Neighbouring-group composition and within-group relatedness drive extra-group paternity rate in the European badger (Meles meles)

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation

Published Version
doi:10.1111/jeb.12473

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:13890644

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Neighbouring-group composition and within-group relatedness drive extra-group paternity rate in the European badger (*Meles meles*)

G. ANNAVI*†‡, C. NEWMAN*, H. L. DUGDALE†§¶, C. D. BUESCHING*, Y. W. SIN*†**, T. BURKE† & D. W. MACDONALD*

*Wildlife Conservation Research Unit, Department of Zoology, Recanati-Kaplan Centre, University of Oxford, Tubney, Abingdon, Oxfordshire, UK
†NERC Biomolecular Analysis Facility, Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK
‡Biology Department, Faculty of Science, University of Putra Malaysia, Selangor Darul Ehsan, Malaysia
§Theoretical Biology, Centre for Ecological and Evolutionary Studies, University of Groningen, Groningen, The Netherlands
¶Behavioural Ecology and Self-Organization, Centre for Ecological and Evolutionary Studies, University of Groningen, Groningen, The Netherlands
**Department of Organismic and Evolutionary Biology, Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA

Keywords: breeding density; European badger; extra-pair paternity; group composition; heterozygosity; inbreeding; mate incompatibility; mating system; promiscuity.

Abstract

Extra-group paternity (EGP) occurs commonly among group-living mammals and plays an important role in mating systems and the dynamics of sexual selection; however, socio-ecological and genetic correlates of EGP have been underexplored. We use 23 years of demographic and genetic data from a high-density European badger (*Meles meles*) population, to investigate the relationship between the rate of EGP in litters and mate availability, mate incompatibility and mate quality (heterozygosity). Relatedness between within-group assigned mothers and candidate fathers had a negative quadratic effect on EGP, whereas the number of neighbouring-group candidate fathers had a linear positive effect. We detected no effect of mean or maximum heterozygosity of within-group candidate fathers on EGP. Consequently, EGP was associated primarily with mate availability, subject to within-group genetic effects, potentially to mitigate mate incompatibility and inbreeding. In badgers, cryptic female choice, facilitated by superfecundation, superfoetation and delayed implantation, prevents males from monopolizing within-group females. This resonates with a meta-analysis in group-living mammals, which proposed that higher rates of EGP occur when within-group males cannot monopolize within-group females. In contrast to the positive meta-analytic association, however, we found that EGP associated negatively with the number of within-group assigned mothers and the number of within-group candidate fathers; potentially a strategy to counter within-group males committing infanticide. The relationship between the rate of EGP and socio-ecological or genetic factors can therefore be intricate, and the potential for cryptic female choice must be accounted for in comparative studies.

Introduction

In socially monogamous pair-breeding and group breeding species, offspring may be fathered by males from outside of the female’s pair or group. This is termed extra-group or extra-pair paternity (EGP/EPP; hereafter EGP), where all, or just a proportion, of litters (broods) may have a nonresident father. EGP is a widespread phenomenon, occurring in 90% of socially monogamous bird species (Griffith et al., 2002) and more than two-thirds of the 26 social group-living mammalian species investigated so far (Isvaran & Clutton-Brock, 2007; Soulsbury, 2010). EGP can play...
an important role in the mating system and the socio-
genetic structuring of otherwise socially segregated pop-
ulations (Young et al., 2007; Schubert et al., 2011), yet
there is limited understanding of the interaction be-
tween socio-ecological and genetic factors in deter-
mining the frequency of EGP (Cohas et al., 2006; Isva-
rann & Clutton-Brock, 2007).

Although males may engage in EGP to increase
breeding success (Westneat et al., 1990), for example
 gaining kleptogamous mating opportunities despite sub-
or- dinate breeding status (Young et al., 2007), the
advantages of EGP to females are less clear (Clutton-
Brock & McAuliffe, 2009). In species where females are
also able to reassess choices through cryptic mecha-
nisms such as superfecundation, superfoetation, delayed
implantation, selective implantation and embryonic
re-absorption (Yamaguchi et al., 2006), mating with
multiple partners might have a selective advantage. For
example, extra group mating combined with within-
group mating might mask extra-group paternity, as a
counter-strategy to infanticide (Agrell et al., 1998).

Mate selection is predicated upon mixed criteria, such
as: (i) which mating partners are available or accessible;
(ii) mate compatibility (i.e. the rejection of unsuitable
mates or unviavle embryos); and (iii) mate quality (e.g.
mate heterozygosity, when this is associated with
increased offspring or grand-offspring fitness) (Jennions
& Petrie, 2000; Kokko & Rankin, 2006; Kempenaers,
2007).

The availability of potential (candidate) partners may
be an important determinant of mate selection. Mate
availability is affected by the rate at which individuals
encounter each other (e.g. Connor & Whitehead,
2005), the potential for female coercion (Smuts &
Smuts, 1993), and the extent to which access to
females might be defended by co-resident males (e.g.
Treves, 1998). For example, EGP could arise if females
mated randomly with whomever they encountered and,
by chance, therefore mated with extra-group
males (Kokko & Rankin, 2006). Under this mechanism,
encounter rates between individuals from different
groups will affect mating opportunities, as a function of
group-range overlap or the rate of intergroup transgres-
sion. Higher encounter rates with neighbouring groups
than with more isolated groups would therefore be
expected to lead to higher rates of EGP with these
neighbouring males.

Without further mate selection refinement (or post-
copulatory selection), EGP might be nonadapative; in
fact, extra-group (or extra-pair) paternity occurs in
many species without advantages being apparent (e.g.
Forstmeier et al., 2011). There are, however, risks asso-
ciated with EGP, such as agonistic encounter with a
same-sex competitor from the group (or pair) visited, or
the possible rejection of extra-group young (Westneat
& Stewart, 2003) if EGP can be detected. EGP can
provide an adaptive tactic through which females seek
to increase offspring production, for example, avoidance
of infanticide when neighbouring-group/immigrant
males pose a threat to infants (Agrell et al., 1998;
Burries et al., 2011) or fertility assurance (Sheldon,
1994; Vedder et al., 2011).

If social mate choice is limited, individuals might
settle for a social group mate with less optimal compati-
bility, but mitigate this with extra group matings (Rich-
ardson et al., 2005). Mate incompatibility may lead to
inbreeding depression (Moore & Ali, 1984; Pusey &
Wolf, 1996), and inbreeding avoidance through EGP
has been shown empirically in some mammal (e.g.
Sillero-Zubiri et al., 1996) and bird populations (e.g.
Brouwer et al., 2011). Moreover, dispersing from the
natal territory involves risk (Van Vuren & Armitage,
1994), with the potential to exacerbate inbreeding. EGP
may therefore have evolved to mitigate inbreeding
(Durrant & Hughes, 2005) as a facet of promiscuity
(Brooker et al., 1990), especially when offspring
dispersal is delayed, or over a short distance (Pusey &
Wolf, 1996). As a consequence, EGP frequency might
correlate positively with the relatedness of breeding
females to the mates available within their group (e.g.
Kingma et al., 2013).

Alternatively, the advantages of EGP for a breeding
population might be due to genetic benefits, such as
the ‘Good-genes-as-heterozygosity Hypothesis’ (Brown,
1997). This posits that ‘general’ allelic diversity
increases fitness, consequently individuals should select
mates to produce the most heterozygous offspring. The
relationship between fitness and heterozygosity, how-
ever, can vary (Britten, 1996; David, 1998; Hansson &
Westergard, 2002; Colman & Slate, 2003; Annavi et al.,
2014). In studies that show positive effects, heterozy-
gosity has been associated with higher offspring sur-
vival rates (Cohas et al., 2009; Mainguy et al., 2009;
Annavi et al., 2014), breeding success (Harrison et al.,
2011), disease resistance (Colman et al., 1999; Whit-
eman et al., 2006) and developmental stability
(reviewed in Kempenaers, 2007). In circumstances
where mate heterozygosity confers fitness benefits to
offspring (Fromhage et al., 2009), EGP rates would be
predicted to correlate with the level of heterozygosity
among within-group males (Cohas et al., 2006).

European badgers (Meles meles) provide an informa-
tive species to address the adaptive benefits of extra-
group paternity. They exhibit a variety of traits that can
lead to multiple-paternity litters (Carpenter et al., 2005;
Dugdale et al., 2007). In the study population examined
here, which typifies populations in south-western Eng-
land, badgers have a polygynandrous mating system
(i.e. they do not have one exclusive social mate;
Dugdale et al., 2007, 2011); up to seven males and
females breed within a social group per year, with a mean of 1.9 breeders of each sex (95% confi-
dence interval: 1.8–2.0; range = 1–7, Dugdale et al.,
2007). Badgers also have low fecundity (i.e. 1–4 cubs
once per year; Macdonald & Newman, 2002; Carpenter et al., 2005; Dugdale et al., 2007), and extra-group paternity accounts for > 40% of offspring in our study population, which has been assigned mainly to males in neighbouring groups (Dugdale et al., 2007; see also Carpenter et al., 2005). An individual’s social group and neighbouring groups therefore contain close relatives (Dugdale et al., 2008). Badgers typically have two mating peaks, a major peak immediately post-partum and a secondary peak in the late summer/autumn (Cresswell et al., 1992; see also Ahnlund, 1980). Females are induced ovulators, and gestation involves several months of embryonic diapause, where delayed implantation uncouples mating and parturition (Thom et al., 2004), and then they give birth fairly synchronously around February (Yamaguchi et al., 2006). Wandel & Graf (1982) discovered that ova produced during delayed implantation may also be fertilized, resulting in superfecundation promoted by superfecundation (Yamaguchi et al., 2006). This extends the opportunity for females to select the most suitable mates, through pre-and post-copulatory mate choice (Andersson & Simmons, 2006; Fisher et al., 2006). Furthermore, a proportion of males extend testicular activity late into autumn – prolonging the mating season (Woodroffe & Macdonald, 1995; Buesching et al., 2009). Crucially, males provide no paternal care to litters (Fell et al., 2006; Dugdale et al., 2010).

Badgers in this population also exhibit high group fidelity, through natal philopatry (Woodroffe et al., 1995). Macdonald et al. (2008) report that 19% of the badgers captured at least four times in this population were found to have dispersed, mainly to adjacent neighbouring social groups. The extent to which each sex solicits extra-group mating is not known in badgers (Dugdale et al., 2011). Whether within-group males actively defend their group territory and/or within-group females is highly equivocal in our study population (Stewart et al., 1997; Kilshaw et al., 2009). Badgers forage solitarily, and both sexes make incursions (Bohm et al., 2009) and temporary visits to other groups (Macdonald et al., 2008), which demonstrates that within-group males are not able to control female access to extra-group males effectively.

Based on a genetic pedigree spanning 23 years of data, here we examine the effects of local socio-ecological (breeding group size and numbers, and proportions of sexes per group) and genetic (breeding group relatedness) factors on extra-group paternity rates. We test whether EGP is more likely when there is [1a] a larger number of neighbouring-group candidate fathers (86% of EGP were assigned to neighbouring-group fathers; Dugdale et al., 2007), [1b] a larger number of within-group candidate or assigned mothers and [1c] a lower number of within-group candidate fathers. We then test whether: [2a] EGP increases with the mean pairwise relatedness between within-group assigned mothers and candidate fathers, consistent with inbreeding avoidance and [2b] EGP correlates negatively with the mean or maximum heterozygosity of within-group candidate fathers (offspring first-year survival probability is positively correlated with paternal heterozygosity in years with low food availability in this population; Annavi et al., 2014).

**Materials and methods**

**Study site and field methods**

This study was based on a high-density population of badgers inhabiting Wytham Woods; a 424-ha site situated 5 km north-west of Oxford, England (51°46′26 N; 1°19′19 W), which has been studied intensively since the 1970s (Kruuk, 1978a,b). A detailed description of the study site (e.g. soil, microclimates and vegetation) is provided elsewhere (Morecroft et al., 1998; Savill et al., 2010). At this study site, there was a mean [95% confidence interval] of 19 ([17, 21]; range = 14–26; Dugdale et al., 2008) mixed-sex social groups (Johnson et al., 2002; Newman et al., 2011), with a mean of 13 ([12–14]; range: 2–51) individuals (including annual cubs) per social group per year (hereafter social-group-year).

Since 1987, this study has attempted to mark all individuals in the population, following a systematic capture–mark–recapture regime (Macdonald & Newman, 2002; Macdonald et al., 2009). Live-trapping was conducted three to four times per annum; generally over 2 weeks in June, September and November, with 1 week of trapping in January of some years (Macdonald et al., 2009). Badgers were caught in mesh-traps, baited with peanuts (Macdonald & Newman, 2002; Macdonald et al., 2009), placed near the entrances of active communal badger dens, termed setts (Noonan et al., 2014). Captured badgers were then transferred to holding cages and transported to a central handling facility and sedated by an intramuscular injection using ketamine hydrochloride at 0.2 mL kg⁻¹ body weight (McLaren et al., 2005). Upon their first capture, all badgers were tattooed with a unique number on the left inguinal region for permanent individual identification. The sex, age-class (cub or adult, based on body size and previous trapping history) and capture location (social group name) of each badger were recorded. For genetic analysis, hair samples and/or blood from the jugular vein (ca 3 mL) were collected from all individuals.

Social group ranges were established using a ‘bait-marking’ technique approximately every 2 years (Kilshaw et al., 2009). The number of social group ranges within this study site has increased steadily (Macdonald et al., 2004) with population density (Macdonald & Newman, 2002; Macdonald et al., 2009). We defined the social group of residence of each individual per year (N = 1165; five unmarked individu-
We genotyped 1170 individuals trapped during 1987
where (Dugdale et al. Details of the DNA extraction, microsatellite character-
genotyping and parentage analysis
sistently from Hardy–Weinberg equilibrium or linkage
alleles per locus. No locus, or pair of loci, departed con-
estimated at 0.45 [0.39, 0.51], with 4.46 [3.79, 5.13]
otherwise stated. Mean observed heterozygosity was
born between 1988 and 2010. Means are provided with
et al. Annavi et al. 2009). For detailed description about the selection of
candidate parents and their social group assignment,
2194 G. ANNAVI ET AL.
als found dead on local roads, for which social group
could not be assigned, were excluded from analyses)
based on their trapping history, according to the follow-
ing rules:
1 Badgers first caught as cubs (N = 839) were consid-
ered to be resident in the social group in which they
were first caught (natal group; N = 709/839), unless they subsequently satisfied the dispersal rules
(N = 130/839) provided by Macdonald et al. (2008).
2 Badgers first caught as adults (N = 326) were
assigned to a social group based on the site where
they were trapped most frequently (N = 273/326),
unless: clear dispersal events (Macdonald et al., 2008)
were recorded (N = 43/326), or they exhibited equal
affiliation to two social groups over their lifetime, in
which case they were assigned to the social group in
which they were captured initially (N = 10/326).
3 In years when females were assigned maternity, they
were allocated to the social group in which their
cubs were born subsequently, if this differed from the
previous historical trapping data (N = 3).
4 Badger social groups often include more than one
sett in their group territory and therefore territories
can undergo fission, that is the splitting of a social
group into two (or more) new distinct social units
(da Silva et al., 1993; Macdonald et al., 2004), divid-
ing the physical range occupied by the former group
(defined by bait-marking). Badgers trapped in the
new social group were assigned accordingly after the
group split (N = 41), unless they were subsequently
retrapped in the former group (N = 173).

Genotyping and parentage analysis
Details of the DNA extraction, microsatellite character-
ization and genotyping methods are presented else-
where (Dugdale et al., 2007; Annavi et al., 2011, 2014).
We genotyped 1170 individuals trapped during 1987–
2010, at 35 microsatellite loci, of which 813 were cubs
born between 1988 and 2010. Means are provided with
their lower and upper 95% confidence values, unless
otherwise stated. Mean observed heterozygosity was
estimated at 0.45 [0.39, 0.51], with 4.46 [3.79, 5.13]
alleles per locus. No locus, or pair of loci, departed consis-
tently from Hardy–Weinberg equilibrium or linkage
equilibrium (Annavi et al., 2011). Two types of geno-
typing error, allelic drop-out rate (e₁ = 0.005) and
stochastic error rate (e₂ = 0.005), were estimated using
PEDANT 1.0 (Johnson & Haydon, 2007).
Parentage was determined sequentially using Master-
Bayes 2.47 (Hadfield et al., 2006) implemented in the R
statistics programme 2.12.2 (R Development Core Team,
2011) and in Colony 2.0 (Wang & Santure, 2009). For detailed description about the selection of
candidate parents and their social group assignment,
see Dugdale et al. (2007); for the MasterBayes and Col-
ony analyses, see Annavi et al. (2014). We were unable
to assign a mother to 16% (N = 130) of cubs and a father to 19% (N = 158).
Offspring that were fathered by males residing within
their natal group in the year of conception were cate-
gorized as within-group offspring (WGO, N = 340; assigned to 125 within-group males), whereas offspring
fathered by males that did not reside in their natal
group were categorized as extra-group offspring (EGO,
N = 315; assigned to 140 extra-group males). Cubs with
unassigned paternity (N = 158) were excluded from this
analysis.

Heterozygosity and pairwise relatedness
Individual standardized heterozygosity was estimated as
the proportion of loci that were heterozygous, divided
by the population mean heterozygosity for these loci
(Coltman et al., 1999) using GENHET 2.2 (Coulon,
2010) in R 2.12.2. The mean and maximum standard-
ized heterozygosity of within-group adult males (can-
didate fathers) was then derived per social-group-year.
Models with mean and maximum within-group candi-
date fathers’ heterozygosity (SH) produced comparable
results overall; therefore, we present results from mean
SH models in the main text (see Tables S1–S4 for the
results from models including maximum SH).
Pairwise relatedness values between females that were
assigned maternity, and their candidate within-
group mates, were estimated using Coancestry 1.0.0.1
(Wang, 2011) and averaged per social-group-year. We
computed and compared two marker-based pairwise
relatedness estimators, to take a comprehensive
approach: the commonly used Queller and Goodnight’s
pairwise estimator (QG; Queller & Goodnight, 1989),
and the Lynch and Ritland’s pairwise estimator (LR;
Lynch & Ritland, 1999), which performs well for most
population compositions in simulations (Cillier et al.,
2006). The QG and LR pairwise relatedness estimators
were highly correlated (Spearman’s rank correlation
coefficient = 0.91, P < 0.001) and yielded similar results
generally. Consequently, we used the QG estimator for
our main analyses (see Tables S3–S6 for the LR estima-
tor).

Distribution of EGO and extra-group mate pairs
(EGMP) across litters
We tested whether the distribution of EGO within each
litter corresponded to an expected distribution, gener-
ated through binomial processes, using a chi-square
goodness-of-fit test. For this analysis, we included only
litters in which all cubs were assigned paternity
(N = 378). The number of litters expected to include
EGO was calculated as:

\[ nC_X \times p^X \times q^{n-X} \times N \]
where \(C = n!/[n-X]!X!\], \(p\) = proportion of EGO in the population, \(q = 1-p\), \(n\) = litter size, \(X\) = number of EGO per litter and \(N\) = number of litters of size \(n\) (Sokal & Rohlff, 1995; Perreault et al., 1997). Using this formula, we also tested whether the distribution of EGMPs (each female’s litter could be sired by EGMPs, within-group mate pairs (WGMPs), or both; where multiple EGMPs or WGMPs, or both a WGMP and an EGMP occurred, the litter was attributable to multiple fathers) differed from that expected under the binomial distribution.

**Statistical analyses**

All analyses were run in R 2.13.2 (R Development Core Team, 2011), and the rate of EGP was investigated at the litter level. We fitted generalized linear mixed models (GLMMs), using the *lmer* function with Laplace approximation in the lme4 0.999375-42 package (Bates & Sarkar, 2007).

We used two measures for EGP (expressed, throughout, as per litter): (i) the number of EGO, and (ii) the number of EGMP. We also investigated EGP from two perspectives: (i) the relative proportion of EGPs (EGO: \(N_{EGO}/[N_{EGO} + N_{WGO}]\); EGMP: \(N_{EGMP}/[N_{EGMP} + N_{WGMP}]\)) in relation to the fixed effects, using a binomial error distribution and a logit link function, and (ii) the absolute number of EGP per litter in relation to the fixed effects by controlling for the total number of cubs (in models including EGO metrics), or the number of mate pairs involved in each litter (in models including EGMP metrics), using Poisson error structure and log link function. Here, we present results from the relative proportion models; these are compared to the absolute models, the results of which are presented in Tables S1–S6 and S8–S10.

We examined the socio-ecological effects of the number of females and males in proximity to the assigned mother, by including the following fixed effects: (i) the number of within-group assigned mothers (or within-group candidate mothers; see Tables S9–S10), (ii) the number of within-group candidate fathers, (iii) the number of neighbouring-group candidate fathers, and (iv) all two-way interactions of the three previous terms.

We included two genetic estimates of within-group candidate parents: (i) the mean (or maximum, Tables S1–S4) heterozygosity of within-group candidate fathers as a fixed effect and (ii) the mean pairwise relatedness of within-group assigned mothers and candidate fathers, as a linear and quadratic effect. To interpret main effects in the presence of interactions and quadratic effects when model averaging (Schieleth, 2010; Grueber et al., 2011), all fixed effects were standardized to a mean of zero and a standard deviation (SD) of two (Gelman, 2008). Female identity, social group and year were included as random effects in all models.

We employed an information-theoretic (IT) approach to select sets of plausible models and to estimate the overall importance of each fixed effect (Burnham et al., 2011). Models were ranked by their QAICc value, such that the top model had the lowest QAICc value (Burnham et al., 2011). If the difference in QAICc between the top model and the second ranked model (\(\Delta QAICc\)) was \(\geq 7\), we considered the top model to be the only plausible model. A model’s relative Akaike weight (\(\omega\)) was calculated as the model’s relative likelihood (\(exp[-0.5 \times \Delta QAICc]\)), divided by the sum of the likelihoods for all models considered (whether plausible or not).

We used the ‘natural average method’ (averaged over all plausible models in which the given parameter was included, weighted by the summed weights (\(\omega\)) of these models; Burnham & Anderson, 2002) to estimate model-averaged parameters. Estimates of fixed effects were averaged over the plausible models, including models with and without the parameter estimates as an interaction and/or quadratic effect. Unconditional standard errors for model-averaged parameter estimates were calculated using the model.avg function in R. The relative importance of each fixed effect was calculated as the total \(\omega\) of all plausible models that included the fixed effect of interest.

To investigate the rate of EGP per litter, measured as EGO and EGMP, we used an unrestricted data set that included all social-group-year data for which all trapped within-group candidate parents were genotyped, comprising 549 cubs (297 = WGO; 252 = EGO), although not all offspring were necessarily assigned both parents (unrestricted data set: no. of litters = 386 [from 198 mothers]; 205 litters were assigned only to WGMP, 170 only to EGMP and 11 both WGMP + EGMP).

We also performed these same analyses using a restricted data set, including only social-group-years (as group compositions differed between years) in which all individuals were genotyped and all offspring were assigned both parents (restricted data set: no. of litters = 239 [from 147 mothers], comprising 345 cubs [174 = WGO; 171 = EGO]; 119 litters were assigned only to WGMP, 112 only to EGMP and 8 both WGMP + EGMP). The restricted data set was smaller than the unrestricted data set and thus had reduced statistical power, but including cubs that were not assigned paternity could bias the EGP rate (all within-group candidate parents were genotyped, consequently cubs that were not assigned a father were likely to be EGO). These analyses ultimately yielded very similar results to the unrestricted data set (Tables S1–S10).

**Results**

**Patterns of EGP**

Of the 502 candidate mothers and 612 candidate fathers trapped between 1987 and 2010, only 228 females (45%) and 201 males (33%) were assigned off-
spring. The mean litter size was 1.46 [1.43, 1.49] (range = 1–5). Forty-eight per cent of assigned paternities were extra-group (315 of 655 cubs), of which 85% were attributable to neighbouring-group fathers (268 of 315 cubs). EGP was detected in 64% of 225 social-group-years and 47% (178 of 378, Table 1a, Fig. 1) of litters, considering only those litters for which all offspring were assigned fathers. Of these 178 litters, 64% included one EGO (94% of them with litter size of 1), 32% included two and 4% three (Table 1a).

The number of EGO within a litter ranged from 0 to 4, with a mean of 0.65 [0.61, 0.69] in the unrestricted data set (restricted data set: 0–3, mean = 0.72 [0.67, 0.77]). Considering only litters that included EGO, 26 litters involved two fathers (16 had two extra-group fathers and 10 had one extra-group and one within-group father). No litters were fathered by more than two extra-group males. The remaining 152 litters were fathered exclusively by one extra-group male (Table 1b).

The number of assigned EGMP per litter ranged from 0 to 2 with a mean of 0.51 [0.48, 0.54] in the unrestricted data (restricted data set: 0–2, mean = 0.54 [0.50, 0.58]). EGO were not distributed evenly among litters: attributing a probability of 0.48 (the mean population EGP rate) to each offspring being assigned as EGO, we observed significant differences in the number of EGO according to litter sizes (per assigned mother, per social-group-year) than expected, according to the binomial probability distribution ($\chi^2 = 75.25$, d.f. = 13, $P < 0.001$, Table 1a). The number of EGMP within a litter had a random distribution ($\chi^2 = 5.54$, d.f. = 4, $P = 0.236$, Table 1b). Sixty-four per cent of females (unrestricted data set; 65% in restricted data set) mated with an extra-group male during their lifetime, with a maximum of five and a mode of one different mating partner pairs.

### Socio-ecological effects of breeding group size

The number of neighbouring-group candidate fathers had a positive effect on both the absolute number and relative proportion of EGP (measured as EGO or EGMP per litter), with both the unrestricted and restricted data sets (Table 2, Fig. 2, Tables S7 and S8 and Fig. S1). Higher numbers of within-group candidate fathers were associated with a lower proportion of EGO and EGMP per litter (Table 2); this ceased to be the case, however, when litters with incomplete paternity assignment were excluded, showing that this effect differs when restricted data are used (Table S7). We also found no associations between the number of within-group candidate fathers and the absolute numbers of EGO and EGMP (Table S8).

A higher number of assigned mothers in the natal group was associated with lower relative proportions of EGO and EGMP per litter (Table 2), when using the unrestricted data set. Using the restricted data set, this effect was only detected in the EGO analysis (Table S7). Models that included candidate mothers, rather than assigned mothers, yielded similar results overall; however, the number of candidate mothers was not significant in the restricted analyses of the relative proportion of EGO and EGMP (Tables S9 and S10).

### Genetic factors

The mean pairwise relatedness between within-group assigned mothers and candidate fathers was associated positively with both the relative proportion and abso-

---

### Table 1: Distribution of (a) extra-group offspring (EGO) and (b) extra-group mate pairs (EGMP) within litters that include only cubs that had both parents assigned. The numbers of litters expected from binomial probabilities are shown in parentheses.

<table>
<thead>
<tr>
<th>Litter Size</th>
<th>No. of EGO per litter</th>
<th>Total litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>132 (129.1)</td>
<td>239</td>
</tr>
<tr>
<td>1</td>
<td>107 (109.9)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>57 (54.1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 (8.3)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200 (166.6)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of mates</th>
<th>No. of EGMP per litter</th>
<th>Total litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>188 (190.2)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>152 (159.8)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12 (10.7)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200 (190.9)</td>
<td></td>
</tr>
</tbody>
</table>

---

![Fig. 1](all.png)

**Fig. 1** Percentage of litters with only within-group offspring (WGO), only extra-group offspring (EGO) and having both WGO and EGO. Data were restricted to litters that include only cubs with both parents assigned ($N = 378$). Values at the top of each bar represent the numbers of offspring.
Table 2  Model-averaged parameter estimates over all submodels with ΔAICc< 7, testing the relative proportion of extra-group offspring (EGO) and extra-group mate pairs (EGMP) in a litter in relation to local group density and composition using the unrestricted data set. WGO = within-group offspring. M = No. of within-group assigned mothers. WGCF = No. of within-group candidate fathers. NGCF = No. of neighbouring-group candidate fathers. Mean SH = Mean standardized heterozygosity of within-group candidate fathers. QG = Queller and Goodnight’s mean pairwise relatedness estimator between within-group assigned mothers and candidate fathers. QG^2 = quadratic effect of QG. *

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>The relative proportion of EGO</th>
<th>The relative proportion of EGMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
</tr>
<tr>
<td>(Intercept)</td>
<td>0.37</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean SH</td>
<td>-0.74</td>
<td>0.42</td>
</tr>
<tr>
<td>WGCF</td>
<td>-1.04</td>
<td>0.44</td>
</tr>
<tr>
<td>M</td>
<td>-0.98</td>
<td>0.35</td>
</tr>
<tr>
<td>NGCF</td>
<td>1.34</td>
<td>0.48</td>
</tr>
<tr>
<td>QG</td>
<td>1.51</td>
<td>0.49</td>
</tr>
<tr>
<td>QG^2</td>
<td>-1.65</td>
<td>0.66</td>
</tr>
<tr>
<td>M*WGCF</td>
<td>1.08</td>
<td>0.73</td>
</tr>
<tr>
<td>M*NGCF</td>
<td>0.85</td>
<td>0.68</td>
</tr>
<tr>
<td>WGCF*NGCF</td>
<td>-0.87</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Full models:
Model EGO
y = – chind(EGO,WGO)
Model < – lmer (y ~ Litter Size + (1|Year) + (1|Social Group) + (1|Mother ID) + M + WGCF + NGCF + Mean SH + QG + QG^2 + M*WGCF + M*NGCF + WGCF*NGCF, family=binomial, REML = FALSE, data=Unrestricted)
Model EGMP
y = – chind (EGMP, WGMP)
Model < – lmer (y ~ Number of mates + (1|Year) + (1|Social Group) + (1|Mother ID) + M + WGCF + NGCF + Mean SH + QG + QG^2 + M*WGCF + M*NGCF + WGCF*NGCF, family=binomial, REML = FALSE, data=Unrestricted).

Discussion
In reality, sexual selection is not predicated upon free choice (Millstein, 2002; Walsh et al., 2002). Although mate selection may relate to perceived quality and compatibility, with realized offspring further honed by postcopulatory mechanisms, the actual availability of mates is also a major contributing factor (Eshel, 1979; Clutton-Brock & McAuliffe, 2009). Capacity to access mates may simply be determined by encounter rate, or it may be limited (actively or passively). Furthermore, although females are typically the choosy sex (Clutton-Brock & McAuliffe, 2009), males can also exhibit prudence (Wedell et al., 2002), and intermale or interfemale competition can restrict free access to mating partners (Preston et al., 2003). As a consequence, ecological, sociological and genetic factors interact to constrain the pace of evolution (Emlen & Oring, 1977).

Extra-group (or extra-pair) copulation is well documented in birds (e.g. Schwartz et al., 1999; Pryke et al., 2010) and mammals (e.g. Cohas et al., 2006). We observed that 48% of offspring in this badger population were assigned extra-group fathers, and the majority of EGP (85%) were attributed to candidate fathers from neighbouring groups. This demonstrates that the social group unit does not correspond to a breeding unit (Carpenter et al., 2005; Dugdale et al., 2007). We also showed that the rate of EGP was associated with the number of each sex in each group, as well as genetic parameters.

Socio-ecological factors
We found that, when the number of candidate males in neighbouring groups was greater, there was a higher rate of EGP. Furthermore, when the number of within-group candidate fathers and also within-group candidate or assigned mothers was higher, the relative proportions, but not the absolute numbers, of both EGO
and EGMP per litter were lower in the unrestricted, but generally not in the restricted, analyses. That the rates of EGO and EGMP were lower in the unrestricted analyses probably arose because cubs with unassigned parents were present in these groups (which were likely to be extra-group parents, because both analyses only included groups in which all resident candidate parents were genotyped); however, including these groups added statistical power to the analyses. The smaller sample size of the restricted data set produced effects in the same direction, but the 95% confidence intervals around these estimates overlapped zero.

The largest socio-ecological model-averaged parameter estimate was attributed to the number of neighbouring-group candidate fathers (Tables 2 and S7–S8). The number of within-group candidate fathers and candidate/assigned mothers, however, also affected the rate of EGP. The lower rate of EGP in groups with more within-group candidate fathers might be a tactic to deter within-group males from killing EGO. In contrast, females could use EGP as a counter-strategy to infanticide committed by extra-group males (Agrell et al., 1998).

Our finding of lower EGP with greater numbers of candidate/assigned mothers in a group is in contrast to Isvaran & Clutton-Brock (2007). Their meta-analysis of 26 mammal species found that higher rates of EGP were associated with larger numbers of within-group females and that lower rates of EGP occurred in species with longer mating seasons. They concluded that within-group males are less able to mate guard when more within-group females are present and when the breeding season is extended. Following a post-partum mating peak in February/March, badgers can continue to mate throughout the summer, occasionally up until December, yet 48% of cubs were EGO. There is little evidence that male badgers mate guard; rather, non-competitive serial copulations with different partners underscore a freely promiscuous system (Dugdale et al., 2007). Moreover, female badgers are able to reject matings (Dugdale et al., 2010), preventing individual males from monopolizing females. Delayed and selective implantation, superfoetation and superfecundation then facilitate cryptic female choice, which should be accounted for in meta-analyses.
In socially paired species, the constraints of limited social mate choice, due to a lack of breeding territories, will lead to unpaired females pairing with any available unpaired male possessing a breeding territory (Richardson et al., 2005). In this high-density badger population, contact occurs frequently between groups (Macdonald et al., 2008) comprising multiple (breeding) males and females (Dugdale et al., 2007, 2008). Females mate promiscuously (Dugdale et al., 2011) and delayed implantation allows them to mate whenever a male is available (Yamaguchi et al., 2006), thus females are unlikely to be constrained by the availability of mating partners. Furthermore, we have found no evidence for clear mating hierarchies in these badgers; that is, male mounting frequency is not related to dominance rank or body condition index, and male mounting frequency does not correlate with paternity success (Dugdale et al., 2011).

If females mate indiscriminately with whomever they encounter, and thus by chance mate with extra-group males (Kokko & Rankin, 2006), then EGP would not necessarily be adaptive. Nevertheless, the correlation we observed with relatedness precludes parentage from being totally random. When the relative proportion of neighbouring-group candidate fathers was higher, the rate of EGP was greater. We assume that greater neighbouring-group mate availability implies a greater likelihood of contact. This type of mating system could be due to nonexclusive use of feeding ranges (Stewart et al., 1997) or facilitated by the relatively high rate of temporary intergroup movements observed in this population, even from a maximum of just four trapping events per year (see Macdonald et al., 2008; Huck et al., 2008; see also Stewart et al., 1997). These visits might serve to ingratiate males with females in adjacent groups and exploit EGMP encounter rate, to broaden mate selection options. Encounter rate-based mating could arise if the genetic basis of a female’s response to her social mate (within-group males) and extra-group males is the same (Within-sex Genetic Correlation Hypothesis; Forstmeier et al., 2011). Alleles for resistance to (group-) infidelity may, however, also convey resistance to (group-) fidelitous copulations, leading to infertility (Arnqvist & Kirkpatrick, 2005).

Genetic factors
We found no evidence that badgers were seeking heterozygosity in their within-group mating partners (heterozygous advantage; Jennions & Petrie, 2000), although we did not test for an environmental interaction. The propensity for males and females to engage in promiscuous mating may be affected by the same set of alleles (Halliday & Arnold, 1987). There is likely to be strong positive selection for alleles that enhance promiscuous behaviour in males (Albrecht et al., 2007). If promiscuous behaviour is heritable (Reid et al., 2011), pleiotropic effects on these sexually selected alleles could also cause promiscuous behaviour to evolve in females, despite antagonistic selection (Rice, 1992) known as the between-sex genetic correlation hypothesis (Halliday & Arnold, 1987; Forstmeier et al., 2011).

The mean pairwise relatedness between within-group assigned mothers and candidate fathers had high relative importance, for both the relative proportion and the absolute number of EGP (Tables 2 and S7 and S8). The rate of EGP increased with greater relatedness between within-group assigned mothers and candidate fathers, showing a negative quadratic effect such that the rate of EGP plateaued with high relatedness. Small sample size, however, prevented us from testing this effect (Fig. 3).

Female preference for immigrant males over residents, when the residents are relatives, is theoretically predicted to occur when inbreeding is costly (Lehmann & Perrin, 2003). Badgers might avoid inbreeding; Annavi et al. (2014) report positive correlations between offspring-first-year survival probability and paternal heterozygosity in this same population in summers with a good food supply (from the proxy of wet conditions, yielding abundant earthworm food). Additionally, assigned within-group parent pairs in this population were less related than randomly assigned within-group pairs (Sin, 2012), but simulations including all potential parent pairs are required to determine whether inbreeding avoidance occurs. Intergroup promiscuity, facilitated by frequent temporary visits between groups (Macdonald et al., 2008), seems a plausible mechanism to avoid inbreeding, circumventing the need for permanent dispersal, and the associated risks.

Conclusions
Studies of the ecological correlates of EGP in mammals have focused on the spatial and temporal grouping of females and males within groups (Ivson & Clutton-Brock, 2007). We highlight that the number of neighbouring males is an important and overlooked parameter, along with the potential for cryptic female choice. From the combined socio-ecological and genetic correlates of EGP in badgers, it is possible that the genetic effects we observed could occur through cryptic female choice, superimposed on a backcloth of random mating. Further research is required to elucidate the costs and benefits of EGP in mammals; for example, the survival, recruitment or life-time reproductive success of WGO versus EGO, and whether there is a genetic basis to EGP.

Acknowledgments
We thank Pierre Nouvellet and Stephen Ellwood for their support in the field, Andy Krupa, Deborah Dawson and Gavin Horsburgh for advice in the laboratory and
Jarrod Hadfield for help with the program MasterBayes. We are also grateful to Jan Komdeur, Marta Szulkin and two anonymous reviewers for comments on the manuscript. Trapping protocols were subject to ethical review and were performed under Natural England Licence (currently 20104655) and UK Home Office (Animals [Scientific Procedures] Act, 1986) Licence (PPL 30/2835). This research was supported by the UK Natural Environment Research Council (NERC) Biomolecular Analysis Facility, Sheffield; People’s Trust for Endangered Species (DWM): Ministry of Higher Education, Malaysia (GA); Netherlands Organisation for Scientific Research (Visitor’s Travel Grant 040.11.232; HLD); NERC (fellowship NE/I021748/1; HLD); and the Croucher Foundation, Hong Kong (YWS).

References


Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 The relationship between the number of neighbouring-group candidate fathers and the mean absolute number of extra-group paternity (EGP) measured as extra-group offspring (EGO) and extra-group mate-pairs (EGMP) per litter using the unrestricted data set.

Figure S2 The relationship between the mean pairwise relatedness (Queller and Goodnight’s estimator) of assigned mothers and candidate fathers within each social-group-year, and the mean absolute number of extra-group paternity (EGP) measured as extra-group offspring (EGO) and extra-group mate-pairs (EGMP) per litter using the unrestricted data set.

Table S1 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the relative proportion and absolute number of extra-group offspring (EGO) in a litter in relation to local group density and composition.

Table S2 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the relative proportion and absolute number of extra-group mate-pairs (EGMP) in a litter in relation to local group density and composition.

Table S3 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the relative proportion and absolute number of extra-group offspring (EGO) in a litter in relation to local group density and composition.

Table S4 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the relative proportion and absolute number of extra-group mate-pairs (EGMP) in a litter in relation to local group density and composition.

Table S5 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the relative proportion and absolute number of extra-group offspring (EGO) in a litter in relation to local group density and composition.

Table S6 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the relative proportion and absolute number of extra-group mate-pairs (EGMP) in a litter in relation to local group density and composition.

Table S7 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the relative proportion of extragroup offspring (EGO) and extra-group mate-pairs (EGMP) in a litter in relation to local group density and composition using the restricted data set.

Table S8 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the absolute number of extra-group offspring (EGO) and extra-group mate-pairs (EGMP) in a litter in relation to local group density and composition using the (A) unrestricted and (B) restricted data set.

Table S9 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the relative proportion and absolute number of extra-group offspring (EGO) in a litter in relation to local group density and composition.

Table S10 Model-averaged parameter estimates over all sub-models with ΔAICc < 7, testing the relative proportion and absolute number of extra-group mate-pairs (EGMP) in a litter in relation to local group density and composition.

Data deposited at Dryad: doi:10.5061/dryad.k2q52

Received 14 February 2014; revised 22 July 2014; accepted 27 July 2014