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Experimental nest site limitation affects reproductive strategies and parental investment in a hole-nesting passerine

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In resource defence mating systems, males monopolize a resource that is of primary importance for breeding females. For secondary cavity nesters, the availability of suitable nesting sites is important in determining the strength of intrasexual competition, whereby phenotypic and behavioural traits will be favoured that enable individuals to gain access to these sites. The traits that are important in male competition may additionally affect mate choice decisions and a female's investment in the current brood. In a field study on blue tits, *Cyanistes caeruleus*, we increased intrasexual competition by experimentally limiting nest sites in experimental plots and compared these plots to control plots. Birds breeding in experimental plots did not differ phenotypically from birds in control plots. However, females that bred in the nest site-limited plots fed their offspring at a higher rate than control females. This result indicates that increased competition for limited resources led to more investment in current reproduction, either because successful females were of higher intrinsic quality or because they adjusted their investment in relation to superior territory or male characteristics.

In birds, most mating systems are based on resource defence (Lack 1968). Males have to compete among each other for suitable territories or limited nesting sites in order to attract mates (Andersson 1994). The intensity of this conflict among males is expected to depend on variation in male quality, on the predictability and the abundance of the environmental resources and on a male's ability to monopolize and defend them. Factors such as reduced habitat complexity and limited food resources are thought to intensify intrasexual competition (Basquill & Grant 1998; Maher & Lott 2000). Limiting factors are expected to differ between species according to their life history and vary depending on the local environment.

In secondary cavity nesters, nest hole limitation, that is, the availability of suitable natural cavities, poses an important selective force determining the strength of inter- and intraspecific competition (von Haartman 1971; Newton 1994). From an individual's perspective, a limitation of suitable cavities will affect a bird's probability of breeding and consequently its current reproductive success. A decrease in nest sites should therefore lead to more intense intrasexual competition (Gustafsson 1988; Newton 1998), so that strategies or traits will be favoured that enable males and females to gain access to these resources. Such traits might be related to physical dominance (e.g. size) or secondary sexual traits that reliably indicate an individual's condition and competitive ability (e.g. plumage colours, Senar 2006) or they might originate from plastic variation in fighting ability or aggressiveness caused by differences in resource-holding potential or motivation (Hurd 2006).

As a result of intense competition, the quality of the defended resources should covary with individual quality and sexual signals (Orians 1980). When territories are limited, there is empirical evidence that territory holders have larger and brighter plumage patches than 'floating' nonbreeders in male red-shouldered widowbirds, *Euplectes axillaris* (Pryke & Andersson 2003a, b), eastern bluebirds, *Sialia sialis* (Siefferman & Hill 2005), male collared flycatchers, *Ficedula albicollis* (Part & Qvarnstrom 1997) and both sexes of rock sparrows, *Petronia petronia* (Pilastro et al. 2003). Such signals indicate a male's vigour or dominance and are important in male competition (Alonso-Alvarez et al. 2004; but see Korsten et al. 2007), but can additionally be used by females to assess a male's ability to provide direct or indirect benefits (Berglund et al. 1996; Borgia & Coleman 2000). When these male sexual signals are related to offspring quality, females should adjust their investment

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in the current brood according to these male traits (the differential allocation hypothesis, Burley 1988; Sheldon 2000).

Limitation of essential resources will also lead to a situation where some individuals that are capable of breeding are prevented from doing so. Selection should then favour alternative strategies that allow subdominant individuals to obtain reproductive success. Floating males can gain paternity via extrapair copulations (Kempenaers et al. 2001), a strategy that is not limited to territory holders. Females, on the other hand, are known to parasitize conspecific broods (Yom-Tov 2001). In eastern bluebirds, a reduction in the number of nestboxes caused a significant increase in the rate of dumped eggs from floating females (Gowaty & Bridges 1991). A similar effect was found in a correlative study on blue tits, *Cyanistes caeruleus*, where a limitation of nesting sites led to occasional cases of intraspecific brood parasitism (Vedder et al. 2007). These studies demonstrate that individuals are able to adjust their investment in reproductive behaviour in a changing environment and highlight the importance of investigating the plasticity in reproductive and life history strategies in relation to variation in environmental conditions.

We performed a nest site limitation experiment in a population of blue tits that breed in nestboxes. Shortly before egg laying started, we removed all nestboxes in the population. In experimental plots, we provided half of the original number of boxes at new locations (on territory boundaries). In control plots, all nestboxes were re-erected so that all pairs obtained a new nest site. We thus created plots with low and high competition for nesting sites within the study population. Blue tits are the smallest secondary cavity nesters in European deciduous forests, and are inferior in competition for nest sites with the great tit, *Parus major*. The presence of great tits can thereby limit the numbers of the subdominant blue tit (Dhondt & Adriaensen 1999). Suitable natural cavities are often rare in European 'managed' forests, and the experimental removal of small-holed nestboxes, which can only be used by blue tits, is likely to have a major impact on the opportunities for breeding in this species.

Our aims in this study were (1) to compare phenotypic traits of males and females that obtained a nestbox after experimental nest site limitation with those in control plots, and (2) to investigate how availability in nesting sites translates into variation in mating strategies and reproductive investment (i.e. brood sex allocation and patterns of parental care).

METHODS

General Methods

The study was conducted in 2005 in a colour-banded blue tit population breeding in nestboxes in a mixed deciduous forest at Kolbeterberg, Vienna, Austria (48°13′17″N, 16°14′12″E). The study area contained a total of 233 nestboxes and was divided into two experimental and two control plots. The four plots were delineated (1) to minimize edge effects within a treatment (e.g. the contact area between the two control and the two experimental plots was close to zero) and (2) to control for habitat heterogeneity within the study area (Valcu & Kempenaers 2008). Splitting up the area into four plots should ensure that differences in habitat quality between experimental and control plots were minimized. In two plots we reduced the number of nestboxes by 50% (experimental plots), whereas the other two plots served as controls (Fig. 1). Control and experimental plots were of similar size (control plots: $C_1 = 11.6$ ha, $C_2 = 13.4$ ha; experimental plots: $E_1 = 11.5$ ha, $E_2 = 13.5$ ha).

Fieldwork

Pre-experimental period

During the pre-experimental period (mid-March–5 April) all nestboxes were checked daily for the stage of nest building. Based on nest-building activity, we identified 78 breeding pairs in our study area (34% box occupation rate). Control and experimental plots contained the same number of breeding pairs (control: $N = 39$; experimental: $N = 39$). We visited each box daily and

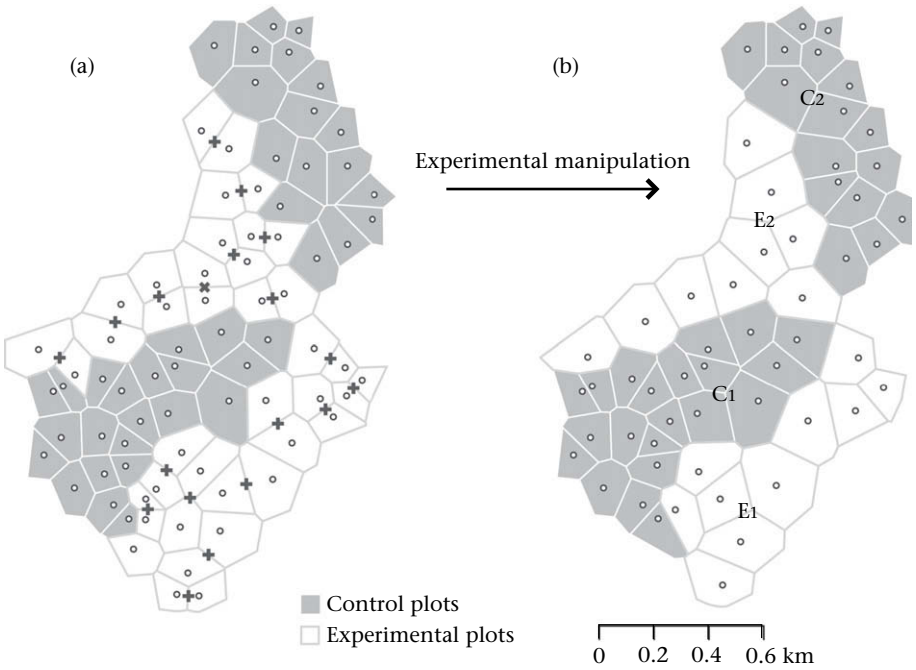

Figure 1. Map of the study site indicating occupied territories (a) before and (b) after the experimental manipulation. In two plots (E_1, E_2; white) we reduced the number of nestboxes by 50% shortly before egg laying started. Circles refer to the original nestboxes; crosses indicate the location of the nestboxes after the experimental manipulation. Two plots (C_1, C_2; grey) served as controls.

recorded the identity of the individuals showing territorial behaviour near the box. Unbanded individuals were trapped with a mist-net trap near the nestbox and marked with a unique combination of three plastic colour bands and a numbered metal ring. In 65% of the boxes we could identify or band both individuals, in 26% of the boxes only one individual was identified and in the remaining 9% of the boxes none of the individuals could be identified or captured. The number of individuals identified (none, one or both) did not differ between the control and experimental plots ($\chi^2_2 = 2.90$, $N = 78$, $P = 0.23$).

Experimental manipulation

The experimental manipulation took place on 5 April, which is the mean date of the first egg in the population for the previous 6 years (1998–2004). In the afternoon all nestboxes (155 empty boxes and 78 boxes where nest building had started) were removed and kept in an opaque plastic bag near the old position. During the following night (from 5 to 6 April) we put up new boxes as follows. In control plots (C_1 and C_2), all active nestboxes that contained nest material were replaced with a new box in exactly the same location. In experimental plots (E_1 and E_2), one new nestbox was placed at the estimated territory boundary of two adjacent occupied territories (Fig. 1). To estimate territory boundaries we used a spatial mapping technique known as Dirichlet tessellation (Adams 2001). Dirichlet tessellation assigns all the space that is closer to a given breeding box to that box rather than to any other box. Thus, in experimental plots breeding opportunities were reduced from 39 to 19 boxes (E_1: 21 → 10, E_2: 18 → 9), whereas in control plots every breeding pair simply received a new box (C_1: 22 boxes, C_2: 17 boxes) at their previous nest site (Fig. 1).

Postexperimental period

A reliable quantification of competitive interactions (e.g. identity of individuals) at the new nestboxes was not feasible and we therefore focused on recording the stage of nest building and egg laying on a daily basis and on determining the identity of the breeding pair. For all breeding pairs, clutch size, hatching success and fledging success were determined by regular nestbox checks. Nestling body mass was measured 14 days after hatching (with an electronic balance, ±0.1 g), with day 1 as day of hatching. On day 14 we also banded nestlings and measured their tarsus (standard technique in Svensson 1992, with slide callipers, ±0.05 mm) and wing length (flattened and straightened, with callipers, ±0.5 mm). On day 18, shortly before the nestlings fledged, we measured the plumage colour of the ultraviolet (UV)/yellow breast, using a UV-sensitive spectrometer (Avantes, AvaSpec-2048, AvaLight-DHS, Avantes, Eerbeek, The Netherlands). In addition, we plucked a tail feather (third from the right) to measure variation in the UV/blue tail feather coloration. Removing a rectrix during moult induces the regeneration of a replacement within a few days and is expected to have negligible fitness costs (Grubb 1995). In the laboratory, the tail feather spectra were measured against a black velvet background with uniform, low reflectance across all wavelengths. Reflectance spectra were measured at two different spots along the erupting tail feather. For a detailed description of the methodology and the analysis of the colour variables see Jacot & Kempenaers (2007). The fledglings' greyish crown coloration was not measured, because birds do not acquire the UV/blue crown until postjuvenile moult in autumn.

From all adults and nestlings we took a 5–100 μl blood sample from the brachial vein for molecular sexing (Griffiths et al. 1998) and paternity analysis. We also collected the embryo from eggs that did not hatch and a piece of tissue from all dead nestlings found inside a nestbox, as a source of DNA. The methods used to analyse paternity have been described for this blue tit population in detail elsewhere (Foerster et al. 2003; Valcu & Kempenaers 2008). Around day 10, we captured all adults in the nestbox and measured tarsus, wing length, body mass and plumage coloration using photospectrometry. In addition, unbanded birds were banded with a unique colour combination consisting of three plastic colour bands (AC Hughes, Middlesex, U.K.) and one numbered metal band (Vogelwarte Radolfzell, Radolfzell, Germany). All birds were fitted with a small transponder (ID 101; length: 11.5 mm; mass: 0.1 g) that was attached to one of the colour rings. To minimize handling time of birds, passive transponders were already glued (UHU plus endfest 300, uhu GmbH, Bühl/Baden, Germany) to a single colour ring in the laboratory and the ring/transponder unit could instantly be fitted to a bird's leg in the field. A similar method has been used in our study population for another experiment, and no adverse effects on adult birds were detected (Johnsen et al. 2005).

Measurement of Parental Investment

Blue tits feed single prey items during each feeding visit to the brood (Cramp & Perrins 1993). The number of feeding events within a 24 h period is therefore expected to be a good indicator of an individual's investment in offspring feeding, even if food quality might differ slightly between each food item.

To quantify parental feeding rates we used a transponder identification system from Trovan RF (Euro I.D., Weilerswist, Germany; for further details see Johnsen et al. 2005). The system consists of an antenna around the nest hole, which is connected to an OEM board/data logger that records the exact time and the unique transponder number whenever a tagged bird passes the nest hole in either direction. The OEM board/logger unit and the 12 V battery were placed in a waterproof plastic bag and mounted on the same tree somewhat lower than, and at the opposite side of, the nestbox. If a bird sits in the entrance hole, the transponder touches the border of the magnetic field and many recordings in rapid succession will occur. To extract reliable feeding rates from these data, we excluded all data points that were less than 3 s apart. Previous video analyses showed that male feeding events can last for a few seconds only and that the cutoff point of 3 s reliably reflects actual feeding events (our unpublished data). All analyses were also performed with a cutoff of 6 s, following a previous study (Johnsen et al. 2005), but this yielded similar results. Feeding effort was measured for a 24 h period, when chicks were between 8 and 16 days old. Feeding rates were recorded for breeding pairs where both parents were fitted with a transponder (12/18 pairs in the experimental plots, 18/38 pairs in the control plots).

Statistical Analyses

All statistical analyses were performed with R2.5.0 (R Development Core Team 2007). The functions lm and glm were used to fit general linear and generalized linear models, respectively; function confint (Venables & Ripley 2002) was used to compute confidence intervals for glm parameters. For glms with binomial error distribution we used the Wald test statistic, which has a chi-square distribution (Fox 2002). The package nlme (Pinheiro et al. 2005) was used to fit linear mixed-effects models, following Pinheiro & Bates (2000). The denominator degrees of freedom of the test statistics of mixed models were computed according to Pinheiro & Bates (2000, page 91). The standard model diagnostics of non-normal errors, nonconstant error variance and the presence of outliers were performed on each of the final models according to Fox (2002).

To investigate treatment effects on adult and offspring phenotypic traits, we selected models in a stepwise fashion using the function stepAIC in the R package MASS (Venables & Ripley 2002). The function stepAIC selects the most parsimonious model based on minimizing the Akaike information criterion (AIC) for adding

and deleting terms (Venables & Ripley 2002). Age (for adult traits) and sex (for offspring traits) and their interaction with treatment were included as covariates.

When analysing treatment effects on brood sex ratio (ratio of males to total number of offspring (males/(males + females))) we tested the two treatments against the predicted 50:50 sex ratio (Trivers & Willard 1973). To test whether the brood sex ratio is significantly different from the 50:50 expectation we ran a glm with binomial error distribution and logit link function on the proportion of males, including only the intercept. An intercept significantly different from zero (corresponding to 0.5, back-transformed from the logit scale) indicates a significant departure from a 50:50 sex ratio (Hardy 2002).

Absolute overall feeding rates of both parents and the relative contribution of each parent to offspring feeding were analysed using a repeated measures ANOVA with male and female feeding rates as repeats (i.e. within-subject factors), the between-subject factor 'treatment', and offspring number and age as covariates. Both covariates are known to affect parental food-provisioning behaviour in our population (brood size: see Johnsen et al. 2005; offspring age: own unpublished data) and are included 'a priori' in the models.

Ethical Note

The experimental removal of nesting sites created a situation where (1) individuals had to compete for nestboxes and (2) a subset of adults were prevented from breeding in nestboxes. Our study mimicked a natural situation, since (1) suitable nesting sites are limited in most if not all natural forests and (2) inter- and intraspecific competition for this scarce resource is expected to be high (Dhondt & Adriaensen 1999). Hatching success and fledging success in 2005 were not lower than in previous years (glmm with binomial error distribution and parents' identity as a random factor, all comparisons between 2005 and 1998–2004: hatching success: $N = 534$, all $P > 0.14$ except in 1998 where $P < 0.01$; fledging success: $N = 531$, all $P > 0.53$ except in 2001 where $P < 0.01$).

When measuring nestlings on day 14, we removed only half of the brood at a time. Measurements were carried out at a distance from the nestbox, such that parents continued feeding the other half of the brood. For the plumage colour measurements of chicks at day 18, whole broods were removed from the natal box for a period of 45–60 min. Adult birds were captured inside the nestbox when nestlings were approximately 10 days old, brought to a nearby site for colour and morphometric measurements and released near their nestbox after approximately 30 min. All field work was done under licence from the Magistrate of Conservation in Vienna and the Magistrate of Forestry and Agriculture.

RESULTS

In the morning following experimental removal of the nestboxes, focal observations indicated intense competition for the new nestboxes in the experimental plots (e.g. more than two individuals inspecting the same nestbox and chasing each other) whereas pairs in the control plots started immediately with nest-building activities. Nest material was found in all but one of the new nestboxes on the following days, so that occupation rate did not differ between the experimental (19 of 20) and control plots (37 of 39). Most individuals (72%) that resumed breeding had previously been identified in the population (71 individuals observed before manipulation were observed among the 99 banded individuals breeding after nestbox limitation). After the nest site limitation, only three individuals (three pairs) moved from the experimental to the control plots, whereas no bird moved from the control plots to the experimental plots. Experimental and control nests did not differ in laying date or in hatching date (Table 1). Thus, seasonal effects can be excluded when further assessing treatment effects.

Table 1
Means ± SE per brood and test statistics for breeding parameters in relation to the nest site limitation

	Experimental groups		*t*	*P*
	Control (*N*=36)	Nest site limited (*N*=19)		
Laying date	0.05±0.18	−0.10±0.21	−0.56	0.58
Hatching date	−0.08±0.14	0.15±0.32	0.49	0.63
Clutch size	11.5±0.4	12.0±0.7	0.60	0.55
Hatchling number	10.5±0.5	9.9±0.8	−0.62	0.54
Fledgling number	9.2±0.7	7.8±1.1	−1.22	0.23

Tests: glm with Gaussian error distribution.

Adult Morphometric Traits

Individuals that bred in the control and experimental plots did not differ in tarsus length, wing length and body mass (Table 2). There was also no difference in residual body mass in females (glm with Gaussian error distribution (ANCOVA): treatment: $t_{43} = -0.95$, $P = 0.35$; covariate tarsus length: $t_{43} = 0.60$, $P = 0.55$) and males (glm with Gaussian error distribution (ANCOVA): treatment: $t_{43} = 0.14$, $P = 0.89$; covariate tarsus length: $t_{43} = 3.38$, $P = 0.001$). Neither the UV/blue crown and tail coloration nor the UV/yellow breast coloration of breeding males and females differed between the experimental and control plots (see Appendix Table A1).

Breeding Success and Offspring Traits

Females from experimental and control plots laid similar-sized clutches, and their broods contained a similar number of hatchlings and fledglings (Table 1). Similarly, there was no difference between the experimental and control plots in hatching success (glm with binomial error distribution and logit link function, with the number of hatchlings as the dependent variable and the total number of eggs as the binomial denominator: control: 87.7 ± 2.6%; experimental: 83.0 ± 4.6%; $\chi^2_1 = 0.21$, $N = 50$, $P = 0.65$) and fledging success (i.e. % of eggs that fledged, glm with binomial error distribution and logit link function, with the number of fledglings as dependent variable and the total number of eggs as binomial denominator: control: 76.8 ± 5.0%; experimental: 65.5 ± 8.3%; $\chi^2_1 = 1.85$, $N = 50$, $P = 0.17$).

Table 2
Means ± SE (body mass, tarsus and wing length), median and confidence interval (age) and test statistics of male and female adult blue tits in relation to the nest site limitation

	Experimental groups		Test	*P*
	Control	Nest site limited		
Males				
Age	2 (1.72–2.28)	2 (1.58–2.42)	χ^2_1=0.03	0.86
Body mass	11.63±0.14	11.63±0.19	t_{44}<0.01	0.99
Tarsus	17.20±0.10	17.20±0.13	t_{44}<−0.01	0.99
Wing	68.03±0.21	68.60±0.26	t_{46}=−1.58	0.12
Females				
Age	2 (1.73–2.27)	2 (1.62–2.38)	χ^2_1=0.09	0.76
Body mass	11.03±0.11	11.10±0.12	t_{49}=−0.35	0.72
Tarsus	16.60±0.09	16.71±0.08	t_{48}=−0.76	0.45
Wing	65.38±0.18	65.68±0.26	t_{49}=−0.96	0.34

Tests: age: Pearson chi-square test with Yates' correction for continuity; body mass, tarsus and wing length: glm with Gaussian error distribution.

There were no detectable treatment effects on chick body mass on day 14 (linear mixed-effect model with box as random intercept: treatment: $t_{47} = 0.41$, $P = 0.68$; sex: $t_{453} = -6.86$, $P < 0.001$; covariate brood size: $t_{47} = -2.54$, $P = 0.01$), tarsus length (linear mixed-effect model with box as random intercept: treatment: $t_{47} = 0.26$, $P = 0.79$; sex: $t_{453} = -8.00$, $P < 0.001$; covariate brood size: $t_{47} = -0.82$, $P = 0.42$) and body condition (linear mixed-effect model with box as random intercept: treatment: $t_{47} = -0.71$, $P = 0.48$; sex: $t_{452} = -2.45$, $P = 0.01$; covariate tarsus length: $t_{452} = 15.69$, $P < 0.001$; covariate brood size: $t_{47}=-3.00$, $P < 0.01$). Neither the UV/blue tail coloration nor the UV/yellow breast coloration of male and female chicks differed between the experimental and control plots (see Appendix Table A2).

Brood Sex Ratio

The mean brood sex ratio (males/(males + females)) did not differ significantly between females breeding in nest site-limited and control plots (glm with binomial error distribution and logit link function, with the number of male chicks as the dependent variable and the total number of offspring as binomial denominator: treatment: $\chi^2_1 = 2.49$, $P = 0.11$; Fig. 2). In a second analysis we tested the two treatments against the predicted 50:50 sex ratio (Trivers & Willard 1973). Overall mean brood sex ratio was 0.53 (95% confidence interval, CI: 0.493–0.575). Pairs in the control plots produced broods that did not deviate from an expected 0.5 sex ratio (0.51, 95% CI: 0.464–0.565; to test against the null model we used a glm with binomial error distribution and logit link function, with the number of male chicks as the dependent variable and the total number of offspring as binomial denominator: $\chi^2_1 = 0.18$, $P = 0.67$; Fig. 2), whereas experimental broods contained significantly more males (0.57, 95% CI: 0.502–0.641; $\chi^2_1 = 4.14$, $P = 0.042$; Fig. 2). To rule out that brood sex ratios were generally male biased, we analysed brood sex ratios from 1998 to 2004 in the same part of the study site. First, we analysed the deviation of mean brood sex ratio from the 0.5 expectancy in any of the previous years. Mean brood sex ratios of birds breeding in the experimental plots never significantly deviated from the 0.5 expectancy between 1998 and 2004 (for details see Appendix Table A3). In a second step we analysed whether the sex ratio in the experimental year differed from that in previous years. The overall analysis is nonsignificant (glm with binomial error distribution and logit link function: year: $\chi^2_7 = 6.62$, $P = 0.47$), but the estimated sex ratio was always smaller than in 2005 and the sex ratio in 2004 was significantly less male biased than in the experimental year (Table 3).

Nest Site Limitation and Reproductive Strategies

Paternity data were obtained for 34 control and 18 experimental broods. The proportion of broods with extrapair paternity did not differ between the two treatments (control: 0.53 ± 0.09; experimental: 0.44 ± 0.12; Fisher's exact test: $N = 52$, $P = 0.77$). The proportion of extrapair young within broods did not differ between the two treatments (control: 0.16 ± 0.04; experimental: 0.11 ± 0.05; glm with binomial error distribution and logit link function, with the number of extrapair chicks as the dependent variable and the total number of offspring as the binomial denominator: $\chi^2_1 = 0.56$, $N = 52$, $P = 0.45$). In control plots, nine of 36 males (25%) sired at least one extrapair chick, while four of 19 males (21%) breeding in the experimental plots sired at least one extrapair young (treatment: Fisher's exact test: $N = 55$, $P = 0.91$). The probability of paternity gain (i.e. number of extrapair young by each male) did not differ between males in experimental versus control broods (glm with Poisson error distribution: treatment: $\chi^2_1 < 0.01$, $N = 55$, $P = 0.94$). The genetic father could not be assigned for 62 extrapair offspring (10% of 588 genotyped young) in 23 broods but the proportion of unassigned extrapair young did not differ between treatments (glm with binomial error distribution and logit link function corrected for overdispersion, with the number of unassigned chicks as dependent variable and the total number of offspring as binomial denominator: $\chi^2_1 = 0.64$, $P = 0.43$). These young may have been sired by floaters, males breeding in natural cavities (ca. 1.8% between 1998 and 2002; Foerster et al. 2003), or males breeding outside the study area.

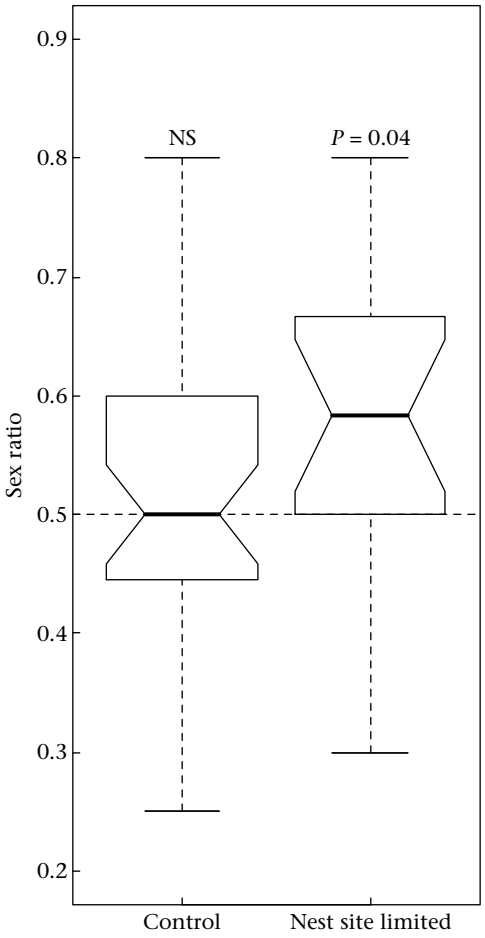

Figure 2. The effect of the nest site limitation on brood sex ratios (males/(males + females)). Box plots are shown with lines at the lower quartile, median and upper quartile values. The notches give 95% confidence intervals for the difference in two medians: a nonoverlap indicates that the medians are statistically different. The whiskers indicate the range of the data.

Table 3
Estimates and test statistics for sex ratios of birds breeding in the experimental plots before the nest site limitation experiment (1998–2004) in contrast to the experimental year 2005

	Estimate	SE	*Z*	*P*
Intercept (2005)	0.30	0.15	2.03	0.042
1998	−0.16	0.19	−0.82	0.41
1999	−0.29	0.18	−1.63	0.10
2000	−0.30	0.18	−1.74	0.08
2001	−0.27	0.18	−1.49	0.14
2002	−0.16	0.17	−0.92	0.36
2003	−0.22	0.17	−1.27	0.20
2004	−0.39	0.18	−2.14	0.032

Tests: glm with binomial error distribution and logit link function.

Eight cases of intraspecific brood parasitism (ISBP) were detected with a median of 2.5 dumped eggs per nest (range 1–10). The proportion of parasitized broods did not differ between control (4/34) and experimental (4/18) plots (treatment: Fisher's exact test: $N = 52$, $P = 0.42$). ISBP was significantly higher in the experimental year than in previous years (1998–2004: $4/536 = 0.75\%$; 2005: $8/52 = 15.38\%$; Fisher's exact test: $P < 0.001$). In all four cases of ISBP between 1998 and 2004, only one chick could genetically not be assigned to the breeding female.

Parental Investment: Daily Feeding Rates

Total offspring feeding rate (joint effort of both parents) did not differ between broods from experimental and control plots (repeated measures ANOVA: between-subject factor 'treatment': $F_{1,26} = 0.76$, $P = 0.39$) while the relative contribution of each parent to offspring feeding differed significantly between treatments (repeated measures ANOVA: within-subject contrast 'sex*treatment': $F_{1,26} = 6.22$, $P = 0.019$; covariate number of offspring: $F_{1,26} = 6.49$, $P = 0.017$; covariate offspring age: $F_{1,26} = 4.86$, $P = 0.037$; Fig. 3). Females from experimental plots fed their offspring relatively more often (mean relative feeding rate: $58.4 \pm 4.9\%$ of total feeding rate) compared to females from control plots ($43.2 \pm 3.5\%$ of total feeding rate). Post hoc tests demonstrate that the significant interaction is driven by a higher female feeding rate in experimental plots (glm with Gaussian error distribution (ANCOVA): treatment: $F_{1,26} = 5.99$, $P = 0.021$; covariate number of chicks: $F_{1,26} = 0.52$, $P = 0.48$; covariate offspring age: $F_{1,26} = 0.46$, $P = 0.50$), and not by lower paternal care (glm with Gaussian error distribution (ANCOVA): treatment: $F_{1,26} = 2.01$, $P = 0.17$; covariate number of chicks: $F_{1,26} = 6.29$, $P = 0.019$; covariate offspring age: $F_{1,26} = 4.45$, $P = 0.045$). When we entered male feeding rate into the model as covariate, the effect of the treatment on female feeding rate remained significant (glm with Gaussian error distribution (ANCOVA): treatment: $F_{1,25} = 4.30$, $P = 0.049$; covariate male feeding rate: $F_{1,25} = 1.19$, $P = 0.29$; covariate number of chicks: $F_{1,25} = 1.28$, $P = 0.27$; covariate offspring age: $F_{1,25} = 1.10$, $P = 0.31$), indicating that a female's investment in parental care is not driven by her mate's feeding pattern.

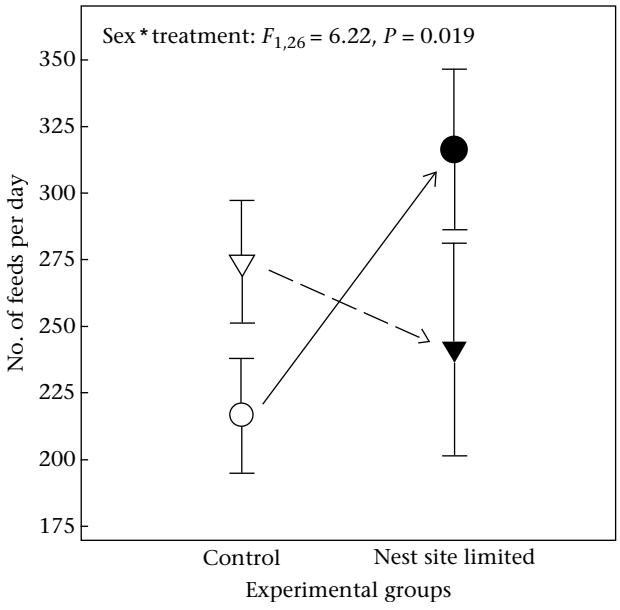

Figure 3. The relative parental offspring provisioning in control and experimental plots. Means are given ±SE. Triangles: males; circles: females.

DISCUSSION

This field study provides experimental evidence that a limitation of nest sites has significant but somehow cryptic effects on the reproductive behaviour of a secondary hole-nesting passerine. The effects are cryptic because there was no evidence that phenotypic characteristics were important in gaining a nest site and because successful birds did not produce more offspring or chicks of higher quality. However, the nest site limitation led to a relatively high incidence of intraspecific brood parasitism over the whole study area and we found a significant increase in maternal investment to offspring feeding in pairs breeding in experimental plots. These findings indicate a sex-specific effect, where only females increased their investment in the current reproductive attempt. We suggest and further discuss that the enhanced reproductive investment either reflects intrinsic aspects of female quality or is due to strategic allocation on the part of the female.

Both pair members are usually involved in territory establishment (Krebs 1982), but the relative investment by each sex and the importance of the cooperation within a pair are little explored. The higher investment of females in reproduction could be explained by intense female–female competition (Kempenaers 1994, 1995), where females of enhanced phenotypic and/or genetic quality outcompeted lower-quality females. The high investment in offspring feeding could thereby reflect a female's intrinsic quality largely independent of any strategic investment. However, females are also expected to optimize or plastically adjust their reproductive investment in relation to their mate's quality or the quality of a male's defended resources ('the differential allocation hypothesis', Burley 1986, 1988; Sheldon 2000).

Such an allocation pattern of females in current reproduction is a valid alternative, because the nest site limitation probably affected territory and male quality. By manipulating the availability of nesting sites, we reduced breeding density, which may have led to an increase in territory size and food availability and thus territory quality in experimental plots. On the other hand, the limitation of nesting sites led to an increase in competitive interactions, whereby high-quality males may have outcompeted other conspecifics. Even though males in experimental plots did not differ phenotypically from males in control plots, females may have altered their investment in relation to unmeasured traits or to the perceived attractiveness of a mate. The increase in competitive interactions among males for limited cavities and the fact that these males have outcompeted other males might have affected a female's perception of a male's quality where successful males were perceived as high-quality mates.

The nest site limitation had two effects on reproduction. First, experimental females invested more in offspring feeding than control females. Large territories might simply contain more food but females may additionally strategically feed more when breeding in a food-rich territory with a high-quality male (differential allocation hypothesis). Previous studies have shown that females altered their feeding behaviour in varying environments (Tripet et al. 2002; Nakagawa et al. 2007) and experimental studies demonstrated that enhanced male attractiveness can induce a female to invest more in offspring feeding (Limbourg et al. 2004; Johnsen et al. 2005). If territory quality was the main factor for differential allocation, one could expect a similar increase in feeding in males. However, males generally feed at a higher rate than females (Johnsen et al. 2005) and they show less plasticity in their feeding pattern (Nakagawa et al. 2007), which reduces the potential to detect a treatment-induced increase in male feeding behaviour. The increase in female feeding rates did not translate into significantly higher total feeding rates, indicating that males might even have slightly reduced their investment in

offspring feeding. Yet, this decrease was not significant and more than 2.5 times weaker than the increase in female feeding behaviour, reflecting at most a partial response to their partner's extra effort.

The second effect of the nest site limitation was that there was a tendency for females from experimental plots to produce male-biased broods. Although this treatment effect was rather small and only marginally significant when tested against the predicted 50:50 sex ratio, such an allocation pattern might be adaptive when nesting sites are limited. The sex allocation theory (Trivers & Willard 1973) provides an explanation for the sex ratio bias. Given that sons inherit their father's 'attractiveness' or 'dominance', females mated with high-quality males should produce more sons, because sons are expected to derive higher fitness benefits (Korsten et al. 2006; Leech et al. 2006; Delhey et al. 2007). Even though males show limited natal dispersal compared to females (Greenwood & Harvey 1982; Matthysen 2005) and intrasexual competition is expected to increase in the following years, the production of 'dominant sons' might be adaptive when paired with a high-quality male, either because the sons will outcompete other males for limited nest sites or because they will simply be more attractive to females (Weatherhead & Robertson 1979). Clearly, more experimental data are needed to demonstrate whether, and under what conditions, females adjust their investment in daughters or sons.

The proximate mechanisms responsible for the observed allocation patterns in females are still unclear. The increased investment of experimental females in the current breeding attempt could be mediated via circulating androgen levels that were elevated during territory establishment and were subsequently transferred via maternal effects to the offspring. Recent studies indicate that an increased testosterone level of a female can bias the primary sex ratio towards males (Veiga et al. 2004; Rutkowska & Cichon 2006) and increase a chick's begging behaviour (Eising & Groothuis 2003; von Engelhardt et al. 2006; Goodship & Buchanan 2006). In line with these studies, the females' increase in offspring feeding may thereby partly be explained by a plastic response to more vigorous begging behaviour of their chicks.

As a consequence of our experimental design, half of the individuals in the experimental plots were prevented from breeding. Floating females that did not manage to monopolize a nestbox in experimental plots might have adopted a best-of-a-bad job strategy by dumping eggs into other females' nests. Such an alternative reproductive strategy has already been found in eastern bluebirds, where an experimental reduction in nesting sites led to an increase in dumped eggs (Gowaty & Bridges 1991). Intraspecific brood parasitism, that is, females laying eggs into another female's nest has only recently been described in blue tits (Vedder et al. 2007). This study corroborates their findings and indicates that brood parasitism is an alternative reproductive strategy in blue tits. Whether this phenomenon is a common strategy of blue tit females is difficult to infer from our experimental set-up, because nest sites were limited just shortly before egg laying. The importance of brood parasitism in secondary cavity nesters can probably only be detected in studies mimicking a natural situation and not under conditions where nestboxes are provided in excess (i.e. most of today's studies on cavity nesters; see also Kempenaers et al. 1995).

To summarize, this study demonstrates how short-term variation in resources, as seen in the limitation of nesting sites, can potentially influence the strategic investment of females in current reproduction. This plasticity in current investment probably reflects an optimal resource allocation, where benefits of an increase in value of the current brood have to be traded against lower survival prospects and future reproduction. Whether such variation in reproductive investment reflects an adaptive strategy is still unclear, and we hope that these intriguing findings will encourage research on the adaptive value and long-term fitness consequences of environmentally induced plasticity in reproductive behaviour.

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APPENDIX

Table A1
Estimates ± SE and test statistics (glm with Gaussian error distribution) for adult male and female plumage colours in the control and nest site-limited plots

	Factor	Estimates	SE	*df*	*t*	*P*
Males						
Tail feathers						
Brightness	Intercept	22.278	1.525			
	Treatment*	0.127	1.090	45	0.116	0.908
Hue	Intercept	373.46	7.742			
	Treatment*	0.725	5.533	45	0.131	0.896
Chroma	Intercept	0.962	0.048			
	Treatment*	−0.023	0.034	44	−0.672	0.505
UV chroma	Intercept	23.679	1.668			
	Age	0.922	0.456	42	2.023	0.049
	Treatment*	0.260	0.851	42	0.306	0.761
Breast feathers						
Brightness	Intercept	30.952	1.701			
	Treatment*	−0.30	1.216	45	−0.435	0.665
Carotenoid chroma	Intercept	0.371	0.057			
	Age†	0.045	0.016	43	2.857	0.007
	Treatment*	0.054	0.029	43	1.862	0.07
UV chroma	Intercept	16.268	0.899			
	Treatment*	−0.249	0.643	45	−0.388	0.7
Crown feathers						
Brightness	Intercept	25.184	2.228			
	Treatment*	1.654	1.592	45	1.038	0.305
Hue	Intercept	375.095	7.574			
	Treatment*	6.492	5.413	45	1.199	0.237
Chroma	Intercept	1.084	0.053			
	Treatment*	−0.017	0.038	45	−0.452	0.654
UV chroma	Intercept	27.523	1.489			
	Treatment*	−0.762	1.064	45	−0.716	0.477
Females						
Tail feathers						
Brightness	Intercept	21.686	1.559			
	Treatment*	0.211	1.125	47	0.187	0.852
Hue	Intercept	403.975	8.737			
	Treatment*	−6.885	6.308	47	−1.091	0.281
Chroma	Intercept	0.829	0.064			
	Treatment*	−0.049	0.046	47	−1.073	0.289
UV chroma	Intercept	21.137	1.068			
	Treatment*	0.663	0.771	47	0.86	0.394
Breast feathers						
Brightness	Intercept	31.648	1.907			
	Treatment*	−1.272	1.377	47	−0.923	0.361
Carotenoid chroma	Intercept	0.525	0.040			
	Treatment*	−0.022	0.029	47	−0.741	0.463
UV chroma	Intercept	13.636	0.929			
	Treatment*	0.781	0.670	47	1.164	0.25
Crown feathers						
Brightness	Intercept	24.456	1.697			
	Treatment*	−0.042	1.225	47	−0.034	0.973
Hue	Intercept	395.69	7.046			
	Treatment*	−3.646	5.087	47	−0.717	0.477
Chroma	Intercept	1.005	0.065			
	Treatment*	−0.067	0.047	47	−1.431	0.159
UV chroma	Intercept	21.967	1.263			
	Treatment*	0.712	0.912	47	0.781	0.439

Only minimal adequate models are shown (for model selection procedure see Methods).
* Estimates are relative to individuals in control plots.
† Estimates are relative to first-year birds.

Table A2
Estimates ± SE and test statistics (glmm with Gaussian error distribution and nestbox as random intercept) for nestling plumage colours originating from control and nest site-limited plots

	Factor	Estimates	SE	*df*	*t*	*P*
Tail feathers						
Brightness	Intercept	19.295	0.595			
	Sex*	−0.862	0.231	370	−3.731	<0.001
	Treatment†	0.008	0.418	40	0.019	0.98
Hue	Intercept	318.541	0.864			
	Treatment†	−0.840	0.615	40	−1.367	0.18
Chroma	Intercept	0.527	0.021			
	Sex*	0.104	0.006	370	16.555	<0.001
	Treatment†	0.002	0.015	40	0.134	0.89
UV chroma	Intercept	26.599	0.176			
	Sex*	1.04	0.060	370	17.358	<0.001
Breast feathers						
Brightness	Intercept	40.898	1.046			
	Treatment†	−0.978	0.746	41	−1.312	0.197
Carotenoid chroma	Intercept	0.565	0.021			
	Treatment†	<−0.001	0.01	40	−0.058	0.95
UV chroma	Intercept	25.0	0.347			
	Carotenoid chroma	−11.326	0.399	375	−28.361	<0.001
	Sex*	−0.163	0.041	375	−3.940	<0.001
	Treatment†	0.094	0.186	40	0.503	0.618

Only minimal adequate models are shown (for model selection procedure see Methods).

* Estimates are relative to male nestlings.

† Estimates are relative to nestlings from control plots.

Table A3
Brood sex ratio (males/(males + females)) of birds breeding in the experimental plots in the years before the nest site limitation experiment

	Sex ratio	Confidence intervals		Brood size ($\bar{X}$±SD)	χ^2	*P*	*N*
		2.5%	97.5%				
1998	0.535	0.474	0.596	10.02±2.66	1.264	0.261	26
1999	0.502	0.454	0.551	10.03±2.47	0.010	0.921	41
2000	0.499	0.453	0.545	9.99±2.45	0.002	0.962	44
2001	0.508	0.457	0.559	8.66±3.66	0.097	0.756	43
2002	0.535	0.492	0.579	10.02±2.59	2.547	0.111	52
2003	0.520	0.477	0.563	9.68±2.62	0.853	0.356	53
2004	0.478	0.428	0.529	9.53±3.34	0.691	0.406	39
All years 1998–2004	0.528	0.483	0.572	9.71±2.86	1.514	0.219	298

To test whether the brood sex ratio was significantly different from the 50:50 expectation a glm with binomial error distribution and logit link function including only the intercept was run separately for each year. When testing all years together we additionally included year as a factor ($\chi^2_6 = 2.536$, $P = 0.865$). The 95% confidence interval (CI) for the intercept was computed according to Venables & Ripley (2002, page 220). The sex ratio and the 95% CI were back-transformed from the logit scale.