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Brood sex ratios, female harem status and resources for nestling provisioning in the great reed warbler (*Acrocephalus arundinaceus*)

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Abstract The theory of parental investment and brood sex ratio manipulation predicts that parents should invest in the more costly sex during conditions when resources are abundant. In the polygynous great reed warbler, *Acrocephalus arundinaceus*, females of primary harem status have more resources for nestling provisioning than secondary females, because polygynous males predominantly assist the primary female whereas the secondary female has to feed her young alone. Sons weigh significantly more than daughters, and are hence likely to be the more costly sex. In the present study, we measured the brood sex ratio when the chicks were 9 days old, i.e. the fledging sex ratio. As expected from theory, we found that female great reed warblers of primary status had a higher proportion of sons in their broods than females of lower (secondary) harem status. This pattern is in accordance with the results from two other species of marsh-nesting polygynous birds, the oriental reed warbler, *Acrocephalus orientalis*, and the yellow-headed blackbird *Xanthocephalus xanthocephalus*. As in the oriental reed warbler, we found that great reed warbler males increased their share of parental care as the proportion of sons in the brood increased. We did not find any difference in fitness of sons and daughters raised in primary and secondary nests. The occurrence of adaptive sex ratio manipulations in birds has been questioned, and it is therefore important that three studies of polygynous bird species, including our own, have demonstrated the same pattern of a male-biased offspring sex ratio in primary compared with secondary nests.

Key words Sex ratio manipulation · Polygyny · Female harem status · Resources for nestling provisioning

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Introduction

Most sexually reproducing species produce both sons and daughters. Given that sons and daughters may confer different costs, Trivers and Willard (1973) suggested that natural selection will favour parents that can adjust their brood sex ratio in response to available resources, and hence optimise their own reproductive success. When resources are abundant, a bias of the brood sex ratio towards the more costly sex is favourable, whereas parents with less resources should bias the brood sex ratio towards the less costly sex. This is particularly so when the reproductive values of sons and daughters show different slopes relative to the resource variable (Trivers and Willard 1973).

In birds, the fitness factors that entice parents to bias their brood sex ratio can be either genetic or environmental (see review in Bensch 1999). Sexually selected traits, like red beak and leg colours in male zebra finches, *Poephila guttata* (inferred from manipulations of leg colour bands; Burley 1986), and large white forehead patches in collared flycatchers *Ficedula albicollis* (Ellegren et al. 1996) seem to result in male-biased brood sex ratios presumably because sons will inherit their fathers' attractive ornament. Several other studies, both correlative and experimental, support the theory that a biased sex ratio can be related to the available resources (Komdeur et al. 1997; Appleby et al. 1997; Kilner 1998; Nishiumi 1998a; Nager et al. 1999).

Females of primary status in polygynous species often have more resources than secondary females for nestling provisioning, because polygynous males predominantly assist the primary female with nestling provisioning (Alatalo et al. 1981; Yasukawa et al. 1990; Bensch and Hasselquist 1994; Sejberg et al. 2000). Assuming that sons are more costly to raise than daughters, or that male fitness is more sensitive than female fitness to the resources during the nestling period, primary females are expected to preferentially invest in sons and secondary females in daughters to maximise the reproductive value of their offspring (Trivers and Willard 1973).

Male quality is positively correlated with brood sex ratio in collared flycatchers (Ellegren et al. 1996), while no such correlation is found in red-winged blackbirds, *Agelaius phoeniceus* (Weatherhead 1983). An alternative is that female quality influences the brood sex ratio. In a recent study of the polygynous oriental reed warbler, *Acrocephalus orientalis*, in Japan, females of primary harem status were found to have a higher proportion of sons in their clutches than did females of lower harem status (Nishiumi 1998a). A similar result has been reported in another polygynous marsh-breeding species, the yellow-headed blackbird, *Xanthocephalus xanthocephalus* in North America (Patterson et al. 1980). In these two studies, male genetic quality is unlikely to explain the difference in brood sex ratios, since primary and secondary females in a harem are mated to the same male. Hence, if adaptive, the bias in brood sex ratio in relation to female harem status probably reflects environmental factors.

One argument against the occurrence of adaptive brood sex ratio manipulations is that the adaptive explanations are either unique or inconsistent between studies (Krackow 1999). In the present study, we examine brood sex ratios in the polygynous great reed warbler, *Acrocephalus arundinaceus*, a species closely related to the oriental reed warbler. Here we seek to repeat the analyses of brood sex ratios in the oriental reed warbler by Nishiumi et al. (1996) and Nishiumi (1998a), to investigate whether a male-biased brood sex ratio in primary nests and a female-biased brood sex ratio in secondary nests is a general pattern in polygynous birds. Furthermore, we investigate whether the difference in brood sex ratios in primary and secondary nests can be explained by an increased willingness of males to assist primary broods containing sons (Nishiumi et al. 1996), or by differential fitness of sons and daughters in primary and secondary nests.

Methods

The study population

We studied great reed warblers at Lake Kvismaren in south-central Sweden (59°10' N, 15°25' E) between 1987 and 1999 (see Bensch and Hasselquist 1991; Hasselquist et al. 1995). The great reed warbler has a polygynous social mating system where 38% of the males pair with two to five females in a season, and 23% of the territorial males remain unpaired (Hasselquist 1998). The breeding population consisted of on average (\pm SD) 32 \pm 6.1 breeding females and 26 \pm 5.6 territorial males per year. The breeding and natal site fidelity is high relative other small passerines; on average 53 \pm 16% of the adults and 16 \pm 4.9% of the chicks return to the breeding site from their wintering quarters in tropical Africa (Hasselquist 1995; unpublished data). The majority of the breeding males and females (>98%) were caught in mistnets and ringed with one aluminium ring and a unique combination of two to three colour rings. During the breeding season, May to July, pair formation of individual males and females, the position of singing males and female nest-building activity were recorded daily. Most nests were located (>95%) and visited at 1- to 4-day intervals. When the chicks were 9 days old they were ringed, weighed, the length of the innermost primary feather was measured and a blood sample

was collected. The blood samples were suspended in SET-buffer and stored at -20° (Hasselquist et al. 1995). DNA was isolated from blood using standard phenol/chloroform-isoamylalcohol extraction (Sambrook et al. 1989). During the years 1989–1990 and 1995–1997, a total of 109 nests were studied to obtain a measure of parental care. Parental feeding rate was recorded and each parent was identified by its unique colour ring combination (Bensch and Hasselquist 1994; Sejberg et al. 2000).

Sexing of offspring

In birds, the female is the heterogametic sex with one W and one Z chromosome, while males have two Z chromosomes. It is not possible to sex great reed warbler nestlings on morphological characters, but with molecular genetic techniques a female-specific region on the W chromosome can be amplified (Griffiths and Tiwari 1993; Lessells et al. 1996). In an earlier study, we sexed the nestlings born in our study area between 1987 and 1993 (Westerdahl et al. 1997) using the oligonucleotide OPA-09 (5'-GGGTAACGCC-3') (Operon Technologies, Alameda, Calif.) for polymerase chain reaction (PCR) amplification of a fragment from the W chromosome (Perkin Elmer, Foster City, Calif.). OPA-09 amplifies five different PCR products (0.5–1.6 kb) from females, one of which is female specific, and four different products from males. The present study adds data on sexing of nestlings from 1994–1997 in addition to the dataset analysed by Westerdahl et al. (1997). In total, we sexed 1,161 fledglings (274 broods) that hatched between 1987 and 1997. Since the blood samples were collected when the chicks were 9 days old we measure the fledgling sex ratio, not the primary sex ratio.

Nestling body mass

Nestling body mass was measured 8–10 days after the first chick in the brood had hatched. Nestling body mass was linearly related to the length of the innermost primary feather within the present study interval of between 10–30 mm (0.38 g/mm primary feather, $r=0.72$, $P<0.001$). The residual mass of each nestling relative to the length of the growing innermost primary feather compensates for age differences in nestling mass, time of day when weighed, and for asynchronous hatching within clutches. The average nestling body mass, adjusted to a weighing at 1200 hours on day 9, was 24.6 g. This average body mass was added to the residual body mass to illustrate the actual weight of the nestlings.

Female harem status

In our study population, a male can have one to five females in his harem. Female harem status was defined as follows: primary females include females of monogamous males and females of primary status in polygynous harems, while secondary females include females of secondary and lower status in polygynous harems. The harem status of a female may change several times over the breeding season (Bensch 1996). We defined settling status (SS) as the status of a female when choosing a territory for the first time in a season, and the female's status when feeding the nestlings as her feeding status (FS).

Analyses of male feeding frequency

We wanted to repeat the study by Nishiumi et al. (1996) and therefore conducted our analyses on male feeding frequency using their criteria. Hence, the following broods were excluded from the analyses of male feeding frequency: secondary broods, primary broods where the social male was mate-guarding another female at the time of nestling feeding in his first nest, and broods with only one chick. These nests were excluded because at such nests, male great reed warblers normally provide no or very little assistance.

However, when the chicks in the primary nest have reached independence, polygynous males may help a lower-status female as if she is the only female on his territory (Bensch and Hasselquist 1994).

Analyses of offspring fitness

Our aim was to study whether there was a difference in relative fitness depending on nest status and offspring sex. The critical test is therefore to examine the interaction term between nest status and sex. We investigated the interaction term between sex and three different parameters that could indicate offspring fitness: body mass, survival and lifetime reproductive success. "Survival" is based on whether or not the offspring was later recorded as a breeding recruit in our study population and "lifetime reproductive success" is the number of lifetime fledglings among these recruits. Hence, of the examined fitness correlates, the last one should be most closely correlated with fitness. In the analysis of lifetime reproductive success, we excluded offspring hatched after 1993, because some individuals from these cohorts were still alive in 1999.

Statistics

We used two-tailed tests unless otherwise stated. One-tailed tests were used when we examined whether our study population showed the same patterns as previously found in the oriental reed warbler in Japan (see Nishiumi et al. 1996; Nishiumi 1998a). We used the female breeding season as independent observations, hence 42 females were included more than once. Their use as independent observations is justified because (1) the same male and female only occasionally remate in successive years (Bensch and Hasselquist 1991) and (2) female feeding status shows no repeatability between years (repeatability, $r=-0.01$, $F_{61,118}=0.87$, $P=0.72$). The analyses of proportion of sons in broods were run in SAS genmod module (logit link function, binomial distribution) with number of sons as the dependent variable and number of chicks in each clutch as the binomial denominator (SAS 1990). The difference between the model and the data is given by the deviance which is distributed as χ^2 (see Crawley 1993). The remaining analyses were performed in SYSTAT 7.0 for Windows (Wilkinson 1997). We report mean \pm SEs if not otherwise stated.

Results

Female harem status and brood sex ratios

Sons (25.16 ± 0.07 g) weighed about 5% more than daughters (24.03 ± 0.07 g) at an age of 9 days ($t_{1,053}=11.8$, $P<0.001$), suggesting that sons need more food resources than daughters. Because great reed warbler males rarely help with nestling provisioning in secondary nests, we expected that food resources per nestling would be lowest in large broods of secondary status, and hence offer unfavourable conditions for male-biased broods. Thus, we expected females in this situation to bias their brood sex ratio towards daughters.

When we considered the females' SS, primary great reed warbler females produced a higher proportion of sons (0.541 ± 0.021) in their clutches than did secondary females (0.487 ± 0.025 ; $\chi^2=3.6$, $P=0.03$, $n=274$, one-tailed test; Fig. 1). When we instead considered the females' FS, this difference was even more pronounced: the proportion of sons for primary females was 0.545 ± 0.019 , that for secondary females, 0.455 ± 0.031 ($\chi^2=6.5$, $P=0.005$, $n=274$, one-tailed test). A multiple-regression model with brood sex ratio as the dependent

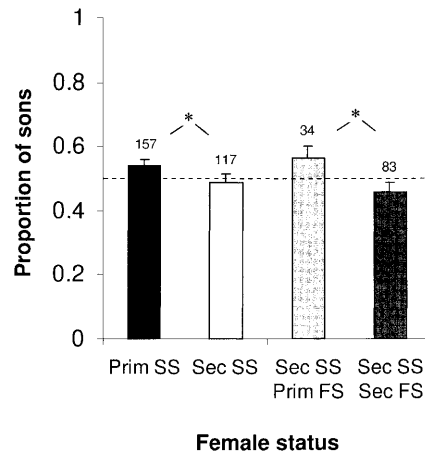


Fig. 1 Proportion of sons in broods of primary (Prim) females (black bar) and secondary (Sec) females (white bar) considering female harem status when settling (SS). Females with secondary settling status are also separated on the basis of if remaining secondary (dark grey bar) when feeding the nestlings (FS) or changing to primary status (light grey bar) (bars show mean \pm SE; * $P<0.05$; numbers above bars refer to sample size)

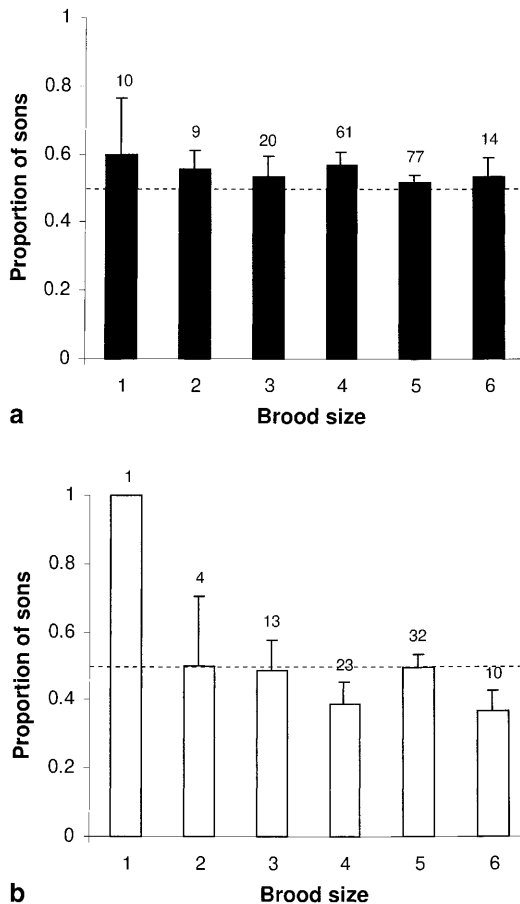


Fig. 2 Proportion of sons in broods of different sizes (one to six chicks) for females of primary settling status (a) and secondary settling status (b) (bars show mean \pm SE; numbers above bars refer to sample size)

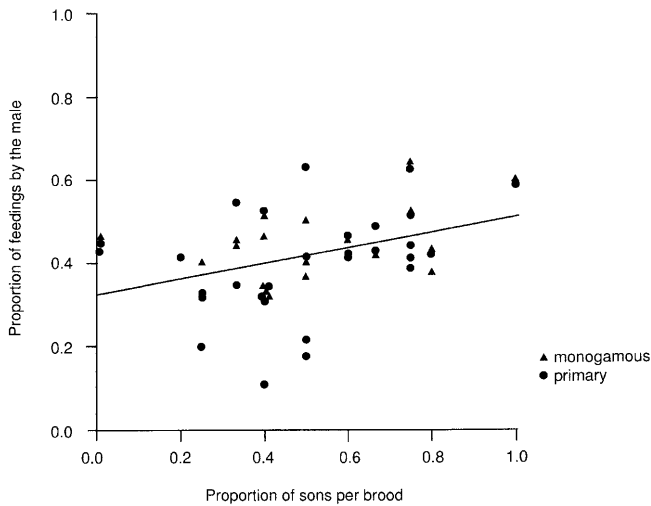


Fig. 3 Relationship between the proportion of sons in the brood and the proportion of feedings by the male. *Triangles* indicate females in monogamous pairs and *circles*, females of primary harem status. For females in monogamous pairs and of primary harem status combined ($n=48$), the correlation is $r=0.32$ ($P=0.013$)

variable, and SS and FS as independent variables, suggested that there is no additional affect of SS ($\chi^2=0.02$, $P>0.5$) when FS is included ($\chi^2=2.9$, $P=0.04$, one-tailed). Interestingly, the females that improved their harem status from secondary SS to primary FS had a significantly higher proportion of sons in their broods than secondary SS females that did not improve their status ($\chi^2=2.7$, $P=0.05$, $n=117$, one-tailed test; Fig. 1). The proportion of sons in the largest brood size group (six chicks), where the risk of starvation is expected to be highest, was on average 0.54 ± 0.05 in primary nests and 0.37 ± 0.06 in secondary nests (Fig. 2). However, the interaction term between brood size and nest status was not significant ($\chi^2=3.3$, $P=0.64$, $n=274$).

We also compared the proportion of sons in broods for individual females that in different years were recorded both at nests of primary and secondary status. The expected pattern was found: each female had on average a higher proportion of sons in broods when of primary status than of secondary status (paired $t_{34}=2.22$, $P=0.016$, one-tailed test).

Male feeding assistance

We repeated an analysis done on male feeding frequencies in the oriental reed warbler (Nishiumi et al. 1996).

Table 1 Results from multiple-regression analyses of independent variables – sex, nest status and their interaction term – on three potential correlates to fitness – body mass, survival and lifetime

Dependent variable	n	Sex	Nest status	Interaction term: sex \times nest status
Body mass	1126	$F=117$, $P<0.001$	$F=5.86$, $P=0.016$	$F=0.08$, $P=0.8$
Survival	1124	$F=0.3$, $P=0.6$	$F=0.9$, $P=0.3$	$F=1.8$, $P=0.2$
Lifetime reproductive success	91	$F=0.3$, $P=0.6$	$F=2.3$, $P=0.13$	$F=0.7$, $P=0.4$

Hence, the following broods were excluded from the analyses: secondary broods, primary broods where the social male was mate-guarding another female at the time of nestling feeding in his first nest, and broods with only one chick. Similar to the observations in the oriental reed warbler, we found that males increased their proportion of feedings with the proportion of sons in the brood (linear regression, $r=0.32$, $t_{48}=2.3$, $P=0.013$, one-tailed; Fig. 3). This effect remains significant even after correcting for brood size (multiple regression: proportion of sons, $t_{48}=2.4$, $P=0.01$, coef.=0.12, one-tailed; brood size, $t_{48}=3.3$, $P=0.002$, coef.=−0.06; total model, $F_{2,45}=8.8$, $P=0.001$).

Fitness of sons and daughters

The difference in sex ratios between primary and secondary nests could be explained in adaptive terms if it is advantageous to raise sons in primary nests and to raise daughters in secondary nests. However, although sons weighed more than daughters, and the average chick mass in secondary nests was lower than in primary nests, there was no evidence of a sex-differential relationship between mass and nest status, as reflected by the non-significant interaction term (Table 1). Because mass may not be a good predictor of survival, we analysed whether there was a sex-differential relationship between survival until breeding age and nest status. Again, the interaction term between sex and nest status was non-significant, suggesting that the proportion of returning sons and daughters was similar for primary and secondary nests (Table 1). Finally, we examined the lifetime reproductive success of recruited offspring. Among the returning sons and daughters, there was no difference in the number of lifetime fledglings, and neither nest status nor the interaction term between nest status and sex were significant (Table 1). Hence, we found no evidence that the relative reproductive value of sons and daughters differs between secondary and primary nests.

Discussion

We found that great reed warbler females of primary harem status had a higher proportion of sons in their broods than females of secondary status. This conforms with the results obtained in the oriental reed warbler (Nishiumi et al. 1996; Nishiumi 1998a). In a previous study of this great reed warbler population, we found that the varia-

reproductive success (which excludes offspring hatched later than 1993 since these years contain individuals still alive)

tion in brood sex ratio did not vary with breeding dates, so the later breeding by secondary females cannot explain their difference in brood sex ratios (Bensch 1996; Westerdaal et al. 1997). Furthermore, individual females had a significantly higher proportion of sons in their broods when they were of primary rather than secondary status, which indicates that environmental factors are important for the observed sex ratios. We therefore suggest that the adaptive explanation for the difference in brood sex ratios between nests of primary and secondary status is that resources for nestling provisioning are higher at primary than secondary nests, mainly due to greater male assistance at primary nests (Bensch and Hasselquist 1994). The result of the present study is particularly important because it confirms a pattern previously found in two other species of marsh-nesting socially polygynous birds, the oriental reed warbler and the yellow-headed blackbird (Patterson et al. 1980; Nishiumi 1998a). Thus, the pattern of a biased brood sex ratio in relation to female harem status may be general among species showing resource defence polygyny.

Explanations for the observed sex ratio pattern

We were not able to obtain an unbiased measure of the primary sex ratio of the broods because we sexed the young when they were 9 days old and in some broods chicks had already died at an earlier age. About 80% of the laid eggs resulted in chicks that reached an age of 9 days, and this proportion was similar for young raised in primary and secondary nests (Bensch 1996). Two alternative mechanisms may explain why primary females have a higher proportion of sons in their broods than secondary females. First, the primary sex ratio may be biased so that primary and secondary females indeed lay clutches of different sex ratios. Alternatively, all females lay clutches with an average primary sex ratio close to unity, with sex-differential mortality during the first week of life resulting in fewer daughters in primary nests and fewer sons in secondary nests. Such sex-biased mortality may also occur after fledging (see Smith et al. 1989; Heeb et al. 1999). Sex-biased mortality in nests could result if body size determines the ability to compete for food: because daughters are smaller than sons, they would be more likely to die from starvation through competition. On the other hand, because males are larger, they may need more food which would make them more vulnerable to starvation during periods of food shortage (Anderson et al. 1993). Hence, our finding of male-biased broods among primary females and female-biased broods among secondary females could result if sibling competition over food is more intense in primary broods and periods of food shortage are more common in secondary broods.

In many bird species, the resources chicks receive in the nest have a profound impact on their future prospects of survival and success (reviewed in Schew and Ricklefs 1998). There could be adaptive reasons for the females

to adjust their brood sex ratios differently depending on their harem status if the reproductive value of sons and daughters is differently affected by nest status. However, we found no such evidence (Table 1). If primary and secondary females invest in optimal brood sex ratios to maximise their fitness, we should not expect to find differences in either survival or lifetime reproductive success of offspring raised in nests of different status. The only way to demonstrate a fitness effect on nestlings due to nest status would be through an experimental manipulation of the proportion of sons and daughters in nests of different status (see Sheldon et al. 1998).

Females that improve their harem status

Females that settle as primary females, and whose nests are not destroyed, will remain in primary status when feeding young. However, many females that settle as secondary females will obtain the primary feeding status as the result of nest failure at the primary females' nests. This seems often to be an active behaviour, as secondary females have been shown to deliberately destroy nests of primary females (Bensch and Hasselquist 1994; Hansson et al. 1997). We found that secondary females that improved their harem status had a significantly higher proportion of sons in their broods than secondary females that did not improve their status (Fig. 1). Perhaps these females could evaluate the probability of improving their harem status and then adjust their broods accordingly.

Brood sex ratio and parental provisioning

Primary females have a higher proportion of sons in their broods than secondary females, either because of an adaptive manipulation of the primary brood sex ratio or because of sex-biased mortality in the brood after hatching. The latter may be caused by different food requirements of sons and daughters, and sons that are significantly heavier than daughters possibly have larger food requirements (Anderson et al. 1993). The male seldom helps the secondary female to feed the nestlings and thus starvation is most likely in large secondary broods (Bensch and Hasselquist 1994). Both the present study and the study of the oriental reed warbler indeed found that the brood sex ratio in nests of secondary status was most strongly female biased in the largest brood size (Nishiumi 1998a; Fig. 2). Hence, these results indicate that food may be limiting in large secondary broods and if sons have a higher nest mortality than daughters due to food shortage, this could explain the female bias in large secondary broods.

Great reed warbler males increase their proportion of feedings with the proportion of sons in the brood, a result that has also been found in the oriental reed warbler (Nishiumi et al. 1996), in red-winged blackbirds (Yasukawa et al. 1990) and in small broods of corn buntings *Miliaria calandra* (Hartley et al. 1999). Whereas

female great reed warblers increased their feeding rate with brood size, the male feeding rate was not related to brood size (Sejberg et al. 2000; Fig. 3). These data therefore suggest that great reed warbler males decide on their level of investment in nestling provisioning, and that the females then adjust their feeding level accordingly and take on the remaining part of nestling care.

Why would males increase their proportion of feedings with an increasing proportion of sons in the brood and not with brood size? Nishiumi et al. (1996) suggested that males increase their feeding effort in male-biased broods more than is needed for nestling survival because fathers preferentially invest in sons. The extra food provided by the males is thus supposed to increase lifetime reproductive success of the nestlings. Sons will benefit more than daughters from the extra food since they have a larger variance in reproductive success. Although male oriental reed warblers increase their proportion of feeding with the proportion of sons in the brood, they do not preferentially feed sons (Nishiumi 1998b). Perhaps more frequent begging calls from the brood can trigger great reed warbler and oriental reed warbler males to increase their feeding effort. Studies from other passerines have shown that sons beg more frequently than daughters (Teather 1992), and that the parents will feed more in response to louder and more intense begging calls (Ottooson et al. 1997; Kilner et al. 1999). Even though we do not know why males feed male-biased broods more than female-biased broods, such a biased feeding pattern by males could be used by primary females to obtain more male care by producing male-biased broods.

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