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The cost of infidelity to female reed buntings

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Females of many socially monogamous bird species accept or even actively seek copulations outside the social pair bond. It has been shown that females profit from extrapair fertilization by increased offspring quality, but extrapair mating may also induce costs to females. We measured parental food provisioning and paternity in the reed bunting, *Emberiza schoeniclus*, a species with biparental brood care and high levels of extrapair paternity (EPP). We found a negative relationship between the proportion of EPP in broods and paternal care across the local population. Individual males adjusted food provisioning to the amount of EPP between sequential broods. Females did compensate for low male food provisioning. The cost of extrapair fertilizations to females is increased nestling mortality due to the social partner's reduction of brood care. If the mixed mating system of the reed bunting is at an evolutionary equilibrium, we expect that genetic fitness for males as well as for females is maximized. Any costs incurred by extrapair mating should therefore not exceed the benefits for both sexes. *Key words:* extrapair paternity, infidelity, parental care, paternal care, reed bunting. *[Behav Ecol 20:601–608 (2009)]*

Extrapair paternity (EPP) is widespread in bird species with biparental brood care (Griffith et al. 2002). Males may increase the number of offspring with EPP, whereas females might profit from EPP by fertility assurance (Lifjeld et al. 2007) or by increasing offspring quality through good and/or compatible genes (Hasselquist et al. 1996; Kempenaers et al. 1997; Foerster et al. 2003; Stapleton et al. 2007; Suter et al. 2007; Fossøy et al. 2008). Extrapair mating can also entail costs to males and females. Due to extrapair copulations (EPC), males risk to lose paternity in their own nests. The costs of EPC to females are more difficult to assess. Female costs go from physical punishment (Valera et al. 2003) to infanticide by the social male (Robertson 1990; Osorio-Beristain and Drummond 2001); alternatively, a female could lose the social male's support in raising the young. The cost of extrapair mating to females will then depend on how easily a female can compensate for the reduced paternal care (Kokko 1999; Houston et al. 2005). Theoretical models predict that males should reduce their paternal care when paternity is in doubt (Trivers 1972). However, males may not be able to identify extrapair young (EPY) directly (Kempenaers and Sheldon 1996), and therefore, a reduction of paternal care toward the whole brood will also affect their own genetic offspring (Davies and Hatchwell 1992). Whether males adjust their paternal care to perceived paternity depends on several factors (Westneat and Sherman 1993; Whittingham and Dunn 2001): 1) the variability in the proportion of EPY between broods, 2) a male's ability to recognize the own genetic offspring or to assess the amount of EPP, and 3) the cost and benefits of male parental care.

Parental care might be quantified from observed feeding frequencies at the nest. In house finches, *Carpodacus mexicanus*, and tree swallows, *Tachycineta bicolor*, it has been shown that parents bring about the same amount of food on each visit, and therefore, the number of visits gives a good estimation of parental care (Nolan et al. 2001; McCarty 2002). Previous studies on paternity and paternal brood provisioning in reed buntings, *Emberiza schoeniclus*, provided mixed results. Dixon et al. (1994)found that male reed buntings adjust their feeding effort to the proportion of EPP in the nest. Buchanan (2001)and Bouwman et al. (2005)could not reproduce this result in similar studies. Replicates are important when drawing general patterns from empirical studies, but replicates of behavioral studies are not common (Kelly 2006). In a conceptual and partial replication of Dixon's study, we addressed the question whether reed bunting males adjust their parental care (i.e., food provisioning) to perceived paternity. We also looked for female compensation of reduced paternal care. To arrive at a conclusive result, we worked with a large number of nests and with long observation times. The aim of this study was to measure the costs that the females incur when engaging in EPC. In an evolutionary stable system of behavior, we might expect symmetry of costs and benefits with regard to EPC for both sexes.

MATERIALS AND METHODS

Study species and study site

The reed bunting is a small-sized (ca., 19 g), sexually dimorphic passerine species. The predominant breeding system is social monogamy with high rates of EPP (O'Malley 1993). Pairs normally raise 2 broods per season, which lasts from April until the end of July. The female builds the nest into the vegetation near the ground. The first clutch usually contains 5 eggs while the second clutch mainly contains 3–4 eggs (Keiser 2007). After nest failures caused by inundation or predation, clutches are often replaced. The males scarcely incubate but contribute an important part of food provisioning of the brood. Nestlings are able to leave the nest 8 days after hatching. Parents continue to feed the fledglings up to 3 weeks after they have left the nest. We conducted fieldwork from the end of March to early August during 2004–2006 on the marshland Grande Cariçaie at the Lake of Neuchâtel near Gletterens (46°54′30″N, 6°56′00″E), Switzerland. Vegetation of the 33.5-ha study site is dominated by dense stands of sedges, *Carex elata*, and fen-sedges, *Cladium mariscus*, mixed with reed, *Phragmites australis*(Aebischer et al. 1996; Keiser 2007).

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General field methods

For orientation, a grid of 50 × 50 m was laid over the study area. From the end of April, we systematically searched for nests, mapped them, and monitored daily to assign the exact hatching date. However, 23 of 186 broods were only found after hatching. In 2005 and 2006, we installed wire fences around the nests to prevent predation caused by foxes. The mesh size (6 × 10 cm) allowed the parents to pass but kept out foxes. We installed fences only after the female had completed the clutch. Adult birds were mist netted either at the nest site or (for some males) with the help of playback near their song posts. Birds were ringed with a unique combination of 3-color rings and a numbered aluminum ring from the Swiss Ornithological Institute. From all birds, we took about 5–50 µl blood from the brachial or leg vein for parental analysis. Blood samples were collected in a 70-µl capillary tube, put on ice in the field, and stored at −18 °C on the same day. Nestlings were bled on day 2 or later and measured and ringed between day 6 and day 8 after hatching (day 0 = hatching day). Mass was measured with a Pesola balance (to nearest 0.1 g). Dead nestlings and eggs that failed to hatch were collected. The social parents of a brood were identified at the nest site by direct observation or with the help of a color video camera installed at the nest. Reed buntings did accept manipulations at nest sites (i.e., fence and camera installation, netting adults, and handling of nestlings) as they came back to the nest within a few minutes after a disturbance.

Video recording

Video recording was performed from late May to late July to document the pattern of food provisioning. We used 15 miniature black and white video cameras (CMOS CLVMPC2, CL-Electronics GMbH, Niederweningen, Switzerland) and 1 color camera (CMOS CLVMPA, CL-Electronics GMbH) that we put into weather-resistant plastic boxes (5 × 5 × 8 cm). Video cameras were fixed on wooden pickets 20–30 cm south of the center of a nest giving a clear view on the nest. The permanently mounted camera box was covered with vegetation. The video recorder (Archos AV100 and Archos AV420, Archos AG, Switzerland), the power supply of the video recorder (Lead battery 6 V 12 Ah, additionally to the built-in accumulator), and the power supply of the camera (Lead battery 6 V 5 Ah) were placed into a plastic box, about 5 m away from the nest. The plastic box was fixed on 2 pickets to avoid inundation. The cameras were installed 1 day after hatching, but recording only started the next day. We recorded the activity at nests continuously from day 2 until the day the first young left the nest. Every day we distributed the video recorders and their storage battery before 8 AM and gathered them after 6 PM. Recorders were programmed to record from 6 AM to 12 AM and from 12 AM to 6 PM. A 6-h film had a size of 1.94 GB and was stored in an AVI format. Films were transferred on external hard disks and connected to Apple Macintosh iMac G4/G5. The lead battery for the video recorders had to be recharged overnight; the lead battery for the camera lasted for the whole nestling period.

Video analysis

We analyzed video recordings in AVI format using Quick Time Pro for MAC OSX. Videos were cut into 1-h segments to display a picture every 4 s in the accelerated view mode. We excluded from the analysis recordings that were made during the capture of adults and the rest of this day. We also excluded the time where we ringed and measured the young and the hour following these manipulations. Due to technical problems, inundation, and predation, the recordings are not complete. For 94 broods, we analyzed a 4-h window between 8 AM and 12 AM between day 5 and day 7 after hatching. For 14 double-brooded pairs, we analyzed the whole recording time between day 2 and day 7. On average, we have 32 ± 7.8 h per brood (range 20–48 h) and 5 ± 3.4 h (range 0–10 h) per day. We analyzed for each day the same number of hours for the first and second brood of a pair.

Molecular methods

DNA was extracted from blood and tissue samples using Peqgold blood DNA isolation kit (Peqlab, Erlangen, Germany). Six polymorphic microsatellite loci were used to determine paternity: Escµ1, Escµ3, Escµ4, Escµ6 (Hanotte et al. 1994), Pdoµ5 (Griffith et al. 1999), and Ppi2 (Martinez et al. 1999). The forward primers were fluorescently labeled. The microsatellite loci were amplified through a polymerase chain reaction (PCR) under the following conditions: all primers 95 °C for 10 min (polymerase activation + template denaturation); Escµ3, Escµ6, and Ppi2, 34 cycles 94 °C for 40 s (denaturation), 52 °C for 30 s (annealing), and 72 °C for 90 s (extension); Escµ1, Escµ4, and Pdoµ5, 34 cycles 94 °C for 40 s (denaturation), 54 °C for 30 s (annealing), and 72 °C for 90 s (extension); and all primers 72 °C for 30 min (final extension) and stored at 4 °C. The mix for the PCR contained the following: 4 µl MilliQ water, 1 µl PCR 10× buffer II (Applied Biosystems Inc., Forster City, CA), 1 µl $MgCl_2$(25 mM), 1 µl deoxynucleoside triphosphate (2 mM), 3 × 0.33 µl primer forward (5 µM), 3 × 0.33 µl primer reverse (5 µM), 1 µl DNA template, and 0.05 µl AmpliTaq Gold Polymerase (Applied Biosystems Inc.). Basic solution for the length determination of microsatellite consisted of formamid (buffer) and length standard ROX500 (Applied Biosystems Inc.) in a ratio of 2500:2. We added 0.7 µl of the PCR product to 25 µl basic solution and let it run on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems Instrument). The alleles were determined with DNA fragment analysis software (GENESCAN version 3.1, Applied Biosystems Inc.). The combined exclusion probability of the 6 microsatellite loci was higher than 0.995 for the first parent and 0.999 for the second parent using the program Cervus version 3.0 (Marshall et al. 1998; Kalinowski et al. 2007).

Statistics

The relationship between paternity and provisioning was investigated in 3 ways: first, across population and years; second, between broods of the same male with and without EPP; and third, between sequential broods of pairs within 1 breeding season. We have data on parental care for 94 broods from 57 females and 51 males. The pairs where social partners changed between years were treated as independent; in total, 68 different breeding pairs were formed. Five males cared for broods of 2 different females within the same breeding season. However, these broods were not overlapping in time. No female had more than 1 male that helped provisioning broods within 1 breeding season. For 11 broods (10 females), no male assistance at nest was observed in the focused 4-h observation window. For 6 (5 females) of them, a social father (partner) was provisioning the brood with food, outside the 4-h observation. For 5 broods (5 females), we could not determine the social father (partner), and therefore, these young could not be categorized as within- or extrapair offspring. For 14 double-brooded pairs, we have data on both paternity and parental provisioning, from day 2 until day 7 after hatching. For the

comparison between sequential broods, each individual was only included once. Pairs remained together for 2 broods on the same territory within a breeding season. The food-provisioning rate is the number of feedings per hour per nestling. A male's share of provisioning is the number of feedings of the male divided by the total number of feedings for the male and female combined. The effect of the proportion of EPP on provisioning was tested in a general linear mixed model (GLMM) for males and females separately. In the model, we included the age of nestlings (4–6 days, fixed factor), brood size (2–5 nestlings, fixed factor), sex ratio (proportion of male nestlings, fixed factor), and individual parent (random effect). We included sex ratio in the initial models because male nestlings are significantly heavier than female nestlings (Suter et al. 2007). We checked whether reduced male provisioning had an effect on the mean mass of nestlings. For this analysis, we only included broods where all hatchlings survived to the day when we took measurements ($n = 69$). Mean mass of nestlings was standardized, separately for each sex, by subtracting the mean value of each age class (day 6–8) from the individual fledgling mass. Fledgling success is the percentage of hatchlings that survived to the day when we took measurements. We excluded nests that failed due to a raising water level or predation from the analysis of fledgling success. Statistical analysis was performed using JMP version 5.0.1 (SAS Institute Inc., Cary, NC), R version 2.4.1 (R Foundation for Statistical Computing, Vienna, Austria), and Excel. In case of nonnormal distribution, the data were transformed to achieve normality or nonparametric tests were used. In case of unequal variances, we applied Welch statistic. Means are shown with the standard errors, all tests are 2 tailed with a significance level of $P < 0.05$.

RESULTS

Frequency and distribution of EPP

Over the 3 years, we genotyped 540 offspring from 143 broods (2004: 147 offspring from 38 broods, 2005: 209 offspring from 56 broods, 2006: 184 offspring from 49 broods). The social father was determined for 506 offspring from 132 broods. On average, there were 39% EPY (2004: 46/139, 2005: 91/199, 2006: 61/168; likelihood ratio test: $G = 6.32$, degrees of freedom [df] $= 2$, $P = 0.05$) and 64% of the broods contained at least 1 EPY (2004: 20/36, 2005: 38/53, 2006: 26/43; likelihood ratio test: $G = 2.72$, df $= 2$, $P = 0.26$). The EPY were not equally distributed among broods; there were 48 entire within-pair broods, 60 mixed broods, and 24 entire extrapair broods. The social father included, up to 3 different males sired young in broods with mixed paternity. In all years, the distribution of EPY among broods differed from what is expected under a binomial distribution (likelihood ratio test: all $P < 0.01$). We have data on 30 pairs that produced 2 broods within 1 breeding season. Differences in the proportion of EPP between first and second brood of the same pair ranged from −1 to 1. EPP increased between 13 sequential broods, decreased between 8 sequential broods, and did not change between 9 sequential broods (likelihood ratio test: $G = 1.35$, df $= 2$, $P = 0.51$). The time between first and second brood and the size of the second clutch did not depend on the proportion of EPP in the first brood (Spearman rank correlation: time until second clutch $r_s = 0.235$, $N = 30$, $P = 0.21$; brood size second clutch $r_s = -0.246$, $N = 30$, $P = 0.19$). The probability of a second brood did not depend on the proportion of EPP in the first brood (logistic regression: $\chi^2 = 1.28$, $n = 56$, $P = 0.26$). No intraspecific brood parasitism was detected as the social mother always corresponded to the genetic mother.

Ecology of food provisioning

The diet of the nestlings was composed of insects and spiders. On films, we could determine the following taxa: Araneae, Odonata, Lepidoptera, Ephemeroptera, Tipulidae, Tabanidae, and Chironomidae. Broods contained on average 3.87 ± 0.11 nestlings ($n = 94$, range 2–5). Females provided nestlings at a higher rate with food than males (mean feeds per hour per nestling: females 1.69 ± 0.08; males 1.01 ± 0.07; Wilcoxon signed-rank test, $Z = -5.58$, $n = 68$, $P = 0.001$). The male's share of provisioning was on average 0.37 ± 0.02 ($n = 68$, range 0–0.67). The feeding rate increased with brood size (Supplementary material, Figure 1). However, the total number of feeds per hour per nestling decreased with increasing brood size (Supplementary material, Figure 2). The provisioning rate increased with the age of nestlings (Supplementary material, Figure 3). The sex ratio of a brood (proportion of male nestlings) had no significant effect on brood provisioning (GLMM: male provisioning $F_{1,32} = 0.01$, $P = 0.93$, $n = 51$; female provisioning $F_{1,29} = 0.24$, $P = 0.62$, $n = 54$; total provisioning $F_{1,16} = 0.01$, $P = 0.92$, $n = 68$); thus, it was not included in the GLMMs below. There was no significant change in the hourly provisioning rates during the day between 8 AM and 18 PM (Supplementary material, Figure 4).

Paternity and food provisioning

Males reduce food provisioning when cuckolded

We have data on paternity and food provisioning for 89 broods from 54 females and 51 males. We found a negative relationship between the proportion of EPP in a brood and a male's provisioning rate (Figure 1). For 10 males, we have both nests with and without EPY. The mean provisioning rate and the mean share of provisioning were lower at nests that contained EPY when compared with nests without EPY (Figure 2).

For 14 pairs with 2 broods within the same season (2004: $n = 4$, 2005: $n = 8$, 2006: $n = 2$), we have data on paternity and

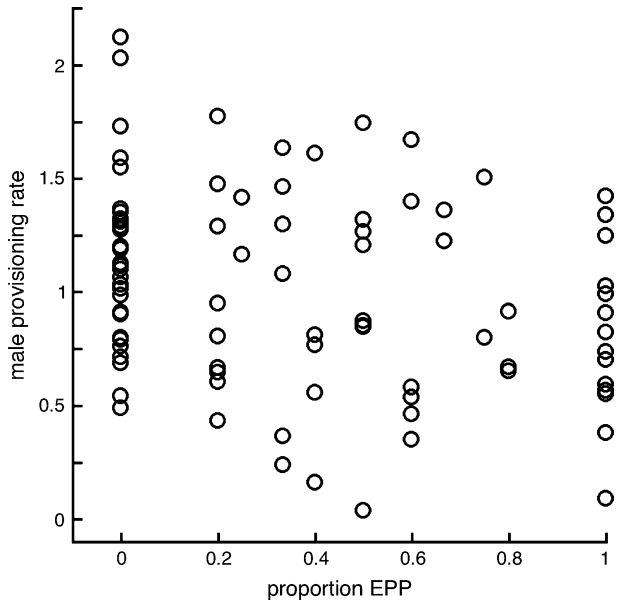

Figure 1
The relationship between proportion of EPP in broods ($n = 89$) and male provisioning rate corrected for nestling age. The relationship was analyzed in a GLMM (GLMM: $F_{1,33} = 4.070$, estimate $= -0.38 \pm 0.64$, $P = 0.04$, $n = 51$). The model includes age of nestlings (4–6 days, fixed factor), brood size (2–5 nestlings, fixed factor), and individual male (random effect).

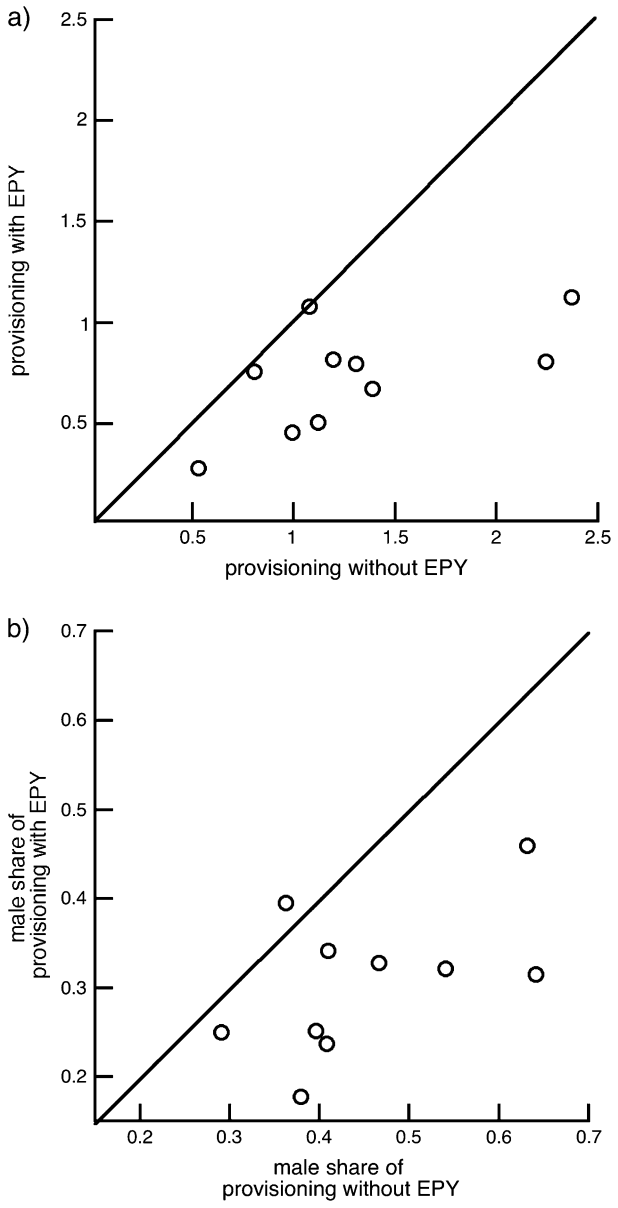

Figure 2
The relationship between a reed bunting male's provisioning at nests with and without EPY. (a) Male provisioning rate in feeds per hour per nestling (paired *t*-tests: $t = -3.95$, df = 9, $P = 0.0034$). (b) Male share of provisioning ($t = -5.155$, df = 9, $P = 0.0006$). The lines indicate identical values.

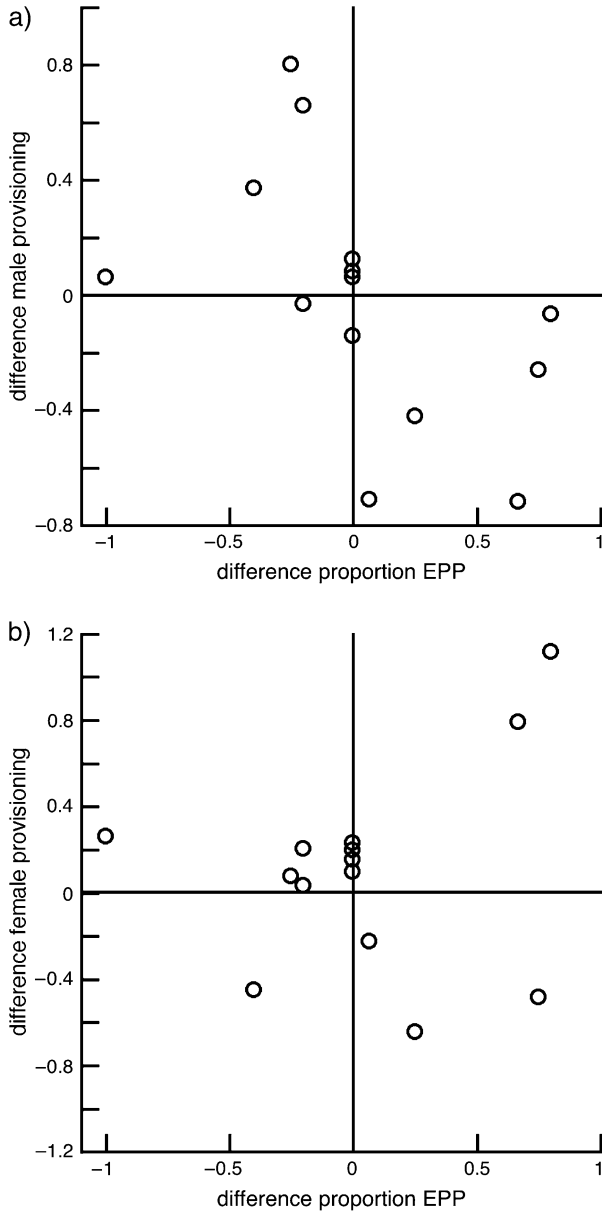

Figure 3
The relationship between difference in proportion of EPP and difference in provisioning rate (feeds per hour per nestling) between sequential broods of the reed bunting. (a) The change of male provisioning rate in relation to the change in the proportion of EPP ($r_s = -0.71$, df = 12, $P = 0.005$); (b) the change of female provisioning rate in relation to the change in the proportion of EPP ($r_s = 0.03$, df = 12, $P = 0.91$).

parental provisioning from day 2 until day 7 after hatching. There was no pattern for a systematic change of the percentage of EPP from the first to the second brood. Proportion of EPP increased between broods in 5 cases, decreased in 5 cases, and did not change in 4 cases (likelihood ratio test: $G = 0.15$, df = 2, $P = 0.93$). We found a negative relationship between the difference of the proportions of EPP and the difference of the provisioning rates for males (Figure 3a). Between broods, males increased their provisioning rate in 4 out of 5 cases where the percentage of EPP decreased and they decreased their provisioning rate in all 5 cases where the percentage of EPP increased (exact binomial test: $n = 10$, $P = 0.02$). For the females, we found no relationship between the difference of the proportions of EPP and the difference of the provisioning rates (Figure 3b).

Females compensate reduced male food provisioning

Females without male assistance at the nest fed at a higher rate when compared with females that had the help of a social partner (mean provisioning rate of females: without male assistance $n = 10$, 2.72 ± 0.20; with male assistance $n = 47$, 1.61 ± 0.09; $t = 4.91$, df = 55, $P = 0.0001$). Brood sizes did not differ between nests with male assistance and nests without male assistance (mean brood size of females: without male assistance $n = 10$, 3.60 ± 0.31; with male assistance $n = 47$, 3.83 ± 0.14; Wilcoxon rank-sum test, $Z = -0.79$, $P = 0.43$). There was no significant difference in total feeding rate between nests without male assistance and nests with male

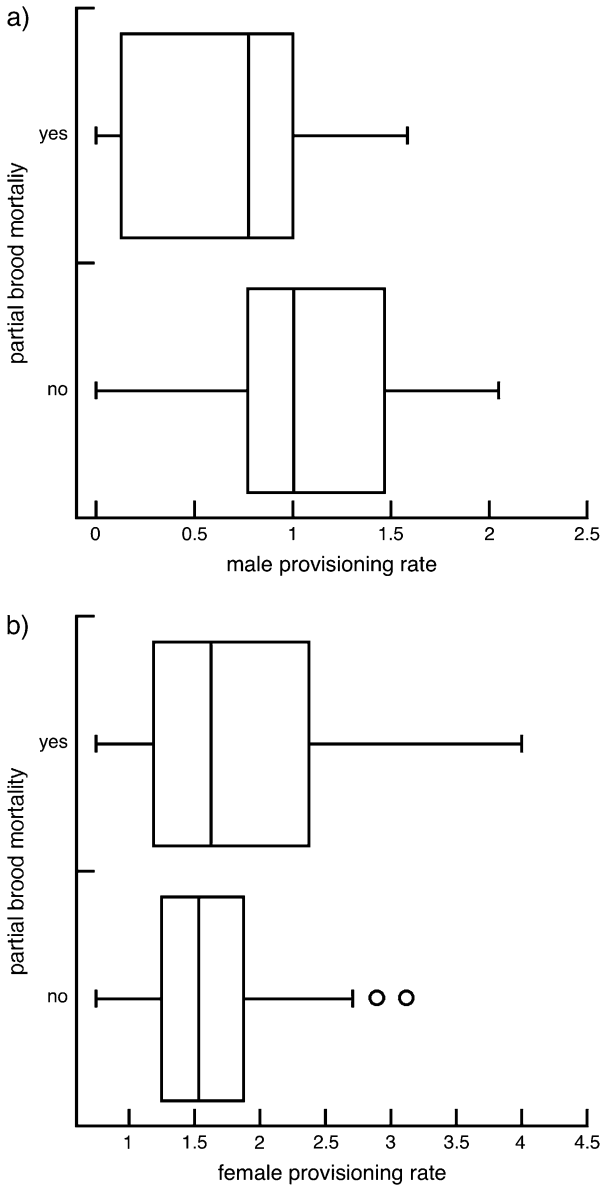

Figure 4
Mean provisioning rates (feeds per hour per nestling) in relation to occurrence of partial brood mortality in reed buntings. (a) Mean provisioning rate of males in relation to the occurrence of nestling mortality (logistic regression: $\chi^2 = 6.15$, df = 1, $P = 0.02$; yes: $n = 14$, no: $n = 43$). (b) Mean provisioning rate of females in relation to the occurrence of nestling mortality (logistic regression: $\chi^2 = 1.58$, df = 1, $P = 0.21$; yes: $n = 15$, no: $n = 45$).

assistance (total mean provisioning rate of nests of females: without male assistance $n = 10$, 2.72 ± 0.20; with male assistance $n = 47$, 2.65 ± 0.12; $t = 0.265$, df = 55, $P = 0.79$). We found a tendency for a positive relationship between the proportion of EPP and a female's provisioning rate (GLMM: $F_{1,30} = 3.36$, estimate = 0.21 ± 0.11, $P = 0.08$, $n = 54$). A male's shared contribution to provisioning had no significant effect on the total provisioning rate of a brood (GLMM: $F_{1,17} = 0.36$, $P = 0.55$, $n = 68$).

The consequences of reduced male food provisioning

In 69 broods, no nestling mortality occurred between hatching and fledging. The nestlings of broods with male provisioning

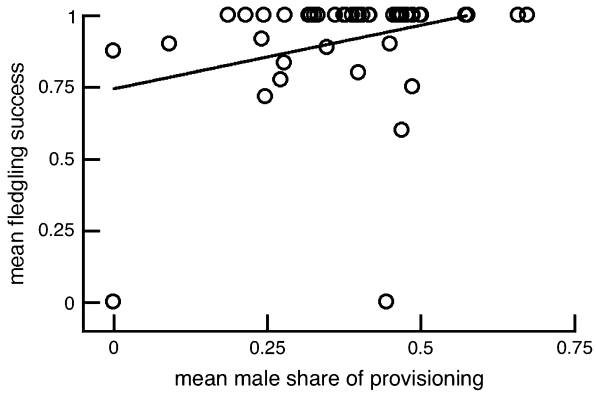

Figure 5
Mean share of provisioning (male provisioning divided by male and female provisioning) in relation to fledgling success (number of fledgling divided by the number of hatchling) in the reed bunting ($r = 0.29$, $n = 48$, $P = 0.04$).

were on average 1.09 g (8%) heavier when compared with nestlings of broods without male provisioning (mean standardized fledgling mass of broods of females: with male assistance $n = 42$, −0.05 ± 0.13; without male assistance $n = 5$, −1.04 ± 0.39, $t = -2.41$, df = 45, $P = 0.02$). There was no significant relationship between the brood size and the standardized mean mass of nestlings ($r = -0.12$, $n = 69$, $P = 0.29$). There was no significant relationship between male provisioning rate and standardized mean mass of nestlings (GLMM: $F_{1,22} = 2.00$, $P = 0.17$, $n = 43$). Also a male's share of provisioning showed no relationship to the standardized mean mass of nestlings (GLMM: $F_{1,22} = 1.79$, $P = 0.19$, $n = 43$). Female provisioning rate was not significantly related to the standardized mean mass of nestlings (GLMM, after log transformation: $F_{1,21} = 0.01$, $P = 0.93$, $n = 47$).

Out of the 186 nests we found, 3% failed due to inundation and 25% were depredated. The following predators were filmed at nests: grass snake *Natrix natrix*, fox *Vulpes vulpes*, wild boar *Sus scrofa*, and water rail *Rallus aquaticus*. For the analysis of fledgling success, we excluded broods that failed due to inundation or predation. The fledgling success in nests with male assistance was higher compared with nests without male assistance (mean fledgling success of broods of females: with male assistance $n = 45$, 0.93 ± 0.03; without male assistance $n = 9$, 0.78 ± 0.06; Wilcoxon rank-sum test, $Z = -2.09$, $P = 0.04$). Across the local population, partial brood mortality was more likely to occur in broods with low male provision rate (Figure 4a), whereas female provisioning rate was not significantly related to nestling mortality (Figure 4b). We also found a positive correlation between mean of a male's share of food provisioning and mean fledgling success (Figure 5).

Females that had no male assistance at nests were as likely to return in the following year as females that had help at the nest (proportion of females returning the following year—2004: with male assistance 60% [6/10], without male assistance 66% [2/3]; 2005: with male assistance 50% [15/30], without male assistance 40% [2/5] [both years: Fisher's Exact test $P = 1$]). We found no significant relation between a female's mean feeding rate (feeds per hour) and return the following year (logistic regression—2004: $\chi^2 = 0.03$, $n = 13$, $P = 0.85$; 2005: $\chi^2 = 0.01$, $n = 35$, $P = 0.93$). Also the female's mean share of provisioning was not significantly related to return in the following year (logistic regression—2004: $\chi^2 = 1.07$, df = 1, $P = 0.30$, $n = 13$; 2005: $\chi^2 = 0.04$, df = 1, $P = 0.85$, $n = 35$).

DISCUSSION

EPP was frequent (39% EPY) in the studied reed bunting population, and males contributed an important part (37%) to food provisioning of broods indicating sexual conflict over paternity and parental care. The male share of provisioning was highly variable ranging from 0% to 67%. We found a negative correlation between EPP in broods and paternal care (i.e., food provisioning) across the local population (Figure 1). However, an adjustment of paternal care to perceived paternity cannot be inferred from this correlation. Individual males could be at the same time successful paternity defenders and good brood providers, whereas "poor males" that lose paternity may also be poor brood providers. We measured differences in the proportion of EPP between broods of the same couple to test for an adjustment of paternal care to perceived paternity. A test between sequential broods of the same couple excludes variances based on individual male, individual female, and territory quality (Dixon et al. 1994; Yezerinac et al. 1996). Paternal investment proved to be inversely related to the degree of EPP, like in earlier studies on an English reed bunting population (Dixon et al. 1994) and in barn swallows (Møller and Tegelstrom 1997). In our study, a difference of 100% EPP resulted in a difference of 0.47 feeds per hour per young, which represents 17% of the total feeding rate. Even if the change was in the predicted direction for 9 out of 10 males, the size of the individual adjustment was not precise and some males also fed at nests where all nestlings were EPY. Therefore, we assume that males are unable to assess their paternity precisely. Our estimation of relative parental effort depends on several factors. We showed that feeding rate increases with the number of nestlings (Supplementary material, Figure 1), but simultaneously, total feedings per nestling decreased (Supplementary material, Figure 2). This might be due to smaller energy requirement for thermoregulation in larger broods, as the ratio between surface and volume becomes more favorable in terms of heat loss with an increasing brood size (Mertens 1969). Judged from dividing the feeding rate (feeds per hour) by the number of young, we have overestimated the relative effort of parents in small broods and underestimated it in large broods. The larger the difference in the number of young between sequential broods the larger the error in the estimated difference in parental effort.

We do not know how or whether reed bunting males assess their share of paternity. A significant positive relationship between the proportion of paternity and a male's mate guarding effort has been found in a Norwegian reed bunting population (Marthinsen et al. 2005). Investment into mate guarding could therefore be a reliable cue on which males assess their paternity. Another reliable cue may be the intrusions by neighboring males because such intrusions are common in reed buntings (O'Malley 1993; Bouwman et al. 2005; Marthinsen et al. 2005). Studies on other species suggest that males estimate their paternity from the frequency of EPC (Møller 1988; Ewen and Armstrong 2000) or from the access to the female during her fertile period (Davies et al. 1992).

It has been argued that only experimentally induced change of paternal care can prove the existence of male adjustment of paternal care to paternity (Kempenaers and Sheldon 1997; Sheldon 2002). Studies with manipulated cues on which males may assess their paternity have produced mixed results (Lifjeld et al. 1998; Whittingham and Dunn 2001). With pairwise analysis of sequential broods, temporary variation in the quality of a male cannot be excluded (Lessells 1994). However, regardless of the reduction of parental care due to a social male's adjustment of paternal care to paternity or due to male quality, EPP will induce costs to females.

We found a tendency for a positive relationship between EPP and maternal care (i.e., food provisioning) across the local population. This finding may indicate a female's compensation for reduced male assistance. Females without help of a social partner were able to compensate completely the shortfall of food provisioning, at least on day 5 after hatching. Thus, females with a social partner would have been able to increase their feeding rate by 68%, corresponding to 1.1 feeds per hour per young on day 5 after hatching. This raises the question: why should males feed at all? Males feed because with paternal care, they increase the survival probability of their genetic offspring and thus their own fitness. In the analysis across the population, we focused on day 5 after hatching. However, food demand increases with the age of the young (Supplementary material, Figure 3), and we observed that young could be fed up to 3 weeks after they left the nest.

We did not find a positive relationship between paternal feeding rate and mean mass of the nestlings of a brood across the population. However, mortality was more likely to occur at nests with decreased male assistance (Figure 4a). Thus, food shortage would rather lead to partial brood mortality than to a decrease in mean mass of the young. Females may have been able to compensate deficient male assistance at day 5 after hatching, but not later when food demand increased. Alternatively, females that have to feed more can invest less time into other brood-caring activities like brooding or removing parasites, activities that we also observed at nests.

Our results confirm the finding of Dixon et al. (1994)and are not in accordance with the findings of Buchanan (2001)and Bouwman et al. (2005). It is possible that relative costs and benefits of parental care were different at different study sites. The mean provisioning rates of reed bunting nestlings were not similar in the 4 studies (England: 2.6 [$n = 26$]; Poland: 2.3 [$n = 45$]; The Netherlands: 2.9 [$n = 29$]; Switzerland: 2.7 [$n = 68$] feeds per hour per nestling [Dixon et al. 1994; Buchanan 2001; Bouwman, et al. 2005; this study; $F_{3,165} = 3.76$, $P = 0.01$]). Thus, different food availability may cause differences in the costs of parental care at different study sites. The proportion of EPP was significantly different among the 4 studies (England: 55% [$n = 216$]; Poland: 44% [$n = 217$]; The Netherlands: 50% [$n = 294$]; Switzerland: 39% [$n = 506$] EPY; $G = 17.84$, df = 3, $P = 0.001$). However, the studies with the highest (England, Dixon et al. 1994) and the lowest (Switzerland, this study) proportion of EPP revealed both a significant negative relation between the proportion of EPP and paternal care. In 3 studies, a significant variation in proportions of EPP between broods of the same male was found (Lessells 1994; Bouwman et al. 2005; this study; no data available for Buchanan 2001).

In previous studies, the estimation of the effort of paternal care in sequential broods was based on relatively short observation times (Dixon et al. [1994]: 9 h; Buchanan [2001]: 2–16 h; Bouwman et al. [2005]: 3 h). During short observation times, weather, disturbances, and variability of individual behavior might increase the variance of observational data (but see Møller 1988). Our estimation of parental effort between sequential broods is based on an average of 32 h dispersed over 6 days. This study was designed as a conceptual and partial replication of Dixon's study, following the arguments of Kelly (2006). A replication is considered successful when the absolute difference of the 2 Fisher's z' values, called Cohen's q, is close to zero. Fisher's z' is based on effect size (Pearson r). The values of this study are as follows: $r_s = -0.53$; standard error of the mean (SEM) = 0.12; $q = 0.46$. The estimated values for Dixon et al. (1994)are the following: $r_s = -0.78$; SEM = 0.03; q is not applicable. The corresponding values for Buchanan are the following: $r_s = -0.42$; SEM = 0.23; $q = 0.60$. For Bouwman et al. (2005), they are as follows: $r_s = 0.09$; SEM = 0.271; $q = 1.13$. Our

replication was more successful than Buchanan's and much more so than Bouwman's. If a true replication of Dixon's study with the same sample size had been performed, the replicability ($P < 0.05$) based on the observed P value of 0.0064 would be about 80% (see Figure 1 in Kelly 2006). It is possible that Buchanan (2001)and Bouwman et al. (2005)did not find an existing relationship for purely statistical reasons or because of the high variances in their data.

Our results imply that the costs of extrapair mating to females is increased nestling mortality caused by the social partner's reduction of brood care. The costs are limited, however, because the female can compensate by feeding more. Females that fed more were not less likely to return to the study site in a subsequent year. Despite these costs, extrapair mating can be an adaptive behavior for females because of long-term benefits, such as the high viability of extrapair offspring (Suter et al. 2007). Theoretical models by Kokko (1999)predict that high frequencies of EPP may occur in situations where females profit substantially from extrapair fertilizations, if females are easily able to compensate for reduced male care, and if males cannot accurately assess female infidelity. Our findings suggest that these conditions are fulfilled in the mixed mating system of the reed bunting. If the mixed mating system is at an evolutionary equilibrium, we would expect genetic fitness for females and males to be maximized and any costs incurred by EPC not exceeding the benefits for both sexes.

SUPPLEMENTARY MATERIAL

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

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