

Egg sex ratio and paternal traits: using within-individual comparisons

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Empirical studies of sex ratios in birds have been limited due to difficulties in determining offspring sex. Since molecular sexing techniques removed this constraint, the last 5 years has seen a great increase in studies of clutch sex ratio manipulation by female birds. Typically these studies investigate variation in clutch sex ratios across individuals in relation to environmental characteristics or parental traits, and often they find no relationships. In this study we also found that clutch sex ratios did not vary in relation to a number of biological and environmental factors for 238 great tit *Parus major* nests. However, interesting sex ratio biases were revealed when variation in clutch sex ratios was analyzed within individual females breeding in successive years. There was a significant positive relationship between the change in sex ratio of a female's clutch from one year to the next and the relative body condition of her partner. Females mating with males of higher body condition in year $x + 1$ produced relatively male-biased sex ratios, and the opposite was true for females mated with lower condition males. Within-individual analysis also allowed investigations of sex ratio in relation to partner change. There was no change in sex ratios of females pairing with the same male; however, females pairing with a new male produced clutches significantly more female biased. Comparisons of clutch sex ratios within individuals may be a powerful method for detecting sex ratio variation, and perhaps female birds may indeed manipulate egg sex but require personal contextual experience for such decisions. *Key words*: body condition, great tits, offspring sex ratios, *Parus major*. [*Behav Ecol* 13:503–510 (2002)]

Natural selection favors individuals that modify investment in male and female offspring when fitness benefits from producing each sex differ (e.g., Charnov, 1982; Fisher, 1930). If offspring sex ratios are representative of the division of resources between sons and daughters, then male-biased sex ratios are expected when the reproductive value of male offspring exceeds that of females and vice versa. Manipulations of the proportions of males and females produced are possible either at the egg stage or through differential investment in offspring during the period of care. Maternal control of egg sex ratios has been convincingly demonstrated in Hymenoptera (e.g., Charnov and Bull, 1977; Herre, 1987), yet investigations of egg sex ratios among birds have yielded disappointing results, despite the potential for egg sex control because of female heterogamety in this taxon.

The interest in egg sex ratio manipulation in birds follows the development of molecular techniques for sexing birds, which previously presented an obstacle to sex ratio studies. These techniques have been mainly used to search for correlational evidence of sex ratio variation in relation to a number of breeding gradients or traits. The premise for such studies is that the reproductive values of male and female offspring may vary with breeding conditions, and hence females benefit from sex-biased investment according to their particular breeding situation. These conditions include timing of breeding, intensity of brood competition (reflected in clutch sizes, brood sizes, or hatching asynchrony), male quality or

attractiveness, female quality, environmental or territory quality, presence or absence of helper offspring, and brood status. Although there is clearly a need for experimental manipulations in further investigations of avian sex allocation, observations of natural sex ratio variation and sex ratio skews can offer a view of natural investment patterns of parents in male and female offspring and the degree to which we can expect sex ratios to be biased in the wild. Furthermore, any particularly consistent, significant trends within species, genera, or even the class should be recognizable.

To date however, evidence for such egg sex ratio biases has been variable. Hatchling sex ratios have been associated with diverse ecological factors or traits in wild bird populations. Sex ratios have been found to vary in relation to resource abundance (Appleby et al., 1997; Komdeur et al., 1997; Korpimäki et al., 2000), timing of breeding (Daan et al., 1996; Dijkstra et al., 1990; Howe, 1977; Lessells et al., 1996; Sheldon et al., 1999; Weatherhead, 1983; Zijlstra et al., 1992), clutch size (Lessells et al., 1996), hatching asynchrony (Lessells et al., 1996), brood status (Nishiumi, 1998; Patterson and Emlen, 1980; Westerdahl et al., 2000), paternal traits (Ellegren et al., 1996; Kölliker et al., 1999; Sheldon et al., 1999; Svensson and Nilsson, 1996; Westerdahl et al., 1997), maternal traits (Blank and Nolan, 1983; Gowaty and Lennarz, 1985; Heg et al., 2000; Nager et al., 1999; Whittingham and Dunn, 2000), harem size (Nishiumi, 1998), and helping activity (Gowaty and Lennarz, 1985; Ligon and Ligon, 1990). In laboratory studies, sex ratio variation has been associated with parental attractiveness (Burley, 1981, 1986) and diet and maternal quality (Bradbury and Blakey, 1998; Kilner, 1998). Many studies, however, report no significant sex ratio biases in relation to a number of variables, even when such associations are sometimes expected (European starling *Sturnus vulgaris*: Bradbury et al., 1997; lesser snow goose *Anser c. caerulescens*: Cooch et al., 1997; Harmsen and Cooke, 1983; corn bunting *Miliaria calandria*: Hartley et al., 1999; western bluebird *Sialia mexicana*: Koenig

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and Dickinson, 1996; blue tit *Parus caroleus*: Leech et al., in press; yellowhammer *Emberiza citrinella*: Pagliani et al., 1999; yellow-headed blackbird *Xanthocephalus xanthocephalus*: Patterson and Emlen, 1980; bluethroat *Luscinia svecica*: Questiau et al., 2000; great tit *Parus major*: Radford and Blakey, 2000; barn swallow *Hirundo rustica*: Saino et al., 1999). It is impossible to estimate how many more studies remain unpublished due to preferential publication of significant results. One study that does uncover a strong primary sex ratio bias in Tengmalm's owl (*Aegolius funereus*) broods can offer no explanation for their observation (Hörnfeldt et al., 2000). Consistent sex ratio trends do not appear to be emerging, though this may be due to inappropriate assumptions on which our expectations of sex ratio biases are based. At present it is difficult to make any generalizations about causes of avian sex ratio variation or about the adaptive nature of skews.

Within species, reports of sex ratio variation have also been inconsistent. In two Swedish blue tit populations, clutch sex ratios have been shown to vary with paternal sexual traits and probability of survival (Sheldon et al., 1999; Svensson and Nilsson, 1996). However, no effect of paternal quality or extra-pair paternity on clutch sex ratio has been found in a British population of the same species (Leech et al., in press). Similarly, in great tits, a positive relationship between hatching sex ratio and male body size has been demonstrated in one population (Kölliker et al., 1999) but not in two others (Lessells CM, personal communication; Radford and Blakey, 2000). Furthermore, hatching sex ratio biases within the same populations have sometimes been inconsistent, with significant sex ratio biases in some years but not others (Koenig and Dickinson, 1996; Korpimäki et al., 2000; Radford and Blakey, 2000).

When studies involve data collected over more than 1 year, data are typically analyzed for all years pooled, with year as a factor in a general linear model (e.g., see Radford and Blakey, 2000, who analyzed years both separately and pooled). We suggest that looking for differences within recaptured breeding individuals across years may be a fruitful alternative approach to analyzing sex ratio variation in wild populations. A within-individual analysis permits identification of factors influencing clutch sex ratio having removed nuisance variables specific to individual birds. Furthermore, as female birds are expected to bias sex ratios according to their particular breeding circumstances, analyzing variation within individual females might present a more powerful method for detecting egg sex ratio skews in relation to the particular breeding environment experienced. This method may provide valuable insight as to individual decisions concerning investment in young of different sexes, yet only two published studies have examined the variance in sex ratio due to variation of a factor within an individual. Westerdahl et al. (2000) examined primary sex ratio variation within female great reed warblers (*Acrocephalus arundinaceus*) breeding in different years and found that individual females had a higher proportion of sons in their brood when they were of primary rather than secondary breeding status. Komdeur et al. (1997) revealed a strong shift in sex ratios of individual female Seychelles warblers (*Acrocephalus seychellensis*) translocated to territories of different quality.

Analysis of sex ratio variation across different breeding attempts also allows investigation of the effect of mate swapping (enforced or chosen), which cannot otherwise be examined. Females might be expected to increase the proportion of sons in their brood if male offspring have higher reproductive values than their sisters do when fathered by a high-quality male. Given that high-quality males are more likely than poorer conspecifics to survive to breed the next year, and also that female great tits may remain faithful to their mate if he is of partic-

ularly high quality (Lindén, 1991), the following consequences for clutch sex ratios can be envisaged. (1) We expect females who mate with the same male to benefit from retaining their high-quality male and produce a higher proportion of sons in their clutches in year 2, but sex ratios of females who change partners not to change over the 2 years. (2) If females are changing males as an active strategy to upgrade their partner, we expect sex ratios of such divorcing females to increase from year 1 to year 2, but those of females who retain their partners to remain constant.

For an island population of great tits, we first analyzed egg sex ratio variation in the traditional between-individual manner and then used a within-individual approach from a set of birds that were caught breeding in more than 1 year. We also explored whether clutches of different sex ratios are of different value to females by looking for associations between sex ratio and hatching success and sex ratio and nest desertion. We encourage similar analyses in other bird populations.

METHODS

Field work

Data were collected from great tits breeding in nest-boxes in 14 separate woodlands on the Swedish island of Gotland (57°10' N, 18°20' E) between 1997 and 1999. For each nest we recorded lay date (first egg), clutch size, hatch date (first egg), and the number of eggs hatching. Parents were caught and ringed (if not already ringed) while provisioning broods between 8 and 14 days after hatching. We measured parental tarsus length (to nearest 0.5 mm), wing length (to nearest 0.5 mm), and mass (to nearest 0.5 g), and we recorded age as either 1 year old or 2+ years. We calculated body condition index of adults as the residual from a linear regression of body mass on tarsus length. Measurements of male birds fitted a linear regression of mass on tarsus length better than mass on tarsus length cubed. There was little difference in fit, however, and we used residuals from mass on simple tarsus length to indicate body condition in analyses. Blood or tissue samples were required from each egg/offspring for sex determination. A 2–10 µl blood sample was taken from 1- or 2-day old nestlings by puncturing the brachial vein and collecting in a capillary, which was then stored in SET buffer at 4°C (1997) or 98% ethanol (1998 and 1999). We collected unhatched eggs 4 days or more after the hatch date of the first egg. (Only on rare occasions do eggs hatch more than 2 days after the first hatch date.) Blastocysts (seen as white spots on the yolk surface) and embryos were dissected out immediately and stored in the same way as blood samples.

Molecular sexing

We determined clutch sex ratios using a polymerase chain reaction (PCR)-based molecular technique from DNA extracted from blood samples or embryonic tissue from unhatched eggs. We used primers P2 and P8 (Griffiths et al., 1998) to amplify introns within the CHD1 gene. PCR products were run on 6% polyacrylamide gel for between 1 and 3 h at 75 W and visualized using silver staining (Promega, 1996). Female nestlings/embryos possessed two different-length copies of the PCR products: CHD1-W from the W chromosome and CHD1-Z from the Z chromosome. Males possessed only one copy, CHD1-Z, because males are homogametic. We sexed 74 individual adults phenotypically, and molecular sex matched in all cases.

Analysis between individuals in 3 years

We analyzed clutch sex ratio variation in relation to a number of factors from pooled breeding data from 238 nests, collected

Table 1
Analysis of proportion of males in broods of great tits from 1997–1999 pooled

	<i>n</i> nests included in analyses	ΔD	Δdf	<i>p</i>
Single factors				
Year	238	1.469	2	>.40
Woodland area	238	5.516	13	>.95
Lay date	197	0.012	1	>.90
Clutch size	227	1.158	1	>.20
Male age	143	0.041	1	>.80
Male tarsus length	173	0.290	1	>.50
Male body condition index	164	0.812	1	>.30
Female age	138	0.678	1	>.40
Female tarsus length	175	0.150	1	>.90
Female body condition index	162	0.302	1	>.50
Interactions with year				
Year * area		29.460	11	>.10
Year * lay date		1.475	2	>.40
Year * clutch size		5.355	2	>.05
Year * male age		1.129	2	>.50
Year * male tarsus length		0.740	2	>.60
Year * male body condition index		0.535	2	>.70
Year * female age		0.096	2	>.95
Year * female tarsus length		2.659	2	>.20
Year * female body condition index		0.185	2	>.90

ΔD is the change in deviance in the model when each factor potentially affecting clutch sex ratio is excluded first from a GLM with binomial errors and logit link. Similarly, Δdf is the change in degrees of freedom when each factor is removed. The associated chi-square test assesses the significance of the change in deviance for each explanatory variable when it was removed from the model.

over 3 years (Table 1). We had data from 276 nests, but 38 individuals breeding in more than 1 year were included only once to avoid pseudoreplication. In each such case the breeding attempt that was included was selected randomly. We measured sex ratio as the proportion of males in a clutch. Because of non-normally distributed error variance and unequal sample sizes, we analyzed the proportional data with a general linear model analysis of deviance, assuming binomial errors, and a logit link function. The response variable was the number of males in a clutch, with the number of eggs sexed as the binomial denominator. Using clutch size as the denominator would lead to overrepresentation of females as not all eggs were sexed, and those we failed to sex would be categorized as “not male” in the analyses. Analyses presented here were weighted according to the amount of information we had for each clutch (i.e., the proportion of a clutch sexed [total sexed/clutch size]). Results did not differ if analyses were repeated on clutches only with complete sex ratio data.

A model including several predictor variables and their second-order interactions with year was first fitted to the data. The significance of a term in the model was determined by assessing the change in deviance after removal of that term, using a chi-square test with appropriate degrees of freedom (Crawley, 1993). Reported in this paper are changes in deviance values after removal of each variable alone rather than sequentially because of the presence of missing cases within the whole data set (see Table 1). A new general linear model was made for each factor, still complete with all other terms and interactions, but excluding cases with missing values for the factor in question. If cases with missing values are not excluded in analyses, the change in degrees of freedom when

the variable in question is dropped from the model is inflated. Results did not differ when variables were removed first from the model or later after stepwise exclusion of other variables. In the latter analyses clutch size was never included simultaneously with other factors in models because number of eggs sexed had been included as the binomial denominator and was not independent of clutch size.

We calculated a heterogeneity factor (HF), the ratio of residual deviance to the residual degrees of freedom, to examine the data for overdispersion. A value of HF < 1 indicates the variance in the data to be less than that expected for a binomial distribution, and HF > 1 indicates more variance than that expected. Here HF = 0.995, and so fitting the data to a binomial model was justified, and scaling analyses by the HF did not change results (Krackow and Tkadlek, 2001). Analyses were carried out using the statistical package GLMStat (Beath, 2000).

Associated questions: analyses with sex ratio as independent variable

We examined the effect of clutch sex ratio on hatching success and nest desertion to investigate whether females with different clutch sex ratios invest differently in those clutches. To test whether hatching success varied significantly with clutch sex ratio, we analyzed the proportion of eggs hatching per clutch with a GLM with binomial errors and logit link. The number of eggs hatching was used as the response variable, with clutch size as the binomial denominator. Sex ratio was included singly as a predictor variable and whether the removal of this term caused a significant increase in deviance was assessed with an *F* test. Model deviance was scaled by the HF (2.81) because the data were overdispersed, hence the application of an *F* rather than a chi-square test. To test whether nest desertion was associated with clutch sex ratio, we carried out a logistic regression analysis of brood desertion in relation to the proportion of males in a clutch. We included year, lay date, and clutch size as potential predictor variables.

Analysis within individuals across 2 years

Due to the fact that females, being heterogametic, have the potential to control clutch sex ratios rather than males, the following analyses were performed on 23 female birds where sex ratios over 2 years were known. Data for individual males where brood sex ratio was known over 2 years is presented for comparison ($n = 23$). Birds were identified which nested in 1998 following 1997 and in 1999 following 1998. First we determined whether clutch sex ratio was repeatable within individuals across years, using a simple regression of sex ratio in the second year against the first. Sex ratio data for females' clutches in year 2 were first arcsine square-root transformed because of their proportional nature (Shapiro Wilks $W = 0.928$; $p = .030$). Other sex ratio data were not transformed (females year 1: Shapiro Wilks $W = 0.964$, $p = .426$; males year 1: Shapiro Wilks $W = 0.982$, $p = .089$; males year 2: Shapiro Wilks $W = 0.975$, $p = .709$). To find the variance in clutch sex ratio due to individual birds, we also ran a general linear model with binomial error structure and logit link including bird ID as a factor, with number of males as the response variable and number of eggs sexed as the binomial denominator. The change in deviance when bird ID was removed from the model indicated the proportion of the variance attributable to between individual differences.

We analyzed the change in egg sex ratio from one year to the next for females, in relation to the change in their own body condition, laying date and clutch size, with linear regressions. Not enough data existed to test for effects of age,

Table 2
Proportion of male eggs laid in each of 3 years and analysis of whether this differed significantly from 0.5 using G tests for goodness of fit

Year	n nests	No. of males	Total chicks sexed	Proportion males	G_i	p
1997	26	93	206	0.45	1.944	>.10
1998	90	389	788	0.49	0.127	>.70
1999	122	536	1040	0.52	0.985	>.30

Sample sizes are greater for 1998 and 1999 because of increased sampling effort in the field.

although it is possible that breeding experience of females may influence clutch sex ratios, especially comparing first-time breeders with others (e.g., Blank and Nolan, 1983; Heg et al., 2000). In the same way, we analyzed the change in females' sex ratio in relation to the change in their partners' quality relative to that of their mate the previous year. Analyses were repeated for males. The independent variable change in sex ratio (sex ratio year 2–sex ratio year 1) was not transformed because data were distributed normally (females: Shapiro Wilks $W = 0.976$, $p = .841$; males: Shapiro Wilks $W = 0.975$, $p = .792$).

Over two breeding attempts, a female may mate with either the same male in both years (same pair) or change her partner (new pair). We compared the clutch sex ratios of same-pair and new-pair females. We repeated these analyses for males. Retaining the male or mating with a new partner may be an active female choice (i.e., divorce) or a passive decision through survival of the male to the next breeding season (i.e., widowed). In this population, over a 3-year period, 24 new-pair females were caught. Of these 24, in only 3 cases was the original male caught breeding elsewhere in year 2. Admittedly, the other 21 males may have been breeding in natural holes, but these data strongly suggest that females mate with a new partner because of male overwinter mortality rather than choice. In this case we expect females mating with a male in good condition (assumed to be of high quality) to produce higher proportions of male eggs in year 2, whereas females pairing with a different male are not expected to change clutch sex ratios (scenario 1 presented in Introduction). We also examined whether there was a difference in the clutch sex ratio of females in the first breeding attempt according to their future mate category, same pair or new pair.

RESULTS

Analysis between individuals in 3 years

A total of 238 nests with known sex ratios were included in the analysis. Of these, 139 nests had complete sex ratio data (i.e., proportion of eggs sexed = 1). If data were missing, it was usually not more than 1 egg per nest; for 204 nests (86%), the proportion of eggs sexed was > 0.8. In total we sexed 2034 individual eggs and nestlings. Overall population sex ratios analyzed at the level of the nestling showed no deviation from a 1:1 sex ratio in any year (Table 2). For the 3 years pooled, the overall sex ratio of broods showed binomial distribution of the sex ratio (residual deviance/residual degrees of freedom = 236/237 \approx 1).

Analysis of the proportion of males per clutch for all 3 years pooled provided no evidence of systematic biases in sex ratio in relation to any factors included in a general linear model with binomial errors. Table 1 lists variables fitted to the model

and the change in deviance and related p value when each term was removed individually from the full model. No variables explained significant variation in the proportion of males in a brood, whether they were removed first from the model or later after stepwise exclusion of other variables which contributed least to the variance.

Associated questions: analyses with sex ratio as independent variable

The proportion of eggs hatching did not depend on the proportion of male eggs in the clutch ($\Delta D = 2.122$, $p > .050$, $df = 1$). Neither was there any indication of the proportion of male eggs affecting the likelihood of nest desertion ($\chi^2_1 = 0.323$, $p = .570$, $n = 238$).

Analysis within individuals across 2 years

There were 51 cases of female birds nesting in one year being recaptured in a subsequent breeding attempt, and 44 cases of male breeding recaptures. Of these, sex ratios of broods in both years were known for 23 females and 23 males (not necessarily paired). One female and two males were caught breeding in all 3 years. In these cases one breeding attempt was selected at random for each individual to be included in the analysis to avoid pseudo-replication in the data set. Not all data (e.g., exact lay dates, adult measures) were available for breeding attempts in both years; therefore sample sizes in analyses may not always equal 23.

A simple linear regression of egg sex ratio of the second breeding attempt against the first suggested that sex ratio was not repeatable across years either for females ($F_{1,20} = 1.094$, $p = .308$) or males ($F_{1,20} = 0.117$, $p = .736$). We examined the variance in sex ratios due to individual birds in a general linear model with binomial error structure, essentially testing whether differences within an individual were smaller than differences between individuals. An insignificant proportion of the variance in sex ratio was accounted for by between-subject differences (females: $\Delta D = 24.41$, $\Delta df = 22$, $p > .30$, $HF = 1.3$; males: $\Delta D = 25.29$, $\Delta df = 22$, $p > .20$, $HF = 1.2$).

The following results involve analyses of the change in sex ratio within individual birds (i.e., sex ratio year 2–sex ratio year 1) with respect to the change in predictor variables from one year to the next. The change in egg sex ratio of female birds was positively related to the change in body condition of the male to which she was mated ($F_{1,9} = 9.698$, $p = .012$; Table 3 and Figure 1a). Applying a Bonferroni correction for multiple comparisons gives a new p statistical threshold of .013, and our result remains significant below this corrected value (Table 3). Females did not alter the sex ratio they produced in response to changes in any other variables—either to their own body condition or with respect to clutch size or laying date (Table 3). In comparison to females, clutch sex ratios of males breeding over successive years did not change consistently with regard to their mate's quality (Figure 1b).

Given this result, we returned to the first analysis between all individuals across all years to test for a relationship between male body condition and clutch sex ratio, but restricting analyses to only females aged 2 years or more (i.e., not their first breeding attempt). Among these 50 females there was no relationship between absolute male body condition and sex ratio ($\Delta D = 0.08$, $\Delta df = 1$, $p > .70$, $HF = 1.04$). It appears that females respond to male body condition relative to previous breeding attempts; at least we could detect no change in sex ratio in relation to absolute male body condition, even for experienced breeders only.

Finally, we compared the sex ratios of same-pair and new-pair males and females. Same-pair females showed no change

Table 3
Comparisons of changes in clutch sex ratio in relation to changes in breeding situation, mate's condition, and own condition in successive breeding attempts of great tits on Gotland

Variable	<i>n</i>	df	<i>F</i>	<i>p</i>
Recaptured females				
Change in laying date	18	1	0.024	.879
Change in clutch size	22	1	1.467	.240
Change in partner's (males) body condition index	11	1	9.698	.012
Change in own body condition index	13	1	0.914	.360
Recaptured males				
Change in laying date	17	1	0.777	.392
Change in clutch size	21	1	0.557	.465
Change in partner's (females) body condition index	20	1	0.010	.922
Change in own body condition index	18	1	1.135	.263

Results of individual regressions.

in their clutch sex ratio ($t = 0.265$, $p > t = 0.398$), but those pairing with a new male showed a decrease in the proportion of males in their clutch ($t = -2.103$, $p < t = 0.034$; Figure 2a). The difference between the proportion of males in broods of same-pair and new-pair females was not statistically significant ($t = 1.498$, $p = .156$, $n = 20$). Clutch sex ratios of same-pair and new-pair males did not differ ($t = -0.590$, $p = .563$, $n = 20$; Figure 2b). There was no difference in clutch sex ratios in the first breeding attempt between same-pair and new-pair females ($t = -1.037$, $p = .309$, $n = 29$).

DISCUSSION

Despite abundant theories and recent interest in egg sex ratio studies in birds, empirical evidence of consistent sex ratio biasing by females is elusive. Identifying factors responsible for sex ratio biases through a correlative approach across individuals is a commonly used method for identifying relationships between variables and primary sex ratio variation. Like many other investigations, we found no correlative evidence of primary sex ratio biasing by great tits over 3 years, from a large data set of nearly 240 broods. Brood sex ratio did not vary in relation to several environmental variables or parental traits (Table 1).

Previous investigations of heterogeneity in great tit brood sex ratios at the egg stage have been published from three different populations. In a Dutch population, brood sex ratios increased with hatching date and hatching asynchrony, where-

as they decreased with increasing clutch size (Lessells et al., 1996). The proportion of sons in broods of a Swiss population of tits increased significantly with increasing male tarsus length, and there was a similar, though nonsignificant, trend with increasing breast stripe size (Kölliker et al., 1999). (Male breast band stripe was not measured in the present study because of time constraints of fieldwork.) Great tit females prefer males with larger breast stripes (Norris, 1990), and male tarsus length correlates with breeding success (Blakey, 1994; Verboven and Mateman, 1997). The relationship between sex ratio and male traits was interpreted as a female response to either male genetic quality or body-size related territory quality (or both). This relationship with tarsus length was not apparent in the present study, despite a much larger sample size ($n = 173$ compared to $n = 57$), nor in the Dutch population.

A third great tit study suggested one potential explanation for the discrepancy in these findings. Radford and Blakey (2000) found significant predictors of brood sex ratio from a correlational analysis based on 5 years of breeding data from British great tits. These included lay date (1993), male age (1998), male tarsus length (1991), female tarsus length (1991 and 1998), and female condition (1991). However, no relationships were consistent across years, and no variables predicted sex ratios when data for all years were combined (Radford and Blakey, 2000). They argued for evaluation of breeding data from several years in order to identify consistent sex ratio biasing and confirm whether sex ratio manipulation is truly a female breeding strategy. The previous sex ratio biases

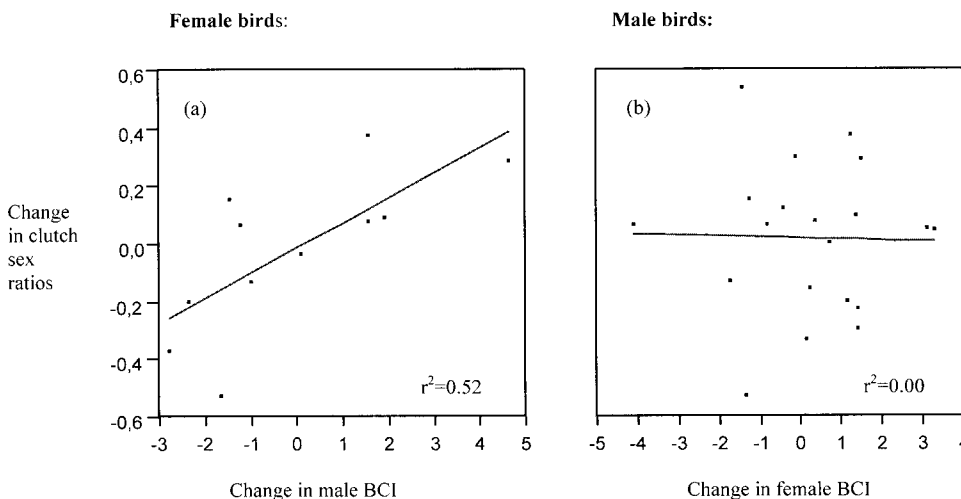


Figure 1
 Change in clutch sex ratio measured as the proportion of sons in a clutch (year 2) minus the proportion of sons in a clutch (year 1) in relation to the change in partner's body condition index, for recaptured breeding (a) females and (b) males. Body condition index is the residual mass from a correlation of mass on tarsus length. Figures show points for both same-pair and new-pair individuals pooled.

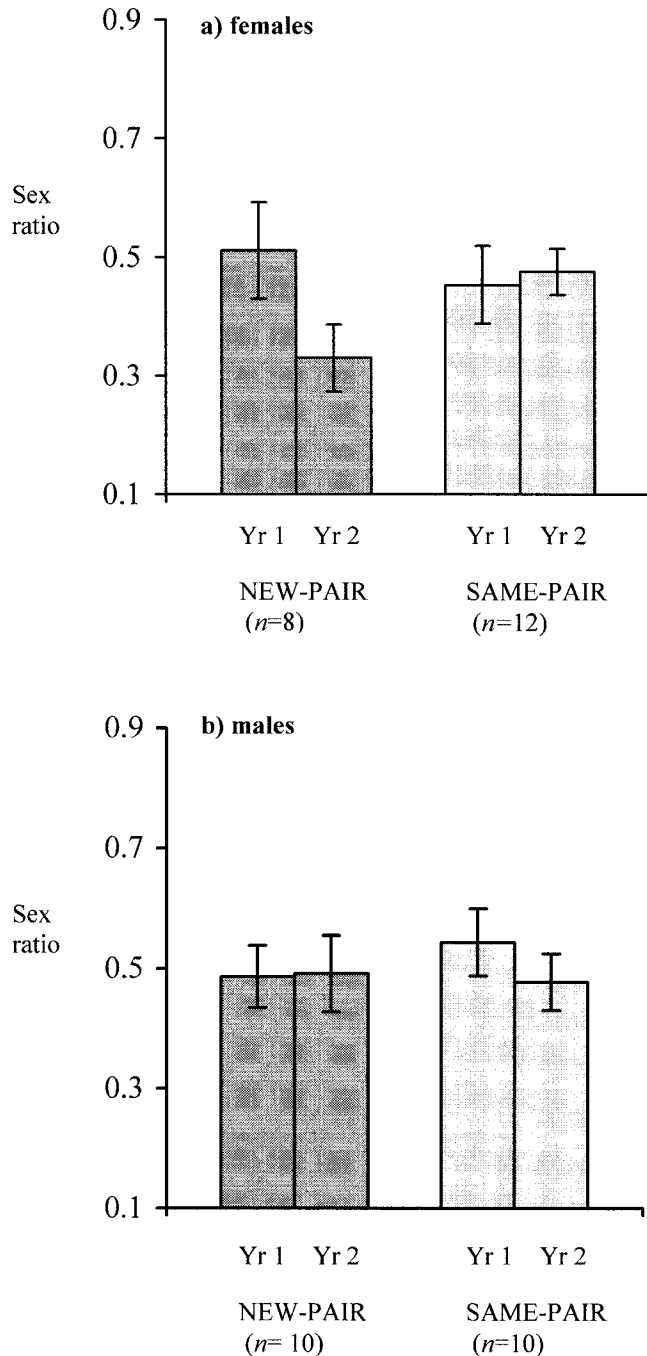


Figure 2
Clutch sex ratios (\pm SE) in first and second breeding attempts among same-pair and new-pair (a) females and (b) males.

reported from the Swiss and Dutch populations examined sex ratio biases over 1 year, and one of these relationships has proved unrepeatable in subsequent years (Lessells CM, personal communication).

These previous studies have all examined sex ratio variation among individuals. As we expect sex-ratio adjustment to be performed by a female in response to her particular situation, adjustment is unlikely to be detected by averaging all individuals of a population because they face various microenvironments. Fine-tuned sex-ratio adjustment in response to particular factors is more likely to be detected when examining variation within female individuals, as done here. By using a with-

in-individual approach, we found interesting results for a sample of great tits in which no variables explained significant variance in observed sex ratios using a traditional analysis with year as an explanatory variable.

We found that females mated to males of better condition relative to their last breeding attempt tended to increase their clutch sex ratios, and females mated with males in relatively worse condition adjusted their brood sex ratios negatively (Figure 1). This relationship between sex ratio and mate's body condition was evident for recaptured females but was absent for recaptured males because in birds the female is heterogametic and thus responsible for egg sex ratios. Our results suggest bidirectional female control of sex ratios. They are complementary to those found by Kölliker et al. and from blue tits (Sheldon et al., 1999; Svensson and Nilsson, 1996), where females were found to adjust sex ratios to increase the proportion of males in their brood with increasing male quality. This could be considered adaptive if high-quality (good body condition) males father high-quality sons relative to daughters. Body condition has been shown to be moderately to highly heritable in the closely related blue tit (Merilä et al., 1999), although how this varies with offspring sex is not known. Heritability estimates are determined from regressions of offspring measures on measures from the same-sex parent (i.e., male-father regressions and female-mother regressions; Falconer, 1981). Male-father regressions have revealed high heritability values for body weight (van Noordwijk et al., 1980) among great tits and similarly high heritability of both weight and tarsus length in another passerine with similar breeding ecology (Gustafsson, 1986). With a significant heritable component of body condition, females mating with males in better condition could potentially increase grand-offspring production through rearing more male offspring in their broods.

If all females modify sex ratios in respect to their partner's condition, one might expect a positive relationship between absolute male body condition and clutch sex ratios, which was not evident from a much larger data set ($n = 164$), even when the analysis was restricted to females aged 2 years or more ($n = 50$). One explanation for this discrepancy may be that females are responding to relative body condition based on that of previous mating events, and without a benchmark females are unable to respond to partner condition. Female control of the sex ratio could then only be expected to evolve if a significant proportion of the female population bred in more than 1 year. In this population, between 35% and 46% of female birds breed in more than 1 year (Oddie and Reim, unpublished data).

There was further evidence of the importance of relative mate quality from analyses of partner fidelity. Same-pair females tended to have higher clutch sex ratios relative to the last breeding attempt than new-pair females, although this result is not statistically significant. Same-pair females showed no change in sex ratio from one year to the next, but new-pair females produced a higher proportion of female eggs. Clutch sex ratios in the first breeding attempt did not differ between same-pair and new-pair females. This further indicates that the differing sex ratios of same-pair and new-pair females is a result of decreasing sex ratios among new-pair females, rather than an increase in sex ratio among same-pair females. The result differs from our prediction of increasing sex ratios among same-pair females and constant sex ratios among new-pair females; however, new-pair females do still show a decrease in sex ratio compared to same-pair females. In great tits, newly formed pairs have lower breeding success than birds previously breeding together (Perrins and McCleery, 1985), and pairs that enjoy high reproductive success together are more likely to remain together (Lindén, 1991). Females with a new partner may be less sure of his parenting

abilities (e.g., food provisioning) than those paired to the same male, and consequently produce more of the relatively smaller sex (females; e.g., Oddie, 2000; Perrins, 1963), which require less food. Alternatively, new-pair females may produce more female offspring in their next clutch because of intrinsic male qualities (i.e., they are mating with inferior males). Perhaps in great tits maintenance of the sex ratio in a second year of breeding is a luxury only afforded by those who mate with the same partner, and the default tactic is to decrease the sex ratio with a new partner.

Together these results suggest that investigating sex ratio variation within individuals over different breeding attempts may provide a more powerful means of identifying factors causing sex ratio skews in birds than a conventional across-individuals approach. These analyses have two advantages. First, within-individual analyses of sex ratios control for any unexplained variation due to individual birds. Second, they allow detection of sex ratio changes relative to previous breeding experiences, if a previous breeding attempt is a prerequisite for sex ratio biasing. Two previous studies have examined repeatability of sex ratios of individual females (Appleby et al., 1997; Westerdahl et al., 1997), and two others have found sex ratios to vary within individuals according to breeding territory (Komdeur et al., 1997) and breeding status (Westerdahl et al., 2000). No studies have considered within-individual variation in relation to more than one determining factor. We found that great tit clutch sex ratios of individual birds were not repeatable across years, suggesting that the proportion of males in a brood is not fixed for each female but varies between breeding attempts. The fact that we also found clutch sex ratio variation among male birds between years may lead us to question any conclusions that could be drawn. At least this variation allows that birds may potentially adjust sex ratios according to environmental or mate characteristics.

One significant limitation of within-individual comparisons is that sample sizes will inevitably be limited due to low numbers of birds caught breeding in successive years. Data presented here suggest that females may adjust egg sex ratios in relation to mate quality (see also Burley, 1981, 1986; Ellegren et al., 1996; Kölliker et al., 1999; Sheldon et al., 1999; Svensson and Nilsson, 1996). It would be interesting to repeat these investigations using within-individual comparisons of primary sex ratios from much larger data sets. We hope this study will encourage further within-individual analyses. Furthermore, we advocate accompanying experimental approaches to demonstrate causal relationships in sex ratio studies—for example, experimental manipulation of partner choice (e.g., partner removal experiments) and its effects on relative sex ratio. However, whether studies are experimental or correlational, we believe that a new within-individual approach could reveal egg sex ratio biases, as females respond to their particular breeding conditions.

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