Extra-pair young in house wren broods are more likely to be male than female

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Sex-allocation theory predicts that females should preferentially produce offspring of the sex with greater fitness potential. In socially monogamous animal species, extra-pair mating often increases the variance in fitness of sons relative to daughters. Thus, in situations where offspring sired by a female's extra-pair mate(s) will typically have greater fitness potential than offspring sired by the within-pair mate, sex-allocation theory predicts that females will bias the sex of offspring sired by extra-pair mates towards male. We examined the relationship between offspring sex and paternity over six breeding seasons in an Illinois population of the house wren (*Troglodytes aedon*), a cavity-nesting songbird. Out of the 2345 nestlings that had both sex and paternity assigned, 350 (15%) were sired by extra-pair males. The sex ratio of extra-pair offspring, 0.534, was significantly greater than the sex ratio of within-pair offspring, 0.492, representing an increase of 8.5 per cent in the proportion of sons produced. To our knowledge, this is the first confirmed report of female birds increasing their production of sons in association with extra-pair fertilization. Our results are consistent with the oft-mentioned hypothesis that females engage in extra-pair mating to increase offspring quality.

Keywords: extra-pair mating; house wren; paternity; sex allocation; sex ratio; *Troglodytes aedon*

1. INTRODUCTION

In many socially monogamous bird species, some fraction of individuals routinely mate outside the pair bond (Griffith *et al.* 2002). It is widely hypothesized that females engage in such extra-pair mating to enhance the fitness potential of their offspring, primarily through the acquisition of beneficial or compatible alleles (Brown 1997; Jennions & Petrie 2000; Zeh & Zeh 2003). This hypothesis has received some, although not unequivocal, support (Akçay & Roughgarden 2007; Kempenaers 2007).

Females may further enhance their fitness by manipulating the sex of their offspring (Pike & Petrie 2003; Alonso-Alvarez 2006). Specifically, sex-allocation theory predicts that females should preferentially produce offspring of the sex with the greater fitness potential (Trivers & Willard 1973; Charnov 1982). Under certain circumstances, extra-pair mating can increase the variance in the fitness of sons relative to daughters, resulting in differential fitness pay-offs from offspring depending on their sex (Webster *et al.* 1995; Møller & Ninni 1998). Thus, in situations where offspring sired by extra-pair mate(s) routinely have greater fitness potential than offspring sired by the within-pair mate, females should

bias the sex of offspring sired by extra-pair mates towards male (Westneat *et al.* 1995; Sheldon & Ellegren 1996).

To date, 22 separate studies have tested for a relationship between offspring sex and paternity in 15 different bird species (see the electronic supplementary material for summary of studies). Only one study, done by Kempenaers *et al.* (1997) on blue tits (*Cyanistes caeruleus*), found a greater male bias among offspring in the event of extra-pair fertilization (Kempenaers *et al.* 1997). However, a follow-up study on the same population that used molecular rather than less-reliable morphological methods to sex offspring failed to confirm these results (B. Kempenaers 2004, personal communication). One other study that used molecular sexing reported a strong, but not significant, tendency towards a greater male bias among extra-pair than within-pair offspring in red-capped robins (*Petroica goodenovii*; Dowling & Mulder 2006). A third study, on tree swallows (*Tachycineta bicolor*), found a significantly greater male bias among offspring in broods *without* rather than with extra-pair offspring, opposite of the prediction described above (Delmore *et al.* 2008). Sample sizes in several studies done to date have been small (e.g. fewer than 150 individuals or 30 broods), which has limited the ability to detect small but potentially meaningful biases in offspring sex ratios. Moreover, many studies have tested for biased sex ratios only at the level of the individual and not at the level of the brood. This is noteworthy because the female bird's ability to control, or at least identify, which of her eggs are fertilized by

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extra-pair partners may be limited. If so, then females engaging in extra-pair mating may simply bias the sex of offspring in the brood at random to increase the chances that at least some extra-pair offspring are male. This would lead to a male bias among offspring at the level of the brood (i.e. brood sex ratios), but not necessarily at the level of the individual (i.e. among extra-pair offspring).

We examined the relationship between offspring sex and paternity in a large sample of broods of the house wren (*Troglodytes aedon*), a small, cavity-nesting songbird. House wrens are an ideal model species for such a study. Extra-pair mating is common in this species, with 27–40% of broods reported to contain one or more extra-pair offspring, depending on the year and population (Soukup & Thompson 1997; Johnson *et al.* 2002; Poirier *et al.* 2004; Forsman *et al.* 2008). In addition, male house wrens that secure multiple, suitable nest sites on their territories sometimes pair socially with two or more females simultaneously (Johnson & Kermott 1991*a*), which further increases the variance in male fitness relative to that of females (Whittingham & Dunn 2005). Competition among males for limited nest sites can be intense, often involving prolonged physical battles (Johnson & Kermott 1990). This further raises the premium on the production of fit sons. Finally, results of several studies suggest that female house wrens have some control over the sex of their offspring (Albrecht 2000; Albrecht & Johnson 2002; Janota *et al.* 2002; Dubois *et al.* 2006; but see Johnson *et al.* 2005).

2. MATERIAL AND METHODS

(a) *Study species*

House wrens are small (10–12 g), insectivorous passerines (biology of species summarized in Johnson 1998). Males and females have identical, drab sets of feathers, coloured primarily with shades of brown. Sexes appear identical in size, although males average a few per cent larger than females in traits such as body, bill and wing length (Johnson 1998). Dimorphism occurs primarily in voice; only males possess a repertoire of complex songs (Platt & Ficken 1987; Johnson & Searcy 1993). House wrens nest naturally in preformed tree cavities but readily use human-made nest-boxes. Males arrive on breeding grounds in early May, defend territories around one or more nest sites and advertise with song for mates. Several studies suggest that females choose a social mate based largely on the quality or quantity of the nest site(s) that he offers, and less, if at all, on his own characteristics (Johnson & Searcy 1993; Eckerle & Thompson 2006). Females typically lay four to eight eggs, which they alone incubate. During incubation, males often invest some time trying to attract a second mate to surplus nest cavities on the outskirts of territories (Johnson & Kermott 1991*b*), and also try to obtain extra-pair copulations by intruding into neighbouring territories (Johnson & Kermott 1989). Fertile females do not appear to seek extra-pair matings off-territory. After the eggs hatch, males help females feed nestlings. Nestlings normally fledge 16–17 days after hatching begins, and one or both parents care for fledglings for another 10–14 days. Females typically raise two broods each season. Mate switching is common between broods, most often as a result of males abandoning first mates during the nestling stage to seek mates elsewhere (Drilling & Thompson 1988).

(b) *Study site and field methods*

Data were gathered during six breeding seasons (1991–1993 and 2004–2006) using a population of house wrens occupying a second-growth deciduous forest bordering the Mackinaw River in central Illinois, USA (40°40′ N, 88°53′ W). This site contained 585 wooden nest-boxes prior to 2004 and 700 boxes thereafter; nest-box density (5.4 boxes ha^{-1}), however, remained constant. Boxes were mounted on 1.5 m metal poles and protected from predators.

We visited boxes every 1–4 days to determine dates of first eggs in active nests, clutch sizes and dates that eggs began hatching (brood day 0). We captured females in boxes during the incubation stage, took a small blood sample to obtain DNA and affixed a numbered aluminium leg ring. We captured, bled and marked males with a unique combination of coloured and aluminium rings during the nestling stage. We took a sample of blood from the nestlings between brood days 7–13 in 1991–1993 and brood days 10–12 in 2004–2006.

(c) *Parentage and sex determination*

Paternity assignments for 1991–1993 offspring were made using minisatellite DNA fingerprint analysis as described in detail by Soukup & Thompson (1997). Assignments for 2004–2006 offspring were made using three to five microsatellite loci as described by Forsman *et al.* (2008). Overall, the probability that an offspring actually sired by an extra-pair male would be misassigned as a within-pair offspring was less than 0.008.

Sexing was done blind with respect to paternity. We sexed individuals by amplifying different-sized, sex-specific introns of the *CHD-1* gene using primers and protocols described either in Khan *et al.* (1998) or Fridolfsson & Ellegren (1999), with modifications described in Johnson *et al.* (2002). Control samples of DNA isolated from birds of known sex were included in all sets of PCRs, and products from control reactions were always electrophoresed alongside PCR products of unknown samples. Failure to determine the sex or paternity of some hatchlings resulted from hatchlings not surviving to bleeding age or failure of DNA to amplify.

(d) *Sample sizes and statistical analyses*

We estimated sex ratios using an optimally weighted estimator that considers broods as independent by adjusting statistics using the within-brood correlation of sexes (Neuhäuser 2004). The asymptotic test based on the weighted estimator was performed to determine whether the overall sex ratio deviated from 0.5. We used the *G* (likelihood ratio) test to determine whether the distribution of brood sex ratios differed from that expected under a binomial distribution.

We compared the sex ratios of within-pair and extra-pair offspring within broods using conditional logistic regression, where female identity was used to define strata (Molenberghs 2002; Norberg 2004; Dietrich-Bischoff *et al.* 2006). The sex of nestlings in broods with at least one extra-pair offspring was compared with that of nestlings in broods containing only within-pair offspring using logistic regression. Here, a conditional model was not applied because the explanatory variables were identical for all nestlings within a brood. For the same reason, a logistic regression was carried out to compare the sex of offspring produced by females that produced at least one brood with extra-pair offspring and females without any extra-pair offspring. In some nests, we did not determine the sex of embryos in all eggs laid because

of failure of eggs to hatch, death of nestlings before we could obtain a blood sample or poor quality of the DNA sample. We did not conduct separate analyses using only broods in which all offspring were sexed. Such a biased sample of broods has been shown not to represent primary sex ratio more accurately than incomplete broods and can, in some cases, be misleading (Krackow & Neuhäuser 2008).

We used ANOVA to test whether, for a given female, the brood sex ratio differed when she did and did not have extra-pair offspring in the brood. Here, nested effects were specified, *viz.* the binary variable, extra-pair offspring (i.e. yes or no for every brood), was nested within the variable female.

We calculated repeatabilities of sex ratios and proportion of extra-pair offspring in the brood for individual females following Lessels & Boag (1987). Repeatabilities were calculated for all broods of the same female and for broods of the same female within years. Significance of the repeatability was determined based on the corresponding confidence intervals given by Krebs (1999). All analyses were performed using SAS, v. 9.1 (2004).

3. RESULTS

Our study included 521 clutches produced by 270 different females. Out of 3291 eggs laid, 3123 (94.9%) hatched. We were able to sex 2517 (81%) out of the 3123 hatchlings. The sex ratio of all 2517 nestlings sexed was 0.503 (male/male + female), which did not differ significantly from 0.5 ($z = 0.32$, $p = 0.75$). The observed distribution of brood sex ratios did not differ significantly from the distribution expected under a binomial distribution ($G_{38} = 33.3$, $p = 0.68$).

We were able to assign paternity to 2345 of the sexed nestlings. Out of these, 350 (14.9%) were sired by extra-pair males. The sex ratio of extra-pair offspring, 0.534, was significantly greater than the sex ratio of within-pair offspring, 0.492 (conditional logistic regression: $\chi_1^2 = 4.22$, $p = 0.040$). When we included the variables year, clutch size and date of hatching in the analysis, nestling sex was not significantly related to these three factors (all $p > 0.05$), while the effect of paternity remained significant ($p = 0.037$). All interactions between paternity and the other main effects (year, clutch size and hatching date) were not significant (all $p > 0.05$).

We found one or more extra-pair offspring in 37 per cent of 476 broods examined. The sex ratio of offspring in broods containing one or more extra-pair offspring, 0.509 ($n = 174$), did not differ significantly from that in broods with only within-pair offspring, 0.496 ($n = 302$; logistic regression: $\chi_1^2 = 1.02$, $p = 0.31$). Owing to hatching failure and early nestling death, we did not determine the paternity of offspring from all eggs in 201 out of the 302 instances in which we found females to have only within-pair offspring. Some of the unsampled eggs or nestlings in these instances could have been sired by extra-pair males. If we exclude these cases, the mean brood sex ratio in the 101 broods for which we did assign paternity to all eggs laid drops to 0.483. However, this ratio also does not differ significantly from the sex ratio of offspring in the 174 broods known to contain one or more extra-pair offspring, 0.509 ($\chi_1^2 = 1.29$, $p = 0.26$).

One potential reason for the relatively small difference in the mean sex ratios of broods with and without extra-pair offspring is that, in addition to biasing the sex of

extra-pair offspring towards male, females with extra-pair offspring may bias the sex of within-pair offspring towards female, thus causing brood sex ratios to remain near parity. To test for this, we compared the mean sex ratios of extra-pair and within-pair offspring within 155 broods in which there was at least one sexed, extra-pair offspring and one sexed, within-pair offspring. The mean difference in sex ratios within broods (within-pair minus extra-pair) was -0.05 (s.d. = 0.52; median difference: 0), which was not significant (sign test: $p = 0.098$; Wilcoxon's signed-rank test: $p = 0.27$). The sex ratio of within-pair offspring in the 155 broods with one or more extra-pair offspring, 0.482, did not differ significantly from that in the 302 broods that contained only within-pair offspring, 0.496 (Welch's *t*-test: $t_{253}: = 0.52$, $p = 0.60$).

Sixty-two females produced at least one brood with extra-pair offspring and one brood without extra-pair offspring. Within females, brood sex ratio did not differ significantly when females did and did not produce extra-pair offspring (ANOVA: $F_{59,164} = 0.99$, $p = 0.50$).

The sex ratio of offspring produced by 123 females that contributed at least one brood to the sample and that had one or more extra-pair nestling in one or more broods, 0.502, did not differ significantly from that of the offspring produced by 141 females that produced only within-pair offspring in their broods (0.503, logistic regression: $\chi_1^2 = 0.03$, $p = 0.96$). This suggests that females producing extra-pair offspring are not predisposed to produce sons.

Repeatability of brood sex ratio was -0.055 when considering all broods produced by the same female, and -0.060 when considering broods of the same female within a given year. Repeatability of the proportion of extra-pair nestlings in the brood was 0.001 and -0.042 across and within years, respectively. None of these repeatabilities differed significantly from 0 ($p > 0.05$).

4. DISCUSSION

We found that house wren offspring sired by extra-pair mates were significantly more likely to be male than were offspring sired by within-pair mates. To our knowledge, this is the first study to find a significant male bias among offspring in the event of extra-pair fertilization, when sexing has been done using molecular methods.

Female house wrens increased the proportion of sons that they produced by 8.5 per cent when eggs were fertilized by extra-pair sperm. This increase may reflect selection on females to produce more sons than daughters among offspring sired by extra-pair mates and is, in fact, the outcome predicted by sex-allocation theory when offspring sired by extra-pair mates routinely have greater fitness potential than those sired by social mates (Westneat *et al.* 1995; Sheldon & Ellegren 1996). However, we must point out, that it is possible that females are not biasing offspring sex based on paternity, but on some other factor that correlates with paternity (Dietrich-Bischoff *et al.* 2006). Such factors could include maternal condition and territory quality, neither of which we assessed. A significant, positive association between maternal condition and proportion of sons in the brood was found in a Wisconsin population of house wrens (Whittingham *et al.* 2002). In Michigan, female house wrens paired to males with multiple nest sites on their territories produced

significantly more sons than did females with mates that controlled a single nest site (Dubois *et al.* 2006).

If we assume that females are indeed biasing the sex of offspring based on paternity, we can ask why the difference in the sex ratios of extra-pair and within-pair offspring was not even larger than that observed (0.534 versus 0.492). In part, this may result from limited ability of females to manipulate offspring sex, given the chromosomal nature of sex determination in birds (Krackow 1995; West & Sheldon 2002). Other explanations are, however, possible. Social, physiological or environmental factors other than paternity may simultaneously be favouring manipulation of offspring sex in directions not strictly parallel to those favoured by paternity, lessening the association between sex and paternity. Alternatively, the *net* selective pressure on females to bias the sex of extra-pair offspring towards male may be less than that we anticipate. For example, even if females engage in extra-pair mating to enhance offspring fitness potential, the benefits of producing high-quality sons may be countered to some degree by the benefits of producing high-quality daughters. This could hold, for example, if quality alleles enhance the survival of female offspring more than male offspring (Saino *et al.* 1999; Grindstaff *et al.* 2001). Enhanced survival could have a large effect on female fitness in a short-lived species such as the house wren in which less than half of adults survive to breed in more than one season (Johnson 1998).

One must also recognize that if the goal is to detect whether females bias offspring sex in relation to paternity to enhance the fitness potential of offspring, then the standard approach of comparing the sex ratio of *all* extra-pair and within-pair offspring or *all* broods with and without extra-pair offspring is less than ideal. This approach implicitly assumes that all females without extra-pair offspring will be prone either to make offspring female or at least not bias offspring sex in any direction. In reality, although, a subset of females without extra-pair offspring may be prone to produce *sons*. This would include females that have within-pair mates that are of high quality and hence capable of siring quality offspring. It may also include females that engaged in extra-pair mating but by chance had none of their eggs fertilized by extra-pair sperm, as well as females that were receptive to extra-pair mating, but failed to encounter suitable extra-pair partners. Inclusion of these females in the sample of females without extra-pair offspring would only narrow the difference in offspring sex ratios among females with and without extra-pair offspring, thus reducing the effect size.

We found a stronger association between offspring sex and paternity at the level of the individual than at the level of the brood, with only the former being statistically significant. The lack of significance may be caused, in part, by the necessarily smaller sample sizes for brood-level than individual-level analyses. Regardless, the question remains: why is the sex ratio of broods with extra-pair offspring not as biased towards male as the sex ratio of individual extra-pair offspring? One possibility is that females engaging in extra-pair mating manipulate the sex of within-pair as well as extra-pair offspring. Specifically, if females bias the sex of individual within-pair offspring towards female to some degree, this could lessen or even eliminate any male bias in the sex ratio of broods with extra-pair offspring.

In summary, our results suggest that females in at least some bird species may bias the sex of their offspring in relation to paternity, producing an excess of sons among offspring sired by extra-pair mates. Our results are consistent with the oft-cited but still controversial (Akçay & Roughgarden 2007; Kempenaers 2007) proposal that females engage in extra-pair mating to increase the fitness potential of offspring. Determining whether offspring sex is typically associated with paternity in birds will require many more studies, each with substantial sample sizes and additional information about male quality and attractiveness. Experimental studies, including studies to confirm the results presented here, should be particularly informative.

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