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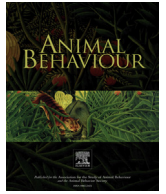


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Spatiotemporal and genetic contingency of extrapair behaviour in a songbird



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Multiple mating to obtain genetic benefits has been championed as the most likely cause of the evolution of polygamy. However, this assumption has been put into question by an increasing number of recent studies, most of which highlight the importance of considering ecological constraints to comprehend variation in extrapair (EP) behaviour. Here, we studied patterns of extrapair paternity (EPP) in the great tit, *Parus major*, using data from 11 nestbox plots that differed in population size and breeding conditions. Specifically, we analysed EPP rates in relation to socioecological variables that could influence the way individuals encountered one another in space and time, we tested whether adults engaged in EPP with more heterozygous, more compatible or phenotypically superior individuals than their social mates and we analysed whether extrapair offspring (EPO) were phenotypically or genetically superior to within-pair offspring. Our results do not provide support for the genetic benefit hypothesis from either the male or the female perspective. EPO were heavier than their within-pair paternal half-siblings, but there was no significant difference between EPO and their within-pair maternal half-siblings in terms of phenotypic quality. Regarding socioecological factors, we found a negative relationship between breeding synchrony and EPP rates both within and among plots, which suggests that males face a trade-off between mate guarding and obtaining EPP elsewhere. Our results show that most males engaged in EPP after the fertile period of their social female despite having to travel long distances: about half of the detected cases of EPP involved individuals from different woodlands. This study indicates that when and where to engage in EPP seem to be more relevant factors than with whom to do it and highlights the importance of considering spatiotemporal constraints at a landscape scale to achieve a better understanding of variation in EP mating behaviour.

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Extrapair paternity (EPP) is a common reproductive strategy in many animal taxa (birds: Griffith, Owens, & Thuman, 2002; reptiles: Uller & Olsson, 2008; mammals: Cohas & Allainé, 2009). However, there is controversy about what prompts 'socially monogamous' individuals, especially females, to be 'unfaithful' (Akçay & Roughgarden, 2007; Forstmeier, Martin, Bolund, Schielzeth, & Kempenaers, 2011). When explaining the motivations of females to engage in extrapair copulations (EPC) several

hypotheses have been raised, although during the last two decades a large body of research has focused on those related to the supposed genetic benefits of EPP (reviewed in Jennions & Petrie, 2000; Slayter, Mautz, Backwell, & Jennions, 2012). Genetic benefits are generally ascribed to three categories: (1) 'good genes', (2) genetic compatibility or (3) production of genetically diverse offspring. According to the 'good genes' hypothesis, females may gain additive genetic benefits if extrapair (EP) mates are of higher genetic quality than their social mates (Cohas, Yoccoz, Da Silva, Goossens, & Allainé, 2006; Hasselquist, Bensch, & von Schantz, 1996; Kempenaers et al., 1992). This hypothesis assumes that male characteristics, such as ornamental traits, but most commonly age and body size, are related to either fertilization success or paternity loss (e.g. Balenger, Johnson, & Masters, 2009; Canal, Potti, & Dávila,

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2011; Cleasby & Nakagawa, 2012). Following this reasoning, females should show a congruent response in their perception of 'top-quality' males and their EP mate choice preferences, in such a way that only a few males would monopolize all EPC. The 'genetic compatibility' hypothesis proposes that offspring viability and female fitness increase when females mate with males whose genomes best complement their own (Mays, Albrecht, Liu, & Hill, 2008). Hence, females may seek to engage in EPC with genetically compatible males (either the most dissimilar or with an optimal level of genetic similarity) in order to increase offspring genetic diversity and reduce the probability that deleterious recessive alleles are expressed and/or increase the probability of heterozygosity at genes experiencing some form of balancing selection (e.g. Brown, 1997; Fossøy, Johnsen, & Lifjeld, 2008; Leclaire, Nielsen, Sharp, & Clutton-Brock, 2013; Suter, Keiser, Feignoux, & Meyer, 2007; Varian-Ramos & Webster, 2012; Zeh & Zeh, 2003). Finally, polyandry may also constitute a female strategy to increase the variability of the genetic composition of their progeny and hence cope with environmental uncertainty and reduce the likelihood of disease ('bet-hedging' strategy sensu Yasui, 1998). Fluctuating natural selection may favour 'bet-hedging' strategies because these can increase the geometric mean fitness through reductions in the variance of mean fitness of each generation (Fox & Rauter, 2003; Williams, 1975).

From the male perspective, the benefits derived from mating with multiple females seem to be more obvious: they can increase their number of descendants without the costs of having to rear them (Trivers, 1972; see also Noble, Keogh, & Whiting, 2013; Sousa & Westneat, 2013; Vedder, Komdeur, van der Velde, Schut, & Magrath, 2011). However, accumulating evidence suggests that males can also be 'choosy' under certain circumstances, for instance, when the costs of EPC increase (Edward & Chapman, 2011). Most paternity studies over the last 20 years have focused mainly on the female's perspective and, consequently, the male's role in this process has frequently been neglected. This bias has led to the misconception that females pursue EPC in a large number of species, for which behavioural evidence is weak (Westneat & Stewart, 2003). For this reason, the adoption of a multiplayer interaction scheme can provide a more reliable and comprehensive approach to studying the significance of EPP, particularly in those species in which it is not clear who promotes EP matings. This kind of approach, taking into consideration the perspective of both sexes, has rarely been applied in the context of genetic benefits studies.

In spite of the considerable amount of effort devoted to exploring the possible indirect benefits of EPC (mostly from the females' point of view), there is actually no consensus about its importance as a selective force driving EP mating behaviour (Parker & Birkhead, 2013; Reid, Arcese, Sardell, & Keller, 2011). A meta-analysis reported that both the 'good genes' and 'compatible genes' hypotheses failed to be supported in more than half of the species studied (Akçay & Roughgarden, 2007). This has prompted a resurgence of nonadaptive (Forstmeier, Nakagawa, Griffith, & Kempenaers, 2014) or new adaptive (Akçay & Roughgarden, 2007; Eliassen & Jørgensen, 2014) hypotheses in recent years and an increase in studies focusing on ecological constraints affecting EP matings such as breeding density and synchrony (e.g. Canal, Jovani, & Potti, 2012; Mayer & Passinelli, 2013; Rubenstein, 2007; Taff, Freeman-Gallant, Dunn, & Whittingham, 2013; Wang & Lu, 2014). In this regard, it has been suggested that one critical determinant of the incidence of EPP is the encounter rate, which largely depends on breeding density and the number of available mates in a certain time window (i.e. breeding synchrony). The influence of breeding synchrony is particularly controversial because it can be argued that EP matings should increase with high levels of breeding

synchrony (since this allows females to assess multiple potential mates that are simultaneously displaying and thus reduce their search costs; simultaneous display hypothesis: Stewart, Westneat, & Ritchison, 2010; Stutchbury, 1998; Stutchbury & Morton, 1995) or decrease (as most males are engaged in mate-guarding duties resulting in a smaller pool of males available to pursue EPC; mate-guarding hypothesis: van Dongen & Mulder, 2009; Saino, Primmer, Ellegren, & Møller, 1997). At the intraspecific level, this relationship may depend on the different socioecological conditions experienced by individuals between populations and also within the same population. With regard to the former, remarkably few paternity studies consider multiple populations with contrasting socioecological conditions at a landscape scale. The few studies that analysed EPP patterns across populations involved a small number of populations (usually between two and four; Griffith et al., 2002) or populations in very similar ecological conditions (but see García-Navas, Ferrer, et al., 2014; Mayer & Passinelli, 2013 for exceptions). Fragmented landscapes, such as those of the Mediterranean region, are well suited for this kind of study because the size and connectivity of patchy populations have been linked to reduced breeding synchrony within and between populations (Banks, Piggott, Stow, & Taylor, 2007). In addition, certain environmental factors (e.g. conspecific density) may determine the reproductive strategies followed by each sex (for instance, in the case of males, to prioritize mate guarding versus EPC), which are expected to vary dynamically.

In the present study, we investigated patterns of paternity in relation to socioecological factors and examined the potential benefits of EPP from both the female and male perspective using a small passerine, the great tit, *Parus major*, as a model system. Our data come from 11 nestbox plots located in small and fragmented woodlands scattered within an area of 150 km² in Montes de Toledo, central Spain. Specifically, we first analysed the association between EPP rates and estimates of population size, breeding density and breeding synchrony in order to infer the phenology and spatial distribution of EP matings. We predicted that EPP rates are positively associated with population size and breeding density if increased proximity among individuals increases encounter rates and mating opportunities (García-Navas, Ferrer, et al., 2014; Mayer & Passinelli, 2013; Westneat & Mays, 2005). According to the mate-guarding hypothesis, the demands of paternity defence constrain males from seeking EPC at the time when most potential EP partners are fertile (Saino et al., 1997). Under this assumption, we predicted that EPP rates are negatively associated with breeding synchrony at the plot level. On the other hand, the simultaneous display hypothesis suggests the opposite: that more synchronous breeding leads to higher EPP rates because the temporal clumping of mating opportunities facilitates mate choice allowing individuals to compare and choose the best option (Stutchbury & Morton, 1995). Second, we tested whether female and male great tits engage in EPP with more heterozygous, more compatible and/or phenotypically superior individuals than their social mates in order to gain genetic benefits for their offspring (Brown, 1997; Jennions & Petrie, 2000; Kempenaers et al., 1992). Finally, we tested whether extrapair offspring (EPO) are phenotypically or genetically superior to within-pair offspring (WPO).

METHODS

Study Species

In spite of being one of the most thoroughly studied species in the world, there is no conclusive evidence on whether EP matings constitute a female- or a male-driven strategy in the great tit (see Appendix Table A1 for a review). Previously, it has been

documented that female great tits actively pursue EPC off-territory, similar to other parid species (Blakey & Norris, 1994; Strohbach, Curio, Bathen, Epplen, & Lubjuhn, 1998) and that males engage in mate-guarding behaviour as a means to avoid paternity loss (Björklund, Møller, Sundberg, & Westman, 1992). However, it is known that males continue singing at high levels during the egg-laying and incubation periods which may be used to attract EP partners (Amrhein et al., 2008). More recent studies in this species have provided evidence for the existence of a link between propensity for promiscuity and behavioural syndromes such as exploratory (Van Oers, Drent, Dingemanse, & Kempenaers, 2008; Patrick, Chapman, Dugdale, Quinn, & Sheldon, 2012) and dispersal behaviour (Szulkin, Chapman, Patrick, & Sheldon, 2012). Thus, there is no a priori reason to think that the pursuit of EPC only falls on one sex in this species.

Study Area and Field Procedures

Fieldwork was conducted during the 2012 breeding season in Montes de Toledo (central Spain). The study area consisted of 11 deciduous (mainly Pyrenean oak, *Quercus pyrenaica*) woodlots ranging in size between 1 and 20 ha (see Fig. 1). In all woodlots, a variable number of nestboxes (5, 40 or 100 boxes) arranged at 30 m intervals are available for hole-nesting passerines (great tits and blue tits, *Cyanistes caeruleus*, being their main occupants). Woodlots are isolated in a matrix of habitat unsuitable for tits, mainly Mediterranean scrubland with low tree cover. Between woodlots there are no nestboxes and breeding density is very low due to the shortage of natural cavities (personal observation; see Appendix Table A2 for more details about the study area).

From the beginning of April on, nestboxes were visited routinely to record those occupied by great tits. Once the nest was complete, nestboxes were inspected every 3 days to determine laying date (i.e. date of first egg laid; 1 = 1 April). For those nests that were not inspected the day the first egg was laid, we estimated laying date by counting back from the observed number of eggs in the nest, assuming that one egg was laid per day. Laying date was standardized to account for differences in mean laying date between plots. Before the expected hatching date (12–13 days of incubation) nests were inspected daily to determine hatching date and the number of hatched eggs. Putative parents (i.e. social pair) were captured by means of spring-traps while feeding nestlings 8 days old, aged (yearling or older) according to plumage characteristics and their body mass (to the nearest 0.1 g), wing and tarsus length (to the nearest 0.1 and 0.01 mm, respectively) were measured. We captured 83% (91/110) and 73% (80/110) of breeding females and breeding males, respectively. On day 13 posthatching, nestlings were banded, measured (tarsus length) and weighed in a way similar to that described above for adults. Numbers of young were determined by counting the nestlings immediately prior to fledging (day 17–19). Adults and nestlings were bled by brachial venipuncture and blood samples (about 20 µl) were stored on Whatman FTA cards (Whatman Bioscience, Florham Park, NJ, U.S.A.). In these study populations, nestling survival (percentage of hatched young that fledge) is high (94%) and thus the unknown paternity status of the few chicks ($N = 7$) that died before blood samples were taken was likely to have a negligible impact on our results. We failed to obtain genetic material from unhatched eggs probably because a high proportion of them were apparently infertile (no sign of development) or embryo mortality occurred at a very early stage of development (personal observation). Overall, we sampled

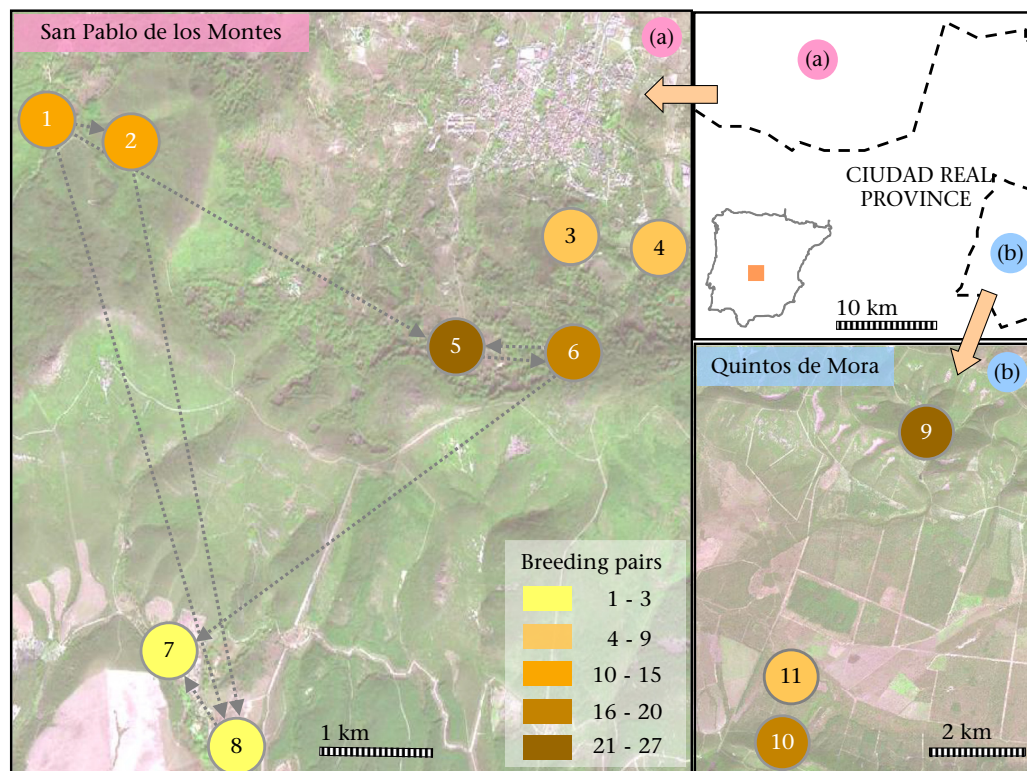


Figure 1. Map of the study area (Montes de Toledo, central Spain) including the location of the 11 nestbox plots (denoted with a circle and their corresponding number: (1) 'Arroyo del Marchés', (2) 'Las Majadillas', (3) 'Ermita', (4) 'Fuente fría', (5) 'Casillas', (6) 'La Morra', (7) 'El Robledillo', (8) 'Fuente Cantarranas', (9) 'Valdeyernos', (10) 'Gil García', (11) 'Las Navas') and a schematic representation of interplot movements by adult great tits detected in the present study, in which the EP male's locality is connected by an arrow to the locality where he gained the EP fertilization. Those cases (11 of 19, see Table 1) in which the identified EP sire belonged to the same plot as its EP partner are not shown.

nearly half (46%; 51/110) of the families present in the study plots. The frequency of double brooding in these populations is very low (1.8% of all the broods in 2012 breeding season) and we only analysed one brood per individual (i.e. no individual was included more than once in our data set). The position of all nests was georeferenced using a GPS device (Garmin Personal Navigator) and distances between them calculated from UTM coordinates.

Microsatellite Genotyping

We genotyped 51 complete families (hatched chicks and both parents sampled) at 15 microsatellite loci (Appendix Table A3). All markers were used for estimating genetic relatedness and heterozygosity, but only a subset of them (nine loci) was employed for parentage analyses (Appendix Table A3; see more below). Genomic DNA was extracted from blood samples and purified using commercial kits (NucleoSpin Blood Kit, Macherey-Nagel GmbH & Co., Duren, Germany) or according to the salt extraction protocol of Aljanabi and Martinez (1997). DNA was quantified with a NanoDrop spectrophotometer (Thermo Scientific Inc., Waltham, MA, U.S.A.) and diluted with TE buffer to obtain working concentrations of 10–50 ng DNA. Amplifications were conducted in 10 μ l reaction volumes containing 1 \times reaction buffer (67 mM Tris-HCl, pH 8.3, 16 mM (NH₄)₂SO₄, 0.01% Tween-20, EcoStart Reaction Buffer, Ecogen, Barcelona, Spain), 2 mM MgCl₂, 0.2 mM of each dNTP, 0.15 μ M of each dye-labelled primer (FAM, PET, NED or VIC), 0.1 U of Taq DNA EcoStart Polymerase (Ecogen) and 1 μ l of template DNA. The polymerase chain reaction (PCR) conditions were as follows: 9 min denaturing at 95 °C followed by 40 cycles of 30 s at 94 °C, 45 s at the annealing temperature (see Appendix Table A3) and 45 s at 72 °C, ending with a 10 min final elongation step at 72 °C. Amplification products were run on an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.) and genotypes were scored using GENEMAPPER 3.7 (Applied Biosystems). Tests for null alleles and deviations from Hardy–Weinberg equilibrium (HWE) were performed using CERVUS 3.0 (Kalinowski, Taper, & Marshall, 2007). Offspring sex was determined by PCR amplification of the CHD1-W and CHD1-Z genes using the primers 0057-F and 0002-R (Round, Hansson, Pearson, Kennerley, & Bensch, 2007).

Molecular Parentage Analyses

A total of 647 individuals (309 nestlings and 338 adults) were used in parentage analyses, for which we selected a subset of nine markers (see Appendix Table A3). These analyses were carried out using a likelihood-based approach in CERVUS. This program assesses the confidence of paternity exclusion using criteria generated through a simulation taking into account allele frequencies in the population, the possible candidate parents and the percentage of missing genetic data and genotyping errors. CERVUS screens candidate individuals and ranks them by the likelihood of their being the nestling's parent. In a first step, maternity was determined for each nestling to check for 'egg dumping'. In five cases (from four broods) we found that nestling genotypes did not match the putative mother (i.e. the social mother did not correspond to the genetic mother) at two or more loci probably as a consequence of intraspecific brood parasitism. Second, paternity was assigned for the remaining 47 nests after setting the mother as a 'known parent' in the analysis and including as candidate fathers all males sampled in the study area during 2011–2013 ($N = 149$). In this way, a chick was considered as extrapair offspring (EPO) if its social father was not in the pool of most likely fathers given by CERVUS, or if there was another sire in the population (nestbox plot) with a better match than the putative father. This is computed by CERVUS as the difference (Δ) in the likelihood ratio (LOD) scores between

the two most likely fathers. CERVUS categorically assigns paternity to a particular candidate father if Δ exceeds a certain threshold, which is computed through simulated paternity inferences (10 000 cycles). 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. We also took into account possible typing errors (1% of loci mistyped) in order to reduce the impact of other potential causes of parent–offspring mismatches (e.g. mutations) that may lead to the exclusion of the true genetic father (Marshall, Slate, Kruuk, & Pemberton, 1998). To minimize erroneous paternity assignments, we followed a conservative criterion and paternity was only assigned if the presumptive father mismatched the nestling's genotype at no more than two loci and had a significant trio LOD score.

Individual Genetic Diversity and Pairwise Relatedness Estimates

Heterozygosity and parental relatedness values were calculated employing seven additional microsatellites (up to a total of 15 microsatellite markers) in order to increase the accuracy of our estimates (e.g. Aparicio, Ortego, & Cordero, 2006). Furthermore, by increasing the number of loci we reduced the possible bias resulting from using the same set of markers to determine paternity and estimate individual heterozygosity, which can favour a link between heterozygosity and EPP (Wetzel & Westneat, 2009). Analyses based on estimates of genetic diversity and relatedness obtained only considering the six microsatellite markers not employed to infer paternity provided analogous results (data not shown). We used the homozygosity by loci index (HL; Aparicio et al., 2006) to estimate individual genetic diversity (heterozygosity). This measure weights the contribution of each locus to the homozygosity value depending on their allelic variability and has been shown to outperform other indices of genetic diversity (Aparicio et al., 2006). HL scores were calculated using an Excel macro written by W. Amos (www.zoo.cam.ac.uk/departments/molecular-ecology/IRmacroN4.xls). We used the program COANCESTRY (Wang, 2011) to calculate Queller & Goodnight's (1989) r , an estimate of relatedness based on gene sharing where a score of -1 denotes two maximally dissimilar individuals and a score of 1 indicates two clones.

Spatial and Temporal Distribution of EPP

To infer the temporal window during which individuals engage in EPP and to achieve a better understanding of their reproductive strategy from an ecological perspective, we examined the distribution of EPP relative to the breeding status of each EP male's social partner. In a first step, we calculated the difference in (non-standardized) laying date (Δ LD) between the nest of the male and that of his EP partner. This was computed by subtracting the social female's laying date from that of the EP female. We then quantified female breeding synchrony by determining the proportion of females in the same plot that were fertile at the same time as the focal female ('synchrony index'). This synchrony index is a surrogate of the temporal availability of EP fertilization opportunities and is calculated as the sum of all females that were fertile for each day of the focal female's fertile period divided by the total number of fertile females. A synchrony index (SI) of 0% indicates a completely asynchronous population with no overlap of fertile periods, whereas an SI of 100% indicates a completely synchronous population. The fertile period of a female blue tit is unknown but there is evidence from other closely related species that females only store sperm for 2 days before the first egg is laid (day -2) onwards (Birkhead, Briskie, Lifjeld, & Slagsvold, 1997). Thus, the fertile period was defined as being from day -2 until the day the

penultimate egg was laid (e.g. Canal et al., 2012). We estimated synchrony at both the plot level (average degree of synchrony for each study plot or population-wide synchrony) and at the individual level (individual breeding synchrony; i.e. the degree of synchrony of the focal female's fertile period with respect to the remaining females in the study plot). Regarding spatial correlates, we used population size (i.e. total number of breeding pairs in each plot) and breeding density (number of breeding pairs per ha) as potential determinants of variation in mating opportunities among study plots.

At the population level (i.e. across plots; $N = 11$ plots), we explored linear relationships between EPP rates and the three socioecological variables described above: breeding synchrony, breeding density and population size. To test the association between EPP occurrence and such variables, we used a logistic regression with a binomial distribution of errors and a logit-link function ('GENMOD' procedure in SAS) including the number of nests with at least one EPO (or the number of EPO) as the response variable, total number of nests (or nestlings) sampled in each plot as the binomial denominator, and a suite of variables (number of breeding pairs, breeding density, population-wide breeding synchrony and standardized laying date) as predictors.

At the within-plot level ($N = 47$ nests), we calculated for each nest the degree of overlap of the female's fertile period with respect to the rest of the breeding plot in order to test whether males were less likely to invest in EPP during periods of high synchrony. We also tested whether standardized laying date had a significant effect on the likelihood of being cuckolded (probability of EPP) within plots. These analyses were performed using generalized linear mixed models (GLMMs) fitted by the Laplace approximation (lme4 package in R; Bates & Maechler, 2009), with a binomial distribution and 'logit-link' function. Nestbox plot was included as a random effect and breeding synchrony and standardized laying date as potential explanatory terms. As these last two variables were highly correlated (see Results), we tested for their effects separately. A similar analysis was repeated by including the number of EPO within the nest as a dependent variable (event) and brood size as a binomial denominator (trial). We obtained similar results when including the proportion of EPO per brood weighted by the number

of chicks as a response variable and by setting the distribution as Gaussian and the link function as 'identity' (analyses not shown).

Finally, we determined the scale at which most EPP events took place in our study system. To that end, we calculated the Euclidean distance between the primary nestbox (social nest) of each EP male and that of the male he cuckolded. Then, we analysed whether this distance varied with the EP mate's timing of breeding relative to that of the social female (three categories: 'before', 'at the same time', 'after'; $N = 19$ nests with EPO in which the cuckolding male was genetically identified; see Table 1). Given our low sample sizes and the non-Gaussian error distribution of dispersal distance data, we used a nonparametric Kruskal–Wallis test. Because a large proportion of known EP sires were not local (i.e. their social nest was located in a different plot; see Results), we also calculated for each focal male ($N = 12$) the number of available females (nesting females) within their origin plot once the fertile period of the social partner ended. In this way, we tested whether males that obtained EPP after the fertile period of their social mate and, thus, were 'freed' from mate-guarding duties, engaged in EPP with females from nests further away because they had difficulty in seeking an EP partner in nearby territories. Lastly, we examined the potential costs in terms of 'paternity losses' of this behaviour, that is, whether those males that (presumably) roam outside of their locality to seek out EP females are more often cuckolded than those that engage in EPC within their breeding site. This was tested using a (2×2) chi-square test and considering those males for which we had complete paternity information (i.e. both within- and extrapair success; $N = 19$).

Genetic Benefits

We used paired t tests to compare the age, phenotypic characteristics (body mass, wing length, tarsus length), genetic diversity and relatedness to the partner of (1) cuckolded males versus their cuckolding males ($N = 19$ pairwise comparisons) and (2) social females versus EP females ($N = 19$ pairwise comparisons). We also compared cuckolded and noncuckolded males and faithful and unfaithful females by using one-way ANOVAs. We defined cuckolded and cuckolding males and faithful and unfaithful females

Table 1
Overview of the EPP cases in which the EP sire was unambiguously identified

Male ID	Plot 1	Plot 2	Distance	N_f	% _f	SLD	ΔLD	EPO	Loss	Period	Female activity
2970663	Gil García	Gil García	380.36	—	—	56	−35	1	1	Before	Prior to laying
2A385660	Valdeyernos	Valdeyernos	650.78	—	—	41	−20	1	0	Before	Prior to laying
2A381863	Arroyo del Marchés	Arroyo del Marchés	172.21	—	—	36	−18	3	0	Before	Prior to laying
2A381904	Las Casillas	Las Casillas	74.60	—	—	34	−11	2	—	Before	Prior to laying
2A381649	Las Majadillas	Las Majadillas	39.82	—	—	24	−5	1	0	Same	Laying
2A381903	Las Casillas	Las Casillas	214.79	—	—	28	−4	5	—	Same	Laying
2A381775	Las Casillas	Las Casillas	157.00	—	—	26	−1	1	0	Same	Laying
2A385527	Gil García	Gil García	286.57	18	100	7	6	1	—	After	Laying/Incubation
2A351433	Las Majadillas	Fuente Cantarranas	4363.68	0	0	42	7	1	0	After	Incubation
2A381679	Arroyo del Marchés	La Morra	4099.69	3	21	23	7	1	0	After	Incubation
2A283695	Gil García	Gil García	146.26	11	61	13	8	1	1	After	Laying/Incubation
2A381611	Arroyo del Marchés	Las Majadillas	899.50	11	79	5	8	1	0	After	Incubation
2A381905	Las Casillas	La Morra	1825.00	9	47	25	9	1	0	After	Incubation
2A381602	Arroyo del Marchés	Fuente Cantarranas	5425.96	11	79	9	12	1	—	After	Incubation
2A381710	Fuente fría	Fuente fría	268.55	2	50	12	14	1	0	After	Incubation
2A363746	La Morra	El Robledillo	3312.62	11	42	27	20	1	0	After	Incubation
2A381913	La Morra	Las Casillas	1334.24	10	37	30	20	1	1	After	Incubation
2A381908	La Morra	La Morra	49.01	19	70	25	28	2	0	After	Rearing
2A381704	Fuente Cantarranas	El Robledillo	1265.30	2	67	10	27	1	—	After	Postfledgling

The following information is given: male identity (genetic father), plot where the social nest of the genetic father is located (plot 1), plot where the nest with EPO sired by the focal genetic father is located (plot 2), distance between the two plots (m), number of available females in the breeding site, plot 1, once the fertile period of the social partner had finished (N_f), percentage of available mates with respect to the total number of breeding females detected in each nestbox plot (%_f), standardized laying date (SLD), difference in laying date between the nest of the male and that of the EP partner (ΔLD), number of EPO, number of paternity losses, nongenetic sires, at their own nest (Loss), time ('before', 'same', 'after') at which the male engaged in EPC with respect to the fertile period of his social partner and activity of the social partner during the period in which EPP events took place (Female activity).

based on data from nests with complete paternity information. In a further step, we explored the existence of differences between EPO ($N = 62$) and WPO ($N = 242$) in tarsus length, body mass and genetic diversity by means of generalized linear mixed models (GLMMs) including sex as a fixed factor (to control for sexual dimorphism) and considering both brood identity (nested within plot) and study plot as random effects. We also used paired t tests to compare (1) maternal half-siblings (EPO versus WPO) raised in the same brood ($N = 25$ pairwise comparisons) and (2) nestlings sired outside the pair bond (EPO) and within the social nest by the same father ($N = 14$ pairwise comparisons). In addition, we examined whether most mixed broods contained EP young sired by different males (multiple EPP cases) and used a chi-square test to analyse whether EPO were randomly distributed over broods as predicted by the genetic diversity hypothesis. Lastly, we also tested whether males that engaged in EPC had a higher realized reproductive success (total number of young sired from both WP and EP fertilizations) than those that did not gain paternity outside the pair bond by means of a one-way ANOVA.

Statistical analyses were done in SAS (SAS Institute, Cary, NC, U.S.A.) and R v. 2.14 (R Development Core Team, 2012) except for nonparametric tests (Pearson correlations, t tests, contingency tables), which were performed using Statistica 7 (Statsoft Inc, Tulsa, OK, U.S.A.). Percentage data were arcsine square root transformed prior to analysis to conform to the assumptions of parametric statistical analyses. Means \pm SE are given.

Ethical Note

The protocols for capturing, handling and taking blood from great tits were approved by the Dirección General del Medio Natural of Junta de Comunidades de Castilla-La Mancha. Ringing permits were granted by the Ringing Office of the Spanish Society of Ornithology (SEO/BirdLife; licence number 520030).

To ensure the safe treatment of adult and nestlings, we used the following protocol when capturing and handling adults and nestlings. Parents were not captured until the nestlings were at least 8 days old to avoid female/male abandonment of nests. Spring-traps were constantly monitored by means of binoculars. No nest was disturbed for more than 60 min; that is, we tried to capture both parents within 1 h and after that time we retrieved the spring-traps from the nest. Some birds were captured twice during the 1 h capture session, confirming that trapped parents resumed their feeding activity after 20–25 min. Nestlings were kept in light-weight bags to prevent them from overheating. On cold or rainy days (which were an exception), nestlings were banded in a warm place (inside a car). The entire handling process lasted less than 15 min and chicks did not exhibit signs of stress during this period.

RESULTS

Patterns of Extrapair Paternity

There was no evidence of null alleles among any of the 15 loci used, nor were there any significant deviations from HWF. We successfully assigned paternity to 53% (33/62) of EPO. The remaining 29 chicks were sired by 'unknown' males (i.e. non-sampled individuals) or paternity was assigned with low confidence (negative or zero LOD scores). Overall, more than half (55%; 26 of 47) of the broods contained at least one EPO, and EPO accounted for 62 of 304 nestlings (20%). The mean number of EPO per brood was 2.08 (range 1–5). Three broods were cuckolded by two different males; in the remaining broods all EPO were sired by a single father. No individual gained EP paternity in two different nests and all EP males identified with paternity analyses were

captured in the year of study (2012). Only 21% (3/14) of males that gained paternity in another nest lost paternity in their own nest. EPO were not allocated randomly among broods and their distribution deviated significantly from a binomial distribution ($\chi^2_5 = 26.23$, $P < 0.01$).

Spatial and Temporal Distribution of EPP

EPP events were distributed throughout the breeding season (Fig. 2). During the period of peak fertility (from day -5 to 0), when the proportion of active nests was highest, we found a relatively low proportion of EPP cases (21% of broods contained EPO; see Fig. 2). This value was lower than expected from a binomial distribution ($P = 0.03$).

At the among-plot level, we found a significant negative association between the population-wide synchrony index and both the proportion of nests with at least one EPO (EPP rate; Fig. 3a, Appendix Table A4) and the number of EPO relative to the total number of nestlings sampled in each plot (EPO rate; Fig. 3b, Appendix Table A4). There was no significant association between these variables and local number of breeding pairs or breeding density (Appendix Table A4). The proportion of EPO (but not the EPP rate) increased significantly with standardized laying date (Appendix Table A4).

Within plots, breeding synchrony did not predict the probability of a nest being cuckolded (estimate \pm SE: -2.51 ± 2.00 , $Z = -1.25$, $P = 0.21$), although, a negative relationship between breeding synchrony and the number of EPO in the nest was found, suggesting that early asynchronous and late asynchronous nests suffered a higher rate of cuckoldry than those nests initiated at the peak of synchrony (estimate \pm SE: -9.65 ± 1.13 , $Z = -8.53$, $P < 0.001$). The time of season at which a brood was initiated (standardized laying date) had no significant influence on the probability of having at least one EPO in the nest (estimate \pm SE: 0.03 ± 0.02 , $Z = 1.29$, $P = 0.19$), but predicted significantly the EPO rate (estimate \pm SE: 0.10 ± 0.01 , $Z = 7.79$, $P < 0.001$). Breeding synchrony was quadratically related to standardized laying date (SLD: $F = 10.73$, $P < 0.01$; SLD²: $F = 11.49$, $P < 0.01$); it increased with a peak in the middle of the season and declined sharply thereafter (Fig. 2).

Males usually gained EPP after the laying period of their social female (Table 1). This occurred in 63% of cases. Most cases (52%) of

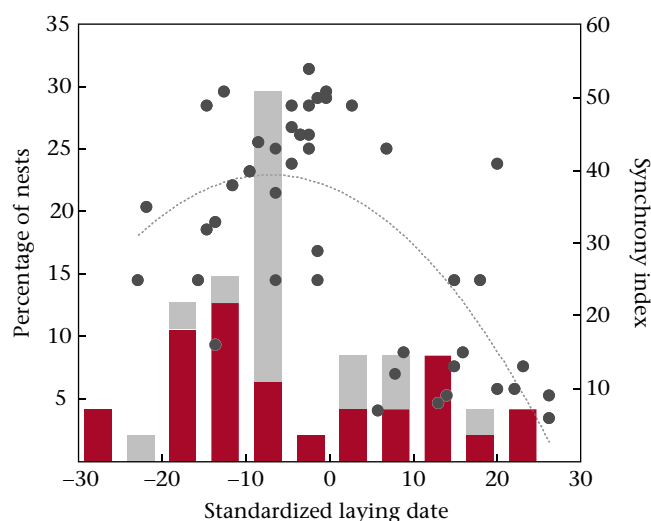


Figure 2. Relative frequency of nests with (maroon bars) and without (grey bars) EPP and breeding synchrony (dots and asymptotic line) in relation to standardized laying date.

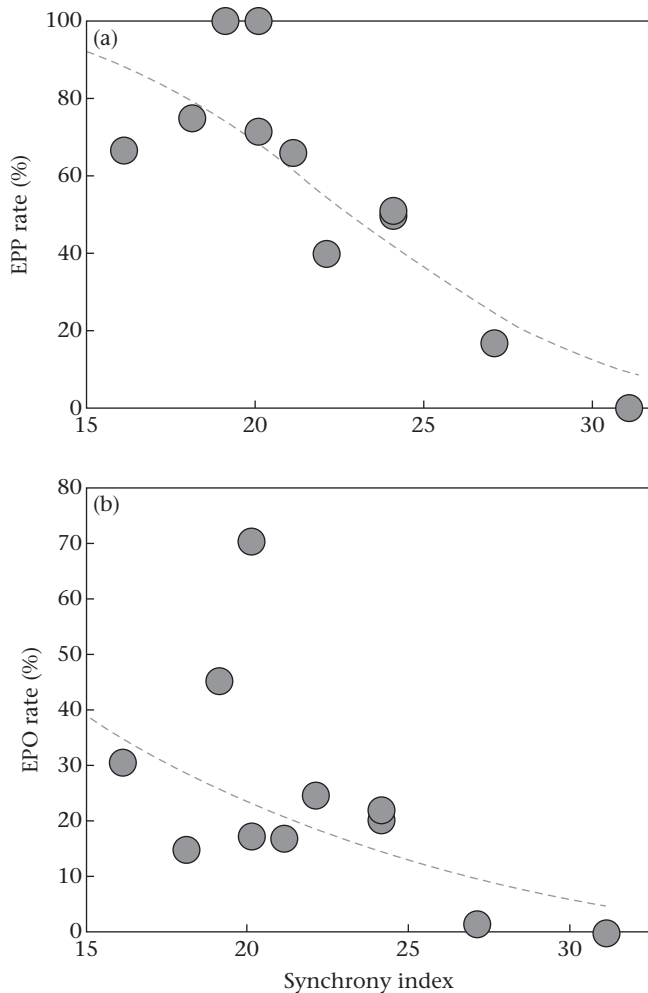


Figure 3. Relationship between the degree of overlap of female fertile periods (population-wide synchrony index) and the observed (a) EPP rate and (b) EPO rate for each study plot ($N = 11$).

EPP detected in the study area took place during the incubation period of the EP male's social female (Table 1). Spatially, EPP events for which the EP male could be identified did not usually occur between individuals from nearby territories (i.e. between the closest neighbours; mean distance: 1314 ± 389 m). Eight of these cases (42%) involved individuals from different plots (Fig. 1, Table 1). EPP events that occurred before or during the fertile period of the social female involved individuals settled in nearby (ca. 200 m) territories. Meanwhile, those that occurred once the social female had already finished her laying period implicated long-distance movements, often between nestbox plots (Table 1). Accordingly, the distance between EP mates differed marginally in relation to the difference in laying date between the social and the EP partner ('before': 319.5 ± 127.5 ; 'at the same time': 137.2 ± 51.4 ; 'after': 1939.7 ± 542.2 m; Kruskal–Wallis ANOVA: $\chi^2 = 5.29$, $P = 0.07$; Table 1).

When exploring the association between the distance travelled by those males without mate-guarding responsibility and the proportion of available females in the breeding locality, despite our small sample size, we found a nonsignificant trend towards males moving further away as the number of mating opportunities at a local scale decreased ($r = -0.47$, $N = 19$, $t = -1.57$, $P = 0.12$).

Of 14 males that engaged in EPC and whose families were sampled, 11 achieved full paternity; only three males that gained

paternity elsewhere were in turn cuckolded by other individuals at their own nest. Those males that (presumably) travelled long distances and gained paternity with EP females from other woodlands were not more likely to be cuckolded than those that engaged in EPC with local females (i.e. within the same plot; 2×2 contingency table: $N = 14$, $\chi^2 = 0.09$, $P = 0.76$).

Genetic Benefits

Female perspective

We did not find differences in phenotypic characteristics, genetic diversity or relatedness to the female between males that lost paternity at their own nest and those that were not cuckolded (Table 2). The proportion of inexperienced breeders did not differ significantly between groups (yearlings accounted for 46% and 33% of cuckolded and noncuckolded males, respectively; 2×2 contingency table: $\chi^2 = 0.55$, $P = 0.46$). In pairwise comparisons, we found that EP males were older than the males they cuckolded (yearling rate, 26% versus 60%, respectively; $\chi^2 = 3.89$, $P = 0.048$), suggesting a female mating preference for older males and/or a limited ability of younger and less experienced males in preventing cuckoldry by means of paternity assurance behaviours. There were no significant differences between social and EP males for the rest of the variables (Table 2).

Extrapair and within-pair offspring did not differ in body size (EPO: 19.27 ± 0.09 mm, $N = 62$; WPO: 19.24 ± 0.04 mm, $N = 242$) or body mass (EPO: 16.97 ± 0.16 g; WPO: 16.59 ± 0.10 g; Appendix Table A5). The results were similar when considering only those broods containing EPO; there were no significant differences in body size (EPO: 19.23 ± 0.11 mm; WPO: 19.29 ± 0.11 ; $t_{25} = -0.39$, $P = 0.69$) or body mass (EPO: 16.99 ± 0.19 g; WPO: 16.80 ± 0.19 ; $t_{25} = 1.16$, $P = 0.26$) between EPO and their maternal half-siblings. Contrary to expected, EPO tended to be less heterozygous than WPO (HL: EPO: 0.31 ± 0.10 ; WPO: 0.26 ± 0.10 ; Appendix Table A5), but this nonsignificant trend disappeared when comparing nest-mates in mixed-paternity broods (EPO: 0.29 ± 0.03 ; WPO: 0.27 ± 0.02 ; $t_{25} = 0.55$, $P = 0.58$).

Male perspective

Males that gained EPP sired more nestlings those that did not (7.21 ± 0.60 versus 4.78 ± 0.40 , respectively; $F_{1,45} = 11.13$, $P < 0.001$). The number of genetic young produced in the social nest did not differ significantly between males that sired EPO and males that sired no EPO (6.00 ± 0.61 versus 4.78 ± 0.40 , respectively; $F_{1,45} = 2.69$, $P = 0.1$), indicating that males engaged in EPP without jeopardizing the fertilization success in their own nests.

Promiscuous females did not differ from monogamous females in size, body mass, heterozygosity or genetic relatedness with the social partner, which indicates that males did not show a preference for EP partners with particular phenotypic characteristics or a superior genetic make-up (Table 3). The proportion of inexperienced breeders did not differ significantly between unfaithful and faithful females (yearling rate: 48% versus 65%, respectively; $\chi^2 = 1.26$, $P = 0.26$). In pairwise comparisons, social and EP females had similar ages (yearling rate: 44% versus 67%, respectively; $\chi^2 = 1.80$, $P = 0.18$) and they did not differ significantly in genotypic or phenotypic attributes (Table 3), suggesting that males did not target more heterozygous or more dissimilar partners to produce EP descendants.

Nestlings sired by the same genetic father outside the pair bond were heavier than those sired within the social nest (EPO: 17.32 ± 0.22 g; WPO: 16.47 ± 0.25 ; $Z_{14} = 2.34$, $P = 0.019$). There were no significant differences in tarsus length (EPO: 19.39 ± 0.17 mm; WPO: 19.11 ± 0.16 ; $Z_{14} = 1.57$, $P = 0.11$) or heterozygosity (EPO: 0.34 ± 0.04 ; WPO: 0.27 ± 0.03 ; $Z_{14} = 0.94$,

Table 2
Comparisons of phenotypic and genetic characteristics between cuckolded and their cuckolding males (pairwise comparisons) and between cuckolded and noncuckolded males

	Male status			Cuckolded versus cuckolding		Cuckolded versus noncuckolded	
	Cuckolded (N=26)	Cuckolding (N=19*)	Noncuckolded (N=21)	t (pairwise)	P	F	P
Tarsus length (mm)	19.75±0.12	19.77±0.13	20.00±0.11	0.28	0.78	1.98	0.16
Wing length (mm)	74.46±0.33	74.48±0.39	74.83±0.36	−0.18	0.85	0.56	0.46
Body mass (g)	17.01±0.19	17.12±0.15	17.35±0.21	1.27	0.22	1.46	0.23
Homozygosity by loci (HL)	0.29±0.03	0.31±0.04	0.24±0.03	1.70	0.11	1.29	0.26
Genetic similarity (r)	0.01±0.04	0.08±0.05	0.03±0.05	1.37	0.19	0.07	0.79

Means ± SE are given.

* For those cases (N = 3) in which the brood was fathered by more than one male, we used the mean values of the EP fathers.

Table 3
Comparisons of phenotypic and genetic characteristics between social (within-pair, WP) and extrapair (EP) partners of males (pairwise comparisons) and between promiscuous (WP with EPP) and genetic monogamous females (WP without EPP)

	Female status			WP versus EP		WP (EPP) versus WP (no EPP)	
	WP (EPP) (N=26)	EP (EPP) (N=19)	WP (No EPP) (N=21)	t (pairwise)	P	F	P
Tarsus length (mm)	19.10±0.07	19.04±0.09	19.08±0.14	−0.55	0.58	0.02	0.87
Wing length (mm)	71.78±0.33	71.63±0.40	71.33±0.54	−0.20	0.83	0.52	0.47
Body mass (g)	16.61±0.24	16.16±0.31	16.19±0.23	−0.87	0.39	1.59	0.21
Homozygosity by loci (HL)	0.30±0.03	0.35±0.04	0.29±0.04	1.96	0.07	0.13	0.71
Genetic similarity (r)	0.01±0.04	0.01±0.04	0.03±0.05	−0.08	0.93	0.07	0.79

Means ± SE are given.

$P = 0.34$) between the two groups. We obtained similar results by using date-corrected measurements (calculated from regression residuals) for both biometric traits (body mass: $Z_{14} = 2.06$, $P = 0.039$; tarsus length: $Z_{14} = 0.59$, $P = 0.55$).

DISCUSSION

Spatial and Temporal Distribution of EPP

The general pattern arising from this study suggests that male great tits tend to engage in EPC after the social female's fertile period. We found that most EPP cases occurred during the incubation period of the EP male's social female, as previously described in other passerines (Canal et al., 2012; Evans, Stutchbury, & Woolfenden, 2008). By engaging in EPC after the social female's fertile period, males may minimize the risk of being cuckolded by another male during the period in which they are absent from their social partner and territory. However, this strategy could be costly if the postponement of this activity implies long-distance travel to gain EPP due to the decreasing likelihood of finding an EP partner in a nearby territory as the season progresses (see more below). In contrast, a few (late-breeding) males were engaged in EPC before the onset of laying of the social female, a pattern similar to that reported in a previous study in this species (Strohbach et al., 1998). The notion that investment in off-territory movements is likely to imply a trade-off between gaining fertilizations elsewhere and losing paternity at home is also supported by the observed negative relationship between breeding synchrony and EPP, both among and within plots. We observed that more synchronous plots had lower EPP rates than those plots with a longer breeding period. Likewise, at the plot level, we found that the more asynchronous the nest, the higher the likelihood that it contained EPO. That is, during periods of low synchrony (mainly late in the season) the few nests that remain active seem to suffer a higher risk of cuckoldry (van Dongen, 2008; Hammers et al., 2009; see also Kristín, Hoi, Valera, & Hoi, 2008).

We did not find any association between the spatial correlates analysed (number of breeding pairs, breeding density) and the incidence of EPP, which can be explained by the fact that a large proportion of EP sires were not local. As previously suggested by some authors (Chuang, Webster, & Holmes, 1999; Mayer & Passinelli, 2013), under these or similar circumstances the EPP rate is expected to be decoupled from the number of potential partners located in nearby territories and thus any surrogate of breeding density is unlikely to accurately reflect EP mating opportunities. The fact that great tits can travel long distances to engage in EPC is somewhat surprising considering that most studies of EPP in small passerines identified the sires of EPO among close neighbours (e.g. Canal et al., 2012; Hill, Akçay, Campbell, & Beecher, 2011; Taff et al., 2013). However, some previous studies have reported long-distance movements of adult birds during the breeding period, which could be for the purpose of EP mating behaviour (Dunn & Whittingham, 2005; Norris & Stutchbury, 2001), and others found long distances between the primary nest of the male and that of their EP partner (Foerster, Delhey, Johnsen, Lifjeld, & Kempenaers, 2003; Leisler, Beier, Staudter, & Wink, 2000; Woolfenden, Stutchbury, & Morton, 2005). This excursion behaviour has also been documented in mammals (e.g. Debeffe et al., 2014; Soulsbury, Iossa, Baker, White, & Harris, 2011). Regrettably, we do not know exactly how EPP events may have arisen in our study and thus we cannot distinguish between three plausible scenarios: males may foray into territories further away, females may visit males settled in different breeding patches or the two sexes could encounter each other at a common site (Reyer, Bollmann, Schläpfer, Schymaında, & Klecack, 1997). Our results indicate short-distance movements during the fertile period of the social partner, long off-territory forays afterwards and an almost total absence of EPP after nestlings hatch in social nests (Table 1). We speculate that these off-territory forays could be linked to the considerable degree of habitat fragmentation of our study area, which could reduce the likelihood of locating additional fertile females as the season progresses in these small populations and

increase the distances travelled by birds to engage in EPP. This pattern contrasts with that reported for blue tits in the same area, in which EPP events occurred at shorter distances (mean distance: 190 m), mostly involving neighbouring individuals (<50 m) and with frequent cases of reciprocal cuckoldry (García-Navas, Ferrer, et al., 2014). This suggests that blue tits are less prone than great tits to move large distances (i.e. to other woodlands) to seek available EP partners. Thus, the results presented here and elsewhere (García-Navas, Ferrer, et al., 2014) illustrate how habitat fragmentation can influence the reproductive tactics of closely related species in a very different manner (see also Evans, Woolfenden, Friesen, & Stutchbury, 2009). Future studies incorporating behavioural data and considering more populations and more extensive paternity analyses could help us improve our understanding of the socioecological constraints of EPP and overcome some of the limitations associated with our approach and the relatively small sample sizes available for plot-based analyses.

Genetic Benefits

Female perspective

So far, the main focus in research on promiscuity in passerines, including the great tit, has been to test the genetic benefits hypotheses. The possible benefits that females obtain by mating multiply have received disproportionate attention taking into account that there is no clear evidence that females actively pursue EPC themselves in most species. In fact, Westneat and Stewart (2003) found descriptions of female pursuit for fewer than 15 bird species, which is an unrepresentative and almost negligible sample. We found no support for indirect genetic benefits of mating with several males (e.g. Augustin, Blomqvist, Szép, Szabó, & Wagner, 2007; Ferreti, Massoni, Bulit, Winkler, & Lovette, 2011). We found no differences in phenotypic or genotypic attributes between the males that lost paternity and those that did not with the exception of a difference in age: in line with previous studies (Lubjuhn, Gerken, Brün, & Schmoll, 2007), we observed that cuckolded males were younger than the cuckolding males. This means that females may show a preference for older individuals that could be the most competitive and/or carry alleles that confer a higher fitness in terms of survival or longevity. Alternatively, younger males may be more prone to paternity loss due to their limited experience in performing paternity assurance behaviours. Neither were there differences between females' social mates and their extrapair males, which implies that female great tits did not engage in EPC with males of higher quality with respect to their social mates (i.e. they did not 'trade up'). At this point, it might be argued that we did not examine two parameters whose importance in extrapair mate choice decisions have been previously suggested in closely related species: plumage colour (Delhey, Peters, Johnsen, & Kempenaers, 2007) and song output (Kempenaers, Verheyren, & Dhondt, 1997). However, these ornamental traits (song, coloration) are frequently related to the genetic quality of the bearer (Kempenaers, 2007; e.g. Ferrer, García-Navas, Bueno-Enciso, Sanz, & Ortego, 2015). Therefore, if female great tits base their choice for EPCs on male ornamentation we should have observed a preference for more heterozygous (ornamented) individuals.

On the other hand, EP matings were equally likely to occur among closely related pairs and those that were more compatible genetically, which does not support the role of EPC as a female strategy to avoid the risk of inbreeding depression (Tregenza & Wedell, 2000), which may be relatively high within small plots. This issue is particularly noteworthy since we have previously reported a significant negative relationship between parental (social) genetic relatedness and hatching success in this study system (García-Navas, Cáliz-Campal, Ferrer, Sanz & Ortego, 2014). Lastly,

the low frequency of EPO within nests and the fact that EPO were usually sired by a single father provides little support to the 'bet-hedging' hypothesis. However, it should also be considered that most EPO may be sired by a single father due to the ability of social males to reduce the chances of being cuckolded even if females aim to increase the variability of the genetic composition of their progeny via EPC with several males. Together, these results are in agreement with a large number of studies that failed to find evidence of genetic benefits of EPP in this (Krokene, Rigstad, Dale, & Lifjeld, 1998; Lubjuhn, Strohbach, Brün, Gerken, & Epplen, 1999; Strohbach et al., 1998) and other species (see Akçay & Roughgarden, 2007 for a review), and reinforce the idea that female EP behaviour may evolve via indirect selection on male behaviour. Accordingly, even though EPC may not supply net benefits for females, they may still express this behaviour through genetic correlation (Forstmeier et al., 2011; Halliday & Arnold, 1987; see also Hsu, Schroeder, Winney, Burke, & Nakagawa, 2014).

Male perspective

While it is increasingly realized that mate choice by males is relatively common in nature (Edward & Chapman, 2011) and the majority (90%) of studies including detailed observations on EP behaviour report the existence of male forays to attempt EPC (Westneat & Stewart, 2003), as far as we know only a few studies (Bollmer, Dunn, Freeman-Gallant, & Whittingham, 2012; Freeman-Gallant, Meguerdichian, Wheelwright, & Sollecito, 2003; Stewart, Hanschu, Burke, & Westneat, 2006; Winternitz et al., 2015) have examined the relationship of female attributes and EPP. Here, we found that males did not show an active preference for more heterozygous or more compatible females when seeking EP partners. Moreover, those males that presumably left their breeding area to engage in EPC did not do so to copulate with females genetically less similar to themselves (i.e. to avoid potential inbreeding depression). However, we found that males produced heavier chicks outside the pair bond but we cannot discern whether this was due to the acquisition of a better partner (mother quality), the effect of the rearing environment (territory quality) or potential maternal effects (differential deposition of nutrients in eggs by laying order and mate quality; see Magrath, Vedder, van der Velde, & Komdeur, 2009). On the other hand, regardless of EP mate quality, mating outside the pair bond has obvious benefits for males as they can increase their reproductive success. We found that cuckolding males had more descendants (about two chicks) than those that did not gain paternity elsewhere, which suggests that the benefits of engaging in EPP may outweigh the associated costs (e.g. Balenger et al., 2009; Canal et al., 2011).

Conclusions

To summarize, our study suggests that certain socioecological factors determining when males are 'time-in' or 'time-out' with respect to EP liaisons may play a key role in shaping EPP patterns (Canal et al., 2012; Westneat & Mays, 2005). Among them, the timing of breeding relative to others may have an important effect on the individual's decisions about when and where to engage in EPC (Currie, Burke, Whitney, & Thompson, 1998). In this sense, variation in the time window of accessibility to fertile females can help to explain why some individuals sired EPO in nearby territories while many travelled long distances to gain EPP. Lastly, because EPP arises from encounters influenced by the behaviour of at least two individuals, whose priorities can vary dynamically, detailed studies about how changes in the social context shape their decision making can provide us with valuable information for a better understanding of such phenomena at both the individual and population level (Westneat & Mays, 2003). Thus, the consideration of the

three parties involved in this game, in either a passive or an active role, constitutes a more appropriate framework than studies only focused on female fitness as a plausible explanation for EPP.

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References

- Akçay, E., & Roughgarden, J. (2007). Extra-pair paternity in birds: review of the genetic benefits. *Evolutionary Ecology Research*, 9, 855–868.
- Aljanabi, S. M., & Martínez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25, 4692–4693.
- Amrhein, V., Johannessen, L. E., Kristiansen, L., & Slagsvold, T. (2008). Reproductive strategy and singing activity: blue tit and great tit compared. *Behavioral Ecology and Sociobiology*, 62, 1633–1641.
- Aparicio, J. M., Ortego, J., & Cordero, P. J. (2006). What should we weigh to estimate heterozygosity, alleles or loci? *Molecular Ecology*, 15, 4659–4665.
- Augustin, J., Blomqvist, D., Szép, T., Szabó, Z. D., & Wagner, R. H. (2007). No evidence of genetic benefits from extra-pair fertilisations in female sand martins (*Riparia riparia*). *Journal of Ornithology*, 148, 189–198.
- Balenger, S., Johnson, L. S., & Masters, B. (2009). Sexual selection in a socially monogamous bird: male color predicts paternity success in the mountain bluebird, *Sialia currucoides*. *Behavioral Ecology and Sociobiology*, 63, 403–411.
- Banks, S. C., Piggott, M. P., Stow, A. J., & Taylor, A. C. (2007). Sex and sociality in a disconnected world: a review of the impacts of habitat fragmentation on animal social interactions. *Canadian Journal of Zoology*, 85, 1065–1079.
- Bates, D., & Maechler, M. (2009). *lme4: Linear mixed-effects models using Eigen and Eigenfaces*. <http://CRAN.R-project.org/package=lme4>.
- Birkhead, T. R., Briskie, J. V., Lifjeld, J. T., & Slagsvold, T. (1997). Breeding cycle patterns of sperm storage in the pied flycatcher (*Ficedula hypoleuca*). *Auk*, 114, 792–796.
- Björklund, M., Möller, A. P., Sundberg, J., & Westman, B. (1992). Female great tits, *Parus major*, avoid extra-pair copulation attempts. *Animal Behaviour*, 43, 691–693.
- Blakey, J. K., & Norris, K. (1994). Do female great tits avoid extra-pair copulations? A comment on Björklund et al. *Animal Behaviour*, 47, 1227–1229.
- Bollmer, J. L., Dunn, P. O., Freeman-Gallant, C. R., & Whittingham, L. A. (2012). Social and extra-pair mating in relation to major histocompatibility complex variation in common yellowthroats. *Proceedings of the Royal Society B: Biological Sciences*, 279, 4778–4785.
- Brown, J. L. (1997). A theory of mate choice based on heterozygosity. *Behavioral Ecology*, 8, 60–65.
- Canal, D., Jovani, R., & Potti, J. (2012). Male decisions or female accessibility? Spatiotemporal patterns of extra pair paternity in a songbird. *Behavioral Ecology*, 23, 1146–1153.
- Canal, D., Potti, J., & Dávila, J. A. (2011). Male phenotype predicts extra pair paternity in pied flycatchers. *Behaviour*, 148, 691–712.
- Chuang, H. C., Webster, M. S., & Holmes, R. T. (1999). Extra-pair paternity and local synchrony in the Black-throated Blue Warbler. *Auk*, 116, 726–736.
- Cleasby, I. R., & Nakagawa, S. (2012). The influence of male age on within-pair and extra-pair paternity in passerines. *Ibis*, 154, 318–324.
- Cohas, A., & Allainé, D. (2009). Social structure influences extra-pair paternity in socially monogamous mammals. *Biology Letters*, 5, 313–316.
- Cohas, A., Yoccoz, N. G., Da Silva, A., Goossens, B., & Allainé, D. (2006). Extra-pair paternity in the monogamous alpine marmot (*Marmota marmota*): the roles of social setting and female mate choice. *Behavioral Ecology and Sociobiology*, 55, 597–605.
- Currie, D. R., Burke, T., Whitney, R. L., & Thompson, D. B. A. (1998). Male and female behaviour and extra-pair paternity in the wheatear. *Animal Behaviour*, 55, 689–703.
- Dawson, D. A., Hanotte, O., Greig, C., Stewart, I. R. K., & Burke, T. (2000). Polymorphic microsatellites in the blue tit *Parus caeruleus* and their cross-species utility in 20 songbird families. *Molecular Ecology*, 9, 1941–1944.
- Debeffe, L., Focardi, S., Bonenfant, C., Hewison, A. J. M., Morellet, N., Vanpé, C., et al. (2014). A one night stand? Reproductive excursions of female roe deer as a breeding dispersal tactic. *Oecologia*, 176, 431–443.
- Delhey, K., Peters, A., Johnsen, A., & Kempenaers, B. (2007). Fertilization success and UV ornamentation in blue tits *Cyanistes caeruleus*: correlational and experimental evidence. *Behavioral Ecology*, 18, 399–409.
- van Dongen, W. F. D. (2008). Mate guarding and territorial aggression vary with breeding synchrony in golden whistlers (*Pachycephala pectoralis*). *Naturwissenschaften*, 95, 537–545.
- van Dongen, W. F. D., & Mulder, R. A. (2009). Multiple ornamentation, female breeding synchrony, and extra-pair mating success of golden whistlers (*Pachycephala pectoralis*). *Journal of Ornithology*, 150, 607–620.
- Double, M. C., Dawson, D., Burke, T., & Cockburn, A. (1997). Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. *Molecular Ecology*, 6, 691–693.
- Dunn, P. O., & Whittingham, L. A. (2005). Radio-tracking of female Tree Swallows prior to egg-laying. *Journal of Field Ornithology*, 76, 260–264.
- Edward, D. A., & Chapman, T. (2011). The evolution and significance of male mate choice. *Trends in Ecology & Evolution*, 26, 647–654.
- Eliassen, S., & Jørgensen, C. (2014). Extra-pair mating and evolution of cooperative neighbourhoods. *PLoS One*, 9, e99878.
- Evans, M. L., Stutchbury, B. J. M., & Woolfenden, B. E. (2008). Off-territory forays and the genetic mating system of the wood thrush. *Auk*, 125, 67–75.
- Evans, M. L., Woolfenden, B. E., Friesen, L., & Stutchbury, B. J. M. (2009). Variation in the extra-pair mating systems of Acadian flycatchers and Wood thrushes in forest fragments in southern Ontario. *Journal of Field Ornithology*, 80, 146–153.
- Ferrer, E. S., García-Navas, V., Bueno-Enciso, J., Sanz, J. J., & Ortego, J. (2015). Multiple sexual ornaments signal heterozygosity in male blue tits. *Biological Journal of the Linnean Society*, 115, 362–375.
- Ferretti, V., Massoni, V., Bulit, F., Winkler, D. W., & Lovette, I. J. (2011). Heterozygosity and fitness benefits of extra-pair mate choice in White-rumped Swallows (*Tachycineta leucorrhoa*). *Behavioral Ecology*, 22, 1178–1186.
- Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T., & Kempenaers, B. (2003). Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, 425, 714–717.
- Forstmeier, W., Martin, K., Bolund, E., Schielzeth, H., & Kempenaers, B. (2011). Female extra-pair mating behavior can evolve via indirect selection on males. *Proceedings of National Academy of Sciences of the United States of America*, 108, 10608–10613.
- Forstmeier, W., Nakagawa, S., Griffith, S. C., & Kempenaers, B. (2014). Female extra-pair mating: adaptation or genetic constraint? *Trends in Ecology & Evolution*, 29, 456–464.
- Fossey, F., Johnsen, A., & Lifjeld, J. T. (2008). Multiple genetic benefits of female promiscuity in a socially monogamous passerine. *Evolution*, 62, 145–156.
- Fox, C. W., & Rauter, C. M. (2003). Bet-hedging and the evolution of multiple mating. *Evolutionary Ecology Research*, 5, 273–286.
- Freeman-Gallant, C. R., Meguerdichian, M., Wheelwright, N. T., & Sollecito, S. V. (2003). Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Molecular Ecology*, 12, 3077–3083.
- García-Navas, V., Cáliz-Campal, C., Ferrer, E. S., Sanz, J. J., & Ortego, J. (2014). Heterozygosity at a single locus explains a large proportion of variation in two fitness-related traits in great tits: a general or a local effect? *Journal of Evolutionary Biology*, 27, 2807–2819.
- García-Navas, V., Ferrer, E. S., Bueno-Enciso, J., Barrientos, R., Sanz, J. J., & Ortego, J. (2014). Extra-pair paternity in Mediterranean blue tits: socio-ecological factors and the opportunity for sexual selection. *Behavioral Ecology*, 25, 228–238.
- Griffith, S. C., Owens, I. P., & Thuman, K. A. (2002). Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, 11, 2195–2212.
- Gullberg, A., Tegelström, H., & Gelter, H. P. (1992). DNA fingerprinting reveals multiple paternity in families of Great and Blue Tits (*Parus major* and *P. caeruleus*). *Hereditas*, 117, 103–108.
- Halliday, T., & Arnold, S. J. (1987). Multiple mating by females: a perspective from quantitative genetics. *Animal Behaviour*, 35, 939–941.
- Hammers, M., von Engelhardt, N., Langmore, N. E., Komdeur, J., Griffith, S. C., & Magrath, M. L. (2009). Mate-guarding intensity increases with breeding synchrony in the colonial fairy martin, *Petrochelidon ariel*. *Animal Behaviour*, 78, 661–669.
- Hasselquist, D., Bensch, S., & von Schantz, T. (1996). Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature*, 381, 229–232.
- Hill, C. E., Akçay, E., Campbell, E., & Beecher, M. D. (2011). Extra-pair paternity, song, and genetic quality in song sparrows. *Behavioral Ecology*, 22, 73–81.
- Hsu, Y.-H., Schroeder, J., Winney, I., Burke, T., & Nakagawa, S. (2014). Costly infidelity: low lifetime fitness of extra-pair offspring in a passerine bird. *Evolution*, 68, 2873–2884.
- Jennions, M. D., & Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, 75, 21–64.
- Johannessen, L. E., Slagsvold, T., Hansen, B. T., & Lifjeld, J. T. (2005). Manipulation of male quality in wild tits: effects on paternity loss. *Behavioral Ecology*, 16, 747–754.
- Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16, 1099–1106.

- Kawano, K. M., Yamaguchi, N., Kasuya, E., & Yahara, T. (2009). Extra-pair mate choice in the female great tit *Parus major*: good males or compatible males. *Journal of Ethology*, 27, 349–359.
- Kempnaers, B. (2007). Mate choice and genetic quality: a review of the heterozygosity theory. *Advances in the Study of Behavior*, 37, 189–278.
- Kempnaers, B., Verheyen, G. R., Vandenbroeck, M., Burke, T., Vanbroeckhoven, C., & Dhondt, A. A. (1992). Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature*, 357, 494–496.
- Kempnaers, B., Verheyen, G. R., & Dhondt, A. A. (1997). Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behavioral Ecology*, 8, 481–492.
- Kristin, A., Hoi, H., Valera, F., & Hoi, C. (2008). The importance of breeding density and synchrony for paternity assurance strategies in the lesser grey shrike. *Folia Zoologica*, 57, 240–250.
- Kroken, C., Rigstad, K., Dale, M., & Lifjeld, J. T. (1998). The function of extrapair paternity in blue tits and great tits: good genes or fertility insurance? *Behavioral Ecology*, 9, 649–656.
- Leclaire, S., Nielsen, J. F., Sharp, S. P., & Clutton-Brock, T. H. (2013). Mating strategies in dominant meerkats: evidence for extra-pair paternity in relation to genetic relatedness between pair mates. *Journal of Evolutionary Biology*, 26, 1499–1507.
- Leisler, B., Beier, J., Staudter, H., & Wink, M. (2000). Variation in extra-pair mating in the polygynous Great Reed Warbler *Acrocephalus arundinaceus*. *Journal of Ornithology*, 141, 77–84.
- Lubjuhn, T., Brün, J., Gerken, T., & Epplen, J. T. (2001). Inconsistent pattern of extra-pair paternity in first and second broods of the Great Tit *Parus major*. *Ardea*, 89, 69–73.
- Lubjuhn, T., Curio, E., Muth, S. C., Brün, J., & Epplen, J. T. (1993). Influence of extra-pair paternity on parental care in great tits (*Parus major*). In S. D. J. Pena, R. Chakraborty, J. T. Epplen, & A. J. Jeffreys (Eds.), *DNA fingerprinting: State of the science* (pp. 379–385). Basel, Switzerland: Birkhäuser.
- Lubjuhn, T., Gerken, T., Brün, J., & Schmolli, T. (2007). Yearling male great tits, *Parus major*, suffer more strongly from cuckoldry than older males. *Journal of Zoology*, 110, 387–397.
- Lubjuhn, T., Strohbach, S., Brün, J., Gerken, T., & Epplen, J. T. (1999). Extra-pair paternity in great tits (*Parus major*) – a long term study. *Behaviour*, 136, 1157–1172.
- Magrath, J. L., Vedder, O., van der Velde, M., & Komdeur, J. (2009). Maternal effects contribute to the superior performance of extra-pair offspring. *Current Biology*, 19, 792–797.
- Marshall, T. C., Slate, J., Kruuk, L. E. B., & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7, 639–655.
- Mayer, C., & Passinelli, G. (2013). New support for an old hypothesis: density affects extrapair paternity. *Ecology and Evolution*, 3, 694–705.
- Mays, H. L., Albrecht, T., Liu, M., & Hill, G. E. (2008). Female choice for genetic complementarity in birds: a review. *Genetica*, 134, 147–158.
- Noble, D. W. A., Keogh, J. S., & Whiting, M. J. (2013). Multiple mating in a lizard increases fecundity but provides no evidence for genetic benefits. *Behavioral Ecology*, 24, 1128–1137.
- Norris, D. R., & Stutchbury, B. J. M. (2001). Extraterritorial movements of a forest songbird in a fragmented landscape. *Conservation Biology*, 15, 729–736.
- Otter, K., Ratcliffe, L., Michaud, D., & Boag, P. T. (1998). Do female black-capped chickadees prefer high-ranking males as extra-pair partners? *Behavioral Ecology and Sociobiology*, 43, 25–36.
- Otter, K. A., Stewart, I. R. K., McGregor, P. K., Terry, A. M. R., Dabelsteen, T., & Burke, T. (2001). Extra-pair paternity among Great Tits *Parus major* following manipulation of male signals. *Journal of Avian Biology*, 32, 338–344.
- Parker, G. A., & Birkhead, T. R. (2013). Polyandry: the history of a revolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20120335.
- Patrick, S. C., Chapman, J. R., Dugdale, H. L., Quinn, J. L., & Sheldon, B. C. (2012). Promiscuity, paternity and personality in the great tit. *Proceedings of the Royal Society of London B: Biological Sciences*, 279, 1724–1730.
- Queller, D. C., & Goodnight, K. F. (1989). Estimating relatedness using genetic markers. *Evolution*, 43, 258–275.
- R Development Core Team. (2012). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org>.
- Reid, J. M., Arcese, P., Sardell, R. J., & Keller, L. F. (2011). Additive genetic variance, heritability, and inbreeding depression in male extra-pair reproductive success. *American Naturalist*, 177, 177–187.
- Reyer, H.-U., Bollmann, K., Schläpfer, A. R., Schymainda, A., & Kleck, G. (1997). Ecological determinants of extrapair fertilizations and egg dumping in Alpine water pipits (*Anthus spinoletta*). *Behavioral Ecology*, 8, 534–543.
- Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J., & Burke, T. (2000). Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Molecular Ecology*, 9, 2226–2231.
- Round, P. D., Hansson, B., Pearson, D. J., Kennerley, P. R., & Bensch, S. (2007). Lost and found: the enigmatic large-billed reed warbler (*Acrocephalus orinus*) rediscovered after 139 years. *Journal of Avian Biology*, 38, 133–138.
- Rubenstein, D. R. (2007). Territory quality drives intraspecific patterns of extrapair paternity. *Behavioral Ecology*, 18, 1058–1064.
- Saino, N., Primmer, C. R., Ellegren, H., & Møller, A. P. (1997). An experimental study of paternity and tail ornamentation in the barn swallow (*Hirundo rustica*). *Evolution*, 51, 562–570.
- Saladin, V., Bonfils, D., Binz, T., & Richner, H. (2003). Isolation and characterization of 16 microsatellite loci in the great tit *Parus major*. *Molecular Ecology Notes*, 3, 520–522.
- Slayter, R. A., Mautz, B. S., Backwell, P. R. Y., & Jennions, M. D. (2012). Estimating genetic benefits of polyandry from experimental studies: a meta-analysis. *Biological Reviews*, 87, 1–33.
- Soulsbury, C. D., Iossa, G., Baker, P. J., White, P. C. L., & Harris, S. (2011). Behavioral and spatial analysis of extraterritorial movements in red foxes (*Vulpes vulpes*). *Journal of Mammalogy*, 92, 190–199.
- Sousa, B. F., & Westneat, D. F. (2013). Positive association between social and extra-pair mating in a polygynous songbird, the dickcissel (*Spiza americana*). *Behavioral Ecology and Sociobiology*, 67, 243–255.
- Stewart, I. R. K., Hanschu, R. D., Burke, T., & Westneat, D. F. (2006). Tests of ecological, phenotypic, and genetic correlates of extra-pair paternity in the house sparrow. *Condor*, 108, 399–413.
- Stewart, S. L. M., Westneat, D. F., & Ritchison, G. (2010). Extra-pair paternity in eastern bluebirds: effects of manipulated density and natural patterns of breeding synchrony. *Behavioral Ecology and Sociobiology*, 64, 463–473.
- Strohbach, S., Curio, E., Bathen, A., Epplen, J. T., & Lubjuhn, T. (1998). Extra-pair paternity in the great tit (*Parus major*): a test of the 'good genes' hypothesis. *Behavioral Ecology*, 9, 388–396.
- Stutchbury, B. J. M. (1998). Female mate choice of extra-pair males: breeding synchrony is important. *Behavioral Ecology and Sociobiology*, 43, 213–215.
- Stutchbury, B. J., & Morton, E. S. (1995). The effect of breeding synchrony on extra-pair mating systems in songbirds. *Behaviour*, 132, 675–690.
- Suter, S. M., Keiser, M., Feignoux, R., & Meyer, D. R. (2007). Reed bunting females increase fitness through extra-pair mating with genetically dissimilar males. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 2865–2871.
- Szulkin, M., Chapman, J. R., Patrick, S. C., & Sheldon, B. C. (2012). Promiscuity, inbreeding and dispersal propensity in great tits. *Animal Behaviour*, 84, 1363–1370.
- Taff, C. C., Freeman-Gallant, C. R., Dunn, P. O., & Whittingham, L. A. (2013). Spatial distribution of nests constrains the strength of sexual selection in a warbler. *Journal of Evolutionary Biology*, 26, 1392–1405.
- Tregenza, T., & Wedell, N. (2000). Genetic compatibility, mate choice and patterns of parentage. *Molecular Ecology*, 9, 1013–1027.
- Trivers, R. L. (1972). Parental investment and sexual selection. In B. Campbell (Ed.), *Sexual selection and the descent of man* (pp. 136–179). London, U.K.: Heinemann.
- Uller, T., & Olsson, M. (2008). Multiple paternity in reptiles: patterns and processes. *Molecular Ecology*, 17, 2566–2580.
- Van Oers, K., Drent, P. J., Dingemans, N. J., & Kempnaers, B. (2008). Personality is associated with extrapair paternity in great tits, *Parus major*. *Animal Behaviour*, 76, 555–563.
- Varian-Ramos, C. W., & Webster, M. S. (2012). Extrapair copulations reduce inbreeding for female red-backed fairy-wrens, *Malurus melanocephalus*. *Animal Behaviour*, 83, 857–864.
- Vedder, O., Komdeur, J., van der Velde, M., Schut, E., & Magrath, M. (2011). Polygyny and extra-pair paternity enhance the opportunity for sexual selection in blue tits. *Behavioral Ecology and Sociobiology*, 65, 741–752.
- Verboven, N., & Mateman, A. C. (1997). Low frequency of extrapair fertilizations in the Great Tit *Parus major* revealed by DNA fingerprinting. *Journal of Avian Biology*, 28, 231–239.
- Wang, C., & Lu, X. (2014). Extra-pair paternity in relation to breeding synchrony in ground tits: an individual-based approach. *Journal of Avian Biology*, 45, 561–565.
- Wang, J. (2011). COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, 11, 141–145.
- Wang, M. T., Hsu, Y. C., Yao, C. T., & Li, S. H. (2005). Isolation and characterization of 12 tetranucleotide repeat microsatellite loci from the green-backed tit (*Parus monticolus*). *Molecular Ecology Notes*, 5, 439–442.
- Westneat, D. F., & Mays, H. L. (2005). Tests of spatial and temporal factors influencing extra-pair paternity in redwinged blackbirds. *Molecular Ecology*, 14, 2155–2167.
- Westneat, D. F., & Stewart, I. R. K. (2003). Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology and Systematics*, 34, 365–396.
- Wetzel, D. P., & Westneat, D. F. (2009). Heterozygosity and extra-pair paternity: biased tests result from the use of shared markers. *Molecular Ecology*, 18, 2010–2021.
- Williams, G. C. (1975). *Sex and evolution*. Princeton, NJ: Princeton University Press.
- Winkel, W., Winkel, D., & Lubjuhn, T. (2001). Paternity analysis of four Great Tit (*Parus major*) broods in unusually close proximity. *Journal of Ornithology*, 142, 429–443.
- Winternitz, J., Promerová, M., Poláková, R., Vinkler, M., Schnitzer, J., Munclinger, P., et al. (2015). Effects of heterozygosity and MHC diversity patterns of extra-pair paternity in the socially monogamous scarlet rosefinch. *Behavioral Ecology and Sociobiology*, 69, 459–469.
- Woolfenden, B. E., Stutchbury, B. J. M., & Morton, E. S. (2005). Male Acadian flycatchers, *Empidonax virescens*, obtain extrapair fertilizations with distant females. *Animal Behaviour*, 69, 921–929.
- Yasui, Y. (1998). The 'genetic benefits' of female multiple mating reconsidered. *Trends in Ecology & Evolution*, 13, 246–250.
- Zeh, J. A., & Zeh, D. W. (2003). Toward a new sexual selection paradigm: polyandry, conflict and incompatibility. *Ethology*, 109, 929–950.

Appendix

Table A1

Summary of published studies on EPP in the great tit (in chronological order)

Study area location	Percentage EPP (broods)	Percentage EPO (chicks)	Method	Main finding	Source
Uppsala, Sweden	50 (of 10)	15 (of 47)	DNA fingerprinting	First study on EPP in this species	Gullberg, Tegelström, and Gelter (1992)
Wuppertal, Germany	47 (of 17)	17 (of 145)	DNA fingerprinting	Association between parental care and number of sired offspring	Lubjuhn et al. (1993)
Bahrdorf, Germany	53 (of 15)	20 (of 114)	Allozymes	Larger social fathers were cuckolded less	Blakey (1994)
Oxford, U.K.	17 (of 94)	14 (of 831)	DNA fingerprinting	Small males were more likely to be cuckolded	Verboven and Mateman (1997)
Vlieland, Netherlands	8.5 (of 82)	3.5 (of 516)	DNA fingerprinting	No association between EPP and male quality	Krokene et al. (1998)
Jomfruland, Norway	27 (of 55)	8 (of 409)	DNA fingerprinting	Females did not choose better quality males for EP matings	Strothbach et al. (1998)
Wuppertal, Germany	40 (of 78)	8.5 (of 681)	DNA fingerprinting	Patterns of EPP were not consistent in successive years	Lubjuhn et al. (1999)
Bahrdorf, Germany	34 (of 265)	7.5 (of 2013)	DNA fingerprinting	Patterns of EPP were not consistent in successive broods	Lubjuhn et al. (2001)
Bahrdorf, Germany	24 (of 25)	5 (of 165)	DNA fingerprinting	Females did not choose their EP partner based on short-term alterations of male behavioural cues	Otter et al. (2001)
Copenhagen, Denmark	39 (of 23)	10 (of 192)	5 microsatellite markers	Low rate of EPP in close nests	Winkel, Winkel, and Lubjuhn (2001)
Bahrdorf, Germany	25 (of 4)	3 (of 34)	DNA fingerprinting	Cross-fostering experiment: no effect of male quality on paternity loss	Johannessen, Slagsvold, Hansen, and Lifjeld (2005)
Oslo, Norway	3 (of 13)	8.5 (of 82)	6 microsatellite markers	Yearlings suffered more strongly from cuckoldry than older males	Lubjuhn et al. (2007)
Bahrdorf, Germany	34 (of 316)	9 (of 2386)	DNA fingerprinting	Personality differences played a role in the mechanism behind EP behaviours	Van Oers et al. (2008)
Arnhem, Netherlands	25 (of 99)	6.5 (of 667)	4 microsatellite markers	Females preferred as EP partners males with wider breast stripes	Kawano, Yamaguchi, Kasuya, and Yahara (2009)
Fukuoka, Japan	25 (of 99)	6.5 (of 667)	7 microsatellite markers	Bold males sired more offspring through EPC, while shy males sired more young at their social nest	Patrick et al. (2012)
Oxford, U.K.	49 (of 164)	13 (of 1185)	5–9 microsatellite markers	No difference in EPP rates between outbreeding and inbreeding females	Szulkin et al. (2012)
Oxford, U.K.	58 (of 40)	11 (of 315)	8 microsatellite markers	Great tits can travel long distances to obtain EPC	Present study
Toledo, Spain	55 (of 47)	20 (of 304)	9 microsatellite markers		

Table A2

Breakdown of extrapair paternity rates and attributes of the 11 nestbox plots in which the present study was carried out

Locality	Study site	Coordinates (x, y)	Size (ha)	N boxes	N pairs	Density	Mean LD	SI (%)	EPP rate	EPO rate	Sampling (%)
1. Arroyo del Marchés	SPM	381911 4377558	25	100	14	0.56	19.1	27	16.7 (6)	2.4 (41)	42.8
2. Las Majadillas	SPM	382428 4377521	8	40	11	1.37	23.3	21	66.7 (6)	17.8 (45)	54.5
3. Ermita	SPM	385865 4376782	2	5	4	2.00	17.0	31	0 (1)	0 (8)	25.0
4. Fuente fría	SPM	386686 4376621	2	5	4	2.00	19.7	16	66.7 (3)	31.6 (19)	75.0
5. La Morra	SPM	381945 4377699	18	100	27	1.50	33.8	24	50.0 (6)	21.0 (38)	22.2
6. Casillas	SPM	381565 4376937	10	40	19	1.90	29.9	24	50.0 (6)	23.0 (39)	31.6
7. El Robledillo	SPM	382428 4377521	2	5	2	1.00	29.5	20	100 (1)	71.4 (7)	50.0
8. Fuente Cantarranas	SPM	382633 4377391	2	5	3	1.50	25.3	19	100 (2)	46.1 (13)	66.7
9. Valdeyernos	QM	385647 4376199	25	100	24	0.96	24.9	18	75.0 (4)	16.0 (25)	16.7
10. Gil García	QM	389938 4374572	20	100	18	0.90	21.5	20	71.4 (7)	18.4 (38)	38.9
11. Las Navas	QM	389856 4375486	7	40	9	1.28	27.2	22	40.0 (5)	25.8 (31)	55.5
Overall				540	135	1.36	24.6	22	57.9 (47)	25 (304)	43.5

The following information is given: site (QM = Quintos de Mora, SPM = San Pablo de los Montes), UTM coordinates, plot size (ha), number of nestboxes (*N* boxes), number of breeding pairs in 2012 (*N* pairs), breeding density (pairs/ha), mean laying date (mean LD), degree of overlap of females' fertile periods in each locality (population-wide synchrony index; SI), occurrence (%) of EPP in each site (EPP rate; number of sampled families in parentheses), number of extrapair offspring divided by the total number of offspring surviving to blood sampling in each site (EPO rate; number of chicks in parentheses) and percentage of sampled nests in each plot.

Table A3

Panel of 15 microsatellite markers used in the present study

Locus	T ^a (°C)	N _A	H _E	H _O	Source
<i>PmaGAn30*</i>	60	7	0.66	0.72	Saladin, Bonfils, Binz, & Richner, 2003
<i>PmaTAGAn86*</i>	60	22	0.86	0.85	Saladin et al., 2003
<i>PmaD105*</i>	55	10	0.83	0.80	Saladin et al., 2003
<i>Titgata39*</i>	50	23	0.92	0.90	Wang, Hsu, Yao, & Li, 2005
<i>Titgata87</i>	60	50	0.89	0.86	Wang et al., 2005
<i>Titgata89*</i>	60	12	0.63	0.62	Wang et al., 2005
<i>Titgata94*</i>	60	12	0.81	0.81	Wang et al., 2005
<i>Ase18*</i>	60	11	0.58	0.55	Richardson et al., 2000
<i>PK11*</i>	52	2	0.18	0.17	GenBank Acc. no.: AF041465
<i>Mcyμ4*</i>	50	6	0.56	0.58	Double et al., 1997
<i>Pca7</i>	60	4	0.33	0.25	Dawson et al., 2000
<i>Pca9</i>	66	11	0.64	0.62	Dawson et al., 2000
<i>Pat-MP2-43</i>	59	13	0.63	0.62	Otter, Ratcliffe, Michaud, & Boag, 1998
<i>PmaD22</i>	60	26	0.90	0.85	Saladin et al., 2003
<i>PmaTGAn33</i>	55	35	0.92	0.90	Saladin et al., 2003

The following information (data based on adult individuals) is given: annealing temperature (T^a), number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosities and original source of the primers or GenBank accession number. A subset of nine markers (denoted with an asterisk) was selected for parentage analyses.

Table A4

GLMs for proportion of nests with EPO and proportion of EPO in relation to socioecological variables at among-plot level (N = 11 plots)

	Estimate	df	Test	P
Proportion of nests with EPO				
Breeding synchrony	−0.303±0.127	1,9	Wald χ^2 : 5.67	0.02
Laying date		1,6	Wald χ^2 : 0.59	0.44
Number of breeding pairs		1,6	Wald χ^2 : 0.03	0.85
Breeding density		1,6	Wald χ^2 : 0.16	0.69
Proportion of EPO				
Breeding synchrony	−0.195±0.017	1,8	Wald χ^2 : 5.67	<0.01
Laying date	0.172±0.049	1,8	Wald χ^2 : 12.56	<0.001
Number of breeding pairs		1,6	Wald χ^2 : 3.30	0.10
Breeding density		1,6	Wald χ^2 : 0.53	0.46

Models were constructed using a binomial error distribution and logit link function. The total number of nests (or nestlings) sampled in each plot was used as an offset. Significant variables retained in the final model are marked in bold.

Table A5

GLMMs for nestling attributes (phenotypic traits and genetic diversity) in relation to paternity status (extrapair or within-pair) and sex

	Estimate	df	Test	P
Offspring tarsus length				
Paternity status		1,286	F=0.01	0.92
Sex	−0.416±0.063	1,270	F=42.43	<0.001
Study plot			Z=1.14	0.13
Study plot (brood ID)	0.153±0.045		Z=3.36	<0.001
Offspring body mass				
Tarsus length	1.118±0.099	1,295	F=125.34	<0.001
Paternity status		1,284	F=1.24	0.27
Sex		1,280	F=2.37	0.12
Study plot			Z=1.25	0.10
Study plot (brood ID)	0.481±0.141		Z=3.39	<0.001
Offspring heterozygosity				
Paternity status		1,294	F=3.23	0.07
Sex		1,288	F=0.09	0.76
Study plot			Z=1.10	0.14
Study plot (brood ID)	0.003±0.001		Z=2.32	0.01

Models were constructed using a Gaussian error distribution and an identity link function. Study plot and brood identity nested within study plot were fitted as random effects. Significant variables are marked in bold.