Facultative sex ratio adjustment in response to male tarsus length in the Varied Tit Parus varius

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During the last decade, evidence from a number of studies has suggested systematic deviations from a 1 : 1 primary sex ratio in birds, in spite of the fact that birds have chromosomal sex determination systems; the mechanism of sex allocation is not fully understood. However, it still remains uncertain whether adaptive manipulations of primary sex ratio occur, especially in Parus species. We studied sex ratio variation in the Varied Tit Parus varius, which is a socially monogamous species similar to the Great Tit P. major and the Blue Tit P. caeruleus. In total, 362 chicks that hatched from 72 broods over 3 years were sexed. Of all nestlings, 51.9% (188/362) were male. The nestling sex ratio did not differ significantly from unity. However, the proportion of sons in each brood was significantly and positively related to the father’s tarsus length. This corresponds with our predictions, given that larger males have higher resource holding potential if tarsus length is a heritable character between fathers and sons.

Under the conditions of random mating and equal quality of parents and offspring, natural selection favours a 1 : 1 primary sex ratio (Darwin 1871, Fisher 1930). However, individual mothers may manipulate primary sex ratio as a conditional strategy. Trivers and Willard (1973) first defined specific conditions under which systematic deviations of primary sex ratio are favoured. The conditions assumed by Trivers and Willard are (1) a variation in body condition of adult females, (2) a correlation between the body condition of the mother during parental investment and the body condition of the young at the end of parental investment, (3) a tendency for the body condition of the young at the end of parental investment to be maintained into adulthood and (4) stronger effects of such adult differences on male reproductive success than on female reproductive success. This original theory of Trivers and Willard has recently been extended to cases of sexually dimorphic birds: if a female pairs with a male that has a heritable secondary sexual character that indicates his reproductive success is higher than that of the average male, and if the variance in reproductive success of males is greater than that of females, then it would be advantageous for that female to produce more sons.

During the last decade, there has been an accumulation of evidence for systematic deviations of this primary sex ratio in birds (see Sheldon 1998, West & Sheldon 2002), although the mechanism of sex allocation is not fully understood (Krackow 1995). The prediction of a positive relationship between sex ratio and a sexually selected character has been supported by some recent studies (forehead patch size in the Collared Flycatcher Ficedula albicollis: Ellegren et al. 1996, UV reflectance in the Blue Tit Parus caeruleus: Sheldon et al. 1999, male survival in the Blue Tit: Svensson & Nilsson 1996, breast band stripe in the Great Tit P. major: Kölliker et al. 1999). However, other studies have found no relationship between brood sex ratio and paternal characters (e.g. tail length in the Barn Swallow Hirundo rustica: Saino et al. 1999, tarsus length in the Great Tit: Radford & Blakey 2000, testosterone level in the Dark-eyed Junco Junco hyemalis: Gringstaff et al. 2001, biometrics, age and mite loads in the Blue Tit: Leech et al. 2001). A recent meta-analysis of 11 studies testing the relationship between sex ratio and mate attractiveness found overall that mate attractiveness had a significant effect on brood sex ratio variation although the effect size was not large (West & Sheldon 2002). Hence uncertainty remains as to whether adaptive deviations of primary sex ratio in response to paternal characters are found in birds. In particular, in Parus species, results have been confusing, with studies both failing to support (the Great Tit: Radford &
Blakey 2000, the Blue Tit: Leech et al. 2001, Oddie et al. in press) and corroborating (the Great Tit: Kölliker et al. 1999, Oddie & Reim 2002, the Blue Tit: Svensson & Nilsson 1996, Sheldon et al. 1999) the Trivers and Willard hypothesis. Evidence is thus far inconclusive, and further work is necessary.

We studied sex ratio variation in the Varied Tit P. varius. This species is socially monogamous, as are both the Great Tit and the Blue Tit. A previous study of this species (Yamaguchi & Kawano 2001) revealed that large males (i.e. males with large tarsi) tend to have a high resource holding potential (RHP) in the study population. Larger males won aggressive interactions and obtained a breeding place more frequently than smaller males (Yamaguchi & Kawano 2001). When males cannot obtain a breeding location, their reproductive success is likely to be zero. Therefore, the variance of lifetime reproductive success of males may be larger than that of females if almost all females are able to find a mate and produce offspring at least once in their lives. Therefore, it should be adaptive for females to adjust sex ratio in relation to the mate’s body size if tarsus length is a heritable character. Tarsus length is heritable in other Parus species (the Great Tit: Riddington & Gosler 1995, Kölliker et al. 1999, the Blue Tit: Merilä & Wiggins 1995, Merilä & Fry 1998, the Willow Tit P. montanus: Thessing & Ekman 1994, Thessing 1999). The Varied Tit is essentially sexually monomorphic in plumage except that the white forehead area and black breast patch of males are larger than those of females (N. Yamaguchi unpubl. data). The variation in male traits did not, however, affect the RHP (Yamaguchi & Kawano 2001), as in other passerine species such as the Collared Flycatcher (white forehead patch; Part & Qvarnström 1997) and Great Tit (breast stripe; Jarvi & Bakken 1984, Lemel & Wallin 1993, Norris 1993). Because the white area on the forehead is roughly rectangular in shape, we calculated its area by multiplying its width by its length. Because the breast black patch is roughly triangular in shape, we calculated its area by half the product of width \times length. In 1999 and 2000, the tarsus lengths of parents and chicks (10 days old) were measured for the analysis of parent–offspring correlation. We marked parents and chicks with metal rings authorized by the Japan Environment Agency, and by unique combinations of coloured plastic rings.

**Molecular sexing**

DNA was extracted from the blood sample using the phenol chloroform extraction technique, and birds were sexed molecularly (Ellegren 1996). Primers 2945F and 3224R were used to amplify 630 base pair (bp) fragments in both sexes, and 2945F and cfr were used to amplify 210 bp fragments that were sex-linked. Amplified products were visualized by electrophoresis in 1.6% agarose gel. Accuracy of molecular sexing was confirmed for 18 individuals (nine males and nine females) that had been sexed previously based on whether they incubated; only females incubate eggs in the Varied Tit (Nakamura & Nakamura 1995). All sexes determined molecularly matched phenotypic sex.

**Statistical analysis**

For the analysis of brood sex ratio and offspring sex, we used generalized linear models (GLM) with binomial errors and a logit link (McCullagh & Nelder 1989). Analyses were performed in the package of ‘R’ (http://www.r-project.org/). We first constructed

**METHODS**

**Data collection**

Varied Tit nests were monitored in 1998, 1999 and 2000 in the Fukuoka City Nature Reserve on Mt Aburayama, Fukuoka, Japan (33°31’N, 130°23’E). We erected 92, 126 and 182 nestboxes in 1998, 1999 and 2000, respectively. We visited each nestbox regularly at 3-day intervals to record clutch size, brood size and laying date. Ten days after hatching, nestlings were caught and blood samples (50–100 µL) were obtained by brachial venipuncture. Parents were also caught when chicks were 10 days old and their tarsus length, white forehead area and black breast patch were measured. The white forehead area and black breast patch were measured because these plumage characteristics may serve as sexually selected characters, as in the Collared Flycatcher (white forehead patch; Part & Qvarnström 1997) and Great Tit (breast stripe; Jarvi & Bakken 1984, Lemel & Wallin 1993, Norris 1993). Because the white area on the forehead is roughly rectangular in shape, we calculated its area by multiplying its width by its length. Because the breast black patch is roughly triangular in shape, we calculated its area by half the product of width \times length. In 1999 and 2000, the tarsus lengths of parents and chicks (10 days old) were measured for the analysis of parent–offspring correlation. We marked parents and chicks with metal rings authorized by the Japan Environment Agency, and by unique combinations of coloured plastic rings.
the initial model that contains all of the factors and interactions with year. Then, via a series of stepwise deletion tests, any non-significant explanatory variables and interaction terms were removed (Hardy 2002). The statistical significance of the terms in the model is determined by calculating the deviance of the model with and without those terms. The deviance is distributed approximately as a chi-squared distribution (Sokal & Rohlf 1995). We used the proportion of sons as the dependent variable, and used brood size as the binomial denominator (we could sex all chicks hatched). When we evaluate the effect of unhatched eggs on brood sex ratio bias, we used clutch size as the binomial denominator.

Kendall’s τ with a blocking variable (Korn 1984) was used for the test of parent–offspring correlation of tarsus length. Data from each year were considered as a block. Because the mean tarsus length of male chicks was slightly but significantly greater than that of females (mean tarsus length: 17.791 mm (sd = ±0.848) for male chicks; 17.527 mm (sd = ±0.872) for females, n = 52 [four broods in which all chicks were females were not included in the analysis]; Wilcoxon’s signed-ranks test: z = −2.601, P = 0.009), we weighted the means for variation in the number of sons and daughters in a brood (Kölliker et al. 1999). Mean chick tarsus length was calculated as the mean of the two sex means of nestling tarsus length.

RESULTS

We sexed a total of 362 chicks that hatched from 72 broods over three different years (16 broods in 1998, 23 broods in 1999 and 33 broods in 2000). There was no nestling mortality before sampling. In 19 broods, some eggs did not hatch (three eggs from three broods in 1998, nine eggs from six broods in 1999 and 11 eggs from nine broods in 2000). Of all nestlings, 51.9% (188/362) were male (Fig. 1). This sex ratio did not differ significantly from unity (binomial test, n = 362, P = 0.28, power = 0.948). When data from each study year were analysed separately, the overall sex ratio still did not differ significantly from parity in any of the three study years (56.4% were sons in 1998, 51.3% in 1999 and 50.3% in 2000).

Over the three years, we caught 59 male parents (ten in 1998, 18 in 1999, 31 in 2000) and 55 female parents (three in 1998, 20 in 1999, 32 in 2000) in the study seasons. Both parents were caught for 47 broods. The proportion of sons in a brood was significantly and positively related to the father’s tarsus length (Table 1, Fig. 2). However, neither the size of

![Graph](image)

**Figure 1.** Frequency distribution of the sex ratio for 72 Varied Tit broods.

**Table 1.** Analysis of a generalized linear model with the proportion of sons as the response variable.

<table>
<thead>
<tr>
<th>Terms</th>
<th>n</th>
<th>df</th>
<th>ΔD</th>
<th>P</th>
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<td>0.192</td>
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<td>Male breast patch size</td>
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<td>Female breast patch size</td>
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<td>Year*Female forehead area size</td>
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<td>Year*Female breast patch size</td>
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<td>Male tarsus length</td>
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<td>9.090</td>
<td>0.003</td>
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</table>

The model is binomial with a logit link. Residual deviance of initial model = 25.500 on 25 df (P = 0.435; Chi-squared test), null deviance = 49.108 on 43 df. Residual deviance of final model = 54.704 on 57 df (P = 0.562; Chi-squared test), null deviance = 63.794 on 58 df. The deviances shown are the changes in deviance when the term is removed from the final model.
Sex ratio adjustment in the Varied Tit

The proportion of sons in a brood was significantly and positively related to the father’s tarsus length as predicted if tarsus is a heritable trait. Female Varied Tits may produce male-biased broods when they mate with large males, because large males tend to have high RHP (Yamaguchi & Kawano 2001). The size of the white forehead area and size of the black breast patch were not related to the brood sex ratio in any way. When the unhatched eggs were assigned to a sex that would be conservative under the null hypothesis of a 1 : 1 sex ratio, the influence of male tarsus length on brood sex ratio remained. Hence, sex-specific mortality of embryos probably did not cause the positive relationship between brood sex ratio and the father’s tarsus length, although there was no significant relationship between male tarsus length and brood sex ratio when unhatched eggs were assigned under the most conservative hypothesis ($H_0$: opposite directional adjustment). There was no significant correlation between male and female tarsus length. Including female tarsus length in the model, the relationship does not change. Hence, the bias in sex ratio was directly linked to male tarsus length and did not arise indirectly via female tarsus length (i.e. due to assortative mating).

Male body size, as estimated from tarsus length, has an important effect on a male’s RHP in the study population (Yamaguchi & Kawano 2001). Larger males tend to obtain a breeding place (a nest-hole) more frequently than smaller males. When males cannot obtain a nest-hole, they are unlikely to reproduce successfully during the breeding season. The study area consists of a mosaic of Japanese cedar plantations and young broad-leaved trees. It is unlikely that many Tits breed in natural cavities because there are few old trees that supply holes for nesting. In male Tits therefore reproductive success depends on their body size and it controls the probability of producing any offspring at all. In female Tits, tarsus length tends to be correlated with clutch size and the number of fledglings ($n = 56, \tau = 0.339, P < 0.01$; Kendall’s rank correlation test, year was
taken as a block). This suggests that reproductive success depends partly on body size in females too. However, we believe there to be less variation in mating success amongst small females than among small males, because the lack of assortative mating observed in this population suggests that body size does not control the probability of female breeding. It would therefore be adaptive that female Tits adjust sex ratio in relation to the mate’s body size if this trait is heritable. However, we could not find a correlation between male tarsus length and mean chick tarsus length, although female tarsus length correlated positively and significantly with mean chick tarsus length. Although we have not found evidence for extra-pair copulation (EPC) in this population, it is possible that it occur; however, it is unlikely that the frequency of EPC would be so high that it significantly obscured the correlation between tarsus lengths of putative father and offspring.

The plumage characteristics of this species (white forehead area and black breast patch) did not affect the RHP (Yamaguchi & Kawano 2001), contrary to findings in the Collared Flycatcher (Part & Qvarnström 1997) and Great Tit (Jarvi & Bakken 1984, Lemel & Wallin 1993). If these characters are heritable, a female Varied Tit that mates with a male possessing a large forehead patch and/or breast patch gains no enhanced reproductive value through biasing her brood’s sex ratio towards sons. Therefore, as we expected, but unlike the Collared Flycatcher (Ellegren et al. 1996) and Great Tit (Kölliker et al. 1999), Varied Tits did not adjust their brood sex ratio in response to plumage characteristics.

As stated above, the results of studies of primary sex ratio in tits have been equivocal. Whereas our study supported the Trivers and Willard hypothesis, as did the studies of Kölliker et al. (1999) and Sheldon et al. (1999), other studies have failed to do so despite their use of large samples (e.g. Radford & Blakey 2000, Leech et al. 2001). It is not known why the facultative manipulation of brood sex ratios appears to occur in some populations but not in others, although the difference in the intensity and/or direction of selection pressures between populations may be an important factor (Dale et al. 1999, Griffith et al. 1999). In Parus species, perhaps maternal sex ratio adjustment in response to male tarsus length does not occur when available nest-holes are abundant in the population, because small males, which have low RHP, could also obtain nest-holes, reducing the amount of variance in reproductive success between individual males. There are probably very few potential nest-holes in our study area (see above). Unfortunately, however, we did not investigate the abundance of natural nest-holes available.

Little is also known about temporal variation in sex ratio adjustment in birds (but see Oddie & Reim 2002). Most studies that find relationships have only been carried out in one year. It is possible, if not actually probable, that interannual variation in the environment (e.g. population density, nesting place abundance, food resource abundance, operational sex ratio and divorce rate) affects sex ratio adjustment. Further investigations of sex ratio adjustment in birds would benefit greatly from estimates of the variance in reproductive success between sexes, and spatial and temporal variation in these differences, in order to arrive at sensible predictions of sex ratio adjustment patterns.

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