

Offspring sex ratio of a forest songbird is unrelated to habitat fragmentation

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## Abstract

We assessed whether the sex ratio of nestling Marsh Tits (*Poecile palustris*) varied with habitat fragmentation by sampling broods from two areas of Britain with contrasting forest patch size, and comparing with published results from extensive forest in Poland. We found no evidence for manipulation of offspring sex ratios at the population level, supporting previous results and suggesting that this trait may be typical of the species. The results are important in helping to understand the origin of biased sex ratios observed among adult Marsh Tits in the declining population in Britain.

## Introduction

Sex-based differences in life history are frequent among birds, resulting in biased selection that skews the sex ratio in populations (Donald 2007). Whether birds have facultative control of the sex allocation of their offspring via a genetic or behavioural mechanism, as a means of addressing or exploiting this bias, is a controversial topic that has produced mixed empirical results (reviewed in Hasselquist and Kampenaaers 2002; Ewen et al. 2004).

At the population level, a biased adult sex ratio may be expected to result in parents producing more offspring of the rarer or more competitively advantaged sex, in order to improve the settling and breeding potential of their progeny (Bensch et al. 1999; Stauss et al. 2005). Under environmental stress, however, such as deteriorating habitat quality, parents may adjust the brood sex ratio (BSR) to favour the sex that is least costly to produce (Hasselquist and Kampenaaers 2002).

Forest fragmentation is a major cause of habitat degradation for woodland birds, and Suorsa et al. (2003) found that fragmentation favoured production of less-costly female offspring by Eurasian Treecreepers (*Certhia familiaris*). In fragmented British forest, adult Marsh Tits (*Poecile palustris*) have a male-biased sex ratio that is not apparent in continuous habitat in Poland's Białowieża Forest (Broughton and Hinsley 2015). Czyż et al. (2012) showed that the Marsh Tit population in Białowieża produced offspring with an unbiased sex ratio, reflecting the adult population. However, it is unknown whether the male bias in British populations of adult Marsh Tits results from a male-biased sex ratio among offspring or from costs associated with female-biased dispersal in fragmented habitat (Broughton et al. 2010; Wesolowski 2015).

We examined the secondary sex ratio of Marsh Tits nestlings in two areas of Britain with contrasting habitat fragmentation, and compared results with those previously reported from the continuous forest of Białowieża (Czyż et al. 2012). A male-biased sex ratio among nestlings in the British populations, increasing with habitat fragmentation, would indicate the origin of the male skew among adults. Conversely, an unbiased sex ratio would suggest sex-

biased mortality of full-grown birds, aiding the understanding the Marsh Tit's long-term decline in Britain (Broughton and Hinsley 2015).

## Methods

The study compared a single 312 ha patch of deciduous forest at Wytham Woods, south-central England (51°46' N, 1°20' W), and 201 ha of fragmented forest patches located 101 km to the north-east, comprising the 160 ha Monks Wood (52°24' N, 0°14' W) and two neighbouring woods of 13 ha and 28 ha (hereafter 'Monks Wood'). Inter-patch distances at Monks Wood were 301-719 m across an arable landscape matrix.

Sex was determined for 190 nestlings from Wytham, comprising 19 complete first broods in 2007 (n = 113 nestlings) and 13 in 2008 (n = 77). At Monks Wood 195 nestlings were sampled from 16 complete first broods in the three forest patches in 2007 (n = 106) and 13 in 2008 (n = 89). Total genomic DNA was isolated from whole blood taken from the brachial vein of nestlings when aged 11-15 days post-hatching, which was approximately 5-9 days prior to fledging. Aside from four cases of later predation causing total brood loss, no mortality was detected after sampling and so the recorded sex ratio was considered to represent that of fledged juveniles entering the populations.

Blood samples were archived on Whatman FTA Classic Cards (GE Healthcare Life Sciences, Maidstone, UK). A 1.25 mm disc was cut from the cards using a Uni-Core punch (Whatman) for DNA extraction using FTA purification reagent (Whatman) and the ZR DNA-Card Extraction Kit (Zymo Research, Irvine, California, USA) according to the manufacturer instructions. The sex identification test employed the P8 (5'-CTCCCAAGGATGAGRAAYTG-3') and P2 (5'-TCTGCATCGCTAAATCCTTT-3') primers (Griffiths et al. 1998) and PCR amplification was conducted in a total volume of 10 µL using the PCR conditions reported by Griffiths et al. (1998).

At least five adult females contributed to both years at Monks Wood, identified from leg rings, with an unknown number at Wytham. However, because Czyż et al. (2012) reported no effect of female age or identity on Marsh Tit brood sex ratios, and also no effect of

habitat, year, brood size or breeding phenology, we included all broods in analyses and limited investigation to the population level sex ratio. We hypothesised that the offspring sex ratio would be more male-biased at the fragmented Monks Wood site than at the single, large forest patch at Wytham, and both sites would deviate from the even sex ratio reported from the extensive forest at Białowieża (Czyż et al. 2012).

We employed a binomial generalised linear model (GLM) in R version 3.0.2 (R Core Team 2013) to test for an effect of site and year (including an interaction term) on sex ratio, with the response variable being the number of males and females in each brood. A power analysis assessed the ability of a binomial test to detect up to a 60% skew towards males among nestlings, using the pwr package (Champely 2015).

## Results

The proportion of male nestlings in broods ranged from zero at both sites to 0.80 at Wytham and 0.86 at Monks Wood (Fig. 1), with medians of 0.50 and respective brood sizes of 2-8 (mean = 5.9) and 1-10 (mean = 6.6). The glm analysis indicated a good fit of the full model, which included site and year effects, with the ratio of residual deviance to 57 degrees of freedom being 0.88. However, none of the explanatory terms were significant (Table 1) and the most parsimonious was the null model (deviance ratio = 0.86) in returning a proportion of 0.48 males with 95% CI of 0.43-0.53. The power analysis produced a curve estimating a 0.98 probability of detecting a proportion of 0.6 males in a binomial test of the population-level data (Fig. 2).

## Discussion

Our results from two British sites replicate those previously reported from Białowieża Forest (Czyż et al. 2012) in finding no evidence of significant manipulation of the sex ratio of Marsh Tit broods. Regardless of the degree of forest fragmentation, the Marsh Tit populations studied appeared to produce male and female offspring in unbiased proportions.

111 These results have two significant implications. Firstly, they indicate that an unbiased sex  
112 ratio among offspring appears to be a general feature of Marsh Tit populations under the  
113 conditions and spatial scales studied to date. Ramsay et al. (2003) obtained similar results  
114 for the closely-related Black-capped Chickadee (*P. atricapilla*), suggesting a more  
115 widespread inability to manipulate offspring sex ratios among the *Poecile* tits. The results  
116 add to a growing literature of unbiased brood sex ratios among birds, which challenges the  
117 reported publication bias against non-significant results in studies of sex allocation  
118 (Hasselquist and Kampenaaers 2002).

119 The second important implication of the results is the indication that the male-biased sex  
120 ratio observed among British populations of adult Marsh Tits (Broughton and Hinsley 2015)  
121 is unlikely to originate in the nest. Instead, differences in mortality or settling success  
122 between males and females must be impacting during the juvenile (post-fledging) or adult  
123 stages. A possible mechanism is the greater costs associated with female-biased dispersal  
124 of juvenile Marsh Tits in fragmented landscapes. The tendency to females to disperse further  
125 than males before settling will often necessitate leaving the natal patch of forest in highly  
126 fragmented landscapes. There are likely to be significant risks of starvation or predation  
127 associated with crossing the landscape between habitat patches, requiring discovery of an  
128 unknown route through favourable corridors that must ultimately lead to a vacant patch of  
129 suitable forest in which to settle (Broughton et al. 2010; Wesolowski 2015). Consequently,  
130 the chances of successful settling are likely to be lower for dispersing females than for  
131 males, which disperse shorter distances that may avoid inter-patch movements.

132 In extensive forest, such as Białowieża, the risks associated with inter-patch dispersal are  
133 avoided by both sexes, which can travel for many kilometres in any direction through  
134 continuous, high quality habitat (Wesolowski 2015). As such, exchange of juveniles across  
135 the habitat should be broadly equal for both sexes, even where dispersal distances are  
136 greater for females. This would result in population-level recruitment with an unbiased sex  
137 ratio, reflecting that of the offspring that are produced.

The insights provided by comparison of offspring sex ratios between Marsh Tit populations in Britain and Białowieża are essential in understanding the potential drivers behind the substantial decline in abundance and range of British Marsh Tits (Broughton and Hinsley 2015). The results provide support for the theory of habitat fragmentation impacting on the recruitment of juvenile females, resulting in unpaired males that are unable to breed and, therefore, reduced population productivity (Broughton et al. 2010). As such, the results provide important evidence supporting the implementation of habitat management that could facilitate inter-patch movement of Marsh Tits, such as creation and conservation of dispersal corridors between fragmented breeding habitat (Broughton and Hinsley 2015).

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Table 1. Significance of deleted terms from a binomial GLM during stepwise deletion using analysis of deviance to compare between the larger and reduced model in each case.

Model	$\chi^2$	df	<i>P</i>
Site * Year (saturated)	0.27	1	0.61
Site	0.51	1	0.48
Year	0.66	1	0.42

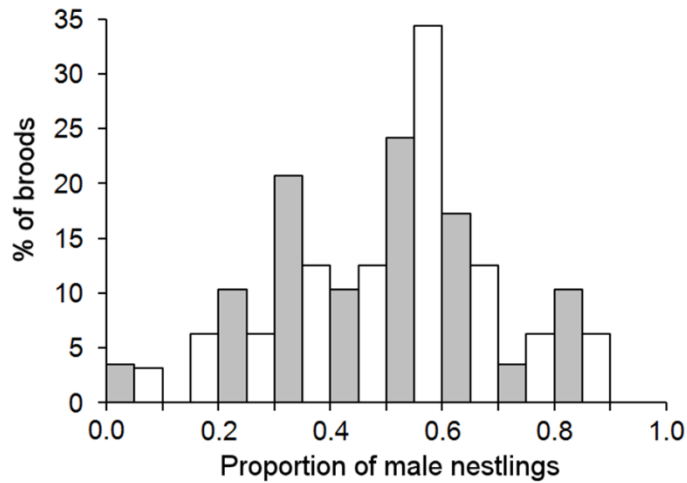
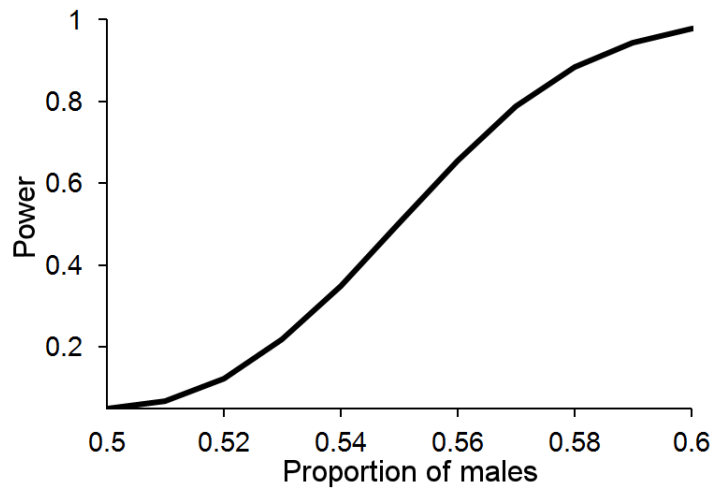


Figure 1. Distribution of the proportion of males in 29 Marsh Tit broods (195 nestlings) at Monks Wood (grey bars) and 32 broods (190 nestlings) at Wytham (white bars) pooled over two breeding seasons.



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235 Figure 2. Curve produced by power analysis indicating the probability (power) of detecting a

236 skew of up to 0.6 in favour of males in a sample of 385 Marsh Tit nestlings.