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## The illusion of monogamy

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## **Costs of extra-pair paternity to females**



## **Male reed buntings do not adjust parental effort in relation to extra-pair paternity**

Karen M. Bouwman, C(Kate). M. Lessells & Jan Komdeur

## Abstract

Parental effort is considered to be costly; therefore males are expected to provide less care to unrelated offspring. Theoretical models suggest that males should either reduce their care to the entire brood, or alternatively distinguish between related and unrelated nestlings and direct provisioning to kin when paternity is in doubt. Reed buntings (*Emberiza schoeniclus*) have been found to have high levels of extra-pair paternity (i.e. offspring of a male other than the male attending the nest; 55% of offspring) and males are therefore under strong selection pressure to adjust their parental effort according to the proportion of extra-pair paternity in their brood. In this study we investigated whether male reed buntings exhibit a reduction in paternal care (incubation and provisioning nestlings) in relation to decreased paternity. We also assess whether males bias their provisioning towards kin. We measured incubation time, provisioning rates and food allocation to individual nestlings using video-recordings at the nests. Microsatellite DNA-analysis was used to analyze the paternity of offspring. In direct contrast to a previous study on the same species, our results provided no indication that males lowered their effort with decreased paternity. Furthermore, in nests of mixed paternity males did not bias their provisioning behavior to kin. It remains to be investigated whether the absence of a relationship between paternity and paternal care can be ascribed to absence of reliable paternity cues or whether the benefits of reducing paternal care did not outweigh the costs in our study population. We found no evidence that the level of paternal care affected male survival or offspring mass, suggesting that both the benefits and costs of any reduction in paternal care would have been low.

develop adjustment of provisioning towards own kin in these species (Johnstone 1997). For that reason paternal adjustment towards kin in mixed broods should be studied in a species with higher levels of extra-pair parentage.

Reed buntings are an excellent species in which to address the question of whether males distinguish between kin and non-kin. They have levels of EPP -55% of nestlings in 86% of nests (Dixon *et al.* 1994)- which are among the highest found in socially monogamous passerines (Griffith *et al.* 2002). They usually form socially monogamous pairs with biparental care during incubating and provisioning and are capable of raising two broods in a single season (O'Malley 1993), and males have been shown to adjust their provisioning rate as a function of their paternity level (Dixon *et al.* 1994). In order to test the generality of behaviors, it is important to compare populations of the same species (Griffith *et al.* 2003). The present study first investigates whether male reed buntings reduce paternal care (incubation and provisioning) in relation to the proportion of EPP in their broods in a population in the Netherlands, as has been shown previously for provisioning rates in a population in England (Dixon *et al.* 1994). If paternity levels are comparable between the two populations, then parental behavior is expected to show a similar response to paternity. Furthermore, possible costs and benefits of reducing paternal care are addressed. Second, this study investigates whether males discriminate between kin and non-kin when allocating food to individual nestlings.

## Methods

### *Data collection*

The reed bunting is a small (males: 19g; females: 17g), sexually dimorphic passerine. Monogamy with biparental care is the main mating system, but polygamy does occur. The breeding season lasts from mid April until August. Pairs are capable of raising more than one successful brood per year, and females quickly reneest if a brood is predated. Nests are built on or just above the ground. Approximately half of the breeding adults return in subsequent breeding seasons, and show high site fidelity (Cramp & Perrins 1994; O'Malley 1993).

In 2001 and 2002, a population of reed buntings was studied in a 13 ha study site, on the island of Noorderplaat (45 ha) in the National Park 'De Biesbosch' in the Netherlands (51°45'N, 4°45'E). The study site had an average density of 2.5 pairs per hectare. Vegetation consisted of a combination of reeds (*Phragmites australis*), soft rush (*Juncus effusus*), hard rush (*Juncus inflexus*) and various species of grass. The height of the vegetation varied from 50 to 300 cm, with most of the vegetation below 150 cm. A grid of which each cell was approximately 20 x 40 meter was laid across the area for mapping territories and nests.

Within our study site adult reed buntings were caught using mist nets. Birds were ringed with a numbered aluminium ring and a specific combination of three color rings for individual recognition. A blood sample (20  $\mu$ l) was taken from the brachial vein and stored in 96% ethanol at room temperature. Nests were located through systematic searches that flushed females off the nest, or through checking territories for any nest-related activities. To minimize the risk of predation, which was mainly by stoats (*Mustela erminea*) and polecats (*Mustela putorius*), nest visits were kept to a minimum. Nestlings were bloodsampled two days after hatching by taking a small blood sample (10  $\mu$ l) from the leg vein.

In both years, video-recordings during incubation and provisioning stage were made between 5 May and 19 July and spread over the day between 7 am and 7 pm. No recordings were made during rainfall. Video-recordings of the provisioning stage were limited to the time-period when the first hatched chick was between four and six days of age. A dummy camera was placed at the nest a day before the video was made to familiarize the birds with the camera. The actual camera consisted of a color mini camera (model AVC56P/F36, size: 3 x 3 x 2 cm) connected to a Sony video Hi8 camera recorder (model CCD-TR840E) with line-in function, which recorded for three hours (90 minute tape on longplay). The camera was placed on a metal wire approximately 30 cm above the nest, giving a clear view of the nest. The Hi8 camera recorder was placed several meters away from the nest behind a bush. After the camera was placed, the adults quickly returned to the nest ( $4.06 \pm 0.45$  min,  $n = 50$ ). Before recording provisioning behavior, the nestlings were weighed and individually marked on their bill, using a non-toxic black marker. The order of the markings was at random, and the sex and paternity of the nestlings were unknown.

Nestlings were weighed again at 7 days of age and given a numbered aluminium ring. This is the latest that offspring can be taken out of the nest without running the risk of premature fledging.

### *Video analyses*

Videos were analyzed using a television (Sony Trinitron) and VCR (Sony SLV-T2000) with real-time display. The total recording time was defined from the time of the first return of one of the parents until the end of the videotape. The time of day, time of season, number of eggs or nestlings in the nest, and age of the first-hatched nestling in days, was known for each recording. Video recordings of incubation behavior had an average length of  $167 \pm 3.27$  minutes ( $n = 63$ ). Of these nests, 48 survived at least until eggs hatched and nestlings could be blood sampled and hence paternity determined (table 4.1). The incubation behavior of 31 different pairs, for which the proportion of EPP in their broods was later determined, was recorded at least once (table 4.1). Incubation was expressed as the number of minutes per hour spent incubating. Video recordings of provisioning



behavior were made at 58 nests (table 1), with an average length of  $177 \pm 1.82$  minutes. The provisioning behavior of 31 different pairs was recorded at least once (table 4.1). Provisioning rate was measured as the number of feeds per nestling, per hour, for each parent. A nestling was considered fed when it swallowed (part of) a food item, therefore during one single visit more than one nestling could receive food. The sex or paternity of the nestlings was unknown when scoring the videotapes.

**Table 4.1.** Sample sizes (number of nests or pairs) of video-recordings.

Year	Total nests	All nests with known paternity	No. of different pairs*	Pairs with double broods	Double broods with D EPP**
Incubation					
2001	14	6	5	1	1
2002	49	42	26	11	10
Provisioning					
2001	17	7	5	1	1
2002	41	39	26	9	8

\* 1 nest per pair; individuals only included once (i.e. excluding remated individuals and secondary females)

\*\* $\Delta$  EPP = Difference in proportion of extra-pair paternity between broods of the same pair

### *Sex determination and paternity analysis*

DNA was extracted from blood samples using salt extraction (Richardson *et al.* 2001). Nestlings were sexed using Griffith's universal PCR method for the sexing of birds (Griffiths *et al.* 1998). The paternity of the nestlings was analyzed using four fluorescently labeled microsatellite markers: *Escu1*, *Escu4*, *Escu6* (Hanotte *et al.* 1994), and *Pdou5* (Griffith *et al.* 1999). PCR amplifications were performed using a Thermolyne amplitron II thermal cycler at an initial hotstart for 90 seconds at 94°, followed by 30 cycles of 1 minute at 94°, 1 minute at annealing temperature and 1 minute at 72°. Annealing temperatures were set at 55° for *Escu1* and *Escu4*, at 52° for *Escu6* and at 50° for *Pdou5*. Each 10-ml mix contained 10-50 ng of DNA, 1.0 mM of each primer, 0.2 mM of each dNTP, 0.05 units of *Taq* polymerase (Advanced Biotechnologies) and 0.625mM MgCl<sub>2</sub> in a supplied reaction buffer (final concentration 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 75 mMTris-HCL, pH 9.0, 0.01% (w/v) Tween). PCR-products were diluted two times. Diluted PCR-products of *Escu1*, *Escu4* and *Pdou5* were multiplexed in a ratio of 2:1:2, after which 1  $\mu$ l of PCR-product (*Escu6*) or multiplex-mixture was mixed with 1.5  $\mu$ l of a loading buffer containing 1.1  $\mu$ l of deionised formamide, 0.18  $\mu$ l

of blue dextran loading dye and 0.22  $\mu\text{l}$  of internal lane standard (ROX500, Applied Biosystems). These samples were denatured by heating at 94° for 3 minutes and then placing directly on ice. One  $\mu\text{l}$  of each sample was electrophoresed using a 10% denaturing polyacrylamide gel on an Applied Biotechnologies (ABI) 377 XL DNA sequencer. DNA fragments were analyzed using DNA fragment analysis software (Applied Biosystems GENESCAN (version 3.1) and GENOTYPER (version 2.5)). Parentage was determined by using a likelihood-based approach in CERVUS (Marshall *et al.* 1998). This program assesses the confidence of paternity assignment through a simulation based on allele frequencies in the population, the number of possible parent-candidates and the number of parent-candidates sampled.

The male and female present at a nest were determined by observations of color-ringed birds during the incubation and nestling period. Potential offspring of the male and female within the territory were identified using CERVUS. First, potential offspring of each female were identified to check for egg dumping; then, potential offspring of each male were identified using the mother as 'known parent' in the analysis. Using a 'known parent' in the analysis increases the confidence level when determining the father. CERVUS was given the choice between two candidate parents: the territory male and one potential, but unknown, extra-pair male. The program calculates the likelihood that the territory male is the actual father by using the natural logarithm of the likelihood ratio or so called LOD score. The territory male is assigned as the father if the LOD-score is positive and rejected if the LOD-score is zero or below. To accept the male as the father, a critical difference is required in LOD scores between the first and the second candidate. The critical values were calculated by entering the following simulation parameters in CERVUS: 10,000 cycles, 2 candidate parents and 50% of candidate parents sampled. We succeeded in obtaining 90% of all potentially available genotypes (i.e. 4 loci  $\times$  (81 adults + 294 nestlings);  $n = 1500$  genotypes). Assigned males were accepted at >95% confidence. The used microsatellite loci had a total exclusionary power of 0.978 and 0.996 for the first parent and second parent respectively.

### *Statistical analysis*

Pairs that reared two broods in a single season were used to relate the difference in proportion of EPP between nests with the difference in paternal care to the entire brood, following Dixon *et al.* (1994). As pairs remained together and on the same territory, no correction is necessary for characteristics of parents or territory. Differences in incubation and provisioning effort between the two broods were compared with the difference in the proportion of extra-pair paternity between the two broods. The incubation and provisioning effort is expressed as the a male's proportion ('share') of male and female effort combined. In addition provisioning effort is expressed as provisioning rate per hour, per nestling, for both males and

females separately, as this was used by Dixon *et al.* (1994), allowing a proper comparison. A power analysis was performed using an expected effect size based on the  $r^2$ -value extracted from the results from Dixon *et al.* (1994) when correlating the difference in male provisioning rate and the difference in extra-pair paternity between nests. This yielded a value of  $r^2$  of 0.57, which corresponds to a very large effect size ( $r = 0.75$ ).

We analysed variance in the proportion of extra-pair young between broods using generalised linear models with binomial errors and a logit link fitted using Proc Genmod of SAS. In such models, statistical significance is often attached to the deviance or change in deviance by comparison with a chi-square distribution. However, the assumption that the deviance or change in deviance is distributed asymptotically as chi-square often fails with low values of the binomial denominator (the number of genotyped nestlings in a brood in our analyses; C.M.Lessells, pers.obs.). We therefore attached significance values to deviances or changes in deviance using randomisation tests (Manly 1997). The general procedure used in these tests was to randomly allocate the measured values across the measured units while maintaining sample sizes per group or sub-group, and then recalculate the deviance or change in deviance for these randomised data. The proportion of 1000 iterations in which the deviance or change in deviance was more extreme than the observed value was taken as the P value. We carried out such tests at three levels: first, we tested whether there was significant variation between all broods in the sample by fitting the null model and carrying out randomisation tests in which the paternity of nestlings (within- or extra-pair) was randomly reallocated across broods while maintaining the observed sample sizes per brood. Second, we tested whether there was significant variation in the proportion of extra-pair young between males (when we analysed data for males with two broods in the same year) or between first and second broods by fitting male identity or brood number (first or second) as explanatory variables in the model. In the randomisation tests we reallocated the observed proportions of extra-pair young in the brood (i.e. the number of extra-pair young and corresponding number of young genotyped) across broods while maintaining the observed sample size of broods per male or for first and second broods. Third, when we analysed data for males with two broods in the same year, we tested whether there was significant variance between the broods within males (i.e. whether there was evidence that the two broods of a male differ more in the proportion of extra-pair young than expected by chance if individual nestlings in both broods have the same probability of extra-pair paternity). In these analyses the relevant test deviance was the residual deviance after fitting male identity as an explanatory variable. The randomisation test was carried out by simultaneously reallocating the paternity of nestlings within each male across his two broods, while maintaining the brood sizes of all broods in the sample.

Broods of mixed paternity were used to investigate whether males bias their provisioning towards own kin. This way no correction is necessary for age of the nestlings. For each brood the average number of feeds per nestling, per hour (provisioning rate) was calculated first for sons and daughters, and then for within-pair and extra-pair nestlings. To avoid pseudo-replication, only one nest was included for each pair. If the provisioning behavior of a pair had been recorded on more than one occasion (i.e. from more than one brood in a season), then the brood in which the numbers of within- and extra-pair young were most similar was included.

Statistical analyses were performed using SPSS 11.0.1 (2001), except for power analysis which was performed using GPOWER (Faul and Erdfelder 1992). All data were tested for normality and data that were not normally distributed were transformed to achieve normality, or non-parametric tests were used. Unless stated, means are expressed with standard errors, probability values are two-tailed and the level of significance was set at  $p < 0.05$ .

## Results

### *Breeding ecology*

Low levels of polygamy were found in both years; 9% of ringed males ( $n = 11$ ) in 2001 and 5% of ringed males ( $n = 39$ ) in 2002 had two females on their territory. Male incubation and provisioning behavior at primary nests were within the range of male incubation and provisioning behavior at monogamous nests (table 4.2). Polygamous males did not incubate or provision at secondary nests. No difference was found between male provisioning behavior at monogamous and primary nests in this study or in that by Dixon *et al.* (1994), therefore no distinction was made in subsequent analyses. Secondary nests of polygamous males are excluded from further analyses.

On average a clutch consisted of  $4.5 \pm 0.07$  eggs ( $n = 125$  nests, range 2-6), with a hatching success of 86%. This resulted in broods containing an average of  $3.92 \pm 0.11$  nestlings ( $n = 79$  nests, range 2-6). Females incubated longer and provisioned nestlings at a higher rate than males (table 4.2; Wilcoxon signed rank test: incubation time:  $Z = -4.86$ ,  $n = 31$ ,  $p < 0.001$ ; provisioning rate:  $Z = -3.63$ ,  $n = 31$ ,  $p < 0.001$ ). Males assisted in incubating eggs in 35% of cases ( $n = 31$  males). There was a significant negative correlation between male and female incubation time ( $r_{\text{spearman}} = -0.82$ ,  $n = 11$ ,  $p = 0.002$ ). No effect was found of clutch size, time of day, day of incubation or day of season on the presence or absence of male incubation (logistic regression: all  $p > 0.5$ ,  $n = 31$ ), on the time spent incubating by male or female (GLM: males (after log transformation): all  $p > 0.2$ ,  $n = 11$ ; females: all  $p > 0.2$ ,  $n = 31$ ), or on the male share of incubation (GLM (after log transformation): All  $p > 0.8$ ,  $n = 11$ ).

The effect of age of nestlings, time of day, day in season, brood size and provisioning rate of partner on provisioning rate was tested, and a significant relationship was shown to occur between age of nestlings and provisioning rate for both females and males (GLM: females (after log transformation): age  $F_{1,31} = 7.03$ ,  $p = 0.01$ , brood size  $F_{1,31} = 4.03$ ,  $p = 0.06$ , rest  $p > 0.4$ ; males: age  $F_{1,31} = 12.03$ ,  $p = 0.002$ , rest  $p > 0.3$ ). A male's share of provisioning was not related to age of nestlings, time of day, day in season or brood size (GLM: all  $p > 0.2$ ,  $n = 31$ ).

**Table 4.2.** Incubation (minutes per hour) and provisioning rate (per nestling per hour) at nests of monogamous males and at primary and secondary nests of polygamous males.

	Total	Male	Female	M/(M+F) ratio
<b>Incubation</b>				
Monogamous ( $n = 29$ )	45.5 ± 1.1	2.7 ± 0.9	42.8 ± 1.2	0.06 ± 0.02
Range (min – max)	(30.5 – 52.1)	(0 – 22.6)	(27.0 – 51.4)	(0 – 0.46)
Primary ( $n = 2$ )	45.3	3.4	41.9	0.08
Range (min – max)	(45.0 – 45.6)	(0 – 6.8)	(38.2 – 45.6)	(0 – 0.15)
Secondary ( $n = 2$ )	45.1	0	45.1	0
Range (min – max)	(38.8 – 51.4)	(0 – 0)	(38.8 – 51.4)	(0 – 0)
<b>Feeding rate</b>				
Monogamous ( $n = 29$ )	2.9 ± 0.2	1.2 ± 0.1	1.7 ± 0.1	0.41 ± 0.02
Range (min – max)	(1.1 – 6.5)	(0 – 2.8)	(0.7 – 3.7)	(0 – 0.67)
Primary ( $n = 2$ )	2.4	0.9	1.6	0.36
Range (min – max)	(2.3 – 2.5)	(0.4 – 1.3)	(1.0 – 2.1)	(0.16 – 0.56)
Secondary ( $n = 2$ )	3.9	0	3.9	0
Range (min – max)	(3.6 – 4.3)	(0 – 0)	(3.6 – 4.3)	(0 – 0)

### *Extra-pair paternity*

In total 294 nestlings from 75 nests were genotyped (2001: 38 nestlings from 10 nests, 2002: 256 nestlings from 65 nests). At 63 nests both male and female members of the breeding pair were caught, and at 12 nests only the male was caught. No cases of intra-specific brood-parasitism were found; all nestlings had genotypes consistent with their being offspring of the female attending the nest, i.e. the putative mother, at a 95% confidence level (2001:  $n = 19$  nestlings, 2002:  $n = 232$  nestlings). The male attending the nest was excluded from being the genetic father at a 95% confidence level for 49.7% of nestlings; thus these nestlings were sired by an extra-pair male (2001: 18/38 nestlings vs. 2002: 128/256 nestlings;  $\chi^2 = 0.017$ ,  $df = 1$ ,  $p = 0.90$ ). On average 80.0% of nests

contained at least one extra-pair young (2001: 6/10 nests vs. 2002: 54/65 nests;  $\chi^2 = 1.62$ ,  $df = 1$ ,  $p = 0.20$ ). Males had no paternity in 22.7% of nests (2001: 4/10 nests, 2002: 13/65 nests), i.e. all offspring were extra-pair. Absolute differences in proportion of EPP between first and second nests of the same pair ranged from 0 to 0.75 ( $n = 21$  pairs). Among the broods produced by 21 pairs for whom two broods in the same year were genotyped, the proportion of extra-pair young in a brood varied significantly between the 42 broods in the sample (deviance = 81.89,  $df = 41$ ,  $p$  (randomisation test) = 0.003). However, individual males did not differ in the proportion of extra-pair young in their broods (change in deviance = 40.04,  $df = 20$ ,  $p$  (randomisation test) = 0.82). Neither did first broods differ systematically from second broods in the proportion of extra-pair young (average % EPP nest 1 and nest 2: 51% and 56%; change in deviance = 0.70,  $df = 1$ ,  $p$  (randomisation test) = 0.48). Nevertheless, there was significant variation between broods within males (residual deviance = 41.86,  $df = 21$ ,  $p$  (randomisation test) = 0.029). This shows that the probability of a nestling having extra-pair paternity differs between the broods produced by a single male in the same year.

### *Paternal care and parental care*

#### CARE TOWARDS ENTIRE BROODS

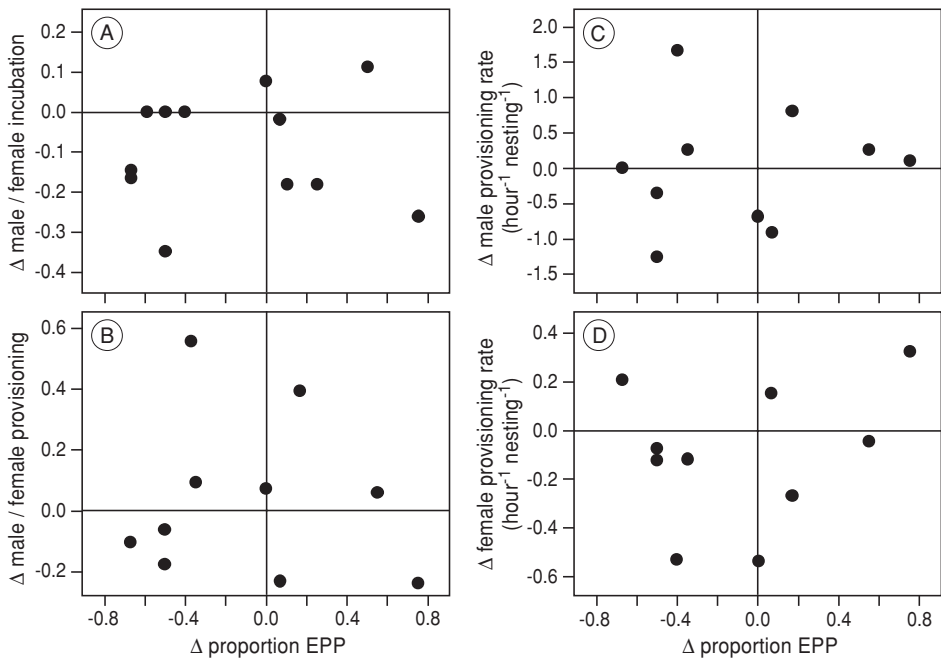
Incubation observations and extra-pair paternity levels were available for twelve pairs (consisting of the same partners remaining on the same territory) that produced two clutches in a single season (table 4.1). Eleven of these pairs had numerically different proportions of EPP in their first and second brood; overall for the 12 pairs, there was statistically significant variation between the two broods within pairs (residual deviance = 25.95,  $df = 12$ ,  $p$  (randomisation test) = 0.040). We found no relationship between the difference in proportion of EPP between broods and the difference in male share of incubation (figure 4.1A). Provisioning observations were available on ten pairs that raised more than one brood in a season (table 4.1). Proportions of EPP differed numerically between the first and second brood in nine of these pairs; overall for the ten pairs there was statistically significant variation between the two broods within the pairs (residual deviance = 22.61,  $df = 10$ ,  $p$  (randomisation test) = 0.013). No relationship was found between difference in proportion extra-pair paternity and difference in male share of provisioning (figure 4.1B) or difference in maternal and paternal provisioning rates per hour per nestling (figures 4.1C and 4.1D; corrected for nestling age). Although the sample size is small, the power analysis showed that a significant relationship similar to the one in Dixon *et al.* (1994) would have been detected with a probability of  $p > 0.85$  for  $\alpha = 0.05$  ( $r = 0.75$ , critical  $t_{(8)} = 2.31$ ,  $n = 10$ ).

Males incubated at four nests and provisioned at eight nests where they had no paternity at all. There were no differences in male share in both incubation and provisioning at nests with no extra-pair paternity compared to nests with at least

one extra-pair offspring (incubation: Mann Whitney U test:  $U = 81.0$ ,  $n_{\text{no EPY}} = 8$ ,  $n_{\text{EPY}} = 23$ ,  $p = 0.59$ ; provisioning:  $U = 72.5$ ,  $n_{\text{no EPY}} = 6$ ,  $n_{\text{EPY}} = 25$ ,  $p = 0.90$ ). Furthermore there was no relationship between male share of incubation and provisioning and the proportion of EPP in the brood among all males (incubation:  $r_s = -0.022$ ,  $n = 31$ ,  $p = 0.91$ ; provisioning:  $r_s = 0.16$ ,  $n = 31$ ,  $p = 0.38$ ).

#### COSTS AND BENEFITS OF PATERNAL CARE

Nestlings of 33 nests were weighed before fledging. In one nest some nestlings died before seven days of age and were therefore excluded from the analysis. No relationship was found between the number of nestlings in the nest and the average mass of nestlings at day seven ( $r = 0.15$ ,  $n = 32$ ,  $p = 0.40$ ). Furthermore there was no correlation between male provisioning rate and average fledging mass (provisioning rate corrected for nestling age;  $r = 0.04$ ,  $n = 32$ ,  $p = 0.83$ ). A negative trend was found between female provisioning rate and average fledging



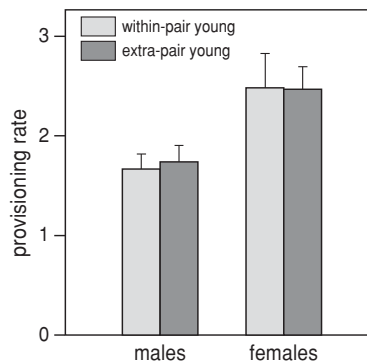
**Figure 4.1.** The relationship between the difference in the proportion of extra-pair paternity (EPP) between two broods of the same pair and (A) the difference in male share of total incubation between two broods ( $r = 0.03$ ,  $p = 0.92$ ,  $n = 12$ ); (B) the difference in male share of total provisioning between two broods ( $r = -0.11$ ,  $p = 0.77$ ,  $n = 10$ ); and the difference in provisioning rate, per hour, per nestling, corrected for nestling age (unstandardized residuals) for (C) males ( $r = 0.09$ ,  $p = 0.80$ ,  $n = 10$ ) and (D) females ( $r = 0.24$ ,  $p = 0.51$ ,  $n = 10$ ).

mass (provisioning rate corrected for nestling age;  $r = -0.32$ ,  $n = 32$ ,  $p = 0.07$ ). Total provisioning rate showed no relationship with average fledging mass (provisioning rate corrected for nestling age;  $r = 0.17$ ,  $n = 32$ ,  $p = 0.36$ ).

Male total parental effort was estimated by adding together a male's share of incubation and provisioning. There was no relation between male total parental effort and his survival to the next year ( $U = 53.5$ ,  $n_{\text{dead}} = 9$ ,  $n_{\text{survive}} = 16$ ,  $p = 0.30$ ).

#### FOOD PROVISIONING TO WITHIN- AND EXTRA-PAIR YOUNG

Video observations of provisioning behavior where the allocation of food to individual nestlings of mixed sex could be observed were made at the nests of 30 different males. Neither males nor females biased provisioning to either sons or daughters (Wilcoxon: males:  $Z = -0.412$ ,  $p = 0.68$ ,  $n = 30$ ; females:  $Z = -0.738$ ,  $p = 0.46$ ,  $n = 30$ ). Therefore no distinction was made in subsequent analysis. Recordings were available for nests belonging to 20 different males, containing nestlings of mixed paternity. At the time of observation extra-pair young had similar mass as within-pair young when correcting for age (GLMM:  $\chi^2 = 0.027$ ,  $p = 0.87$ ,  $n = 88$  nestlings in 20 nests). Extra-pair young received the same number of feeds as within-pair young (figure 4.2).



**Figure 4.2.** Average provisioning rate per hour per nestling ( $\pm$  SE) directed to either within-pair or extra-pair young by males and females in broods of mixed paternity (Wilcoxon: males:  $Z = -0.85$ ,  $n = 20$ ;  $p = 0.39$ ; females:  $Z = -0.024$ ,  $n = 20$ ;  $p = 0.98$ ).



## Discussion

### *Paternity and paternal care: care towards entire broods*

For males to adjust their paternal care towards the entire brood in relation to their paternity, theoretical models require three assumptions to be met (Westneat & Sherman 1993; Whittingham *et al.* 1992). First, levels of extra-pair paternity should vary between breeding attempts of the same male, allowing males with low paternity to achieve higher reproductive success in another brood. Several studies have shown varying levels of paternity between nests of the same male (e.g. Dunn *et al.* 1994; Yezerinac *et al.* 1996); in two different populations of reed buntings significant variation was found in paternity levels between nests of the same male in the same season (Dixon *et al.* 1994; Lessells 1994; this study).

Second, males should be able to assess their share of paternity. The actual level of paternity in broods can be measured relatively easy using molecular techniques; however, the certainty of paternity from a male point of view cannot be measured (Kempnaers & Sheldon 1997). Different studies trying to experimentally decrease certainty of paternity generated different results, as some did (Sheldon & Ellegren 1998) and others did not (Kempnaers *et al.* 1998) find a decrease in paternal care. In the latter case it is not possible to determine whether the certainty of paternity was not decreased by the experiment or whether there was no response in paternal care to the successfully manipulated certainty of paternity (Wright 1998). When studying paternal investment in relation to paternity, it is important to know whether paternity cues are available, as no adjustment of paternal care can be expected if males cannot assess their paternity (Whittingham & Dunn 2001). In other species, males have been shown to judge their share of paternity using access to the female during her fertile period (Davies *et al.* 1992), frequency of extra-pair copulations (Ewen & Armstrong 2000; Møller 1988), and absence of female during egg-laying (Sheldon *et al.* 1997). It has not yet been investigated which cues reed bunting males use to assess their paternity, therefore it remains unknown whether this assumption is actually met. As significant variation was found in levels of paternity between broods of the same male, information may be available from which males can derive their certainty of paternity (Lessells 1994). Male reed buntings guard their mates during the fertile period, but only part-time and both males and females were often seen to leave their territory, e.g. to forage. Males were often seen intruding into a fertile female's territory. However, due to the secretive behavior of females, we do not know whether females also make forays into other territories in search of extra-pair males. Intruding males were usually chased by the territorial male, if he was present, while the female seemingly did not pay any attention. We never witnessed extra-pair copulations. Thus possible cues may be (i) absence of the female during her fertile period, (ii) the number of intruding males into the territory or (iii) how the female reacts to these males.

Third, the benefits of reducing paternal care should outweigh the costs. The benefits of reducing care for the male may be decreased mortality (Nur 1984; Yezerinac *et al.* 1996), and/or increased opportunities for additional matings (Magrath & Elgar 1997; Smith 1995; reviewed in Magrath & Komdeur 2003). The costs of reducing parental care seem obvious in terms of decreased survival of offspring (Bart & Tornes 1989; Wolf *et al.* 1988). Monogamous males may not be able to afford to reduce parental effort, as all offspring would suffer, including a male's own offspring (Davies *et al.* 1992). This would however not be the case if males can distinguish between related and unrelated offspring and provide more care towards kin. In the present study, no evidence of benefits or costs of reducing care was found. Males did not appear to benefit through decreased mortality when providing less paternal care. Neither was there any relationship detected between fledging mass and male provisioning rate. Possibly our measure of paternal care is not a good representation of paternal investment, thereby failing to show an effect. Potentially, when providing less care, males may gain a reproductive benefit through increased extra-pair fertilizations. These can occur throughout the breeding cycle as reed buntings breed asynchronously and are multibrooded. More needs to be known about the effect of paternal effort on offspring fitness and on a male's reproductive success resulting from other activities than parental care, such as extra-pair mating behavior, to adequately address the third theoretical requirement.

Male reed buntings in an English population have been shown to decrease their provisioning rates when their paternity is reduced (Dixon *et al.* 1994). The change in provisioning is however very marginal: when paternity is reduced by 100% from one nest to the next – a change greater than expected from binomial variation (Lessells 1994) –, provisioning rates only decrease by approximately 0.1 feed per nestling, per hour. This would be a change of 4%, when comparing this to an average provisioning rate of 2.6 feeds per hour per nestling (Dixon 1993). Females do not show any compensation for the decrease in male provisioning rates, possibly because the decrease is very small. As male reed buntings provide care even when they have no paternity in the brood, they may be prone to making large paternity assessment errors.

The absence of a relationship between paternity and paternal care may be due to the absence of reliable cues to assess paternity, or to benefits of reducing care not exceeding costs (Whittingham & Dunn 2001). The reason why males differed in their provisioning behavior as a function of their paternity levels between the populations in England and the Netherlands remains unclear. The sample size in our study ( $n = 10$ ) was similar to that of the English population ( $n = 13$ ; Dixon *et al.* 1994), allowing a proper comparison. The power analysis showed there was a high probability of detecting a relationship of comparable strength to the one found in the English population. Furthermore, the levels of extra-pair paternity

were similar for both populations (this study: 50% of nestlings vs. Dixon *et al.*: 55% of nestlings:  $\chi^2 = 1.04$ ,  $df = 1$ ,  $p = 0.31$ ) and in both populations significant variation in proportions of EPP between broods of the same male was found (Lessells 1994; this study). In both populations, females do not change their provisioning rate in relation to paternity, which may be expected since no egg dumping appears to occur. The difference in female provisioning rate between two nests within a breeding season varied from 0 to 0.23 feeds per hour per nestling in the English population (Dixon *et al.* 1994), compared to a range from 0.05 to 0.54 feeds per hour per nestling in the Dutch population. Males seemed to have a larger variability in provisioning rate in the Dutch population, as the difference in male provisioning rate between two nests ranged from 0 to 0.21 feeds per hour per nestling in the English population (Dixon *et al.* 1994), compared to a range from 0 to 1.6 feeds per hour per nestling in the Dutch population. A range similar to the one in the Dutch population was found in a Polish population, namely 0.8 to 1.8 feeds per hour per nestling (Buchanan 2001). When comparing the proportions of EPP and provisioning rates of two broods of the same pair within the same season, male reed buntings in Poland also failed to show a significant decrease in provisioning rate with reduced paternity ( $p = 0.14$ ,  $n = 13$ ; Buchanan 2001).

Variability in paternal care in response to paternity between populations was also found in other species; e.g. barn swallows (*Hirundo rustica*; Møller 1988; Smith & Montgomerie 1992), pied flycatchers (*Ficedula hypoleuca*; Alatalo & *et al.* 1983; Lifjeld *et al.* 1998) and red-winged blackbirds (Weatherhead *et al.* 1994; Westneat 1995). Whittingham and Dunn (2001) suggest that the absence of cues to assess paternity is not likely to be the cause of variability in paternal care in response to paternity between populations. A difference between populations in males' ability to assess paternity may arise through local environmental conditions. For instance differences in food availability between populations could influence paternity cues such as time-spent mate guarding. The average nestling provisioning rates of reed buntings, as a measure for the food availability, was similar between the three populations (England: 2.6 ( $n = 26$ ) vs. Poland: 2.3 ( $n = 45$ ) vs. The Netherlands: 2.9 ( $n = 29$ ) feeds per hour per nestling (Buchanan 2001; Dixon 1993; this study). Food availability is therefore not expected to influence differences in paternity cues in this species. However, Whittingham and Dunn (2001) argue that local conditions also drive the relative costs and benefits of paternal care, which are expected to be of greater importance.

#### *Food provisioning to within- and extra-pair young*

In the absence of a relationship between paternity and overall paternal care in the reed bunting, discrimination against non-kin may still occur. As female reed buntings are related to all the offspring in the nest, they are not expected to bias

their provisioning behavior (as shown in our results). However, males do experience high levels of cuckoldry, but fail to show any bias when allocating food to individual nestlings. In addition, males have been observed to provision at nests in which they had no paternity at all (Burke *et al.* 1989; Dixon *et al.* 1994; this study). Furthermore, no relationship was found between paternal effort and male survival to the following breeding season and between male provisioning rate and fledging mass of nestlings. Therefore, the costs for a male to provision to unrelated offspring or the benefits for related offspring to be recognized as kin by the father may not be high enough to generate kin discrimination (Johnstone 1997). This study confirms previous studies of a lack of kin recognition in parental care (reviewed by Kempenaers & Sheldon 1996).

To conclude, this study found no indication that male reed buntings decreased their paternal effort in relation to paternity, both between nests of the same male in one season, and between individual nestlings. The availability of cues to assess paternity and costs and benefits of reducing care, which are often neglected (Yezerinac *et al.* 1996), play a crucial role in the relationship between paternity and paternal care. This study included two aspects of costs and benefits of reducing care, namely fledging mass and male survival in relation to paternal effort; however, other aspects (e.g. fledging survival and recruitment, male extra-pair fertilization success) also need to be addressed to understand the trade-off between paternal care and other activities (such as self-maintenance or extra-pair mating behavior). Furthermore, the need to study more than one population per species is stressed, as local circumstances may play an important role in variability in paternal care in relation to paternity.

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## Introduction

Social monogamy is the most common mating system in birds and both sexes of the breeding pair are often involved in providing parental care at different stages of the breeding cycle (Lack 1968; Silver *et al.* 1985). However, molecular paternity analysis has shown that extra-pair paternity (EPP; i.e. offspring of a male other than the male attending the nest) is a widespread phenomenon in birds (Birkhead & Møller 1992; Griffith *et al.* 2002; Westneat *et al.* 1990). As parental effort is considered to be costly (Williams 1966) males are expected to provide less care to offspring sired by other males (Trivers 1972).

Theoretical models, developed to show how males should alter their parental effort when paternity is in doubt, predict three main outcomes. Early models, which assume that parentage is, on average, the same for all matings, that there is no paternity assessment and the only cost of paternal care is missed opportunities of re-mating (Grafen 1980; Maynard Smith 1978) indicated that paternal effort may scarcely or not at all be affected by paternity. After adjusting the assumptions, for instance by giving males the capability of assessing their paternity, other theoretical studies predict that males should reduce paternal care to the brood when certainty of paternity is low (Westneat & Sherman 1993; Whittingham *et al.* 1992; Xia 1992). Finally, some studies predict that extra-pair paternity may not only affect total paternal effort, but also the allocation of care among individual offspring, such that males discriminate against non-kin (Johnstone 1997; Westneat & Sherman 1993).

The adjustment of paternal effort in relation to paternity in an entire brood has been studied in many species (Whittingham & Dunn 2001). Several studies found no adjustment of paternal effort with decreased paternity (e.g. Dickinson 2003; Kempenaers *et al.* 1998; Peterson *et al.* 2001; Westneat 1995; Whittingham *et al.* 1993; Whittingham & Lifjeld 1995), while others have supported the prediction (e.g. Burke *et al.* 1989; Lifjeld *et al.* 1998; Neff 2003; Neff & Gross 2001; Sheldon & Ellegren 1998). For instance in the reed bunting (*Emberiza schoeniclus*), when comparing two broods, in the same season, from the same male, but with different proportions of EPP, high proportions of EPP resulted in lower provisioning rates by the territory male (Dixon *et al.* 1994).

Only two studies have looked specifically at food allocation to individual nestlings in broods of mixed paternity; one on red-winged blackbirds (*Agelaius phoeniceus*; Westneat *et al.* 1995); and one on the common yellowthroat (*Geothlypis trichas*; Peterson *et al.* 2001). Both studies failed to show that males biased their provisioning behavior towards genetic offspring compared to extra-pair offspring. Given the observed level of EPP in both species (red-winged blackbird: 25% of offspring (Westneat 1995); common yellowthroat: 20% of offspring (Peterson *et al.* 2001)), selection pressures may not have been strong enough to