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Maternal correlates of brood sex ratio variation in the lekking lance-tailed manakin *Chiroxiphia lanceolata*

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Theory predicts that overall population sex ratios should be around parity. But when individual females can receive higher fitness from offspring of one sex, they may benefit by biasing their brood sex ratios accordingly. In lekking species, higher variance in male reproductive success relative to that of females predicts that male offspring gain disproportionately from favorable rearing conditions. Females should therefore produce male-biased broods when they are in a position to raise higher quality offspring: i.e., in better body condition or when they reproduce earlier in the breeding season. To investigate these hypotheses, we studied brood sex ratios of lance-tailed manakins *Chiroxiphia lanceolata*. We found that overall sex ratios and mean brood sex ratios were not different from random expectation. Brood sex ratios were not related to laying date or female body condition. However, we detected a quadratic relationship between brood sex ratios and maternal age: both young (1–2 years) and old (8+ years) females produced female-biased brood sex ratios. This relationship was most clear in a year also distinguished by early rainy and breeding seasons. We suggest that breeding inexperience in young females and senescence in older females is the most plausible explanation for these results, and that the relationship between female age and brood sex ratio is mediated by environmental conditions.

Adjustment of offspring sex is a classic example of individual adaptive adjustment to the environment. Fisher (1930) originally highlighted the potential for selection to affect sex ratios by demonstrating that balancing selection generally maintains a 50:50 sex ratio: the overproduction of one sex leads to higher reproductive success of the rarer sex. Parents that have a heritable tendency to produce the rarer sex would be under positive selection, until sex ratios equilibrate. However, under certain conditions, natural selection favors the overproduction of one sex even when the population is at an overall 50:50 sex ratio (Trivers and Willard 1973, Hardy 1997). For example, whenever sexes differ in their development and degree of benefit from parental investment, parents in good condition should produce more offspring of the sex that gains more fitness from better rearing conditions (the Trivers-Willard hypothesis, Trivers and Willard 1973). Conversely, parents in worse condition should produce more offspring of the sex that loses relatively less when in a bad condition. Though the original evidence supporting this hypothesis came from mammals (Trivers and Willard 1973), several studies have found sex ratio adjustment in relation to female condition in birds. Female zebra finches *Poephila guttata* (in captivity; Bradbury and Blakey 1998), lesser black-backed gulls *Larus fuscus* (Nager et al. 1999), tree swallows *Tachycineta bicolor* (Whittingham and Dunn 2000), and yellow-legged gulls *Larus cachinnans* (Alonso-Alvarez and Velando 2003) produced male-biased brood sex ratios when in relatively better condition.

The first-cohort advantage hypothesis (Wright et al. 1995) is a modification of the Trivers-Willard hypothesis predicting that offspring sex ratios in early broods should be biased towards the sex with the greatest increase in lifetime reproductive success relative to later-born individuals of the same sex (Dijkstra et al. 1990, Daan et al. 1996, Anderson et al. 2003). In birds, this could be the sex in which early-born individuals are more likely to breed in the next year. Early-born male European kestrels *Falco tinnunculus* were more likely than late-born male fledglings to breed in their second year after hatching (whereas laying date did not affect the probability that female fledglings bred in the following year). As predicted by the first-cohort advantage hypothesis, early broods were male-biased (Dijkstra et al. 1990). Similar results were found in common sandpipers *Actitis hypoleucos* (Anderson et al. 2003).

Though these studies were all conducted on monogamous bird species, the Trivers-Willard hypothesis (Trivers and Willard 1973) and the first-cohort advantage hypothesis (Wright et al. 1995) were originally developed for polygynous species. Lekking represents an extreme form of polygyny in which males display in certain areas and females visit these areas primarily for the purpose of mating (Höglund and Alatalo 1995). Because a few males seem to monopolize most matings, there should be much higher variance in reproductive success among males of lekking than of monogamous species; therefore selection for adaptive sex ratio changes in lekking species should also be more extreme. However, only a few studies have investigated sex ratio allocation in lekking bird species (Clout et al. 2002, Thuman et al. 2003, Pike and Petrie 2005).

In this study we examined brood sex ratios of the lance-tailed manakin *Chiroxiphia lanceolata*. This lek-breeding manakin is a small (15.5–22 g), mainly frugivorous passerine that occurs in lowland forests of Central and northern South America. Females may start breeding in their second year after hatching (EHD unpubl. data), whereas males do not have the opportunity to breed at least until they attain adult plumage in their fourth year, and may require many additional years to gain breeding status. Males form cooperative alliances for the performance of courtship displays, and with rare exceptions only the alpha male of each display partnership has a chance to mate. Alpha males are usually in their fifth year after hatching or considerably older (DuVal 2007a). Females build nests, lay clutches of one or two eggs and raise young without male assistance.

Because of the extreme variance in male reproductive success and the constancy in female reproductive success expected in a lek mating system (Pike and Petrie 2005), male offspring are thought to gain disproportionately from favorable rearing conditions. Females should therefore produce male-biased broods when they are in a position to raise higher quality offspring: i.e., in better body condition or when they reproduce earlier in the breeding season. We tested the predictions of two non-exclusive hypotheses. First we examined the first cohort advantage hypothesis (Wright et al. 1995), which predicts changes in brood sex ratio in relation to laying date. As life history characteristics of lance-tailed manakins could be interpreted as selecting for either male- or female- biased early broods (see Discussion), we test for any deviation from parity. Second, we examined the Trivers-Willard hypothesis (Trivers and Willard 1973) which, applied to lance-tailed manakins, predicts that sex ratios will become more male-biased with increasing female condition.

Studies on other lek mating species report a positive correlation of female body condition and brood sex ratio. When female captive peafowl *Pavo cristatus* and kakapo *Strigops habroptilus* were in relatively good condition, they produced male-biased broods, while females in poorer condition produced female-biased broods (Pike and Petrie 2005, Clout et al. 2002). Similarly, female ruffs *Philomachus pugnax* in poor condition had female-biased broods and females in good condition had unbiased broods (Thuman et al. 2003).

Another type of female condition that may affect the costs and benefits of raising offspring is maternal age or experience. In the arctic skua *Stercorarius parasiticus* and the Seychelles warbler *Acrocephalus sechellensis*, experienced parents are more successful in raising young (Davis 1976, Komdeur 1996). In many more species, female age has been linked to brood sex ratio (Blank and Nolan 1983, Sheldon et al. 1999, Saino et al. 2002, Griffith et al. 2003, Dowling and Mulder 2006). The strong correlation between age and breeding experience makes them difficult to address separately; increasing age and experience had the same (positive) effect on brood sex ratio in at least one case (Heg et al. 2000), but advanced age may also lead to decreased brood sex ratios due to female senescence (Husby et al. 2006). As age may affect the outcome of raising young, we also investigated the influence of maternal age on brood sex ratio.

The adaptive adjustment of brood sex ratios is a controversial phenomenon. Palmer (2000) suggested that selective reporting of only positive or expected results and the lack of repetition of published studies might bias the overall view of sex ratio adjustment. A meta-analysis of published studies found a weak trend supporting facultative adjustment of sex ratio only under some biological or temporal conditions (Ewen et al. 2004, as revised by Cassey et al. 2006). This revised analysis and previously presented studies suggest that adaptive sex ratio adjustment does occur (Hasselquist and Kempenaers 2002). We tested hypotheses regarding adaptive sex ratio adjustment in multiple years of a long-term field study. This approach offers one means of controlling for type I error thought to exist in studies of sex ratio adjustment.

Methods

Study area and field methods

The study was conducted from 2000 to 2006 on Isla Boca Brava, Chiriquí, Panama (8° 12′N, 82° 12′W), on a 46 ha area of mostly secondary growth dry tropical forest (see DuVal 2007b for detailed site description). Field seasons generally lasted from March to late June, but were shorter in 2003 and 2004. Lance-tailed manakins start breeding in March and continue through June and perhaps longer (DuVal 2007b).

Throughout each field season, we caught adult birds by setting mist nets near male display sites or near known nests to target unbanded nesting females. In total, we conducted 3,256 mist net hours (one 12 m net open for one hour). We marked captured birds with one aluminum leg band and a unique combination of three plastic colored leg bands, took 5–80 µl blood from the brachial wing vein for genetic analyses, and recorded morphological measurements before release. Measurements relevant to this study were weight and tarsus length. Females captured as adults were assigned a minimum age, scored as 2nd year in the first year of capture, and 2nd year + i for the following years, where i is the number of years elapsed since the first year of capture (DuVal 2005b). Adult sex ratios were estimated on mist net captures of post-fledging individuals.

Throughout every field season, we looked for nests by searching understory vegetation. We monitored nests every two days to determine dates of egg laying, hatching, and fledging, and to classify nests as successful (nestlings fledged) or failed (nestlings not fledged). Because of high nest predation rates, we took one small (ca. 5 µl) blood sample three days after hatching to allow genetic testing of sex and paternity, then banded chicks and took a second, larger sample on day 12. When eggs remained unhatched for more than 20 d (lance-tailed manakin eggs typically hatch 18 d after laying; EHD unpubl. data), we opened them and took tissue samples of visible embryos.

From 2000 until 2006, we determined the sex of 94.8% of 427 offspring from 94.8% of 269 nests for which maternal ID was known. These were nests of 143 individual females. Overall, 66.7% of nests were completely sampled (i.e. all eggs). In species with small brood sizes, such as the lance-tailed manakin, one-chick broods may frequently occur and have a significant influence on brood sex ratio calculations. In total, 32.5% of broods had only 1 chick, and 64.4% of these may have resulted from post-zygotic sex ratio adjustment (i.e. nests were previously observed with two eggs; Table 1). To control for the possibility that the full dataset reflects a mix of primary and secondary sex ratios, we repeated key analyses using only completely sampled nests.

Sex determination

We stored blood samples in 1 ml Longmire's Solution or Queen's Lysis Buffer, and embryo samples in ethanol. We extracted DNA from blood samples with the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences) according to the manufacturer's protocol (direct method for blood), except that we used 50–100 μl blood-Queens/Longmires solution instead of 5 μl whole blood. For embryo samples, we used the DNeasy Tissue Kit (Qiagen Inc.) according to the manufacturer's protocol for purification of total DNA from animal tissues.

We amplified extracted DNA with polymerase chain reaction (PCR) using the P2/P8 primer set binding at the two CHD genes (Griffiths et al. 1998). This approach has been previously demonstrated to accurately identify sex in this species (DuVal 2005b). Each PCR reaction contained 1 μl DNA solution (5–20 ng/μl), 5.75 μl H_2O, 0.8 μl $MgCl_2$, 1.0 μl NH_4 buffer, 0.2 μl dNTPs, 0.5 μl of each primer (primer P8 labeled with fluorescent dye), and 0.25 μl *Taq* polymerase per sample. DNA was amplified in a thermal cycler (GeneAmp PCR System 2700, Applied Biosystems) using the following program: initial denaturing step at 95° C for 3 min, followed by 28 cycles of 94° C for 30 s, 50° C for 30 s, and 72° C for 45 s, followed by 45 s at 72° C. After completion the products were kept at 4° C.

PCR products were visualized on an ABI 3100 sequencer (3100 Genetic Analyzer, Applied Biosystems, Inc.), and alleles were identified in the program GeneMapper 3.7 (Applied Biosystems 2004). Females were identified by two peaks at around 350 and 390 base-pairs (bp), while males had one peak at around 350 bp. One known adult male and one known adult female were included in each sequencer run as positive controls.

Statistical analyzes

Brood and offspring sex ratios were analyzed for the years 2000 until 2006, with data from 2004 excluded because we sampled only three complete nests during a short field season in this year. Unless indicated otherwise, we excluded multiple nests of the same females in a data set by using only the first observed nest for that female.

First, we tested whether the actual offspring sex ratio deviated from an expected ratio of 50:50 using G-tests with William's correction (Sokal and Rohlf 1995). Then, we tested whether chick sexes were randomly distributed among clutches (i.e. whether individual brood sex ratios differed from random) by applying a randomization test (GLIM program provided by C. Lessells), described in detail elsewhere (Korsten et al. 2006).

We investigated the relationship between brood sex ratios and variables of interest using generalized linear models (glm) with binomial error distribution, unless otherwise specified. For each analysis, we fitted the maximal model containing all relevant candidate variables, and then simplified the model by step-wise elimination of the least significant variable (Wilson and Hardy 2002; excluding terms with $P > 0.10$). The removal of a variable was only accepted when the new model was not significantly different from the non-simplified model, as assessed by likelihood ratio tests ($P > 0.10$). We present the variables considered in the maximal model, and the results only of the final model after simplification, with Wald χ^2 values and probabilities.

We normalized laying date within each year by subtracting the year's average laying date, and then dividing by the year's standard deviation. We examined the effect of laying date and chick sex on recruitment using all nests known or thought to have fledged, and defined the outcome of a nesting attempt as 'recruited' when at least one of the two chicks from the nest was seen in the study area in the next year. Recruitment analyses considered chicks from nests in 2000–2005, as data from chicks in 2006 were not yet available.

We analyzed sex ratios in relation to female body condition using capture data for all females caught in the same year they were observed nesting. We applied a glm of brood sex ratio on female body mass, female tarsus length, and year. Though we employed glms for statistical analyses (following Darlington and Smulders 2001), we graphically represent data on female body condition as the residuals of a linear model of body mass on tarsus length for ease of visualization. The residuals approach yielded the same results as the previous glms in all analyses.

Table 1. Number of lance-tailed manakin nests that had only one chick (32.5% of nests over all years).

Nest found with	No. of nests 2000	No. of nests 2001	No. of nests 2002	No. of nests 2003	No. of nests 2005	No. of nest 2006
Only one egg	1	1	2	1	4	3
Two eggs, one unhatched (infertile?)	2	0	0	0	3	4
Two eggs, but later only one chick	2	7	14	5	14	5
Only one chick	1	2	1	1	2	2
One egg lost due to poor nest construction	2	0	0	0	2	0
Proportion of one-chick broods	0.33	0.31	0.41	0.21	0.39	0.23

Table 2. Mean brood sex ratios and number of individual females observed nesting for lance-tailed manakins in the study area from 2000 to 2006. The first nest from each female per year is considered in yearly totals, whereas the first nest from each female across the entire study period is considered in the 'all years' total. Randomization test showed chick sexes were randomly distributed among broods (after Bonferroni correction). *based on randomization test with 10,000 iterations.

Year	No. of nests	No. of chicks	No. of males	Mean brood sex ratio	SE	P-value* randomization test
2000	23	39	19	0.46	0.09	0.03
2001	25	41	19	0.50	0.08	0.76
2002	35	55	29	0.53	0.07	0.39
2003	31	55	21	0.39	0.07	0.66
2005	56	87	35	0.37	0.05	0.69
2006	49	86	45	0.52	0.05	0.90
All years	143	243	113	0.45	0.03	0.31

Finally, we considered the effect of female age on brood sex ratio, testing for nonlinear effects by including age as a quadratic term. When comparisons included multiple years, we used generalized linear mixed models fitted via penalized quasi-likelihood (glmmPQL), with female ID as a random factor. Full models were simplified by sequentially excluding non-significant terms.

We performed analyses in R 2.3.1 (R Development Core Team 2006; packages: car, Design, effects, lattice, lme, MASS) using a significance level of $\alpha = 0.05$. Power analyses carried out for non-significant results were conducted using the program G*Power 3 (Faul et al. 2007). We report all values as means $\pm$ SE and sex ratios as proportion of males.

Results

Population sex ratio

We captured a total of 618 different adult individuals ($n = 1{,}179$ capture events) of which 295 were males ($n = 554$ capture events). Thus, the overall population sex ratio was 0.48 and not significantly different from 0.5 (G-test: $n = 710$, $G = 1.3$, $P > 0.05$). However, when each year was examined separately there was a significant female bias in adult birds captured in the year 2005 (sex ratio $= 0.34$, $n = 123$, $G = 12.5$, $P < 0.005$; for captures excluding target netting of females at nests: sex ratio $= 0.39$, $n = 90$, $G = 4.5$, $P < 0.05$). It is unlikely that this was due to the low adult capture intensity in 2004; the female bias persisted also in the sex ratio of adult recaptures (sex ratio $= 0.34$, $n = 50$, $G = 5.2$, $P < 0.03$; in other years, range of sex ratios $= 0.51$–0.56, $n = 43$–162 recaptures per year, $0.02 < G < 1.88$, all $P > 0.05$), suggesting differences in overall prevalence of females or in female movement patterns in 2005 compared to other years of the study.

The overall sex ratio of all sex-genotyped offspring was not biased (sex ratio $= 0.46$, $n = 395$, $G = 3.1$, $P > 0.05$). Separating the analysis by year showed that there was a strong female bias in the year 2005 (2005 sex ratio $= 0.38$, $n = 100$, $G = 5.8$, $P < 0.03$; no other year significantly different from 0.5).

Brood sex ratio

The mean brood sex ratio was 0.45 ± 0.03 ($n = 143$ nests, considering the first nests observed from each female). Brood sex ratios did not differ significantly between years (Table 2; glm: $n = 143$, $\chi^2_5 = 5.6$, $P = 0.35$). Chick sexes were randomly distributed among clutches for all years combined and for single years except for the year 2000, which had significantly more single-sex nests than expected by chance (randomization test, $n = 10{,}000$ iterations, Table 2). However, this is quite weak and applying Bonferroni corrections, this significance does not hold. Furthermore, individual females showed no consistency in sex ratios of repeated broods, both within years (glm: sex ratio versus female ID, $n = 32$, $\chi^2_{13} = 4.57$, $P = 0.98$, sex ratios of first and second broods were uncorrelated: $r_s = -0.04$, $P = 0.81$, $n = 16$ females), and across years (glm: sex ratio versus female ID, $n = 131$, $\chi^2_{52} = 13.99$, $P = 1.0$; sex ratios of first broods in first two years observed were uncorrelated: $r_s = 0.03$, $P = 0.81$, $n = 52$ females).

Does brood sex ratio vary with laying date?

Nest sex ratio was unrelated to normalized egg laying date (glm: $n = 143$, $\chi^2_1 = 0.01$, $P = 0.94$), indicating no changes in brood sex ratios in the course of the breeding season. The original model also included year, but this variable had no significant influence ($P = 0.37$) and was excluded during model simplification. Using a medium effect size ($w = 0.3$), this analysis has a power of 0.95 (Post-hoc analysis in G*Power 3, χ^2 test, $n = 143$ nests, $df = 1$). Restricting the analysis to completely sampled nests did not change the result (glm: $n = 103$, $\chi^2_1 = 0.1$, $P = 0.74$).

The probability that a chick recruited in the study area in the next year was also independent of laying date (glm; individual fledglings: $n = 206$, $\chi^2_1 = 2.1$, $P = 0.15$; nests: $n = 129$, $\chi^2_1 = 2.3$, $P = 0.13$).

Does brood sex ratio vary with female body condition?

Brood sex ratios were not related to female body condition (Fig. 1; glm: female body mass, $n = 114$, $\chi^2_1 = 1.6$, $P = 0.21$; tarsus length, $n = 114$, $\chi^2_1 = 0.1$, $P = 0.73$; year, $n = 114$, $\chi^2_5 = 8.6$, $P = 0.13$). All interactions (two- and three-way) were non-significant, and thus were excluded from the final model. Using a medium effect size of $w = 0.3$, this analysis has a power of 0.89 (Post-hoc analysis in G*Power 3, χ^2 test, $n = 114$ nests, $df = 1$). Similar to the results for laying date, considering only completely sampled nests did not change the results (glm: female body mass, $n = 76$, $\chi^2_1 = 0.3$, $P = 0.57$; tarsus length, $n = 76$, $\chi^2_1 = 0.9$, $P = 0.35$).

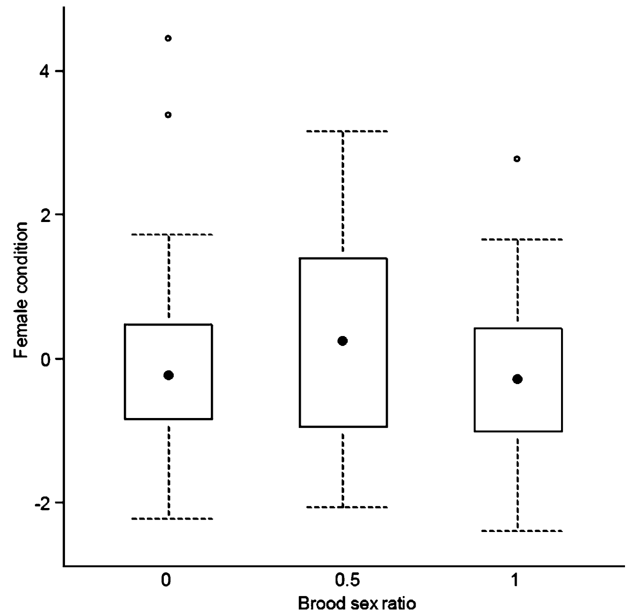

Fig. 1. Brood sex ratios in relation to female body condition. For practical purposes, body condition was calculated as the residual of the linear regression of female body mass on tarsus length. For formal statistical analyses, see text. Boxes enclose the 25–75% interval of the data, with vertical lines indicating data within 1.5 interquartile ranges of these limits.

Does brood sex ratio vary with female age?

We first examined whether females exhibited a directional change in brood sex ratios in sequential breeding attempts. Individual females did not produce more sons (or more daughters) over time, neither within years (glmmPQL with female ID as random factor: n = 32 broods by 16 females, $t_{15} = -1.0$, $P = 0.33$), nor between years (glmmPQL: n = 131 broods by 52 females, $t_1 = -1.0$, $P = 0.34$). Similarly, sex ratios of repeated broods did not vary in relation to individual females' nest sequences (females sampled in multiple years, glmmPQL: $P > 0.5$ for all analyses, nest sequence defined as first nest versus all later, first versus second, or as years between nests).

We then examined whether brood sex ratios varied with female age and number of chicks. We restricted the analysis to the years that had the greatest resolution of maternal ages (2005 and 2006) due to the cumulative nature of age estimation in these long-lived birds. We found a significant quadratic relationship between maternal age and brood sex ratio: young and old females produced female-biased broods (n = 101 broods by 84 females; Table 3). This relationship remained significant when we included only extreme sex ratios (all male versus all female broods, glmmPQL: n = 62, maternal age^2, $t_4 = -1047.2$, $P < 0.001$), and a similar but non-significant trend was present when only two-chick nests were considered (glmmPQL: n = 69 nests; maternal age^2 $t_6 = -1.152$, $P = 0.18$). When data from 2005 and 2006 were analyzed separately, the trend persisted in both years but remained significant only in the 2005 subset (Fig. 2. in 2005, glm: maternal age, n = 53, $\chi_1^2 = 5.25$, $P = 0.02$; maternal age^2, n = 53, $\chi_1^2 = 5.90$, $P = 0.02$. In 2006, glm: maternal age, n = 48, $\chi_1^2 = 1.49$, $P = 0.22$; maternal age^2, n = 48, $\chi_1^2 = 1.79$, $P = 0.18$). In 2005, there was also a significant effect of number of chicks on brood sex ratio, with single-chick nests being more female-biased than two-chick nests (glm: number of chicks, n = 53, $\chi_1^2 = 5.25$, $P = 0.02$); this variable was excluded from the 2006 analysis during model simplification.

Table 3. Influence of maternal age and number of chicks on brood sex ratio in 2005 and 2006. ''Maternal age'' was the females's minimum age based on years elapsed since first capture (see Methods). The initial model also considered year, but this term was excluded after model simplification. Generalized linear mixed model fitted by penalized quasi-likelihood (glmmPQL, binomial error), with female ID as a random effect. N = 101 broods by 84 females.

Model term	Estimate	SE	df	t	P
Number of chicks	0.69	0.38	14	1.80	0.09
Maternal age	1.23	0.43	14	2.93	**0.01**
Maternal age^2	−0.12	0.05	14	−2.78	**0.01**

Discussion

Neither laying date nor female condition was related to brood sex ratios in lance-tailed manakins. However, the proportion of male offspring varied over years and was significantly lower than 50% in one year, 2005. Our results suggest that this might be due to a female bias in broods of young and old females.

As in several other studies of bird species (Dijkstra et al. 1990, Ramsey et al. 2003, Pike and Petrie 2005) the overall population sex ratio in lance-tailed manakins did not differ from parity (Fisher 1930). This was found in adults as well as in offspring, and was consistent in individual years with one exception (the year 2005). Over all years and within years, sexes were randomly distributed among broods. Because adjustments of brood sex ratios at the individual level may still produce unbiased sex ratios overall, we examined whether individual females produced consistent

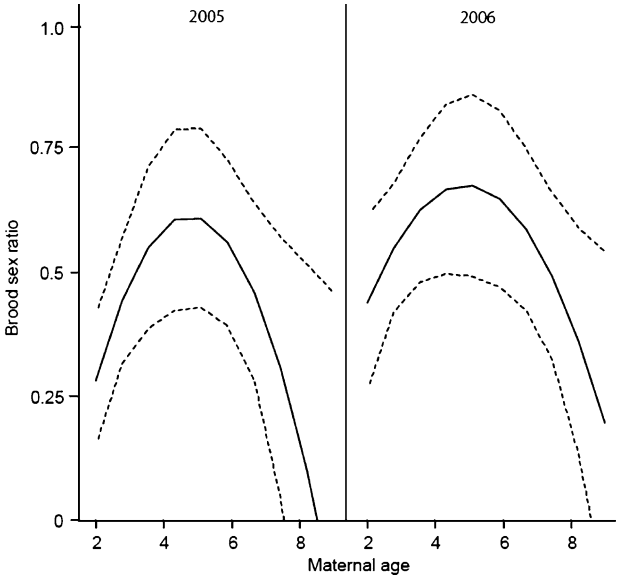

Fig. 2. Effect plot of lance-tailed manakin brood sex ratios in relation to female age in 2005 and 2006. Dashed lines show 95% confidence limits.

offspring sex ratios in multiple breeding attempts. Such consistency would indicate that sex ratio adjustments occur in relation to variables that do not change much over years (e.g. female body size). However, we found no obvious repeatability of sex ratios in different broods of individual females, neither within, nor among years.

Individual brood sex ratios have been shown to vary with many biotic and abiotic factors that may change rapidly for individual females (e.g. Gowaty and Lennartz 1985, Sasvári and Nishiumi 2005; others reviewed in Hasselquist and Kempenaers 2002). One plausible explanation for the lack of sex ratio repeatability reported here is that individual females are able to adjust brood sex ratios in response to variable conditions.

We previously hypothesized that species with lek mating systems should show more pronounced adaptive sex ratio biases than observed for monogamous species. We found no support for the hypothesis that females adjust brood sex ratios in relation to laying date. Similar to our results, the polyandrous spotted sandpiper *Actitis hypoleucos* did not show significant sex ratio changes over the breeding season (Anderson et al. 2003). This is in contrast to studies of generally monogamous European kestrels (Dijkstra et al. 1990) and common sandpipers (Anderson et al. 2003). The role of mating system in selecting for sex ratio adjustment in relation to laying date remains unclear, and is an interesting avenue for future research.

A variety of other factors could lead to unbiased sex ratios in relation to laying date. Even within a species, small differences in environmental or social conditions may affect sex ratio adjustment: in two populations of collared flycatchers *Ficedula albicollis* only one population adjusted sex ratios in relation to laying date (Rosivall et al. 2004). Differences in the life history characteristics of male and female offspring (e.g. growth rate or age at first breeding) which affect the relative benefits of early birth likely underlie seasonal variation in brood sex ratios (Daan et al. 1996).

In the lance-tailed manakin, male and female life history characteristics vary considerably. Males do not attain breeding status at least until their fourth year after hatching, and form complicated social alliances to perform courtship displays (DuVal 2007c). Early-born males could reap long-term reproductive benefits through increased ability to compete for later reproductive status relative to their late-hatched peers. Such an advantage could come from the extra time to increase body condition, or from more opportunities to observe adult courtship displays during their hatch year; first year males have female-like plumage and sometimes attend courtship displays that are directed towards them as if they were females (DuVal 2005a, DuVal pers. obs.). However, other aspects of lance-tailed manakin biology suggest that females could benefit from producing female-biased early broods: female lance-tailed manakins may breed in their second year, and so early-born females could be more likely to breed in their second year than late-born females. In contrast, males have many years to compensate for deficiencies in raising conditions before they have the opportunity to breed (Gorman and Nager 2004). It is possible that chicks of both sexes receive comparable benefit from early birth compared to their late-born peers, which would result in no sex ratio bias in relation to laying date.

We found no support for the hypothesis that females adjust brood sex ratios in relation to body condition. This is in contrast to studies of several other bird species (Bradbury and Blakey 1998, Nager et al. 1999, Whittingham and Dunn 2000, Alonso-Alvarez and Velando 2003, Pike 2005), including three lek-mating species (Clout et al. 2002, Thuman et al. 2003, Pike and Petrie 2005). Similar to the studies on sex ratio adjustment in relation to laying date, these studies also suggest interspecific variation in the occurrence of sex ratio adjustment in relation to female body condition.

Similar to Koenig and Dickinson (1996), we detected departure from a 50:50 brood sex ratio in one year of our study. In 2005, the proportion of female offspring was significantly higher than that of male offspring. Though it is possible that deviation in one year may be due to chance (Type I error), we highlight it here because 2005 was also distinguished from other years by differences in environmental and sampling conditions. First, the local rainy season was relatively early (75.9mm; 243% more rain in March 2005 than on average over the 32 previous years; Direction of Hydrometeorology of Empresa de Transmisión Eléctrica S. A.) which may have resulted in increased food availability, unusually dense vegetation, or other differences in chicks' rearing environments. Second, breeding was earlier relative to previous years (median laying date: 24 March in 2005 vs. 10–23 April for 2000–2002 with similarly long field seasons). Third, relatively more active nests were genetically sampled in 2005 (67% of all discovered, active nests; in other years: $44 \pm 11\%$) than in other years, indicating that more nests survived until day 21 when genetic samples were taken.

This increase in nest survival suggests that 2005 was a relatively good year for manakin nesting. If this was the case, why were broods female- rather than male-biased, when favorable conditions lead to male biases in other species? It is possible that the apparently favorable conditions increased the nesting success of females that normally would not have been successful in nesting, but that environmental conditions were unable to fully compensate for these females' relatively low nesting ability. The observed sex ratio differences in 2005 reflect a female bias in one-chick-broods and in relation to maternal age (see below). The tendency toward reduced clutch size in female-biased nests further suggests that some females might make the best of a bad situation by adapting their brood sex ratio, or by reducing their brood size when not able to raise a full clutch (Lobato et al. 2006).

We found a significant quadratic effect of maternal age on brood sex ratio in data from the later years of the study, when sampling included females in at least their eighth year after hatching: young and old females produced female-biased broods. A relationship of maternal age to brood sex ratio has been shown in a variety of species. Most commonly, such studies have demonstrated an increase in the production of male offspring with increasing maternal age (e.g. Blank and Nolan 1983, Heg et al. 2000, Griffith et al. 2003), though at least one study has shown a non-linear relationship of age and sex ratio consistent with senescence (*Passer domesticus*; Husby et al. 2006). Saino et al. (2002) also reported decreases in sex ratio with increased maternal age, but this effect was observed in repeated broods of individual females. We found no evidence for such a within-individual trend. This, in combination with variation in the strength of the relationship between age and sex ratio in consecutive years, suggests that the mechanism underlying such a quadratic relationship of age and sex ratio could be environmentally mediated rather than strictly senescence-based. Environmental mediation of this relationship could also explain why other studies have failed to detect effects of maternal age on brood sex ratio (e.g. Juola and Dearborn 2007).

The correlation of age and experience complicates the interpretation of results in longitudinal studies of breeding behavior. Parental experience also positively influences the outcome of nesting attempts in several species (Coulson 1966, Davis 1976, Komdeur 1996), and Heg et al. (2000) found that both maternal age and experience had a significant effect on brood sex ratios. We were unable to separate the effects of age and experience in this study, as all young birds were inexperienced, but long-term data on the success of consecutive nesting attempts by individual females may shed more light on the interaction of these two factors in the future.

The Trivers-Willard hypothesis (1973) could explain the relationship of age and brood sex ratio if middle-aged females produce higher-quality male offspring than do young or old mothers. Offspring fitness benefits of maternal age remain to be tested, but could result from combined effects of inexperience in younger females and senescence in older females. Young and old females invest in offspring of the sex with the lowest variance in later reproductive success, presumably because the reproductive success of female offspring is less likely than that of males to be impaired by suboptimal conditions experienced in the nest. Several additional ecological and life-history factors may influence the production of female rather than male offspring. In many species, offspring of one sex are relatively easier to raise and more likely to survive to maturity than offspring of the other sex due to sexual dimorphism: male chicks are larger and require additional resources in great-tailed grackles *Quiscalus mexicanus* (Teather and Weatherhead 1988), or vice versa in great frigatebirds *Fregata minor* (Juola and Dearborn 2007). This seems unlikely in lance-tailed manakins, as there is no easily observed size dimorphism in chicks, and males are actually smaller than females as adults (EHD unpubl. data). Alternatively, mothers may reduce competition over resources by producing more of the sex that disperses further, especially in years of high population density, as suggested by the local resource competition hypothesis (Clark 1978, Hardy 1997). This is more likely to explain female-biased sex ratios of young than old females, however, as young females will face more years of competition with their offspring than older females who are closer to the end of their reproductive life.

In conclusion, we found no consistent influence of multiple hypothesized factors on brood sex ratios over a period of six years. Lack of consistent trends is a common situation in studies of brood sex ratios, and multiple explanations have been suggested: females might have limited ability to influence the outcome of chromosomal sex determination, selective pressures might not be sufficient to elicit sex ratio biasing in some years or populations, sex ratio might be related to untested variables, or sex ratio could be affected by multiple different factors which cancel each other out (Koenig and Dickinson 1996, Ramsey et al. 2003, Budden and Beissinger 2004, Rosivall et al. 2004). We detected a female bias in brood sex ratios of young and old females, suggesting that under certain conditions factors such as maternal age might lead to sex ratio adjustment. As parental age and correlated experience positively influence success in rearing offspring (e.g. Davis 1976, Komdeur 1996, Heg et al. 2000, Husby et al. 2006) the influence of these two variables on reproductive success, as well as on manipulation of offspring sex ratios offers an interesting avenue for future long-term research. A critical next step is to examine the realized reproductive benefits of offspring produced by females of different ages.

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