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Original Article

Local offspring density and sex ratio affect sex allocation in the great tit

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The expected fitness gain for offspring of a given sex may depend on local population sex ratio and density. Knowing the influence of such social factors on brood sex ratios may contribute considerably to the understanding of sex allocation in higher vertebrates. For 3 consecutive years, we manipulated sex ratios and densities of juvenile great tits (*Parus major*) within forest plots and investigated how next year's brood sex ratios were affected. For yearling female breeders, we considered the treatment of the plot of fledging and settlement and for experienced female breeders the treatment of the breeding plot. Experienced females produced more female offspring at increased plot densities and more males at reduced densities. Yearling female's brood sex ratio was not affected by the density treatment but was positively related to the natural density in the plot of fledging. The plot sex ratio treatment affected sex allocation differently for yearling and experienced females. Yearling females produced more females when fledged from male-biased plots and more males when fledged from female-biased plots. Experienced females produced more females in control plots and unbiased brood sex ratios in male- and female-biased plots. Our results show that experimental changes in local sex ratio and density generate social or environmental cues that are used in sex allocation. We cannot show that sex allocation was adaptive. However, natural and experimental plot density and experimental plot sex ratio related differently to sex allocation for yearling and experienced females, which suggest that several sex allocation scenarios might act simultaneously. *Key words:* frequency dependence, local competition, sex-biased dispersal, social environment, Trivers and Willard hypothesis. *[Behav Ecol]*

INTRODUCTION

In each breeding attempt, individuals are confronted with the decision of how many male and female offspring they should produce in order to maximize their fitness. According to Fisher (1930), the rarer sex in a population will always have higher chances of finding a suitable partner than the more common sex and should thus be produced in excess until the sex ratio is equal again. At the individual level, an equal sex ratio is expected if costs of total investment during parental care are the same for both sexes, and if sons and daughters provide their parents with equal fitness benefits after the offspring become independent (Charnov 1982; Hardy 2002).

A number of situations has been described, which should lead to different fitness benefits for each offspring sex and thus are expected to result in biased sex ratios. First, the Trivers–Willard (1973) hypothesis predicts that mothers in above-average condition (status, territory quality, or body characteristics) should produce more of a given offspring sex when 1) mother's condition correlates to offspring condition at the end of parental care; 2) condition of young endures into adulthood; and 3) condition differentially affects the reproductive success of sexes (Trivers and Willard 1973). Second, when one sex requires more resources for rearing, parents in lower condition might simply be restricted to produce the "cheaper" sex to reduce rearing costs and the risk of brood failure (Myers 1978; Gomendio et al. 1990). Third, sex-biased social interaction between relatives, such as competition and cooperation among kin (local resource competition, Clark 1978; local resource enhancement, Emlen et al. 1986) can alter the costs and benefits associated with producing each sex. Accordingly, individuals should produce more of the dispersing sex under conditions of intensified local competition and more of the helping sex when resources are abundant (Clark 1978; Emlen et al. 1986). Finally, in species living in heterogeneous environments, producing the philopatric sex in good-quality habitat and the dispersive sex in bad-quality habitats might provide highest fitness (Julliard 2000).

All the above hypotheses have been applied to explain variation in sex ratios in higher vertebrates, with variable success (Komdeur and Pen 2002; West et al. 2002). Results from sex ratio studies are often conflicting, even in the same species

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(Lessells et al. 1996; Kölliker et al. 1999; Radford and Blakey 2000; Stauss et al. 2005). This lack of empirical support for theoretical predictions might be explained by environmental unpredictability and the complex life histories of the study species (Cockburn et al. 2002; Komdeur and Pen 2002; West et al. 2002; West and Sheldon 2002). Several selection pressures on sex allocation may act simultaneously (e.g., Forsman et al. 2008) or operate only at a particular spatial scale (e.g., the local territory but not the local population; Doligez et al. 2008). In addition, previously unconsidered selection pressures may also be essential (e.g., competition between the sexes; Le Galliard et al. 2005a). Consequently, the identification of important selection pressures on sex allocation is an ongoing challenge, and simple predictions for sex allocation in natural systems are not easily generated.

Selection pressures that play a role in the hypotheses presented above are related to effects of local population density and population sex ratio (Alonzo and Sheldon 2010). Operational sex ratios have been experimentally altered to test for Fisher's (1930) frequency-dependent sex allocation (Le Galliard et al. 2005b; Allsop et al. 2006; Warner and Shine 2007). Local density and local sex ratio may also be used by parents as information to assess habitat suitability for the production of a certain sex (Warner and Shine 2007; Forsman et al. 2008). The local resource competition hypothesis has been tested by relating population growth (Hjernquist et al. 2009) or den availability (Johnson et al. 2001) to brood sex ratio. These results suggest that parents bias the offspring sex ratio toward the sex that competes least with its parents on a local scale. Furthermore, in cases where one sex dominates the other during competition, and in species with sex differences in natal dispersal, variation in local density and sex ratio should alter the costs and benefits of producing male and female offspring (Le Galliard et al. 2005a).

Studies on adaptive sex allocation have frequently focused on birds because, in contrast to mammals, females are the heterogametic sex and are assumed to have control over the sex ratio of the eggs (Krackow 1995; Pike and Petrie 2003; Rutkowska and Badyaev 2008). Several studies have experimentally confirmed that primary sex ratio is adjusted to individual parental traits to produce offspring of the sex with higher fitness prospects (Komdeur et al. 1997; Nager et al. 1999; Sheldon et al. 1999; Kalmbach et al. 2001). However, in other studies no adaptive sex allocation was found in spite of appropriate selection pressures (Bensch et al. 1999; Leech et al. 2001; Le Galliard et al. 2005b; Uller and Olsson 2006).

In the present study on great tits, we manipulated social cues that are likely to elicit a response in brood sex ratio produced by females, according to current sex allocation theory as outlined above. For this, we experimentally changed the local population density and sex ratio of nestlings at the level of forest plots in 3 consecutive breeding seasons (2005–2007) and investigated whether the plot treatment affected the brood sex ratio produced by females in the subsequent year (2006–2008).

In great tits, males are believed to benefit from familiarity with the local habitat when competing for available territories (Drent 1983; Sandell and Smith 1991). This is probably why they show smaller natal dispersal distances than females (Greenwood et al. 1979; Tinbergen 2005). Females, on the other hand, may have higher reproductive success when dispersing further (Greenwood et al. 1979). Females are significantly smaller than males already as nestlings (4–8% difference in body weight; Nicolaus et al. 2009), which might incur that they need fewer resources for raising than males (Oddie 2000; Nicolaus et al. 2009) and they are generally subordinate to males in competition for food and roosts (Kluyver 1957; Drent 1983). Furthermore, reproductive success varies more in male than in female great tits due to extra-pair paternity (11.9%–14.2% between 2004 and 2008 in our study population; see Lubjuhn et al. 2007; Brommer et al. 2010 for extra-pair paternity rates in other populations) and reduced parental condition translates into reduced offspring condition (Gallizzi et al. 2008). Moreover, brood sex ratio has been shown to be positively related to male tarsus length (Kölliker et al. 1999; but see Radford and Blakey 2000) and change in male body condition (Oddie and Reim 2002). Given the life history of the great tit, our experiment might affect sex allocation through the following (not mutually exclusive) scenarios (Table 1). 1) Increased competition (high-density and male-biased sex ratio) negatively affects female condition (e.g., lower body condition, dominance rank, or territory quality) and females consequently produce more female offspring according to hypothesis by Trivers and Willard (1973) or because female offspring presumably need less resources for rearing (Myers 1978). 2) Females produce the sex that was less abundant in the plot the previous year according to hypothesis by Fisher (1930). 3) Females produce the dispersing sex (females) when competition for local resources is increased, that is, under high densities and male-biased sex ratios. 4) Density and biased local sex ratios convey information about habitat quality or plot suitability for the production of a given sex. In this case, the brood sex ratio should be biased in the same direction as the plot sex ratio (Warner and Shine 2007) and more males should be produced in high-density plots (Julliard 2000; Doligez et al. 2008; Forsman et al. 2008). The first 3 scenarios predict about the same patterns of brood sex ratio in response to the experimental changes (Table 1), and it will thus become difficult to pinpoint a single adaptive explanation because the underlying proximate physiological mechanisms might even be the same. However, scenario 4 predicts the opposite of the other 3 scenarios (Table 1) and can thus be excluded if the results support scenarios 1–3. We did not aim at showing that any brood sex ratio response to the experimental changes in density and sex ratio is adaptive, but simply to test whether the patterns fit the patterns predicted by the 4 adaptive sex ratio scenarios.

Table 1

Four scenarios of predictions for how the experimental alteration of plot density and plot sex ratio in year *t* could affect brood sex ratios of female great tits in year *t* + 1

Sex allocation theory	Treatment	Prediction	Experienced females	Yearling females
Condition dependence	Higher plot density	More females	Yes	Yes*
	Male-biased plot sex ratio	More females	Partly	Yes
Frequency dependence	Higher plot density	/		
	Male-biased plot sex ratio	More females	Partly	Yes
Dispersal and local resource competition	Higher plot density	More females	Yes	Yes*
	Male-biased plot sex ratio	More females	Partly	Yes
Quality of breeding habitat	Higher plot density	More males	No	No
	Male-biased plot sex ratio	More males	Partly	No

The corresponding results are shown for experienced and yearling females.
*Brood sex ratio in year *t* + 1 is not affected by density treatment in year t but correlates with natural density in year *t*

We investigated whether the plot treatment affected the brood sex ratio produced by individual females breeding in the subsequent year (2006–2008). We separately analyzed the brood sex ratio of yearling female breeders that had undergone the treatment in their year of birth and the brood sex ratio of experienced female breeders that had been subjected to the treatment as adults. As natal dispersal of juveniles between plots was high (70%), we analyzed for yearling female breeders whether the brood sex ratio produced the following year was affected by the manipulation of either the plot of fledging or the plot of settlement. Experienced female breeders in our population rarely changed plots between breeding seasons (5%), and we thus analyzed whether variation in their brood sex ratio was related to the plot they bred in the previous year and whether these effects were caused by individual adjustment of the brood sex ratio or by selective disappearance.

METHODS

Study species and study area

We studied great tits, a small hole breeding passerine that readily breeds in nest-boxes. Our study was conducted in the Lauwersmeer area that is situated in the northeast of the Netherlands (53°23 N, 6°14 E). In our study area, great tits are resident and juvenile males start to establish a territory in autumn (Drent 1983). In February 2005, we extended the existing study area by establishing 12 nest-box plots, where in some woodlots already existing boxes were rearranged and in others they were newly put up. Each plot consisted of 50 nest-boxes in a regular 50 m grid. The woodlots primarily consisted of deciduous forest and were separated by at least 300 m of open grassland or forest patches without boxes. For more information on breeding parameters in the study population during 2005–2007, see Nicolaus et al. (2009).

Field methods

From the beginning of April, we checked nest-boxes weekly to determine the start of egg laying (where necessary, we back calculated assuming one egg was laid per day). Clutch size was determined at the onset of incubation. Before the expected hatching date, nest-boxes were checked daily to determine the exact hatching date (day 0). We sampled a small quantity of blood (ca 5–10 µL) from all nestlings when they were 2 days old by piercing their tarsal vein (with a Microlance 0.3 × 13 mm, 30 G ½) and clipped the end of their toe nails in a unique combination for later identification (St. Louis et al. 1989). Blood was stored in 100% ethanol (1.5 ml tubes). Between day 3 and 5, molecular sex determination was performed (see below) such that on day 6 after hatching, the sex of all nestlings was known. Eggs that had not hatched until day 6 and nestlings that had died before day 2 were collected and frozen for later sex determination. On day 6, nestlings received a uniquely numbered aluminum band (issued by the Dutch "Vogeltrekstation") and were exchanged between broods of the same age to achieve brood- and plot-level manipulations (see section "Experimental design"). Nestlings were transported within 30 min by car in small boxes and were kept warm using cotton wool and heat packs. We exchanged at least one nestling in every brood to have comparable levels of disturbance. On day 7, parents were caught using spring traps in the nest-boxes and their weight (to the nearest 0.1 g) and tarsus length (to the nearest 0.1 mm) were measured. Unbanded parents were fitted with an aluminum band and a unique combination of 3 color bands. At day 14, all nestlings were additionally provided with 3 color bands in a unique combination with the aluminum band. As nestlings leave the nest about 20 days after hatching, we performed nest-box checks from day 19 onward to determine successful fledging.

Molecular sexing

DNA was extracted from blood or tissue samples using the Chelex method described by Walsh, Metzger, and Higuchi (1991). Sex of the young was determined following Griffiths et al. (1998). The polymerase chain reaction products were separated by electrophoresis on a 2% agarose gel. For unhatched eggs, DNA extraction was attempted if there was a visible embryo present. Of all 543 unhatched eggs collected during 2005–2008, only 117 (22%) could be successfully sexed. Of the 8783 nestlings from which we obtained a blood sample or a tissue sample, only 55 (0.6%) could not be assigned to a specific sex. Of all sexed nestlings from 2005 to 2007, 609 were seen again as breeding birds the following year and in all cases the observed phenotypic sex was in accordance with the molecular sex.

Experimental design

Plot-level manipulation

We simultaneously manipulated plot density (number of nestlings per plot) and plot sex ratio (proportion of male nestlings per plot) of nestling great tits in 12 plots for 3 years (2005, 2006, and 2007) by manipulating broods within plots on day 6 of nestling age (see section "Brood-level manipulation"). The 3 plot sex ratio treatments were male-biased (75% male young), female-biased (25% male young), and control (50% male young). The 2 plot density treatments were high (increased number of nestlings per plot) and low (reduced number of nestlings per plot, Figure 1). In a crossed design, this resulted in 6 plot sex ratio–density treatment combinations ranging from male-biased high-density to female-biased low-density plots (Figure 1). Each of these treatment combinations was randomly allocated to 2 plots each year at the

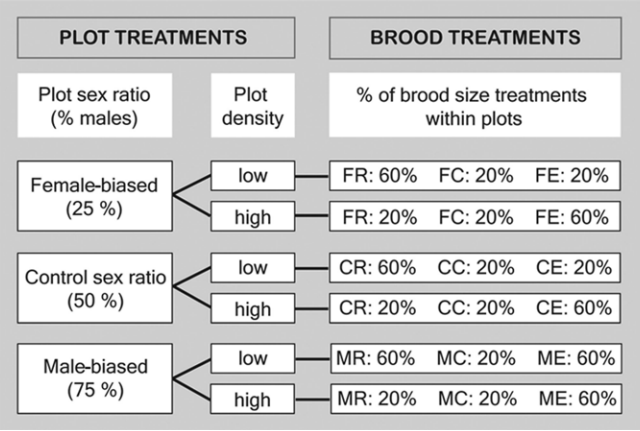

Figure 1
Experimental treatments applied to plots and broods of great tits during the 3 years (2005–2007). Plot sex ratio (female-biased, control, and male-biased) and density (low or high density) of juvenile great tits were simultaneously manipulated on the plot level resulting in 6 different plot treatment combinations. The sex ratio treatment for all broods within a plot was the same as the plot treatment. The density treatment on the plot level (low or high density) was achieved by manipulated 60% of the broods within a plot toward the desired treatment keeping 40% of the nests as controls for the other treatments. R: reduced brood size, C: control brood size, E: enlarged brood size.

start of the breeding season to prevent masking of treatment effects by plot effects (plot quality, distance to other plots, or degree of isolation). No plot was allowed to have the same combination of treatments in consecutive years. The 2 plot density treatment groups did not differ in natural density of nestlings on day 6 (independent t-test: $t = 1.01$, degrees of freedom [df] = 34, $P = 0.321$; mean ± standard deviation [SD]: high density = 141.4 ± 31.5; low density =152.7 ± 35.2) and the 3 plot sex ratio treatments did not differ in natural plot sex ratio of nestlings on day 6 (Kruskal–Wallis test: $H_{2,36} = 4.17$, $P = 0.124$).

Brood-level manipulation

First broods (broods that started less than 30 days after the start of the very first brood in that year) within plots were manipulated on day 6 after hatching to attain the brood treatment that corresponded to the plot treatment (Figure 1). Brood sex ratio and brood size was manipulated by transferring nestlings of known sex between broods of the same hatching date (for details see supplementary material, "How experimental treatments were assigned to plots, broods and nestlings").

For the sex ratio treatment, we manipulated all broods in the plot in the direction of the plot treatment (Figure 1), thus broods were manipulated to contain on average 25% male nestlings in female-biased plots, 50% males in control plots, and 75% males in male-biased plots (Table 2, Figure 1). The plot density treatment was achieved by applying 3 different brood size treatments (reduced, control, enlarged) in different proportions in the 2 density treatment categories. Hence, to achieve a high-density treatment, the majority of broods per plot (60%) was increased, whereas 20% of broods were decreased as opposite treatment controls and 20% were manipulated to real controls (average brood size for a year). These controls were used to study interaction effects between plot density and brood size on breeding output as presented in Nicolaus et al. (2009). Thus, the effects of plot density and brood size can be estimated independently, whereas the effects of plot sex ratio and brood sex ratio are confounded.

Brood size treatments were assigned randomly to nests within plots before clutch completion. We excluded broods with less than 3 nestlings (2005: 2 broods; 2006: 2 broods; 2007: 5 broods). Control broods were manipulated to contain the "average" brood size (based on average clutch size) for a given year. Due to yearly variation in average brood size, experimental control brood size centered around 7 or 8 nestlings in 2005 and 2007 and 9 nestlings in 2006. Broods assigned to the "enlarged" treatment were manipulated to contain a brood size of "average"+3 nestlings (10–12 depending on the year) and broods in the reduced treatment received a brood size of "average"−3 nestlings (4–6 depending on year).

We allowed variation in the final brood size within the 3 brood size categories and variation in experimental brood sex ratios within plots because the number of available synchronous broods, their natural brood sizes, and brood sex ratios varied. However, we needed to bias as many broods as possible to achieve the treatment at the plot level. A few broods remained unmanipulated (2005: 6/249; 2006: 2/168; 2007: 4/252) because we lacked other synchronous broods to achieve the assigned treatment. Average brood size, brood sex ratio, plot density, and plot sex ratio before and after manipulation per year and per brood or plot treatment category are presented in Table 2. We only manipulated first broods, second broods, and replacement broods of known first broods after failure were left unmanipulated. The experiments complied with the current Dutch law (DEC nr. 4114B).

Success of plot- and brood-level manipulations

The plot sex ratios and densities before and after manipulation are based on data from all first broods on day 6 in a plot (manipulated and unmanipulated). The

Table 2

Overview of average natural (top in cell) and experimental (bottom in cell) values per treatment group for brood sex ratio, brood size, plot sex ratio, and plot density for 2005, 2006, and 2007 in the great tit study population

	2005		2006		2007	
Treatment group	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*
Female-biased broods	0.46 ± 0.18 0.22 ± 0.07	79	0.48 ± 0.16 0.24 ± 0.08	60	0.44 ± 0.18 0.22 ± 0.06	90
Control broods	0.48 ± 0.19 0.49 ± 0.07	89	0.51 ± 0.17 0.49 ± 0.06	57	0.52 ± 0.17 0.51 ± 0.07	82
Male-biased broods	0.51 ± 0.18 0.76 ± 0.07	75	0.50 ± 0.17 0.79 ± 0.07	49	0.51 ± 0.19 0.78 ± 0.06	75
Reduced brood size	7.39 ± 1.69 5.22 ± 0.52	106	8.74 ± 1.42 5.88 ± 0.32	61	6.86 ± 1.50 4.69 ± 0.52	106
Control brood size	7.75 ± 1.80 8.05 ± 0.65	60	9.18 ± 1.43 8.81 ± 0.65	38	7.28 ± 1.65 7.58 ± 0.70	57
Enlarged brood size	8.06 ± 1.62 10.83 ± 0.59	77	9.06 ± 1.25 11.87 ± 0.42	67	7.86 ± 1.40 10.39 ± 0.49	84
Female-biased plots	0.49 ± 0.02 0.24 ± 0.005	4	0.47 ± 0.06 0.24 ± 0.02	4	0.46 ± 0.01 0.25 ± 0.02	4
Control plots	0.47 ± 0.02 0.49 ± 0.008	4	0.50 ± 0.02 0.49 ± 0.01	4	0.51 ± 0.04 0.50 ± 0.03	4
Male-biased plots	0.50 ± 0.03 0.74 ± 0.019	4	0.49 ± 0.04 0.79 ± 0.03	4	0.52 ± 0.06 0.76 ± 0.02	4
Low plot density	156.83 ± 28.27 137.83 ± 26.35	6	134.33 ± 48.73 119.67 ± 48.90	6	166.83 ± 19.57 143.00 ± 22.02	6
High plot density	161.50 ± 16.28 181.16 ± 17.32	6	118.00 ± 27.62 132.67 ± 32.67	6	144.83 ± 34.50 168.67 ± 42.97	6

Sample sizes for the brood treatments indicate numbers of broods, whereas sample sizes for the plot treatments indicate number of plots.

plot sex ratio treatment was very successful in changing the proportion of male nestlings because all manipulated broods within plots had a sex ratio bias in the direction of the plot bias (supplementary Figure S1A), whereas some variation in brood sex ratios within plots remained (supplementary Figure S2). Thus, over all years, the 3 plot sex ratio treatment groups differed significantly in the final plot sex ratio of young at day 6, at day 14, and at fledging (supplementary Table S1; see also Michler et al. 2011a). The 3 plot sex ratio treatment groups also differed significantly in the plot sex ratio of fledglings in each study year separately (Kruskal–Wallis test: for each year, $H_{2,12} > 9.80$, $P < 0.008$). The natural and the final experimental plot sex ratios of nestlings at day 6 were not correlated (Spearman rank correlation, female-biased plots: $r_s = 0.42$, $n = 12$, $P = 0.174$; control plots: $r_s = 0.40$, $n = 12$, $P = 0.199$; male-biased plots: $r_s = 0.25$, $n = 12$, $P = 0.430$).

The density experiment reduced the number of nestlings in low-density plots on average by 19.17 ± 9.83 SD individuals (ca 13%) and increased the number of nestlings in high-density plots on average by 19.39 ± 11.38 SD (ca 14%; supplementary Figure S1B), resulting in a significantly different change in number of nestlings between the treatment groups (independent *t*-test: $t = -10.88$, df = 34, $P < 0.001$). Over all years, the 2 plot density treatment groups differed in final number of young at day 6 but not anymore at day 14 and at fledging (supplementary Table S1). The density treatment groups differed in the number of fledglings in 2005 (independent *t*-test: $t = 8.00$, df = 2, $P = 0.018$) but not in 2006 and 2007 (independent *t*-test 2006: $t = -0.22$, df = 10, $P = 0.827$; 2007: $t = 0.66$, df = 10, $P = 0.523$). Over all years, however, the nestling densities per plot before and after manipulation were strongly correlated ($r_c = 0.773$, $n = 36$, $P < 0.001$), probably because plots show high variation in natural nestling density also within years (supplementary Figure S4).

We observed color-banded juveniles in the postfledging phase in 2005 and 2006 to investigate how long experimental changes in plot sex ratio and plot density lasted (supplementary Figures S5 and S6). Due to high levels of juvenile movements, the differences in plot sex ratio of fledglings disappeared in July in 2005 but lasted until October in 2006 (supplementary Figure S5). In 2005, the differences in plot densities between the experimental high- and low-density plots disappeared already soon after fledging (supplementary Figure S6). Nonetheless, the plot sex ratio treatment seemed to have even lasted until winter because the sex ratio of birds roosting in nest-boxes was still biased (Nicolaus et al. 2012)

Data selection and statistical analyses

Primary brood sex ratio

We aimed at determining the primary brood sex ratio of first broods of all females that had experienced a manipulation in the previous year as juvenile (yearling female) or adult (experienced female, see section "Treatment effects on sex allocation of yearling and experienced female breeders"). Primary sex ratio is defined as the sex ratio of all eggs laid in a clutch. Of the total 908 first broods laid from 2005–2008, 67.3% (611) had a known primary sex ratio. This related to 94.2% (7509/7969) of all eggs laid in first clutches that we were able to sex successfully. The sexing of unhatched eggs was not very successful (only 21% sexed successfully). Thus, because of the unreliability of egg sexing, we based the brood sex ratio on the sexing results of the day 2 samples of nestlings (sexing success 99.4%). This could potentially cause biased sex ratio estimates, because for our analyses (described below) 65 broods of yearling females and 43 broods of experienced females had incomplete primary sex ratios (not all eggs laid hatched and were sexed). However, we repeated all analyses using a reduced data set that only included clutches with complete primary brood sex ratio (146 clutches of yearling females and 117 clutches of experienced females, where all eggs had hatched and were sexed). The analyses that were based on complete primary brood sex ratios gave the same results as the analyses based on the large data set with all brood sex ratios (complete and incomplete combined) and are thus not further mentioned.

Treatment effects on sex allocation of yearling and experienced female breeders

We conducted separate analyses for 2 categories of birds that may be affected differently by the experiment. Yearling female breeders ($n = 211$ breeding events) are locally born females that experienced the treatment in year t (2005–2007) as juveniles and bred in the nest-box area in year $t + 1$ (2006–2008). Experienced female breeders are females that experienced the treatment in year t as breeding adult and returned in the next year to the same plot (excluding 9 females that changed plots). If an individual bred in more than 2 consecutive years ($n = 42$), we chose one breeding event randomly, which resulted in 160 breeding events. This random sampling procedure was repeated 3 times but all analyses gave qualitatively the same results.

We analyzed brood sex ratio (proportion of male young in the brood at day 2) in a binomial response model with logit-link function and 2nd-order penalized quasi-likelihood estimation procedure with the number of young in the brood at day 2 as denominator. Because there was a hierarchical structure in the data set, we used linear multilevel analyses in MLwiN 2.0 (Rasbash et al. 2004). For yearling females, we included the 4 levels: plot, cohort (all broods within a plot in a given year), brood (as for 35 out of 170 broods there was more than 1 individual from the previous year), and individual breeding event. For experienced females, we included the 3 levels: plot, cohort, and individual breeding event. For all other analyses, we used STATISTICA version 7 (StatSoft, Inc. 2004). In some analyses, variation for the random effects was 0, which indicates that there was no variation in brood sex ratio on these levels, because the fixed effects explained all variation on the corresponding level or because one of the underlying levels already explains most of the variation. Excluding levels with 0 estimates from the analysis did not change the results and therefore we always kept them in the models.

We tested whether characteristics of a plot in year t affected brood sex ratios in year $t + 1$ (plot of fledging for yearling and plot of breeding for experienced females). We analyzed the plot sex ratio treatment as a categorical variable with 3 categories: female-biased, control, and male-biased (control plot as reference category). We analyzed the density treatment (categorical with low density as reference) to investigate causal effects of the change in nestling density. Because the natural and final experimental nestling densities were correlated, we also investigated the natural density of nestlings per plot as (continuous) density variable that might relate to competition for resources such as food, roosts, or territories. The natural density was based on the number of nestlings per plot before swapping on day 6 and was centered on the overall population average. In the models, we also controlled for natural plot sex ratio (based on nestling sex ratio at day 6 per plot).

In addition, we examined whether competition in the nestling phase or the brood manipulation had an effect on sex allocation the following year. We therefore tested the significance of the experimental brood size categories (with control as reference) and also tested for natural brood size before

Table 3

Analysis of brood sex ratio (year $t + 1$) of *yearling* female great tits (2006–2008) examining the effects of density treatment, plot sex ratio treatment for plot of fledging and plot of settlement (year t), brood size treatment and relative experimental brood sex ratio (deviance of final brood to final plot sex ratio, year t) as well as correlations with natural plot sex ratio and plot density of the plot of fledging and settlement (year t), natural brood size, and natural brood sex ratio (year t)

Explanatory variable	β (SE)	χ^2	df	P
Final model				
Intercept	−0.205 (0.216)	0.899	1	0.343
Plot change	0.045 (0.133)	0.116	1	0.733
Year 2006	−0.017 (0.155)	0.222	2	0.895
Year 2007	−0.061 (0.134)			
Plot of fledging natural density	−0.005 (0.002)	6.066	1	0.014
Plot of fledging natural sex ratio	1.427 (1.544)	0.855	1	0.355
Plot of fledging density treatment: high	−0.113 (0.120)	0.890	1	0.345
Plot of fledging sex ratio: female-bias	0.297 (0.124)	9.958	2	0.007
Plot of fledging sex ratio: male-bias	−0.187 (0.164)			
Plot of settlement natural density	0.00005 (0.002)	0.001	1	0.975
Plot of settlement natural sex ratio	1.903 (1.515)	1.577	1	0.209
Plot of settlement density treatment: high	0.096 (0.106)	0.810	1	0.368
Plot of settlement sex ratio: female-bias	0.116 (0.137)	2.417	2	0.299
Plot of settlement sex ratio: male-bias	0.188 (0.124)			
Natural brood sex ratio	0.277 (0.311)	0.794	1	0.373
Natural brood size	−0.035 (0.036)	0.936	1	0.333
Relative experimental brood sex ratio	0.450 (0.662)	0.552	1	0.457
Brood size treatment: reduced	0.050 (0.142)	0.128	2	0.938
Brood size treatment: enlarged	0.029 (0.130)			
Random effects				
$\sigma^2 \pm$ SE plot	-	-	-	-
Cohort	-	-	-	-
Brood	-	-	-	-

Reference categories for the categorical variables are control sex ratio treatment, low density, year 2005, and control brood size, respectively. Model estimates are from the binomial response modeling procedure in MLwiN, $n = 211$.

manipulation (at day 6). As the plot sex ratio manipulation was done by manipulating the sex ratio of all broods in a plot in the same direction (see also Figure 1), it is not possible to disentangle plot sex ratio from brood sex ratio effects using brood sex ratio treatment categories. Therefore, to analyze the brood sex ratio manipulation, we fitted the relative experimental brood sex ratio (brood sex ratio after manipulation − plot sex ratio after manipulation) while keeping the plot sex ratio treatment in the model and also controlled for natural brood sex ratio before manipulation (related to relative experimental brood sex ratio in a generalized liner model corrected for year: likelihood ratio $\chi^2 = 3.36$, df = 1, $P = 0.067$).

For yearling females, we performed a similar analysis on brood sex ratio as for the experienced females and tested the same variables using the same procedures. In addition, we analyzed 4 extra variables: whether individuals had changed plot from fledging until breeding ("yes" or "no" with "no" as reference category), the natural density, the density and plot sex ratio treatment of the plot of settlement in year t. We also tested whether individuals that changed plot from fledging till breeding differed in their response to the density and plot sex ratio treatments from those that did not change plot by testing the corresponding interactions. This was never the case, so those results are not further mentioned. Wald tests were applied to determine the significance of explanatory variables in the full model. The full model contained all main variables described above. We also applied a backward elimination procedure on the full model, which revealed the same significant effects. Significance was assessed using an alpha level of 0.05.

Effects of competition on sex allocation can also be mediated through effects on parental condition. However, we did not analyze the effects of parental condition in year $t + 1$ on brood sex ratios, because we generally measured parents' weight at day 7 of nestling age, which is considerably later than the determination of brood sex ratio. Therefore, condition in year $t + 1$ may not only be affected by the treatment in year t (previous year) but also by the investment in the current brood in year $t + 1$ (egg laying, incubation, and nestling provisioning).

Does adjustment or selective disappearance shape brood sex ratio of experienced females?

We investigated whether 2 potential mechanisms, selective disappearance and sex ratio adjustment, could have generated differences in brood sex ratio produced by experienced females the next year in relation to the experimental treatment. Therefore, we first tested whether the patterns found were caused by facultative sex ratio adjustment from one year to the next. Alternatively, we tested whether experienced females differed in their local return rate to the next year depending on the treatment and the natural sex ratio they produced in year t. This would indicate that selective disappearance of experienced females could have shaped our results.

We analyzed the change in sex ratio of the same individual from year t to year $t + 1$ in relation to the manipulation. Change in sex ratio was z-transformed and analyzed in a normal response model. The analysis was otherwise performed in the same way as the one explained in the section "Treatment effects on sex allocation of yearling and experienced female breeders."

To detect selective disappearance as a result of the manipulations, we analyzed local return rate to the following breeding season for all females that bred in the years 2005–2007 and whose broods had been manipulated. For females with more than 1 breeding event in those years, we selected 1 at

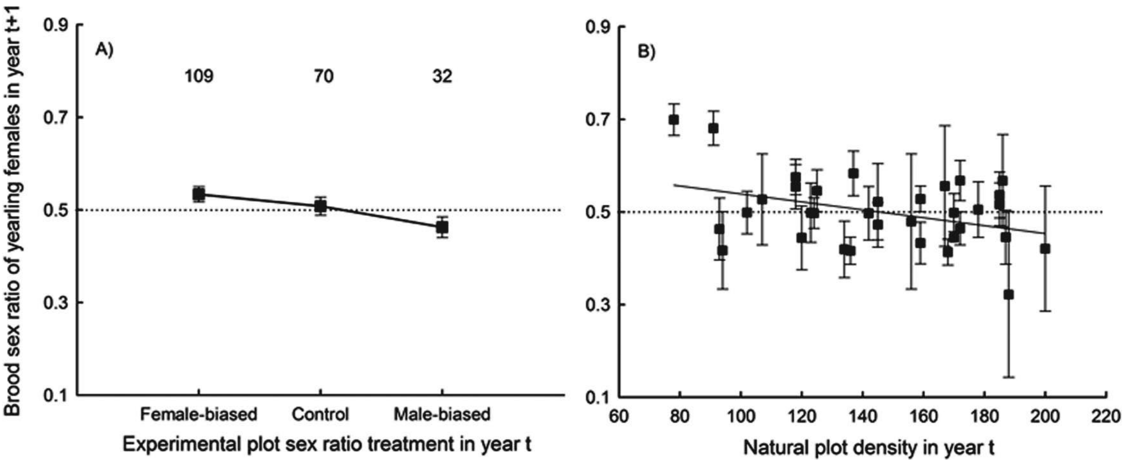

Figure 2
(A) The brood sex ratio of *yearling* female great tits produced in year $t + 1$ is affected by the sex ratio treatment of the plot of fledging in year t. Averages and standard errors per treatment group were calculated using the raw data from the years 2006–2008. Sample sizes of females per treatment group are given as numbers in the panels. (B) The brood sex ratio of *yearling* females produced in year $t + 1$ is related to the natural density of young in the plot of fledging (year t), $n = 211$. Averages and standard errors per plot and year are based on raw data for the years 2006–2008. Dashed lines indicate 50% brood sex ratio (for statistical analysis, see Table 3).

Table 4
Analysis of brood sex ratio ($t + 1$) of *experienced* female great tits (2006–2008) examining the effects of density treatment, plot sex ratio treatment, brood size treatment and the relative experimental brood sex ratio (deviance of final brood to final plot sex ratio) and correlations with natural plot sex ratio, natural plot density, natural brood size, and natural brood sex ratio (year t)

Explanatory variable	β (SE)	χ^2	df	*P*
Final model				
Intercept	−0.137 (0.194)	2.189	1	0.139
Year 2006	−0.097 (0.175)	0.724	2	0.696
Year 2007	0.058 (0.156)			
Plot of breeding natural density	0.001 (0.003)	0.071	1	0.790
Plot of breeding natural sex ratio	−0.862 (2.103)	0.168	1	0.682
Plot of breeding density treatment: high	−0.374 (0.141)	7.023	1	0.008
Plot of breeding sex ratio: female-bias	0.354 (0.166)	6.579	2	0.037
Plot of breeding sex ratio: male-bias	0.392 (0.166)			
Natural brood sex ratio	0.315 (0.331)	0.904	1	0.342
Natural brood size	0.016 (0.039)	0.169	1	0.681
Relative experimental brood sex ratio	−0.263 (0.574)	0.210	1	0.647
Brood size treatment: reduced	−0.031 (0.154)	0.739	2	0.691
Brood size treatment: enlarged	0.089 (0.163)			
Random effects				
$\sigma^2 \pm$ SE plot	-	-	-	-
Cohort	0.026 (0.030)	0.747	1	0.387

Reference categories for the categorical variables are control sex ratio treatment, low density, year 2005, and control brood size, respectively. Estimates are derived from the binomial response modeling procedure in MLwiN, $n = 160$.

random resulting in 494 breeding events with a known primary brood sex ratio. We used a hierarchical model with a binominal error structure and a logit link. The hierarchical structure again consisted of plot, cohort, and individual breeding event. Year was included as fixed factor. We tested whether return rate was associated with the same variables as were found to affect the brood sex ratio in year $t + 1$ and their interaction with the natural brood sex ratio in year t.

RESULTS

The overall sex ratio of all day 2 young that could be sexed (first, second, and replacement clutches) did not differ from an equal sex ratio in any of the years studied (chi-square test for observed and expected numbers, 2005: 1161 females, 1124 males, $\chi^2 = 0.599$, df = 1, $P = 0.439$; 2006: 1082 females, 1057 males, $\chi^2 = 0.292$, df = 1, $P = 0.589$; 2007: 1194 females, 1183 males, $\chi^2 = 0.051$, df = 1, $P = 0.821$; 2008: 971 females, 959 males, $\chi^2 = 0.075$, df = 1, $P = 0.785$).

Treatment effects on sex allocation by yearling females

Plot of fledging

The brood sex ratio produced by yearling female breeders in year $t + 1$ was significantly affected by the plot of fledging sex ratio treatment in the previous year (year t) and significantly associated with the plot of fledging natural density (Table 3). Yearling females that had fledged from male-biased plots produced more female-biased broods and those that had fledged from female-biased plots produced more male-biased broods the following year (Table 3, Figure 2A). In addition, yearling females that had fledged from plots with naturally high densities of young produced more females and

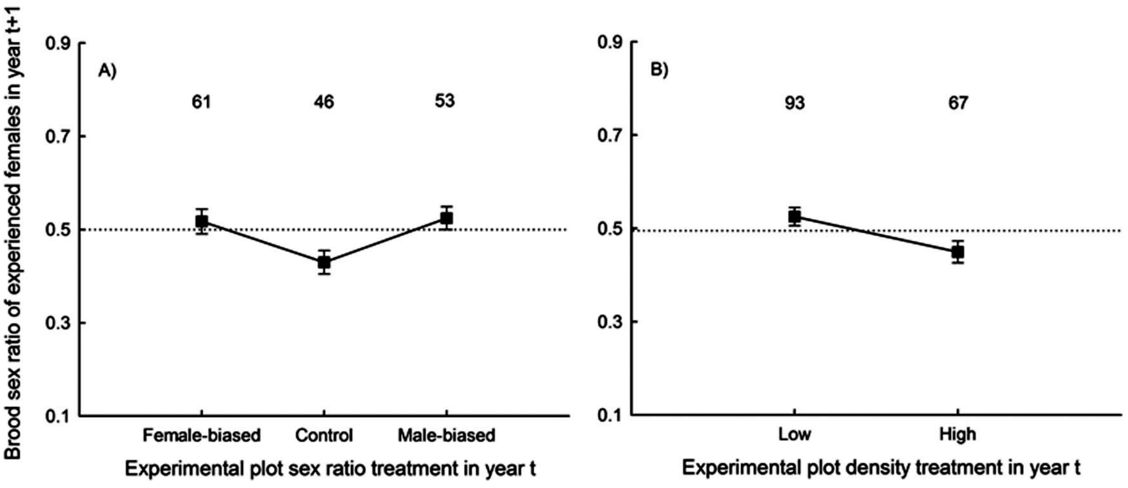

Figure 3
The brood sex ratio of *experienced* female great tits in year $t + 1$ is affected by (A) the plot sex ratio treatment in the breeding plot in year (t) and (B) the plot density treatment in the breeding plot in year t. Averages and standard errors per treatment group are based on raw data for the years 2006–2008. Sample sizes of females per treatment group are given as numbers in the panels. Dashed line indicates 50% brood sex ratio (for statistical analysis, see Table 4).

those that had fledged from plots with naturally low densities produced more males (Table 3, Figure 2B). In contrast, the plot of fledging density treatment had no significant effect on the brood sex ratio produced the next year (Table 3). The effect of the plot sex ratio treatment and the correlation with natural density of the plot of fledging did not vary between years (including all underlying variables, year × natural density: $\chi^2 = 1.969$, df = 2, $P = 0.381$; year × sex ratio treatment: $\chi^2 = 1.323$, df = 4, $P = 0.857$).

None of the natural or experimental brood characteristics (brood size, brood sex ratio) of the brood they fledged from explained significant variation in the brood sex ratio produced by yearling females in year $t + 1$ (Table 3). The brood characteristics were also not significant in a model only containing the intercept (natural brood sex ratio: $\chi^2 = 1.154$, df = 1, $P = 0.283$; relative experimental brood sex ratio controlling for natural brood sex ratio: $\chi^2 = 1.379$, df = 1, $P = 0.240$; natural brood size: $\chi^2 = 0.001$, df = 1, $P = 0.975$; brood size treatment controlling for natural brood size: $\chi^2 = 0.223$, df = 2, $P = 0.637$). This indicates that the effect of the sex ratio treatment was more likely due to the plot manipulation rather than the brood manipulation, although a role of the latter cannot be excluded.

Plot of settlement

The brood sex ratio produced by yearling females in year $t + 1$ was not affected by the plot of settlement sex ratio or density treatment in year t and did not correlate with the natural density in the plot of settlement the previous year (Table 3).

Treatment effects on sex allocation by experienced females

The brood sex ratio produced by experienced females was significantly affected by the plot sex ratio treatment in the previous year (Table 4). Experienced females that had bred in control plots produced more female-biased broods, whereas females that had bred in female- and male-biased plots produced unbiased brood sex ratios (Table 4, Figure 3A). The density treatment in the previous year also affected the brood sex ratio produced the next year. Experienced females that were subjected to an experimentally increased nestling density in the previous year produced more female-biased broods in the next year, whereas females that had experienced an experimentally reduced nestling density produced more male-biased broods (density treatment effect in Table 4, Figure 3B). The natural plot density in the previous year did not correlate with the brood sex ratio in year $t + 1$ (Table 4). The effects of the plot sex ratio and the plot density treatments did not vary between years (including all underlying variables, sex ratio treatment × year: $\chi^2 = 1.354$, df = 4, $P = 0.852$, density treatment × year: $\chi^2 = 1.236$, df = 2, $P = 0.539$).

Neither the natural nor the experimental brood characteristics in the previous year explained significant variation in the brood sex ratio produced by experienced female breeders in the full model (Table 4) or a model only containing the intercept (natural brood sex ratio: $\chi^2 = 0.953$, df = 1, $P = 0.329$; relative experimental brood sex ratio controlling for natural brood sex ratio: $\chi^2 = 0.075$, df = 1, $P = 0.784$; natural brood size: $\chi^2 = 0.026$, df = 1, $P = 0.872$; brood size treatment controlling for natural brood size: $\chi^2 = 0.055$, df = 2, $P = 0.814$). This indicates that the effects found were more likely caused by the plot and not by the brood manipulation.

Sex ratio adjustment and selective disappearance

Experienced female breeders significantly shifted their brood sex ratio toward more females the next year when they had bred in a high-density plot the previous year and toward more males when they had bred in a low-density plot (Table 5). Furthermore, females that bred in control plots significantly shifted their brood sex ratio toward more females the following year (Table 5).

Local return rate to the next year of experienced females that produced a certain brood sex ratio (year t) was not differentially affected by the density treatment (density treatment × natural brood sex ratio year t: $\chi^2 = 2.443$, df = 1, $P = 0.118$; density treatment: $\chi^2 = 0.591$, df = 1, $P = 0.442$; natural brood sex ratio: $\chi^2 = 3.893$, df = 1, $P = 0.048$) or the plot sex ratio treatment (plot sex ratio treatment × natural brood sex ratio: $\chi^2 = 1.024$, df = 2, $P = 0.599$; plot sex ratio treatment: $\chi^2 = 0.437$, df = 2, $P = 0.804$; natural brood sex ratio:

Table 5

Analysis of change in brood sex ratio between year *t* and *t* + 1 of *experienced* female great tits (2006–2008) examining the effects of density treatment, plot sex ratio treatment, brood size treatment and the relative experimental brood sex ratio (deviance of final brood to final plot sex ratio) and correlations with natural plot sex ratio, natural plot density, natural brood size, and natural brood sex ratio (year *t*)

Explanatory variable	β (SE)	χ^2	df	*P*
Final model				
Intercept	−0.122 (0.167)	0.540	1	0.462
Year 2006	−0.131 (0.174)	0.988	2	0.610
Year 2007	0.045 (0.160)			
Plot of breeding natural density	0.0001 (0.002)	0.001	1	0.975
Plot of breeding natural sex ratio	−0.443 (2.122)	0.044	1	0.834
Plot of breeding density treatment: high	−0.394 (0.142)	7.681	1	0.006
Plot of breeding sex ratio: female-bias	0.356 (0.167)	6.252	2	0.044
Plot of breeding sex ratio: male-bias	0.371 (0.165)			
Natural brood sex ratio day 2	−3.696 (0.352)	110.299	1	<0.001
Natural brood size day 6	0.016 (0.041)	0.148	1	0.700
Relative experimental brood sex ratio	−0.119 (0.592)	0.041	1	0.839
Brood size treatment: reduced	0.042 (0.160)	0.812	2	0.666
Brood size treatment: enlarged	0.133 (0.148)			
Random effects	-	-	-	-
σ^2 ± SE plot				
Cohort	0.017 (0.032)	0.283	1	0.595
Individual	0.527 (0.065)	65.826	1	<0.001

Reference categories for the categorical variables are control sex ratio treatment, low density, year 2005, and control brood size, respectively. Estimates are derived from the normal response modeling procedure in MLwiN, *n* = 160.

χ^2 = 0.007, df = 1, *P* = 0.933). There was thus no evidence that the density or plot sex ratio treatment selectively affected local return rate of females depending on their natural brood sex ratio in year *t*.

In summary, our results showed that experienced female breeders adjusted their brood sex ratio in the following year toward females when the plot density of nestlings had been increased and toward males when the plot density of nestlings had been reduced. Brood sex ratio produced by yearling females in the next year was not affected by the density treatment but showed a negative correlation with the natural density in the plot of fledging. Apart from the density treatment, the plot sex ratio treatment also affected brood sex ratios the next year but differently for experienced and yearling females. Yearling females produced female-biased broods when the sex ratio in the plot of fledging had been male-biased and male-biased broods when it had been female-biased. Experienced females produced female-biased broods in control plots but unbiased brood sex ratios in the other sex ratio treatment categories. Yearling females did not alter their brood sex ratio to the density or sex ratio treatment that the plot of settlement had received the previous year.

DISCUSSION

We tested whether experimental changes in plot density and plot sex ratio affected subsequent sex allocation decisions of great tit females in a 3-year experiment. Our results suggest that experimental alterations of local density and sex ratio in one year can have long-term effects on brood sex ratios produced the next year. Thus, the manipulated social cues triggered changes in sex allocation.

The effect size of the density effect (calculated with z-values according to formula (11) in Nakagawa and Cuthill 2007) was 0.24 and the effect size of the plot sex ratio treatment was 0.16–0.18, which is not very strong compared with effect size found for studies on helper effects on sex allocation in birds (0.397) but similar to effect sizes found for effects of mate quality on sex allocation (0.187–0.205; West and Sheldon 2002). Thus, the effects found in our study are not very strong but similar to effect sizes found by other experimental studies on sex allocation.

The question remains whether the brood sex ratios produced by the females in our system were indeed adaptive. We could not test whether this was the case with our experimental setup. For that, brood sex ratios of females would need to be manipulated on an individual level in the year that the females produced the brood sex ratio. Subsequent fitness consequences of sex allocation could then be estimated (Radersma R, Ubels R, van der Velde M, Tinbergen JM and Komdeur J, unpublished data). This was not possible in our experimental set up because in 2 of the 3 study years (2006 and 2007) where we measured the sex allocation response, we had to manipulate broods again to achieve the plot-level manipulation. Indeed, it is possible that brood sex ratios produced by females were not adaptive. Sex ratios might be constrained if 1) the physiological mechanism that evolved in response to natural selection cannot respond to changes in the corresponding environmental or social cues, 2) the cues are mismatched to the temporal or spatial scale the sex allocation mechanisms evolved on, or 3) if more than one adaptive mechanism operates at the same time (Wild and West 2007).

In the following, we will first discuss how successful the experimental design was in manipulating offspring densities and sex ratio both on the temporal and on the spatial scale. We will highlight what implications this has for the interpretation of the results. Then we will discuss to what extent our results are consistent with the predictions of the 4 possible adaptive sex allocation scenarios (Table 1) presented in the introduction and the problems that arise when several scenarios would act simultaneously.

Complications of the experimental design: manipulation effects on the spatial and temporal scale

Our experiment is one of the few large-scale experiments that manipulated the social environment in a free-ranging population. However, the interpretation of the results is hampered by the change of the social environment in space and time after the experiment.

Spatial scale

We manipulated sex ratio and density of nestling birds on the scale of nest-box plots with an average size of 10.29 ha ± 1.39 SD situated at least 300 m apart. We expected that competition for resources such as food, roosts, mates, and space would take place mainly within these plots because during the nestling phase great tit parents were shown to have feeding ranges of 0.33–0.44 ha (Naef-Daenzer 2000) and fledged great tit families had home ranges of around 8.3 ha (Naef-Daenzer and Grüebler 2008; Matthysen et al. 2010). We also expected birds to compete or gather information in adjacent plots because natal dispersal distances were often larger than distances between plots (males: 1.09 km ± 1.01 SD, females: 1.43 km ± 1.01 SD; Nicolaus et al. 2012). Furthermore, in the postfledging phase, juvenile great tits have been observed at an average distance of 1307.58 m ± 23.25 SE from the nest-box of fledging (Michler et al. 2011b). This suggests that yearling birds could experience competition and gather information on a larger scale than a single plot. Breeding dispersal between plots was rarely observed (males: 10/210, females: 15/270). This may help to explain why yearling and experienced females were differently affected by the density and sex ratio treatment in the plot of fledging/breeding because yearling and experienced females may not experience social stimuli on the same spatial scale.

Temporal scale

Dispersal of young after fledging altered the experimental bias in plot density and sex ratio over time (supplementary Figures S5 and S6). The adaptive scenarios 2, 3, and 4 (Table 1) assume that cues such as plot density and sex ratio are linked to selection pressures that are also present in the subsequent year. The experimental changes in plot sex ratio of juveniles were detectable as bias in sex ratio of fledglings until October in one year and even seemed to have lasted until winter because the sex ratio of birds roosting in nest-boxes was still biased (Nicolaus et al. 2012) but whether also the plot sex ratio of breeding birds was biased the next year is not known. With respect to plot density, some autocorrelation between years did exist (supplementary Table S2). Thus, the assumption of autocorrelation of plot characteristics between years is partly fulfilled, although this has only theoretical value because, as already mentioned, we could not measure whether brood sex ratios were adaptive. More importantly, the consistency of plot characteristics over time indicates that the social/environmental cues that birds reacted to could have had their impact anywhere between June and the following breeding season.

Interpretation of sex ratio pattern in the light of the 4 adaptive scenarios

Three out of the 4 adaptive scenarios are roughly in accordance with the patterns found in our study (scenarios 1–3, Table 1). However, effects of both the plot density and the plot sex ratio on subsequent sex allocation differ between experienced and yearling females, and we found effects of the natural plot density as well. This suggests that none of the 4 adaptive explanations can be exclusively applied to our brood sex ratio results.

The experimental changes in density and sex ratio could have resulted in changes in physiological state, body condition, dominance status, or resource holding potential, in accordance with scenario 1 (Table 1). At high natural density and male-biased sex ratio, yearling females may have experienced high competition levels and consequently had lower condition. As a result, they may have produced more female offspring the next year because they lacked resources to produce the larger male offspring (Myers 1978) or because they had lower than average condition which, when translated to their offspring, might provide relatively higher fitness prospects for daughters than sons (Trivers and Willard 1973). Previous experimental studies have suggested that the proximate mechanism of sex allocation involves changes in female body weight (Nager et al. 1999; Kalmbach et al. 2001; Whittingham et al. 2005), changes in plasma glucose level (Cameron et al. 2008), and hormonal changes (Love et al. 2005; Rutkowska and Cichoń 2006; Bonier et al. 2007). In our study, we could not adequately test whether brood sex ratio adjustment in response to plot characteristics was mediated by changes in female body condition or other physiological traits, because we lacked measurement of female condition at the right time.

Yearling females' brood sex ratio was related to natural plot density but not affected by the density treatment. A possible explanation for this is that yearling females reacted to a variable linked to natural nestling density, which was not manipulated. One such variable is breeding pair density (correlated to natural nestling density controlling for year: $r_c = 0.78$, $n = 36$, $P < 0.005$), which might modulate competition pressure for yearlings. Unlike yearling females, experienced females were affected by the density treatment. This could be because juveniles dispersed too quickly after fledging to experience the changes in density for long enough (supplementary Figure S5). Adults are more restricted than juveniles in their postfledging dispersal (Hinde 1952; Saitou 1979) and therefore may be more susceptible to changes in plot density. Thus, experienced and yearling females probably experience different environmental or social cues.

With regard to scenario 2, previous studies in higher vertebrates that investigated sex allocation in response to operational sex ratio provided inconclusive results (Bensch et al. 1999; Le Galliard et al. 2005b; Allsop et al. 2006; Warner and Shine 2007). Most authors found that there was no evidence for frequency-dependent sex allocation (Bensch et al. 1999; Le Galliard et al.2005b; Allsop et al. 2006) and Warner and Shine (2007) even found that more of the same sex was produced, suggesting that other mechanisms are more relevant than pure frequency dependence.

So far, several descriptive studies in mammals and birds have demonstrated that sex ratios are skewed toward the sex, which is less philopatric (Silk and Brown 2008) if densities are high or local competition increased (Hewison and Gaillard 1996; Johnson et al. 2001; Hjernquist et al. 2009) as suggested by scenario 3. Also in great tits, one study found that fledgling sex ratio was female-biased in years with high local breeding pair density (Drent 1984), which, however, might have been also due to differential nestling mortality.

Experienced females showed a mixed pattern with more females being produced in control plots and more males in female- and male-biased plots. This pattern is only partly consistent with scenario 4 but rather suggests that at a certain experimental plot sex ratios, one sex allocation scenario might become more important than another. For instance, the production of the philopatric sex (scenario 3) or the minority sex (scenario 2) could be more important at female-biased plot sex ratios. However, if male-biased plot sex ratios indicate a high plot quality (more resources for production of the expensive sex) or indicate that the plot is suitable for the production of males (scenario 4), it might become more important to invest in males (Warner and Shine 2007). A similar though reversed pattern was found by Forsman et al. (2008), who showed that pied flycatchers adjusted brood sex ratio to the local density of great tits, with more females being produced at low and high densities. This pattern could be explained if flycatchers used increasing density as indicator of high-quality habitat and produced the least dispersing sex (Julliard 2000), but the advantage of producing sons might be outweighed at given densities by the increasing costs of competition.

Large-scale manipulation in wild populations may alter both ecological (high density = abundant resources) and social stimuli (high density = high competition for resources) for birds. Such effects may act simultaneously, yet affect sex allocation in opposite directions. In such a case, it is also likely that net effects on sex allocation are rather small (this study), nonlinear (Forsman et al. 2008) or that even no consistent bias in brood sex ratio is observed (Radford and Blakey 2000; Leech et al. 2001). Furthermore, because we manipulated plot density and plot sex ratio, the ecological and social cues may not agree (high density indicates high competition but not high food abundance) and consequently, sex allocation in response to manipulated plot characteristics would not yield adaptive brood sex ratios. Other studies have investigated brood sex ratio patterns in the light of several adaptive scenarios (Rathburn and Montgomerie 2005; Neto et al. 2011) and also found support for several scenarios (Neto et al. 2011) but the implications of that for the general picture are normally not given. That the results from empirical sex ratio studies on vertebrates often do not match theoretical predictions can also be attributed to the difficulty to quantitatively integrate predictions for several adaptive scenarios (but see Wild and West 2007). Moreover, it is often difficult to measure key variables or cues because we do not know what animals actually measure (Alonzo and Sheldon 2010). For instance, how can we measure whether individuals interpret sex-specific densities as increased competition or as social information? We need new approaches to tackle such problems.

CONCLUSIONS

We showed experimentally that local density and sex ratio have effects on sex allocation of both parent and offspring female great tits. Although, we cannot provide one exclusive adaptive explanation or the mechanism underlying our results, we provide evidence that local density and sex ratio are important cues for avian sex allocation. The patterns found are largely consistent with the idea that females produced the offspring sex that would yield highest fitness prospects in a given social setting or for a certain female condition. Nonetheless, we cannot provide unequivocal evidence that such a behavior would produce adaptive brood sex ratios in a natural situation. Empirical sex allocation studies very often do not attempt to show the adaptive value of the sex ratio response (but see Komdeur 1998; Le Galliard et al. 2008), which is probably due to the various difficulties associated to this task (e.g., studying fitness effects requires study periods longer than a single year for most vertebrate species). In cases like our study system, where several mechanisms probably act simultaneously on sex allocation, it might even be an impossible undertaking. Nonetheless, we think experimental studies on sex allocation should be continued. Studies on island populations where dispersal is limited might provide further insight but also replication of well-designed experiments between species or populations with similar but slightly differing life histories should be evoked. For example, comparative experimental studies between species with and without sexual dimorphism but that both show sex-biased dispersal. Experimental designs are needed that adequately estimate the fitness consequences of sex ratio decisions such that the relative importance of a given adaptive sex allocation mechanism can be established for a given system. Future studies should first test whether individuals adjust brood sex ratios to given individual, social, or environmental cues, and then brood sex ratio should be manipulated on the individual level to measure fitness consequences (survival and reproduction of adults and young).

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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