Original Article

Extrapair paternity in Mediterranean blue tits: socioecological factors and the opportunity for sexual selection

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The frequency of extrapair paternity within populations has been hypothesized to be related to ecological and social factors, which in turn can determine the impact of extrapair paternity on the opportunity for sexual selection. Here, we use the blue tit *Cyanistes caeruleus* as study species to assess both issues. In particular, we analyze patterns of extrapair paternity in 12 nest-box plots that greatly vary in local population size, level of nest-box aggregation, and breeding density. We found a significant positive relationship between extrapair paternity rate and local population size. Within study plots, neither local breeding density nor synchrony had an effect on the occurrence of extrapair paternity. Most extrapair males engaged in extrapair copulations with neighbouring females, probably in order to avoid paternity losses. Individuals that travelled larger distances to gain extrapair paternity likely did so because the social females of most of them had not yet begun their fertile period and, thus, within-pair paternity was not at risk. Variance in male reproductive success was mostly produced by variance in within-pair success, which in turn was primarily influenced by mate quality. Extrapair success contributed substantially to variance in male reproductive success (26%), but its effect was smaller than expected. Bateman gradients showed positive slopes (β_{ss}) for both males and females. However, the lack of a positive covariance between within-pair and extrapair success suggests that the effect of extrapair paternity on the strength of sexual selection was limited. This fact can be explained by the spatial distribution of extrapair fertilizations, which points to the absence of directional female mating preferences in this study system and, thus, not leading to "big winners" and "big losers."

Key words: **Bateman gradient, breeding density, *Cyanistes caeruleus*, fitness components, promiscuity, realized reproductive success, variance in male fitness.**

INTRODUCTION

Sexual promiscuity (i.e., extrapair paternity, EPP) is a widespread phenomenon in socially monogamous species (birds: Griffith et al. 2002; mammals: see Cameron et al. 2011 and references therein). Determining the ecological factors that affect this reproductive strategy within and among species and populations remains a major challenge for behavioral ecologists and evolutionary biologists. What conditions favor the occurrence of genetic polygamy in wild populations? The frequency of EPP likely depends on ecological circumstances affecting the rate of encounters between females and extrapair males and the timing at which they take place. The spatial and temporal distributions of mates are 2 often-cited factors thought to influence the frequency of EPP (Cohas and Allainé 2009; Westneat and Stewart 2003).

First, variation in breeding density is traditionally evoked as an explanation for intraspecific variation in the rate of EPP. However, studies testing for a relationship between these 2 variables have yielded mixed results in a variety of taxa (fishes: e.g., Grant et al. 1995; mammals: e.g., Ims 1988; birds: reviewed in Mayer and Pasinelli 2013). Early studies of extrapair copulations (EPCs) in birds suggested that the greater the proximity of individuals, the higher the EPC rate (Gibbs et al. 1990). Along the same lines,

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Westneat and Sherman (1997) found in a comparative study that there was a general trend for high-density populations to have a higher rate of EPP than conspecific populations at lower densities. Higher breeding densities are likely to reduce searching costs for extrapair mates but may also entail costs in the form of increased number of territorial intrusions by potential cuckolding males (Bouwman 2006). The relationship between breeding density and rates of EPP has been tested experimentally in a small number of studies. Charmantier and Perret (2004) manipulated nest-box density and found that the proportion of extrapair young (EPY) increased with the number of neighbors within 100 m around the nest. Stewart et al. (2010) reached similar conclusions, but they advertised the importance of considering spatial and temporal factors jointly (see below).

Variation in breeding synchrony is the other classic ecological explanation for inter- and intraspecific variation in the rate of EPP. The influence of breeding synchrony is particularly controversial because it can be argued that extrapair matings should either increase with high levels of breeding synchrony (because it allows females to assess potential extrapair mates that are simultaneously displaying; Stutchbury and Morton 1995) or decrease (as fewer males are free from mate-guarding duties and available to pursue extrapair matings; Thusius et al. 2001). Few intraspecific studies have provided significant support for a direct relationship between synchrony and the rate of EPP and, to date, the evidence for either hypothesis has been equivocal or at least inconclusive (Griffith et al. 2002; LaBarbera et al. 2010). Density and breeding synchrony may also interact and lead to complex effects that can hide or confuse simple relationships between these factors and EPP (Thusius et al. 2001; Stewart et al. 2010). For instance, the synchrony of females breeding in neighboring territories (local synchrony) is likely to increase with the number of neighbors or population density, variables that by themselves are also expected to affect rates of EPP (see above).

Variation in reproductive success linked to variation in mating success constitutes the "raw material" of sexual selection (Emlen and Oring 1977). Thus, extrapair matings are likely to be an important path through which sexual selection can act in socially monogamous species. However, a high percentage of EPP does not necessarily imply an increase in the variance of male reproductive success due to the influence of several factors (Freeman-Gallant et al. 2005; but see Albrecht et al. 2007) such as the temporal and spatial distribution of extrapair mating opportunities (Shuster and Wade 2003). When ecological or social factors restrict males to siring extrapair offspring only with nearby females, this can limit the degree to which extrapair matings increase variance in male reproductive success (Whittingham and Dunn 2005). On the other hand, the intensity of sexual selection can strongly increase if a small number of males is responsible for most of the EPP events registered in the population (asymmetrical paternity exchanges) (Webster et al. 2001).

Here, we examine EPP in a socially monogamous passerine, the blue tit *Cyanistes caeruleus*, a species in which the proportion of fertilizations resulting from copulations outside the social bond is moderate (see García del Rey et al. 2012 and references therein). In this work, we present data on EPP rates from twelve nest-box plots located in central Spain. This study is part of a project aimed to study the effects of environmental conditions and the genetic diversity on reproduction, survival, and dispersal of blue tits in a fragmented Mediterranean landscape. Our approach included analyses within and among study plots, a procedure that has been rarely applied in this kind of studies (see Mayer and Pasinelli 2013

for an exception). The aim of this study is 2-fold. First, we examine the distribution of EPP in the study plots and test for relationships with different social, spatial, and temporal variables. According to the density hypothesis (Westneat and Mays 2005), increased number of neighbors and proximity among individuals increases encounter rates and mating opportunities. Thus, we predict that levels of EPP should be positively related to local population density and size (assessed at the level of each study plot), and levels of EPP within plots should be positively related to local breeding density (assessed at the level of the territory). We also test whether breeding synchrony constitutes an underlying constraint for EPP in such plots. Secondly, we analyze the effect of EPP on the potential for sexual selection in our study system. This was done by examining the components of variation in male reproductive success. Specifically, we evaluated the relative influence of the number of mates, the number of young produced per mate, and the proportion of young sired per mate on the variance in male reproductive success. Given that comparisons of variance (apparent vs. realized) can be misleading about the effect of EPP on the opportunity for sexual selection (Webster et al. 2007), we calculated Bateman gradients to complement our analyses (Bateman 1948; see Webster et al. 2007; Krakauer 2008; Balenger et al. 2009; Ryder et al. 2012).

MATERIALS AND METHODS
Study site and field methods

The fieldwork was carried out in 2012 in 2 nearby localities at Montes de Toledo (central Spain): Quintos de Mora (39°24′23″N, 4°4′19″W) and San Pablo de los Montes (39°32′44″N, 4°19′41″W). Twelve oak-dominated woodlots of different size (ranging from 1.3 to 25.5 ha) and supplied with a variable number of nest-boxes were chosen as the study area (see Figure 1 and Table 1). In this region, deciduous woodlands are "habitat islands" in a matrix of unsuitable habitat (grasslands and scrublands with low tree cover) for blue tits due to natural and anthropogenic processes that have contributed to habitat fragmentation resulting in mosaic landscapes characteristic of the Mediterranean basin (Blondel and Aronson 1999). Density of breeding pairs and the mean distance between nests greatly varied among sites, the latter mainly due to the different spatial distribution of nest-boxes in each study plot (arranged in grids or transects) (Table 1). Breeding density outside nest-box plots is low (authors, unpublished data). Most of the study plots (with the exception of plots 1, 2, 11, and 12, see Figure 1) show a moderate level of connectivity although the habitat connecting plots is heterogeneous and discontinuous. Due to the presence of topographic features such as mountain ranges (potential barriers for dispersal in blue tits) and the small body size and sedentary behavior of this species, one would expect to find a very limited number of movements between plots during the breeding season. Previously, we have found that these and other nearby localities may behave as isolated populations and show significant genetic differentiation among them (Ortego et al. 2011; Ferrer et al. 2012). The geographic position of each nest-box was recorded using a GPS, and distances among nest-boxes were estimated from UTM coordinates. The size of each study plot was calculated using a Geographic Information System (ArcView GIS 3.2) and a layer from a land cover database (Spanish forest inventory) including information on the surface covered by oak woodlands.

From early April to mid-June, we monitored the breeding activity of blue tits in these study areas. Daily inspections of nests provided information on basic reproductive parameters such as laying
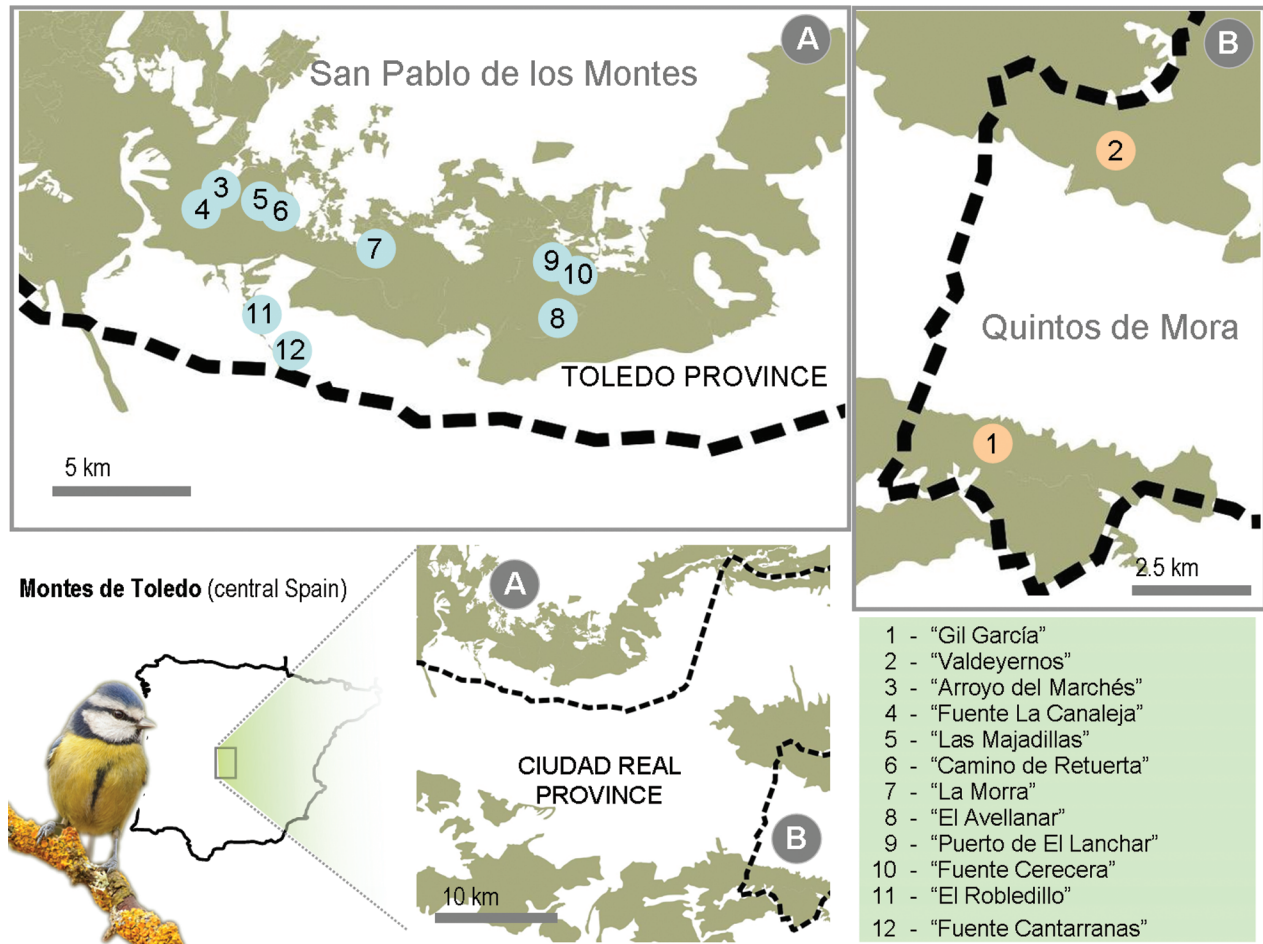
Figure 1

Map showing the location of the different nest-box plots used to study the promiscuous behavior of blue tits in Montes de Toledo (Toledo Province, central Spain). The shaded area represents the surface covered by *Quercus* species (both evergreen and deciduous woodlands). Thus, these localities are not composed by a single, continuous, and homogenous habitat type but include patches constituting an optimal breeding habitat for blue tits (Pyrenean oak *Quercus pyrenaica* woodlands) interspersed with areas that can be cataloged as unsuitable habitat for this species (semiopen areas with low tree cover). See Supplementary Material for more details.

date, clutch size, hatching date, brood size, and number of fledged young. Adult birds were captured by means of spring traps when feeding nestlings 8–9 days old. At capture, parents were sexed and identified with metal rings if they were not already ringed. A small blood sample (ca. 20 μL) was taken from the brachial vein of birds and stored on FTA reagent-loaded cards (Whatman Bioscience, Florham Park, NJ) for parentage analyses. On day 13 posthatching, nestlings were banded, and blood was sampled in a similar way. In our study areas, nestling survival (proportion of hatched young that fledge) is high (95.7%), and thus, we considered unnecessary to take blood samples from chicks at earlier ages.

Microsatellite genotyping

Genomic DNA was extracted from blood samples and purified using commercial kits (NucleoSpin Tissue Kit, Macherey-Nagel GmbH & Co., Duren, Germany) or according to the salt extraction protocol by Aljanabi and Martínez (1997). DNA was quantified with a NanoDrop spectrophotometer (Thermo Scientific Inc., Whaltman, MA) and suspended in TE buffer to obtain working concentrations of 20–50 ng DNA. We genotyped individuals using 11 microsatellite markers (*Pca2*, *Pca3*, *Pca4*, *Pca7*, *Pca9*, *Pocc1*, *Ase18*,

Pat-MP2-43, *PK11*, *PK12*, and *Mcyµ4*). More details about genotyping (PCR conditions, etc.) are indicated in García-Navas et al. (2009).

Molecular parentage analyses

For paternity analyses, we used the maximum likelihood method implemented in CERVUS 3.0 (Kalinowski et al. 2007). CERVUS calculates a likelihood ratio for each candidate father–offspring pair over all loci given the genotypic data of the population and calculates Δ, the difference in the likelihood ratio (LOD) scores between the most likely father (i.e., the candidate father with the highest LOD score) and the second most likely father. CERVUS categorically assigns paternity to a particular candidate father if the difference in Δ exceeds a certain threshold. This threshold is calculated via computer simulation of paternity inferences (Meagher 1986). We simulated 100 000 offspring using allele frequencies observed in our population. According to our own empirical data and preliminary maternity analyses with CERVUS, we set 95.3% of loci typed and 1% of loci mistyped. We set 90% of candidate fathers sampled, according to the mean capture rate of territorial males in the study plots (Table 1). We first assessed whether the social mother

Table 1

Attributes of the 12 plots (deciduous woodlands or remnant woodlands) in which this study was carried out: number of available nest-boxes ($N°$ nest-boxes), type of arrangement, area, number of breeding pairs in 2012, density of breeding pairs, mean number of neighbors within a 100 m radius (local density), proportion of unhatched eggs (%), proportion of fledged young (%), percentage of males sampled (Captured ♂), number of families genotyped in each study plot (n), and EPP rate: number of families with at least one EPY and occurrence of EPP in each site (%, in parentheses)

Study plot	Locality	$N°$ nest-boxes	Type[a]	Area (ha)	$N°$ pairs	Density (pairs/ha)	Local density	Hatching success	Nestling survival	Captured ♂ (%)	n	EPP rate
"Gil García"	Quintos de Mora	100	Grid	20.1	44	2.19	6.51	68.0	100	70	8	3 (37.5)
"Valdeyernos"	Quintos de Mora	100	Transect	25.5	42	1.65	3.90	64.7	100	85	11	7 (63.6)
"Arroyo del Marchés"	San Pablo de los Montes	80	Transect	22.2	34	1.53	3.70	77.9	95.1	100	28	16 (57.1)
"Fuente La Canaleja"	San Pablo de los Montes	20	Transect	3.00	7	2.33	2.57	77.5	88.1	86	3	1 (33.3)
"Las Majadillas"	San Pablo de los Montes	20	Transect	4.60	9	1.96	5.11	65.0	82.0	89	5	1 (20.0)
"Camino de Retuerta"	San Pablo de los Montes	20	Grid	2.40	7	2.92	2.28	70.1	95.5	71	2	1 (50.0)
"La Morra"	San Pablo de los Montes	80	Grid	11.4	19	1.67	2.80	74.3	90.0	91	5	1 (20.0)
"El Avellanar"	San Pablo de los Montes	20	Grid	3.10	13	4.19	3.67	90.3	100	75	4	1 (20.0)
"Puerto de El Lanchar"	San Pablo de los Montes	5	Grid	1.80	4	2.22	1.00	100	100	50	1	0 (0.0)
"Fuente Cerecera"	San Pablo de los Montes	5	Grid	1.30	2	1.54	0	100	100	100	2	0 (0.0)
"El Robledillo"	San Pablo de los Montes	5	Grid	1.90	4	2.10	0	79.2	92.8	100	2	0 (0.0)
"Fuente Cantarranas"	San Pablo de los Montes	5	Grid	1.70	2	1.18	0	70.0	100	100	1	0 (0.0)

[a]Nest-boxes were arranged at 35- to 50-m intervals at all sites, but the type of distribution was different (grid vs. transects) leading to differences in the level of aggregation of nest-boxes (e.g., number of available nest-boxes in a 100 m radius) among sites. Transects consist of 2 lines with >100 m distance between them.

was the actual genetic mother. In 15 cases (3.6%), we found that nestling genotypes did not match the putative mother at ≥3 loci, and thus, they were considered cases of intraspecific brood parasitism and excluded from subsequent analyses. For final paternity analyses, we set the mother as "known parent" and the pool of candidate fathers included all breeding males sampled that year and in previous years in each woodland (Quintos de Mora: 183 individuals from 2008 to 2012, San Pablo de Los Montes: 110 individuals from 2011 to 2012). CERVUS allows the assignment of paternity at different confidence levels (CL), and we present here results based on paternity inferences obtained at the 95% CL. All offspring assigned at the 80% CL were also assigned at the 95% CL, and thus, our results are identical for these 2 CL typically considered in most paternity studies (Jones and Ardren 2003).

Ecological and social contingency of extrapair behavior

We assessed the influence of spatial and temporal factors on the occurrence of EPP both at large and small scales. First, we examined the existence of differences in levels of EPP among study plots. Our study system included nest-box plots that greatly differed in local population size, population density, and level of aggregation of nest-boxes, making it appropriate to test the relationship between these parameters and EPP rates across the studied sites (Table 1). Within study populations, we analyzed the effects of local breeding density and local breeding synchrony on the frequency of EPP. We only took into account the social and ecological circumstances 100 m around the focal nest (about 3 territories, see Bouwman 2006; LaBarbera et al. 2010). Most extrapair sires (>70%) come from neighboring territories to that of the cuckolded male (see Results), and for this reason, we consider that this "local" approximation is more realistic and probably more biologically meaningful than the calculation of density and synchrony measures considering the entire breeding area (population-wide synchrony index). For each male, we calculated the number of potential mates (i.e., number of breeding females) in a 100 m radius. We also estimated the number of synchronous females at a local scale (≤100 m). We defined accessible and synchronous females as those whose fertile period overlapped with that of the focal social female. We assumed that females became fertile 2 days prior to the laying of the first egg and stayed fertile until they laid the penultimate egg (Vedder et al. 2010). For these analyses, we included all males identified as extrapair sires regardless of whether their family was genotyped or not ($n = 26$).

Male reproductive success and the opportunity for sexual selection

In order to consider the influence of EPP on the strength of sexual selection in this study system, we compared the relative variance in apparent and realized male reproductive success (I_s/I_{app}). EPP increases sexual selection in males when it increases variation in male fitness due to some males being more successful at achieving extrapair offspring than others (nonrandom mating). Only as a reference, an I_s/I_{app} ratio greater than 1 is interpreted as evidence that EPP can potentially increase sexual selection in males (Arnold and Wade 1984; Gibbs et al. 1990; Kempenaers and Schlicht 2010). Meanwhile, when all individuals in the population obtain roughly the same number of mates and extrapair males simultaneously gain and lose paternity, the effect of EPP on the overall reproductive success should be diluted (i.e., gains equal losses; Ketterson et al.

1998; Freeman-Gallant et al. 2005). Apparent reproductive success was estimated by simply counting the number of fledglings in the social nest, and actual or realized reproductive success for each male was based on the total number of fledglings sired by him (considering both losses of paternity in his own nest and extrapair chicks sired elsewhere).

Subsequently, in order to identify which component/s of male fitness contributed most to I_s, we divided total variance in reproductive success into 2 additive components: extrapair success (E) and within-pair success (W), along with their covariance (Cov(W,E)) following the model described in Webster et al. (1995). W and E can be expressed as a function of the number of social or extrapair mates (M), average number of young produced per mate (N), and the proportion of these young sired by the focal male (P). Components contributing at least 15% of the total variance in reproductive success are usually thought to be important (Webster et al. 2001; Schlicht and Kempenaers 2010).

Finally, we calculated sexual selection (Bateman) gradients for both males and females by correlating actual reproductive success (number of genetic young) with mating success (number of mates with whom the male/female produced offspring). The slope of this regression between male or female fitness and their ability to obtain mates (β_{ss}) indicates the expected number of offspring produced for each additional mating partner and provides a direct measure of the strength of selection acting on mating success (Jones et al. 2002; Jones 2009; Krakauer et al. 2011). According to Bateman's hypothesis, the gradient should be steeper in males than in females (Bateman 1948; Wade 1979; but see Gowaty et al. 2012).

Statistical analyses

We used a logistic regression with a binomial distribution of errors and a logit-link function ("GENMOD" procedure in SAS) to analyze EPP rates among populations in relation to local population size, density of breeding pairs, mean number of neighbors within a 100 m radius, and type of nest-box arrangement. We included the number of nests with at least 1 EPY as the response variable (event) and total number of nest sampled in each population as binominal denominator (trial). To analyze the factors influencing the occurrence of EPP (probability of having at least 1 EPY) within populations, we employed generalized linear mixed models (GLMMs) with a binomial distribution and logit-link function as implemented in the "lmer" procedure of the "lme4" library (Bates and Maechler 2009), an R package. Study plot was included as a random effect, and laying date, clutch size, local number of females, and local number of fertile females as explanatory variables. As these last 2 variables were highly correlated (Pearson correlation; $r = 0.77$, $n = 65$, $P < 0.001$), we tested for their effects separately. A similar analysis was repeated by including the number of EPY within the nest as dependent variable and brood size as binomial denominator (binomial error distribution and log link function). We also employed generalized linear models to analyze whether the distance travelled by extrapair sires varied depending on the extrapair mates' timing of breeding relative to that of the social female. To that end, we calculated laying date differences (ΔLD) between the social and the extrapair mate. Laying date differences were calculated by subtracting the social female's laying date from that of the extrapair female. Accordingly, we created 3 categories: "before" ($-7 < \Delta$LD; observed range from -17 to -7 days), "at par" ($-7 \leq \Delta$LD ≤ 7, observed range from -1 to $+3$ days), and "afterwards" (ΔLD > 7, observed range from $+7$ to $+45$ days). We chose 7 days as cutoff among categories because 7 is the modal clutch size in our

populations. In addition, we related foray distances to male reproductive success to test for the effectiveness of nonterritorial forays as a tactic to avoid being cuckolded. For those cases in which males gained paternity in more than one nest (4 broods had illegitimate young from 2 extrapair sires), we computed the mean distance for each individual. For this analysis, we excluded males that gained paternity (i.e., those identified as extrapair sires) but whose families were not located (thus, nonidentified as social males, $n = 6$) or not sampled ($n = 6$). Hence, we only included males for which we gathered information within and outside the pair bond ($n = 20$). Nonparametric tests were used to compare the net output (i.e., gains minus losses) between cuckolding males that were cuckolded ("gains and losses") and cuckolding males that avoided being cuckolded ("gains only") and between noncuckolding males that avoided being cuckolded ("no gains or losses") and noncuckolding males that were cuckolded ("losses only"). Statistical analyses were done in R v. 2.14 (R Development Core Team 2012) and SAS 9.1 (SAS Institute, Cary, NC), except nonparametric tests (sign tests, Pearson correlations, and contingency tables), which were performed using Statistica 7 (Statsoft Inc., Tulsa, OK). Means ± standard errors (SE) are given.

RESULTS

EPP rate

We genotyped a total of 417 chicks and 130 adults for 65 breeding pairs. We successfully assigned paternity to 80% (53/66) of extrapair offspring. The remaining 13 chicks were sired by "unknown" males, that is, nonsampled individuals. Six of the 32 males identified as extrapair sires were captured as breeding adults in previous years but not during 2012. We could not discern if these males also bred in 2012, and we failed to capture them or detect their nest or if, alternatively, they did not breed in 2012 (i.e., nonbreeders or floaters). Six families whose social fathers were identified as extrapair sires elsewhere were not genotyped, and thus, these males were not included in the analyses of male reproductive success (see more below). Overall, nearly half of the nests (46%, 30/65) contained at least one EPY and 16% (66/417) of all offspring genotyped were sired by a male other than the social father. There was no difference in the number of eggs laid between clutches with and without EPY (see below). There were no paternity exchanges among study plots.

Influence of plot size, breeding density, and synchrony on EPP

The EPP rate per study area (i.e., number of nests with at least one EPY in relation to the total number of nest sampled in each study plot, $n = 12$) was positively associated with local population size (estimate on logit scale: 0.03 ± 0.01 per breeding pair, Wald $\chi^2 = 5.10$, P $= 0.02$; Figure 2), but not with density of breeding pairs (Wald $\chi^2 = 0.23$, P $= 0.63$), mean number of neighbors within a 100 m radius (Wald $\chi^2 = 0.31$, P $= 0.58$), or type of nest-box arrangement (Wald $\chi^2 = 1.64$, P $= 0.20$). Local population size correlated with local density (i.e., number of neighbors; $r = 0.90$, $n = 10$, $P < 0.001$), but not with population density (measured as number of pairs per hectare) ($r = 0.12$, $n = 10$, $P = 0.71$). Population density and local density were not significantly related ($r = 0.11$, $n = 10$, $P = 0.73$).

Within study plots, the occurrence of EPP was not associated with laying date (with EPP: 19.0 ± 1.5, without EPP: 23.8 ± 1.7,
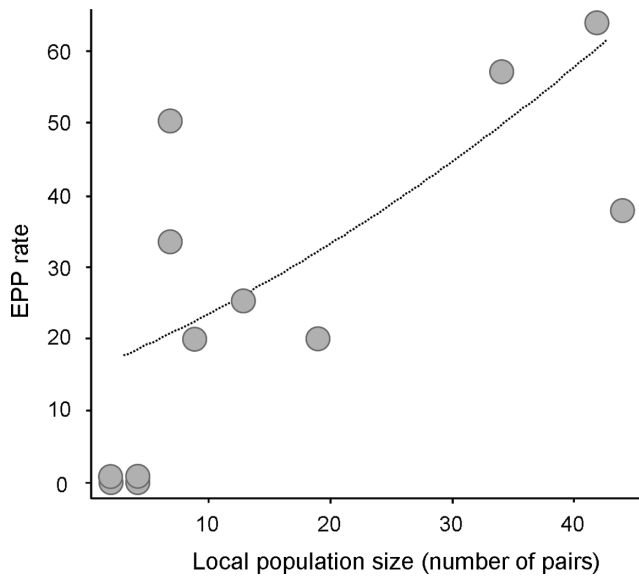
Figure 2

Extrapair paternity rate (percentage of nests with at least one EPY) in relation to local population size (number of blue tit pairs in the plot). The binomial logistic regression employed weights populations (study plots) with sample size (the number of analyzed nests in each plot).

Figure 3

Mean distance (±SE) between extrapair male nest-box and that of their extrapair partner in relation to the difference in laying date between the social and the extrapair partner ("before," $n = 8$; "at par," $n = 9$; "after," $n = 9$; see Materials and Methods for more details).

$\mathcal{Z} = -1.42$, $P = 0.15$), clutch size (with EPP: 9.1 ± 0.3, without EPP: 8.4 ± 0.3, $\mathcal{Z} = 1.60$, $P = 0.10$), breeding density (number of local females; with EPP: 3.5 ± 0.3, without EPP: 3.5 ± 0.4, $\mathcal{Z} = 0.83$, $P = 0.40$), or breeding synchrony (number of synchronous females; with EPP: 2.5 ± 0.3, without EPP: 2.3 ± 0.3, $\mathcal{Z} = -0.54$, $P = 0.59$) at a local scale. Similarly, the proportion of EPY within the brood was not significantly related to any of such variables: laying date ($\mathcal{Z} = -1.47$, $P = 0.14$), clutch size ($\mathcal{Z} = -0.12$, $P = 0.90$), local breeding density ($\mathcal{Z} = 0.50$, $P = 0.61$), or local breeding synchrony ($\mathcal{Z} = -0.55$, $P = 0.58$).

Seeking an extrapair partner: When and where?

Laying date of social mates did not differ significantly with respect to that of the extrapair mate ($t_{25} = -0.45$, $P = 0.65$). This was due to the fact that some males attained EPP before their social female had started to lay ($n = 8$, $\Delta LD = -12.7 \pm 3.0$) and others did so afterward ($n = 9$, $\Delta LD = +15.9 \pm 3.8$). For a few individuals ($n = 9$), the laying date of the social female nearly or perfectly matched that of the extrapair mate ($\Delta LD = -0.6 \pm 1.2$). Those males that engaged in EPP before their social females started to lay did so with females settled at more distant territories ("before," range: 52–846 m; "at par," range 22–266 m; "afterwards," range: 31–466 m; $F_{2,23} = 5.81$, $P < 0.01$; Tukey test "before" vs. "at par," $P = 0.01$; "before" vs. "afterwards," $P = 0.03$; "at par" vs. "afterwards," $P = 0.89$; Figure 3).

Nearly half of extrapair sires (11/26) engaged in EPP with the female from the nearest nest-box regardless of whether the fertile period of these females coincided with that of the social partner (in 5 out of 11 cases both premises were met). Four out of 7 males that did not engage in EPP with the closest female but did so with females breeding at nearby territories (i.e., within 100 m) attained EPP in synchronous nests with respect to their social nest. Finally, only 3 out of 8 males that sired EPY far beyond the vicinity of their territory (>100 m) did so with females whose fertile period
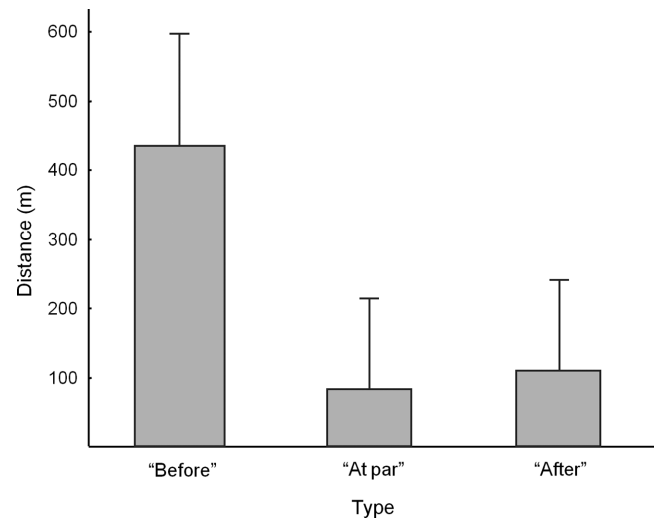
overlapped with that of their social female. The latter was mainly due to the fact that such males seemed to opt for gaining additional paternity before the beginning of their social female's fertile period (see above, see also García-Navas et al. 2013). Overall, there was no significant association between the male's decision of whether or not to travel a large distance to engage in EPP and the decision of whether or not to do it with a female whose fertile period overlapped with that of the social partner (2×2 contingency table; $G^2 = 0.72$, $P = 0.39$).

Neither the number of synchronous mates in the surroundings of the male territory nor the number of fertile females at such a local scale had an influence on the males' propensity to seek extrapair mates among their neighbors or beyond the vicinity of their territory (number of available mates: Wald $\chi^2 = 0.64$, P = 0.42, number of fertile mates: Wald $\chi^2 = 0.11$, P = 0.74). That is, those males that travelled longer distances to engage in EPCs did not seem to do so because they had no option in the vicinity of their territory. We also found that those individuals that gained but did not lose paternity (gains only) travelled slightly longer distances to engage in EPPs compared with those that gained paternity but were also cuckolded (gains only: 266.1 ± 61.6 m, gains and losses: 81.2 ± 68.8 m; $F_{1,18} = 4.04$, $P = 0.059$).

Male reproductive success

We examined the annual reproductive success of 65 male blue tits. Twenty of these 65 males sired EPY and account for 62% (20/32) of the total number of males identified as extrapair sires. Variance in realized reproductive success was larger than variance in apparent reproductive success, and thus, we obtained an I_s/I_{app} ratio slightly larger than 1 ($4.66/4.33 = 1.08$). Extrapair success moderately increased variance in reproductive success among male blue tits (see Table 2). The major source of variance in male fitness was productivity of the social mate (N_w) and the number of extrapair mates (M_e) (Table 2). Within-pair and extrapair success were not significantly related (Pearson correlation; $r = -0.13$, $n = 65$, $P = 0.28$). The covariance between within-pair and extrapair success was

Table 2

Variation in the number of young sired (actual reproductive success, T) by male blue tits (n = 65)

|	Range	Mean	Variance	S_{var}	$T_a$var (%)	CI
var(T)	1–11	5.73	4.66	0.14	100	
var(W)	1–11	5.17	4.04	0.12	86.80	75.5 to 92.7
var(E)	0–6	0.57	1.22	0.04	26.10	16.9 to 38.0
Cov(W,E)			−0.60	−0.02	−12.90	−20.1 to 11.5
Within-pair sources of variance						
var(M_w)		1.00	0	0	0	
var(N_w)	1–11	5.91	3.39	0.10	72.88	60.3 to 81.7
var(P_w)	0.02–1	0.88	0.97	0.03	20.85	11.9 to 31.4
Extrapair sources of variance						
var(M_e)	0–2	0.37	2.55	0.08	54.69	45.8 to 65.4
var(N_e)	1–9	5.95	0.12	0.02	2.58	0.2 to 11.1
var(P_e)	0.06–1	0.31	0.62	0.02	13.49	7.2 to 24.4

S_{var} is the standardized variance computed and weighted from the model described by Webster et al. (1995). $T_a$var means the percentage of the total variance in T_a explained by each component. CI denotes the 95% lower and upper confidence limits for the proportional contribution of fitness components. The variance in realized reproductive success is partitioned into 2 components (calculated and weighted according to Webster et al. 1995): within-pair (W) and extrapair (E) reproductive success, along with their covariance. Both terms (W and E) were further partitioned into variance due to the number of mates (M), female productivity or number of young produced per mate (N), and the proportion of young sired by the male (P). Not all covariance and remainder terms that contributed to the total variance are shown; therefore, W and E do not sum to 86.8% and 26.1%, respectively.

negative but accounted for only 13% of the variance in realized reproductive success (Table 2). Thus, the strength of the trade-off was weak, indicating that individuals seeking extrapair matings do not necessarily lose paternity in their own nest to a similar degree (see below).

Actual reproductive success was higher in males that gained paternity than in those that did not ($F_{1,63}$ = 7.71, P < 0.01; Figure 4). The number of genetic young produced within the social nest (within-pair offspring) did not differ significantly between males that sired extrapair offspring and males with no extrapair offspring (4.9 ± 0.4 vs. 5.3 ± 0.30, respectively; $F_{1,63}$ = 0.33, P = 0.56), indicating that males engaged in EPP without jeopardizing the fertilization success in their own nest. Males that lost and did not lose paternity did not significantly differ in the total number of genetic offspring produced ($F_{1,63}$ = 1.75, P = 0.19; Figure 4). Males that were cuckolded sired a similar number of extrapair offspring compared with those that did not lose paternity (0.4 ± 0.2 vs. 0.7 ± 0.2, respectively; $F_{1,63}$ = 1.56, P = 0.21). Apparent reproductive success did not differ significantly between cuckolded males and noncuckolded males (5.0 ± 0.4 vs. 5.3 ± 0.3, respectively; $F_{1,63}$ = 0.53, P = 0.47).

When considering the net output (gains minus losses), we found that those individuals that gained paternity and were not cuckolded yielded more fledglings than those that gained paternity but also lost paternity ("gains only": +2.2, n = 8; "gains and losses": −0.6, n = 12; Z = 2.47, P = 0.013). Likewise, the final outcome for those males that lost paternity and did not obtain EPP was significantly lower than in those that neither obtained EPP but ensured to be the genetic fathers of all of their offspring in their own nest ("losses only": −1.8, n = 21; "no gains or losses": 0, n = 24; Z = 3.17, P < 0.01). Males that achieved EPY were not more likely to lose paternity in their own nests, suggesting that males did not experience a trade-off between obtaining EPP and securing paternity in their own nest (G^2 = 0.25, P = 0.62).

On average, males produced 1.2 additional offspring for each additional mate obtained. The realized reproductive success of males was strongly tied to the number of mates (r = 0.35, t_{63} = 2.99, P < 0.01; Figure 5). In the case of females, we also obtained a positive Bateman gradient (r = 0.34, t_{63} = 2.92, P < 0.01; Figure 5). Female reproductive success was significantly related to the number
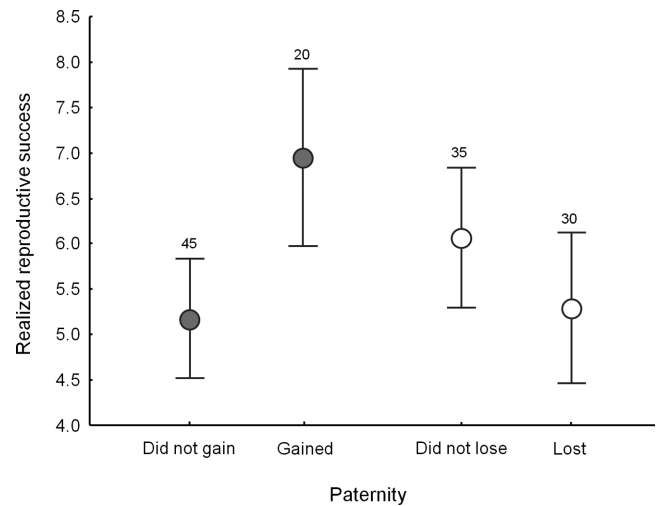
Figure 4

Realized reproductive success (number of genetic young, mean ± SE) of male blue tits in Montes de Toledo depending on whether or not they gained paternity and whether or not they lost paternity. Sample sizes are given above the bars.

of mates even after controlling for female clutch size ($F_{1,61}$ = 4.17, P = 0.02; clutch size: $F_{2,61}$ = 14.07, P < 0.001). The slopes of males and females were not significantly different from each other (ANCOVA: F = 0.07, P = 0.76).

DISCUSSION

The documented rate of EPP in our study system lies within the range reported for other blue tit populations (see Table 2 in García del Rey et al. 2012). At the metapopulation level, we found that the prevalence of EPP was associated with local population size, but not with estimate of density, number of breeding pairs per hectare, or average number of neighbors within a 100 m radius (i.e., local density). Local population size and population density were significantly correlated, but the latter did not turn out to be a good
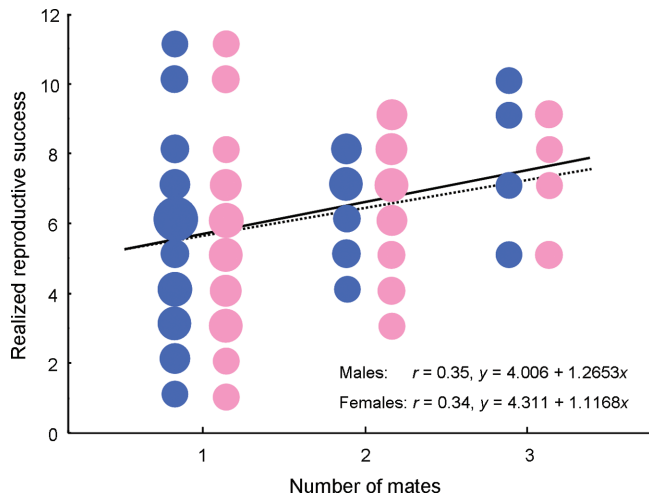
Figure 5
Bateman gradients for male (empty) and female (filled) blue tits illustrating the relationship between number of mates and reproductive success. The data set contained only individuals whose own social families were analyzed. The size of each circle represents the number of occurrences at each intersection (range: 1–14).

predictor of EPP occurrence. This suggests that not only the spatial aggregation of individuals per se may be important when determining the existence of differences in EPP rates among populations, but other circumstances inherent to population size may be more relevant factors. A similar argumentation has been put forward to explain why insular populations usually have lower rates of EPP than their mainland counterparts (Griffith 2000). Although no robust evidence has been found for a relationship between ecological parameters and EPP rates (Griffith et al. 2002), the lack of a consistent association between these variables does not necessarily reflect the total absence of dependence among them. Hence, it may be that such factors are important under circumstances that do not occur in our study system. Alternatively, small population sizes could reduce the frequency of EPP irrespective of local density. This may be the case if individuals are not willing to invest in extrapair mating effort when the pool of candidate mates is too reduced. This contrasts with previous studies suggesting that the occurrence of EPP is not primarily constrained by the availability of fertile females (Westneat and Mays 2005; Lindstedt et al. 2007; LaBarbera et al. 2010). Nonetheless, it should be noted that most of these studies were carried out in populations breeding in natural nests, whose distribution in space is much more heterogeneous and unpredictable (Westneat and Mays 2005; Mayer and Pasinelli 2013). When exploring the geographic distribution of EPP events, we observed that most confirmed extrapair sires were territorial neighbors (but some resided as far as 850 m apart). Clearly, this spatial configuration could have an impact on the way in which EPCs contribute to increasing female fitness (via direct or genetic benefits) in these populations. For instance, in a study on Austrian blue tits, Foerster et al. (2003) reported that EPCs with nonneighboring mates (up to 1.3 km away) accounted for 50% of all EPY, and they found that females increased their offspring's heterozygosity by EPC when mating to more distantly breeding males. However, the interpretation of our results may not be easily generalized to larger populations in less isolated habitats. Most of this kind of studies are carried out in "standard"-sized populations, and

little is known about the evolutionary and ecological significance of small populations for this species. Our findings provide interesting information about the potential influence of habitat fragmentation on reproductive strategies (e.g., EPP patterns) in less-mobile vertebrates like blue tits and highlight the possibility that results derived from relatively large study populations may be less generalizable than commonly thought.

On the other hand, ecological contingencies such as breeding density or breeding synchrony can drive patterns of sexual selection by facilitating or constraining the expression of mating preferences. Unfortunately, our sample size is too small to examine how plot size affects the opportunity for selection even though we think it is timely that behavioral ecologists begin to take more into account the ecological context (e.g., spatial heterogeneity) in our studies (Formica and Tuttle 2009, Gordon 2011). For example, Ryder et al. (2012) found that the strength of sexual selection did not differ between 2 habitat types at the landscape scale, and instead, they observed that breeding density influenced sexual selection on male phenotypes. In another recent study, Taff et al. (2013) addressed the role of nests distribution on the opportunity for sexual selection and strength of selection in common yellowthroats *Geothlypis trichas*. They found that total variance in reproductive success was highest at low density and was mostly explained by variation in within-pair success. In contrast, at high density, both within-pair and extrapair successes contributed substantially to variance in reproductive success (Taff et al. 2013).

We found that total variance in reproductive success increased only moderately as a consequence of EPPs. Previous studies with similar proportions of EPY (16–18%) on single-brooded monogamous species found higher values of I_s/I_{app} ratio than reported here (Sheldon and Ellegren 1999; Albrecht et al. 2007). However, I ratios from many of these studies may have been overestimated because of inadequate sampling of the male population (Freeman-Gallant et al. 2005). For instance, some studies failed to identify extrapair sires for half of the EPY, which can inflate the standardized variance in male reproductive success. Another limitation in such studies is that the presence of socially unpaired males with no apparent and low realized fitness is generally underrepresented, which can bias the estimated variance (Shuster 2009). In this regard, 2 recent studies have made a great effort to maximize the number of identified sires and estimate the number of "floaters" in the population by means of sibship analysis, confirming that the noninclusion of this pool of individuals overestimates the role of EPP due to artificial low variance in apparent success (Lebigre et al. 2012; Schlicht and Kempenaers 2013). Therefore, we must be cautious when interpreting our results because some nests within the study plots were not sampled, and thus, we were unable to estimate accurately the total paternity gains for all males and assign all EPY to extrapair sires (Freeman-Gallant et al. 2005). Taking as reference previous studies with Austrian blue tits (Kempenaers et al. 1992; Schlicht and Kempenaers 2013), the impact of extrapair fertilizations (EPFs) on variance in male reproductive success was less pronounced in our populations. However, the 2 study systems are not fully comparable as a large portion of the Viennese population is socially polygynous, whereas in our study area, this type of mating is uncommon. Both polygyny and EPFs could contribute to boosting the potential for sexual selection in that population and others (Schlicht and Kempenaers 2013; see also Vedder et al. 2011).

In our study system, the majority of variance in male reproductive success was attributable to within-pair success (W), which emphasizes the importance of male traits influencing the success

of achieving a high-quality social partner (Webster et al. 2001). Our results are in line with previous studies in which it has been suggested that social mating success may contribute more to variance in reproductive success than extrapair matings in monogamous bird species (Freeman-Gallant et al. 2005; Whittingham and Dunn 2005; Poesel et al. 2011). Variation in female fecundity (N_w) contributed substantially to the variance in W. Blue tits can lay large clutches (range: 5–13 eggs), and the percentage of EPY found in mixed-paternity broods is generally low (~30%; Schlicht and Kempenaers 2013). Thereby, differences among males in relation to mate quality may swamp other sources of variation. On the other hand, extrapair success (E) explained more than one quarter of the total variance in male reproductive success, a high proportion in comparison to other blue tit studies (15%: Schlicht and Kempenaers 2013). When splitting this term (E) into different fitness components, we found that most of the variance in extrapair success was related to the number of acquired extrapair mates, which means that variation in reproductive success due to EPP is linked to (extrapair) mating success, thus reflecting the opportunity for sexual selection arising via EPP (Schlicht and Kempenaers 2010). However, the interpretation of our results (fitness contributions) must be taken cautiously because the limitations imposed by our modest data set (Schlicht and Kempenaers 2010).

Meanwhile, an increase in I_s does not necessarily entail an increase in selection on specific phenotypic traits because fitness variance may be stochastic (Westneat 2006; Schlicht and Kempenaers 2013, 2010). What we have reported here is that our study system has the necessary substrate on which sexual selection can occur. The fact that a fairly large proportion of males achieved EPFs and that this did not influence the probability of being cuckolded (almost half of the males that fathered EPY lost paternity themselves) may be indicative of a lack of congruent female preference for a particular male phenotype—with respect to extrapair mates—in these study plots. Such ambiguity in female selection of extrapair mates could be the result of hasty choice during extrapair matings or a limited number of mating options (e.g., only 2–3 vacancies). Previous studies have found that individual heterozygosity and blue crown coloration were positively correlated between social mates (Andersson et al. 1998; García-Navas et al. 2009). More than half of the EPFs (69%) occurred between individuals that were separated by less than 100 m (i.e., those individuals present in neighboring territories). Such circumstances (i.e., constrained mate choice) could lead to a breakdown of the association between male quality and extrapair success because a male may constitute the "best option" within a very limited pool of candidates but their quality may be low in comparison with the whole population.

Males that travelled longer distances to engage in EPCs generally were individuals whose females had not yet began their fertile period (i.e., the extrapair female was earlier than the social partner). This suggests that males may be more willing to move away from their territory when they have nothing yet to defend. This conservative strategy may have to do with the potential risks derived from leaving the nest surroundings to search for extrapair mates during the social partner's fertile period, which could be exploited by other males to gain paternity with the unguarded female (Chuang-Dobbs et al. 2001). However, intrusions into neighboring territories are not free of costs because they may lead to reciprocal exchange of paternity between nearby nests and increased loss of paternity (Freeman-Gallant et al. 2005; García-Navas et al. 2013). In fact, we found that those individuals that achieved a higher net reproductive success ("gains only") tended to engage in EPCs away from their

territory, which may indicate that obtaining EPFs with a female earlier than their own partner, and at some distance from the territory, seems to be a good strategy in this system. The observed pattern is the opposite to that frequently reported for some migratory passerines, in which timing constraints (and protandry) can lead to males opting for guarding the social female during her fertile period and seeking additional paternity afterwards (e.g., wood trushes *Hylocichla mustelina*: Evans et al. 2008; pied flycatchers Ficedula hypoleuca: Canal et al. 2012a,b). However, both tactics ("before" or "after," both observed in our study areas) share the same premise: securing within-pair paternity when it is most at risk. At this point, it should be stressed that these results are based on a small sample size and, thus, should be interpreted with caution.

At first glance, it seems that the benefits that cuckolding males obtained through EPFs were not offset by losses in their own families. However, this does not mean that they were immune to cuckoldry. In fact, we found a negative covariance (Cov(W,E)) indicating that males may be constrained in seeking EPCs by the need for mate guarding during the high-risk period. In addition, we found that one third of males sired EPY, that is, extrapair success was not biased toward only a small subset of individuals, which could increase the opportunity for selection generated by EPFs (Webster et al. 2001; Whittingham and Dunn 2005). This contrasts with previous studies on dark-eyed juncos *Junco hyemalis* (Ketterson et al. 1998) and eastern kingbirds *Tyrannus tyrannus* (Dolan et al. 2007), where it was found that EPFs showed a clumped distribution and males that gained EPP tended to not suffer paternity losses.

When examining Bateman gradients, we found that our results apparently do not support the original Bateman's hypothesis because we obtained positive slopes for the gradients of both males and females. The observed slopes were very similar, which can be interpreted as both sexes experiencing similar selective pressures with regard to traits that are associated with acquiring additional mates. However, in the case of females, the interpretation of strong Bateman gradients raises many potential complications. For example, the interdependence between female fecundity and female mating success makes it difficult to determine whether selection is acting on traits related to fecundity rather than on those that have to do with mate acquisition ("fecundity selection" sensu Clutton-Brock 2009; Gerlach et al. 2012). On the other hand, it is important to note that Bateman gradients should be measured on the right time scale so that males do not automatically have more matings than females (Gerlach et al. 2012). Thus, positive Bateman gradients should be examined prudently (attending to the particular circumstances of each case; e.g., mating system, life history) and preferably together with other methods (Klug et al. 2010; Kokko et al. 2012), but not necessarily its existence, particularly in females, needs to be justified by alternative mechanisms. In fact, the number of studies reporting female Bateman gradients that are nearly identical to those of males appears to be increasing (see Tang-Martínez 2010 for a review on this topic), providing evidence that females can increase their fitness as they increase the number of mates (Jennions and Petrie 2000; Wiebe and Kempenaers 2009; Korpimäki et al. 2011).

In summary, in our study populations, male blue tits have 3 main avenues through which they can increase their reproductive performance: acquiring a fecund social female, securing paternity in their own nest, and acquiring extrapair mates. Our results may indicate that EPP slightly increases the variance in male reproductive success and, thus, supports the view that EPP provides additional opportunity for sexual selection to act (Yezerinac et al. 1995; Albrecht et al. 2009; Balenger et al. 2009; Poesel et al. 2011). However, the effect of EPP

on the potential for sexual selection was less than would be expected according to the observed EPP rate (percentage of broods with at least one EPY: 46%) and the substantial contribution of extrapair success to the total variance in male reproductive success (26%). This was mainly due to 1) the high contribution of social success (specifically, female fecundity), 2) the absence of a positive covariance between within-pair and extrapair success (which reflects a lack of strong directional selection on males through EPP), and 3) the apparently conservative strategy adopted by males when pursuing EPCs (reluctance to stray from their territory) probably as a means to reduce the risk of being cuckolded.

SUPPLEMENTARY MATERIAL

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

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