

Sex ratio adjustment in relation to paternal attractiveness in a wild bird population

(sexual selection/sex allocation/*Ficedula albicollis*)

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ABSTRACT When the relative fitness of sons and daughters differs, sex-allocation theory predicts that it would be adaptive for individuals to adjust their investment in different sexes of offspring. Sex ratio adjustment by females in response to the sexual attractiveness of their mate would be an example of this. In vertebrates the existence of this form of sex ratio adjustment is controversial and may be confounded with sex-biased mortality, particularly in sexually size-dimorphic species. Here we use PCR amplification of a conserved W-chromosome-linked gene to show that the sex ratio within broods of a natural population of sexually size-monomorphic collared flycatchers *Ficedula albicollis* is related to the size of their father's forehead patch, a heritable secondary sexual character implicated in female choice. Experimental manipulations of paternal investment, which influence the size of this character in future breeding attempts, result in corresponding changes in the sex ratio of offspring born to males in those breeding attempts. In contrast, manipulations of maternal investment have no effect on future sex ratios, and there is no relationship between variables predicting the reproductive value of the brood and nestling sex ratio. Analysis of recruitment of offspring reveals similar patterns of sex ratio bias. The results suggest that female collared flycatchers are able to adjust the sex ratio of eggs ovulated in response to the phenotype of their mate. This finding is most consistent with "genetic quality" models of sexual selection.

Fisher (1) first offered a theoretical explanation, in terms of frequency-dependent selection, of why equal investment in sons and daughters was likely to be an evolutionarily stable strategy. Modifications of Fisher's assumptions reveals that under some circumstances it is adaptive to bias investment in favor of one or other sex of offspring (2, 3). In many instances, the relative fitness of sons and daughters will differ depending on some feature of the reproductive attempt they arise from. For example, if males are larger than females as adults, sons may be relatively more influenced by nutrition during rearing than daughters (4). In such cases, it would be adaptive for individuals to vary their investment in differently sexed offspring in relation to the feature which influences the relative fitness of sons and daughters. One way in which this might be achieved is to adjust the sex ratio of offspring (3–5). A particular situation in which sex ratio adjustment would be adaptive is where sons inherit characters determining their attractiveness from their fathers, and where these characters have a greater influence on the fitness of sons than on daughters. In such a case, females mated to attractive males might be expected to adjust the sex ratio of their offspring in favor of sons. It is well established that sex ratio adjustment in relation to external factors (e.g., host size in parasitoid wasps) occurs in some taxa (6). However, it has been suggested that

chromosomal sex determination (as found in birds and mammals) may act as a constraint to sex ratio adjustment, since random segregation might prevent adjustment of the sex ratio of gametes (7, 8). Burley (9, 10) presented evidence suggesting that in aviary populations of zebra finches *Taeniopygia guttata*, sex ratio adjustment occurred in response to artificial attractiveness (determined by colored leg-bands) of both males and females. However, Burley was unable to determine the stage at which sex ratio differences arose (9, 10), and indeed sex-biased mortality can give rise to similar patterns without the need to invoke adaptive sex ratio adjustment *per se*, because it may create a correlation between maternal condition and offspring sex ratio (11).

With the advent of molecular genetic markers for determining sex (12–14) it is possible to determine the sex ratio for large numbers of families of wild vertebrates before substantial mortality has occurred. Birds are an ideal system in which to investigate sex ratio adjustment by females, since females are the heterogametic sex (3) and hence the sex of an offspring is determined by the sex chromosome that it inherits from its female parent. In this paper we report relationships between offspring sex ratio (determined by PCR amplification of a W-chromosome-linked gene) and the size of a male secondary sexual character involved in female choice, in a natural population of the sexually size-monomorphic collared flycatcher *Ficedula albicollis*. We use the dependence of this character on previous reproductive effort (15), coupled with experimental manipulations of reproductive effort, to demonstrate that females manipulate the sex ratio of their offspring in relation to their mate's phenotype.

METHODS

A long-term study of the population biology of the collared flycatcher has been conducted on the Swedish island of Gotland since 1980. The birds nest in boxes scattered through deciduous woodlots, which they return to in early May following a spring migration from Africa (16). Males are usually monogamous, but some males (5–15%) attract two or more females to separate boxes (16). Adult males are conspicuously black and white (females are dull gray-brown and white), with a large white forehead patch, the size of which is determined during a partial moult in the winter quarters. This trait, which appears to be a "badge of status" (17, 18), seems important in sexual selection: it is positively related to lifetime reproductive success, and to a male's likelihood of being polygynous (15). Further, experimental manipulations of paternal effort in males cause changes in its size in subsequent years, suggesting that it indicates condition, an argument supported by the

Abbreviation: SSCP, single-stranded conformational polymorphism.

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smaller patch size displayed by males born in nests where brood size was experimentally increased (15). These findings demonstrate that this character is relatively plastic, and sensitive to prior reproductive effort. However, a substantial component of patch size is also genetically determined, as shown by father-son regression in combination with cross-fostering experiments (19).

Sex Determination. Blood samples (5–40 μ l by brachial venipuncture) were collected from broods of 8- to 9-day-old nestlings in 1994. Adults associated with these nestlings were caught while feeding nestlings at 13 days of age, blood samples taken as for nestlings, and adult band numbers recorded, and for males, forehead patch area measured as described (15). Sex determination was performed using a PCR-single-stranded conformational polymorphism (SSCP) assay (Fig. 1), by amplifying a highly conserved gene located on the avian W-chromosome (H.E., unpublished data). This gene exists in two copies in the genome, one of which is autosomal; both are amplified by primers P2 and P3 (described in ref. 20). Sequence analysis of the 69-bp interval between P2 and P3 revealed that the two copies differ at five nucleotide positions (H.E., unpublished data), a situation that allowed separation of the two copies using SSCP analysis. PCR was performed with genomic DNA chelex extracted from whole blood samples; each reaction consisted of 40 cycles of 15 s at +94°C, 20 s at +50°C, and 25 s at +72°C, and contained 4 pmol of primer in a 15 μ l volume. Amplification products were electrophoresed in non-denaturing 8% polyacrylamide gels (SE600; Hoefer) at a constant temperature of +20°C. Either four (males) or seven (female) distinct bands were seen after silver staining (Fig. 1); the different bands represent different single-stranded, heteroduplex, or homoduplex variants of the two copies of the gene. The autosomal copy of the gene served as a positive control for PCR amplification. Two controls were available for sex determination. For all families the putative parents were analyzed on the same gel as the nestlings, and the adults were always sexed correctly ($n = 79$ pairs). Further, all of 32 nestlings (17 females, 15 males) recruited to the study area in 1995 had been correctly sexed from gels by this method before they returned.

Manipulation of Parental Effort and Classification of Mating Status. Parental effort of males and females was experi-

mentally manipulated as described (21). Briefly, manipulations consisted of changing brood size or clutch size by one or two eggs, by swapping small nestlings, or eggs between nests paired by laying date and clutch size. In our study population, very few pairs breed together between years: in only 6% of cases where both pair members survive the winter do they breed together in the following year (22). This therefore enabled us to assess the effect of experimental manipulations on each sex of parent independently. There is no evidence that males subject to brood manipulation in one year are more likely to breed in following years with females given similar experimental manipulations (Fisher exact test, $P = 1.0$). Females were classified as to their mating status, based upon trapping adults on a single day late in the nestling phase, as follows. If a female's mate was caught feeding nestlings at only her nest box, that female was regarded as monogamous. Polygyny was recorded if either (i) a female's mate was caught feeding nestlings at two different nest boxes, or (ii) if no male assisted a female with feeding, since males often feed the young of only their primary female in this species (16). As a result, there will be some misclassifications with respect to mating status, but the effect of these is to make statistical tests conservative (15, 16). Recruits were defined as birds banded as nestlings which subsequently bred within the study area; age was determined from banding records, and reproductive variables (clutch size, laying date) from weekly nest-box checks (16).

Statistical Analysis. Analyses of sex ratio variation were performed using GLIM with binomial errors and a logit link (23); the brood was the unit of analysis, and no adult entered the analysis more than once. The effect of adding a new variable to the model is assessed by the change in deviance, which is distributed approximately as χ^2 , with degrees of freedom specified by the change in degrees of freedom before and after the test variable is added. For significant relationships we calculated the deviance ratio (change in deviance/model deviance before addition of variable), which gives the variance in the dependent variable explained by the added variable (corresponding to r^2). In cases where we had clear *a priori* expectations about the ordering of group means we employed ordered heterogeneity tests (24). These are a general class of tests combining information about ordering of means

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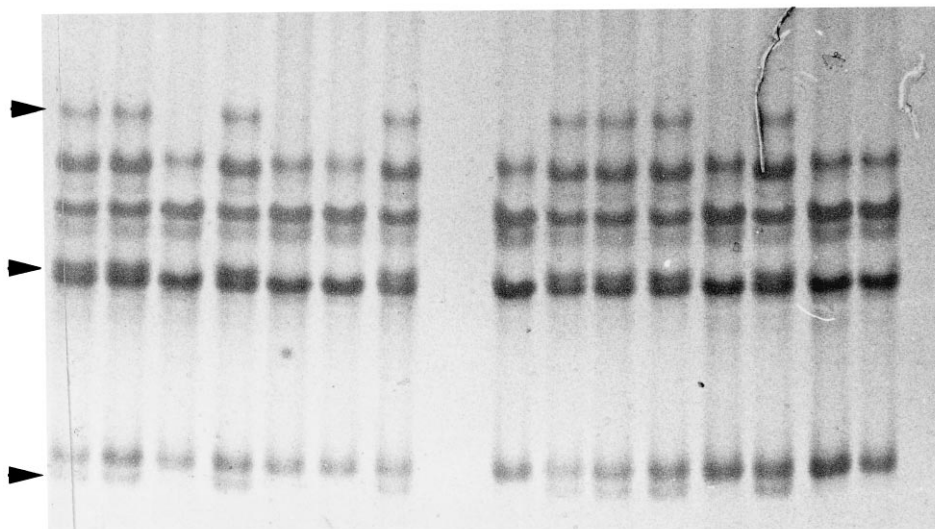


FIG. 1. Sex determination of collared flycatchers using a PCR-SSCP assay. On this gel adult birds of known sex were analyzed (M, male; F, female; -, negative control without template DNA). Arrows indicate female-specific bands.

expected under the tested hypothesis that is independent of that tested by nondirectional heterogeneity tests such as ANOVA (24).

RESULTS

We have recently identified a highly conserved gene on the avian W-chromosome which may provide a universal sexing system for birds (H.E., unpublished data). Here we detected this gene by a PCR-SSCP assay, which allowed us to determine the sex of nestling collared flycatchers unequivocally. Using data from 79 broods of flycatchers sampled in 1994, we found a significant positive relationship between a male's forehead patch size and the proportion of his offspring that were male [Fig. 2; $\chi^2 = 6.837$, $df = 1$, $P < 0.01$; the minimal model is $\ln(\text{number of males in brood}/\text{number of females in brood}) = -1.697 + 0.0196(\text{forehead patch area})$]. The population sex ratio did not differ from equality (264 males: 275 females: $G = 0.225$, $df = 1$, $P > 0.5$; 95% CI for proportion males is 0.448–0.532). In contrast, none of a number of variables which have previously been found to be correlated with offspring sex ratios in wild bird populations (25–29) showed any relation to sex ratio in this species {no relationship between brood sex ratio (proportion males) and laying date ($\chi^2 = 1.34$, $df = 1$, $P > 0.2$), clutch size ($\chi^2 = 0.14$, $df = 1$, $P > 0.9$), male age ($\chi^2 = 2.39$, $df = 6$, $P > 0.8$), male body size [tarsus length ($\chi^2 = 0.39$, $df = 1$, $P > 0.5$)] or female age ($\chi^2 = 1.18$, $df = 5$, $P > 0.9$)}.

Confirmation of the correlational relationship presented in Fig. 2 requires experimental manipulation of the size of the male forehead patch. However, experimental manipulations of secondary sexual characters are often difficult to perform, and manipulations may also not adequately reflect costs of development or maintenance (30). Furthermore, since such traits are expected to be individually optimized (31, 32), manipulations may create discordance between the manipulated trait value and other phenotypic characters. For example, among unmanipulated birds there might be a correlation between song rate and forehead patch size; experimental manipulations of just one character would create males with combinations of trait values not normally encountered by females. The demonstration of the dependence of male forehead patch size on prior reproductive investment (15) suggests a way to investigate the relationship between this trait and sex ratio experimentally. Accordingly, we analyzed the relationship between

experimental manipulations of male parental effort and the sex ratio of their offspring in the breeding attempt in the year after manipulation. We found the predicted relationship (Fig. 3): males with experimentally increased reproductive effort in 1993 had more daughters in 1994 than did males with experimentally decreased reproductive effort [$\chi^2 = 3.267$; $df = 2$; ordered heterogeneity test (24): $r_s P_c > 0.8$, $k = 3$, $P < 0.035$, deviance ratio = 0.136]. In contrast, there were no effects apparent on sex ratio of offspring produced by females in relation to prior manipulations of reproductive effort ($n = 22$ females subject to identical manipulations in 1993; $\chi^2 = 0.612$, $df = 2$, $r_s P_c > 0.1$, $k = 3$, $P > 0.4$), despite such manipulations being known to influence future reproductive parameters (e.g., clutch size) in females (21). This analysis does not formally establish a relationship between male forehead patch size and offspring sex ratio, although it does show that females adjust offspring sex ratio in response to some feature of their mate's phenotype. Since the experimental manipulation also caused a change in male forehead patch size ($r_s P_c = 0.781$, $k = 3$, $p < 0.04$; analysis as in ref. 15) the difference in sex ratio with respect to experimental treatment is consistent with the suggestion that sex ratio is adjusted in response to forehead patch size. It is also possible that part of the effect of the experimental manipulations seen in Fig. 3 is caused by the manipulations of paternal effort influencing other, unmeasured variables (e.g., singing rate), which are themselves also correlated with the sex ratio of nestlings produced by females.

Are biases in sex ratio among nestlings reflected in similar biases among offspring that survive to breeding age? Low recruitment rates, typical of small passerine birds, and the fact that some recruitment does not occur until 2 years of age (16) prevent us from testing this hypothesis with the nests analyzed above. However, we analyzed a different and much larger set of birds, which had been subject to similar experimental manipulations between 1983 and 1992 (15, 21). We found the same negative relationship between the degree of experimental manipulation of brood (or clutch) size and the sex ratio of offspring that recruited successfully to the breeding population from the breeding attempt in the year following experimental manipulation (Fig. 4A; $\chi^2 = 5.25$, $df = 2$, $r_s P_c > 0.90$, $k = 3$, $P < 0.02$; deviance ratio = 0.076). A further sex bias in recruitment was observed (Fig. 4B) when we classified females in breeding attempts between 1980 and 1992 as to whether they were mated to a monogamous male or to a polygynous male.

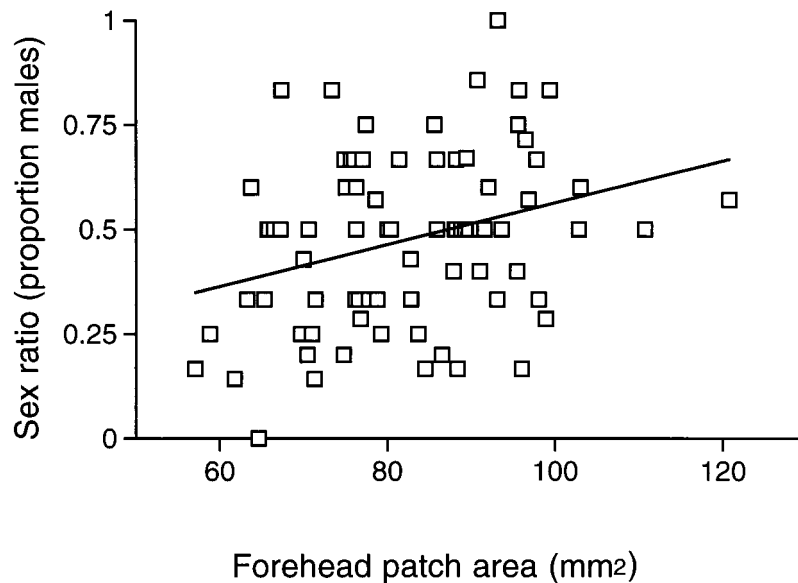


FIG. 2. Relationship between the size of a male collared flycatcher's forehead patch and the sex ratio (proportion males) within his brood. Deviance ratio (corresponding to r^2) = 0.083.

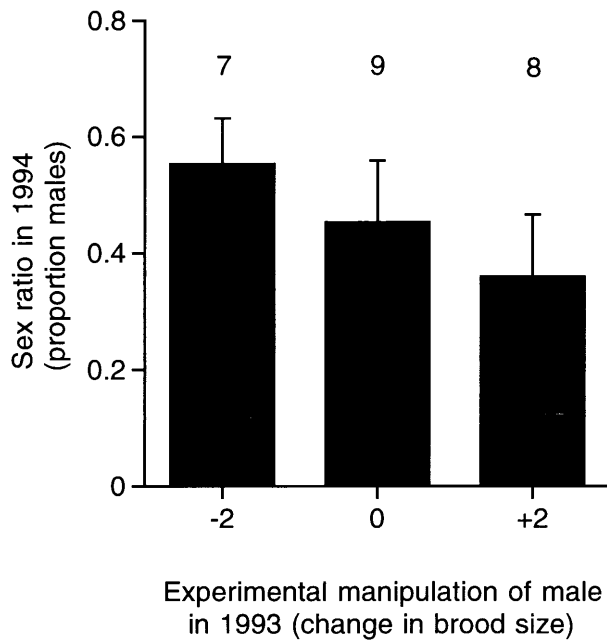


FIG. 3. Sex ratio (proportion male) of offspring born to males in 1994 that had their reproductive effort experimentally manipulated in the previous year (1993). Bars show mean + SE; numbers above bars indicate sample sizes.

Females mated to polygynous males produced more male recruits than females mated to monogamous males ($\chi^2 = 6.814$, $df = 1$, $P < 0.01$). The excess of males produced by females mated to polygynous males is apparently not consistent with sex-biased mortality, since most studies of birds and mammals (11) show that male offspring suffer higher mortality than females in poor environments, such as those experienced in the nests of females mated to polygynous males (16). This argument requires caution however, since most previous studies of mortality in nestling birds (11) have concerned sexually dimorphic species, and it is possible that mortality patterns might be different in the absence of sexual dimorphism. Notwithstanding this, the results again suggest sex ratio adjustment by females in relation to their mate's phenotype, since polygyny is associated with a large forehead patch in this population (15).

Any mortality between ovulation and sampling means that it is not possible to estimate the primary sex ratio within broods, and exclusion of broods from analyses on the basis of mortality having occurred is also flawed, since mortality may be confounded with brood sex ratio (33). Limited mortality (usually due to one egg not hatching or a small nestling dying) occurred in 24/79 (30.4%) of broods that we studied. However, four observations suggest that the patterns we find for nestling sex ratios are due to females altering the sex ratio of eggs ovulated rather than being due to a special case of sex-biased mortality caused by some factor that covaries with the size of their mate's forehead patch. (i) It is difficult to imagine an environmental cause of mortality which affects male and female nestlings differently and that is correlated with the size of a male secondary sexual character, yet is not correlated with timing of breeding, usually the strongest predictor of nestling prospects in small birds (34, 35). (ii) There is no relationship between male forehead patch size and the difference in fledging condition (weight at 13 days of age corrected for body size) of males and females reared in the same nest ($F = 0.007$; $df = 1$, 68; $P = 0.93$; the smaller sample size compared with Fig. 2 is because some broods were single sexed, and not all young were measured). If the sex ratio variation associated with male forehead patch size were the result of either males

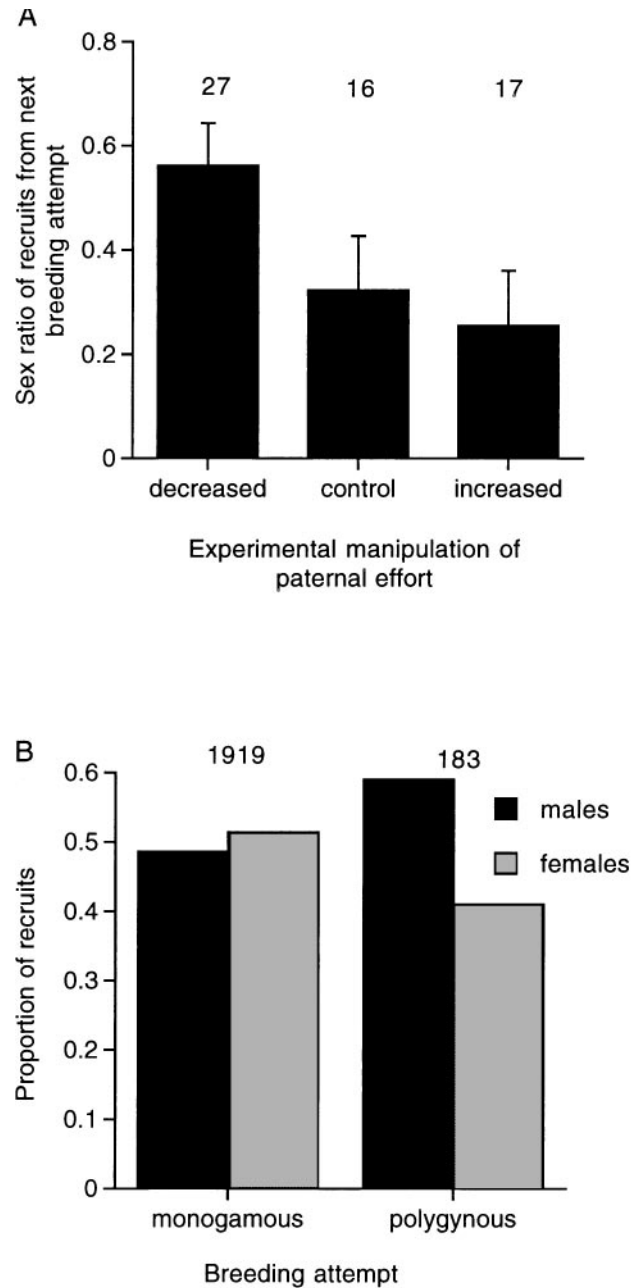


FIG. 4. (A) Relationship between experimental manipulation of male reproductive effort and the sex ratio of recruits from the following breeding attempt by those males (mean + SE; numbers above bars are the number of experimental males). (B) Relationship between breeding status of female and the proportion of male and female offspring recruited from breeding attempts; numbers give sample size (number of breeding attempts).

or females preferentially feeding one sex of nestling, dependent on male forehead patch size, a positive relationship would be expected between these two variables. (iii) In some cases (Figs. 3 and 4A) male nestlings would be required to suffer higher mortality than female nestlings in poor environments, whereas in others (Fig. 4B) mortality would need to be higher for females. (iv) The most conservative assumption possible is that all mortality of nestlings born to males with a patch size greater than that which predicts a sex ratio of 0.5 involved females, whereas all mortality involved males for nestlings sired by males with a patch size smaller than this. Making this assumption, the relationship between inferred sex ratio and patch size is still positive, although no longer statistically

significant [analysis as for Fig. 2: $\chi^2 = 2.003$, $df = 1$, P (one-tailed) < 0.10]. Consequently, we conclude that the patterns that we observe for nestling sex ratios are most consistent with females adjusting the sex ratio of eggs ovulated, rather than resulting from sex-biased mortality.

DISCUSSION

As predicted by sex allocation theory (3–5), we find that the sex ratio within broods of nestling collared flycatchers is apparently adjusted in relation to the phenotype of their father. Further, the sex ratio of offspring born to males is partly dependent on the males' previous (experimentally manipulated) level of parental investment, presumably mediated through a trade-off between paternal effort and secondary sexual character size (15). In both cases, as predicted by theory (3), we find that sex ratio bias occurs in both directions. The mechanism behind the paternal effort secondary sexual character trade-off is unknown, although recent work on birds has suggested that suppression of the immune system resulting from increased energetic expenditure may explain similarly long-term effects of experimentally increased effort (36). The results presented here also suggest that chromosomal sex determination may be less of a constraint to adaptive adjustment of offspring sex ratios than has been argued (7, 8). We suggest that our data are most consistent with adjustment of the sex ratio at the stage of gametes, although we note that a conclusive demonstration of this requires that all broods are sampled before any mortality has occurred (33).

The mechanism by which female birds might adjust the primary sex ratio (the sex ratio of gametes) is unknown, although it has been suggested that hormonal interactions with sex chromosomes might cause differential rates of follicle maturation (29). However, given that the sex of an egg is not determined until the final meiotic division, only hours before ovulation (37), it seems more likely that some form of segregation distortion may occur. In the present species, the high rate of "divorce" between years, and the short interval between pairing and laying (mean interval = 9.4 days; T. Pärt, personal communication) imply that females would be able to influence this mechanism over rather short time scales (in the order of a week). Although it is now clear (this study and ref. 25) that chromosomal sex determination does not completely prevent primary sex ratio adjustment, most studies (refs. 25–28 and this study) find rather weak relationships. This is despite the theoretical prediction that, even with small relative fitness differences between the sexes, large shifts in sex ratio may be expected (38). There are several possible explanations for this discrepancy. Given that the genetic composition of sex chromosomes differs, it has been argued that intragenomic conflict will be particularly prevalent between sex chromosomes, and indeed most known cases of segregation distortion involve loci on sex chromosomes (39, 40). One likely outcome of this form of conflict is a series of adaptations at the genetic and cellular level, resulting from coevolution between the different chromosomes, which have the result of reducing the extent to which segregation distortion can occur (40). Thus, previous evolution of the sex chromosomes, or meiotic processes, due to intragenomic conflict, may constrain the extent to which females could distort segregation. Alternatively, the difference in relative fitness of sons and daughters dependent on their father's patch size may be relatively small. A small difference, coupled with the phenotypic plasticity of patch size (15) acting to introduce error into any estimation of the genetic component of patch size, might reduce selection for sex ratio adjustment in relation to this character.

A recent study (41) of another passerine bird (the blue tit *Parus caeruleus*) has also concluded that females manipulate the primary sex ratio of their offspring in relation to the phenotype of their mate. In this study, females mated to males

which survived the following winter produced male-biased broods. Another study of blue tits (42) found that a male's subsequent overwinter survival was correlated with attractiveness, as determined by his success in obtaining extra-pair copulations. In the study of blue tit sex ratios, as in this work, other aspects of the environment were apparently unrelated to offspring sex ratios. This suggests that sex ratio adjustment relative to the female's mate's phenotype is not likely to be explained by differences in the fitness of sons and daughters resulting from extrinsic factors associated with that mate.

Sex ratio adjustment by female collared flycatchers in relation to the size of a secondary sexual character of their mate would be adaptive if, for instance, large patched males tended to possess higher quality territories or provide higher quality parental care, and the fitness of sons relative to daughters was affected by the amount of parental investment received (3, 4, 11). However, we found no relationships between brood sex ratios in this species, and other variables found to correlate with sex ratio in sexually size-dimorphic bird species (25–28). This, together with the absence of an effect of manipulation of female reproductive effort on future sex ratio, suggests that the relative fitness of sons and daughters is not greatly affected by environmental differences experienced during the nestling stage. Consequently, our finding that brood sex ratios are apparently adjusted by females only in relation to a secondary sexual character of their mate is most readily understood if females obtain genetic benefits (43, 44) for their offspring from mating with those males, and if the magnitude of those benefits is correlated with the size of the secondary sexual character. This would be so whether the secondary sexual character acted as a Fisherian trait (43), where the character simply increases sexual attractiveness, or whether it acts as an indicator of genes conferring higher viability (44). In either case, the requirement is simply that the fitness of a son carrying genes for a large patch is greater than that of a daughter carrying the same genes. Given that (i) male reproductive success is positively related to patch size and (ii) variance in lifetime reproductive success is greater for males than for females, as in this population (15, 16), this is likely to be true. The results of this study are thus most consistent with the idea that females obtain genetic benefits for their offspring by basing mate choice decisions on the size of a male secondary sexual character. Future studies of sex allocation in systems, like this, where environmental differences may have little influence on the relative fitness of sons and daughters have the potential to provide novel insights into patterns and processes of sexual selection.

Note Added in Proof. The gene used for sexing collared flycatchers has recently been identified (45). It is an avian homologue to a previously recognized mammalian gene, CHD, which appears to play a global role in gene regulation. The avian CHD gene is W-linked in probably all nonratite birds and should serve as a general tool for gender identification in such birds.

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