% CI=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” section), we predicted that sex ratios 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 4). 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 ($F_{1,185}=0.20$, $P=0.65$), broods produced earlier in the season contained more males than broods produced later in the season ($F_{1,185}=7.30$, $P=0.01$). In

Table 4 Sex ratios across years

Year	# broods	Average brood sex ratio (\pm SE)	No. chicks	No. of female chicks (%)
1998	37	0.45 (\pm 0.05)	101	44.6 (45)
1999	43	0.33 (\pm 0.05)	128	32.0 (41)
2000	40	0.52 (\pm 0.05)	122	52.5 (64)
2003	9	0.59 (\pm 0.09)	23	57.5 (13)
2004	25	0.46 (\pm 0.05)	74	45.9 (34)
2005	35	0.48 (\pm 0.05)	110	48.2 (53)
Total	189	0.47 (\pm 0.02)	558	44.8 (250)

This data includes multiple broods from females within and between years

addition, females with auxiliaries produced significantly fewer sons than females without auxiliaries ($F_{1,185}=12.72$, $P<0.001$). 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). We also examined the brood sex ratios of individual females when they had and lacked an auxiliary and found a non-significant 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, $t=1.83$, $P=0.084$). Unfortunately, our sample size of females for which we had complete broods produced with and without helpers was small ($N=20$), and so these non-significant results should be interpreted with caution.

We also found considerable year-to-year variation in the primary sex ratio, ranging from 32% to 57% males. Despite having only 6 years of data, we found a significant negative relationship ($R^2=0.78$, $r=-0.88$, $F_{1,4}=13.82$, $P=0.021$) between the proportion of groups with auxiliaries and male bias in the primary sex ratio (Fig. 2a). Since this effect

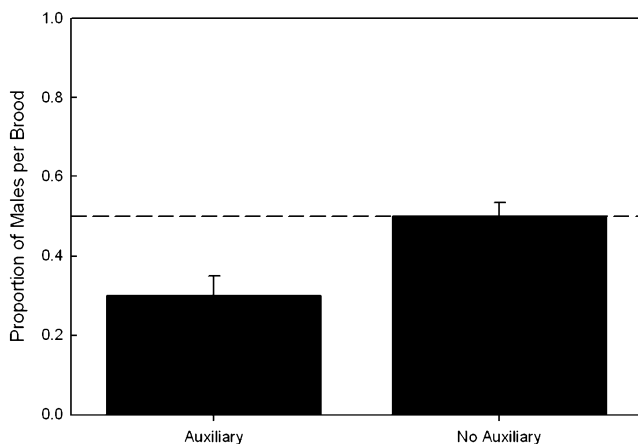


Fig. 1 Effect of presence of an auxiliary on brood sex ratios. Column values are least squared means from the GLMM. Error bars represent one standard error. The horizontal dashed line shows an equal 50:50 sex ratio

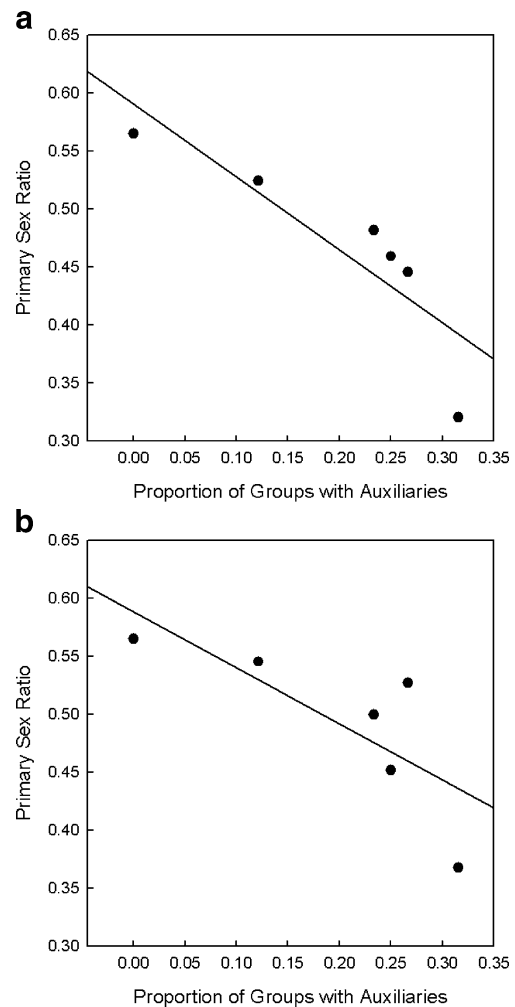


Fig. 2 Relationship between primary sex ratio and proportion of groups with auxiliaries between years for (a) all complete broods and (b) broods without auxiliaries

could be due to females with auxiliaries producing more daughters, we reran the regression using only offspring from females without auxiliaries. The second analysis was non-significant ($R^2=0.61$, $r=-0.78$, $F_{1,4}=6.23$, $P=0.067$) but still showed a negative association between proportion of groups in the population 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, and there was no evidence that nestling condition was improved by auxiliaries. Females with auxiliaries produced more nests in a season than females

without auxiliaries. A high number of nests could be a result of female productivity or could be a result of renesting due to depredation of nests. In either case, females with and without auxiliaries did not differ in the more relevant measures of reproductive success; number of successful nests produced or total number of young fledged per year. Auxiliaries do make many feeding visits to the nest (unpublished data), suggesting that they should enhance the reproductive success of the adults they are assisting, but any such enhancement of parental reproduction appears to be minimal. 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; unpublished 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.

This 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 suggests that such benefits to 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, indicating that breeding opportunities are indeed limiting in the red-backed fairy-wren (unpublished data). 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 cause a false correlation between presence of a helper and reproductive success. It is also possible that helpers provide a benefit only on the poorest of territories (e.g., Blackmore and 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 rejected from a group when they are unneeded, 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 (unpublished data), as would be expected if some males are not allowed to remain 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.

Offspring sex ratios

The lack of benefit provided by male auxiliaries in the red-backed fairy-wren, combined with the relatively limited male 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, which 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 very strong (Wild 2006), particularly 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. In

cooperatively breeding species, only biases toward the helping sex have been reported prior to this study (e.g., Ligon and Ligon 1990; Allaine et al. 2000). Indeed, 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.

Given the extreme natal philopatry displayed by males in this species, local competition seems a likely explanation for the primary sex ratio bias, we observed in this study. 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. 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 population-wide primary sex ratio of the fairy-wren is biased towards males (Cockburn and Double 2008). However, this difference in primary sex ratio 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 also found evidence suggesting possible facultative adjustment of brood sex ratios by females with auxiliaries, as females with auxiliaries were more likely to produce daughters than were females without auxiliaries. These results might arise if having multiple auxiliaries reduces direct fitness of breeders (e.g., Komdeur 1994). However, since nearly all auxiliaries in the red-backed fairy-wren help only for 1 year before breeding independently (unpublished 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 model (1973; see Rubenstein 2007). Because extra-pair paternity is prevalent in the red-backed fairy-wren (Webster et al. 2008), yearly male reproductive success is likely to be much more variable than that of females (Webster et al. 2007) as some males may sire many offspring with numerous females while others, including both breeding males and auxiliary males, may sire none at all. In contrast, females are limited by the number of clutches they can produce in 1 year. Consequently, we might expect females with auxiliaries to be more likely to produce sons, yet we found the opposite pattern. If female mortality during the first winter were dramatically higher than male mortality in the same period, this could increase the variance in lifetime reproductive

success in females. Because female dispersal makes estimating female survival difficult in the first year, it is impossible to unequivocally determine which sex has higher variance in lifetime reproductive success, but it seems unlikely that female mortality in the first year is sufficiently high to offset the variability in male reproductive success caused by extra-pair mating behavior.

Thus, the possible facultative adjustment of sex ratio in the red-backed fairy-wren may be best explained by females with auxiliaries reducing the number of males produced as a means to avoid direct competition for breeding opportunities between sons (i.e., the local competition hypothesis). 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 and Dickinson 1996; Komdeur et al. 1997; Legge et al. 2001; Doutrelant et 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 et al. 1997; Legge et al. 2001).

It appears that competition for breeding opportunities may be high in this population. In an unpublished removal experiment, we found that when the dominant male is removed from a group, the available breeding position is filled within hours, usually by an auxiliary male from a nearby group. It is possible that competition may also explain the sex ratio bias seen across the season. Earlier broods were more male biased than later broods. It is possible that males fledged earlier are more mature at the start of the following breeding season and are thus better able to compete for mates. Thus an early born male has a better chance of reproducing in his first year than a male born later in the season.

The importance of local competition is also supported by the correlation between the annual population sex ratio and the 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 (albeit non-significant) relationship when only the broods of females without auxiliaries were considered. Although it is possible that there may be some other factor that simultaneously increases the probability of son remaining on the natal territory and causes females to produce female-biased broods, this correlation suggests that females may 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 and 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 is likely to play a larger role in determining offspring sex ratios.

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 in which the benefits of auxiliaries are low 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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