University of Groningen

Male reed buntings do not adjust parental effort in relation to extrapair paternity

Bouwman, KM; Lessells, CM; Komdeur, J; Lessells, C(Kate). M.

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Bouwman, KM., Lessells, CM., Komdeur, J., & Lessells, CK. M. (2005). Male reed buntings do not adjust parental effort in relation to extrapair paternity. *Behavioral Ecology*, *16*(3), 499-506. https://doi.org/10.1093/beheco/ari021

Male reed buntings do not adjust parental effort in relation to extrapair paternity

Karen M. Bouwman,[a] **C(Kate). M. Lessells,**[b] **and Jan Komdeur**[a]

[a]Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 AA Haren, the Netherlands and [b]Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 40, 6666 ZG Heteren, the Netherlands

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 extrapair paternity (EPP, 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 EPP 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 toward 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. *Key words: Emberiza schoeniclus*, extrapair paternity, kin recognition, parental care. *[Behav Ecol 16:499–506 (2005)]*

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 extrapair paternity (EPP; i.e., offspring of a male other than the male attending the nest) is a widespread phenomenon in birds (Birkhead and 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 that the only cost of paternal care is missed opportunities of remating (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 the certainty of paternity is low (Westneat and Sherman, 1993; Whittingham et al., 1992; Xia, 1992). Finally, some studies predict that EPP may not only affect total paternal effort but also the allocation of care among individual offspring, such that males discriminate against nonkin (Johnstone, 1997; Westneat and Sherman, 1993).

The adjustment of paternal effort in relation to paternity in an entire brood has been studied in many species (Whittingham and 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 and Lifjeld, 1995; Whittingham et al., 1993), while others have supported the prediction (e.g., Burke et al., 1989; Lifjeld et al., 1998; Neff, 2003; Neff and Gross, 2001; Sheldon and 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 toward genetic offspring compared to extrapair 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 develop adjustment of provisioning toward own kin in these species (Johnstone, 1997). For that reason, paternal adjustment toward kin in mixed broods should be studied in a species with higher levels of extrapair parentage.

Reed buntings are an excellent species in which to address the question of whether males distinguish between kin and nonkin. They have levels of EPP—55% of nestlings in 86% of nests (Dixon et al., 1994)—that 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

Table 1

Sample sizes (number of nests, pairs, or broods) of video recordings

Year	Total nests	All nests with known paternity	Number of different pairs[a]	Pairs with double broods	Double broods with ΔEPP[b]
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

[a] One nest per pair; individuals only included once (i.e., excluding remated individuals and secondary females).

[b] ΔEPP = difference in proportion of EPP between broods of the same pair.

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 nonkin when allocating food to individual nestlings.

METHODS

Data collection

The reed bunting is a small (males: 19 g; females: 17 g), 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 renest 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 and 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 less than 150 cm. A grid of which each cell was approximately 20 × 40 m 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 aluminum ring and a specific combination of three-color rings for individual recognition. A blood sample (20 μl) 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 blood sampled 2 days after hatching by taking a small blood sample (10 μl) 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 0700 and 1900 h. 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 4 and 6 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 × 3 × 2 cm) connected to a Sony video Hi8 camera recorder (model CCD-TR840E) with line-in function, which recorded for 3 h (90-min tape on long play). 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 ± 0.45 min, $n = 50$). Before recording provisioning behavior, the nestlings were weighed and individually marked on their bill, using a nontoxic 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 aluminum 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 ± 3.27 min ($n = 63$). Of these nests, 48 survived at least until eggs hatched and nestlings could be blood sampled and hence paternity determined (Table 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 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 ± 1.82 min. The provisioning behavior of 31 different pairs was recorded at least once (Table 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.

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 polymerase chain reaction (PCR) method for the sexing of birds (Griffiths et al., 1998). The paternity of the nestlings was analyzed using four fluorescently labeled microsatellite markers: *Esc*μ1, *Esc*μ4, *Esc*μ6 (Hanotte et al., 1994), and *Pdo*μ5 (Griffith et al., 1999). PCR amplifications were performed using a Thermolyne amplitron II thermal

cycler at an initial hot start for 90 s at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at annealing temperature, and 1 min at 72°C. Annealing temperatures were set at 55°C for *Esc*μ1 and *Esc*μ4, at 52°C for *Esc*μ6, and at 50°C for *Pdo*μ5. Each 10-μl mix contained 10–50 ng of DNA, 1.0 μM of each primer, 0.2 mM of each deoxynucleoside triphosphate, 0.05 units of *Taq* polymerase (Advanced Biotechnologies, Columbia, MD), and 0.625 mM $MgCl_2$ in a supplied reaction buffer (final concentration 20 mM $(NH_4)_2SO_4$, 75 mM Tris–HCl, pH 9.0, 0.01% [w/v] Tween). PCR products were diluted two times. Diluted PCR products of *Esc*μ1, *Esc*μ4, and *Pdo*μ5 were multiplexed in a ratio of 2:1:2, after which 1 μl of PCR product (*Esc*μ6) or multiplex mixture was mixed with 1.5 μl of a loading buffer containing 1.1 μl of deionized formamide, 0.18 μl of blue dextran loading dye, and 0.22 μl of internal lane standard (ROX500, Applied Biosystems, Foster City, CA). These samples were denatured by heating at 94°C for 3 min and then placing directly on ice. One microliter of each sample was electrophoresed using a 10% denaturing poly-acrylamide gel on an Advanced 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, extrapair male. The program calculates the likelihood that the territory male is the actual father by using the natural logarithm of the likelihood ratio, the 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 second candidates. The critical values were calcu-lated by entering the following simulation parameters in CERVUS: 10,000 cycles, two candidate parents, and 50% of candidate parents sampled. We succeeded in obtaining 90% of all potentially available genotypes (i.e., 4 loci × [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 and second parents, 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 provision-ing effort between the two broods were compared with the difference in the proportion of EPP between the two broods. The incubation and provisioning effort is expressed as 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 EPP between nests. This yielded a value of $r^2 = .57$, which corresponds to a very large effect size ($r = .75$).

We analyzed variance in the proportion of extrapair young between broods using generalized 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; Lessells CM, personal observation). We therefore attached significance values to deviances or changes in deviance using randomization 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 subgroup and then recalculate the deviance or change in deviance for these randomized 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 randomization tests in which the paternity of nestlings (within- or extrapair) 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 extrapair young between males (when we analyzed 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 randomization tests, we reallocated the observed proportions of extrapair young in the brood (i.e., the number of extrapair young and the 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 analyzed 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 extrapair young than expected by chance if individual nestlings in both broods have the same probability of EPP). In these analyses, the relevant test deviance was the residual deviance after fitting male identity as an explanatory variable. The randomization test was carried out by simulta-neously 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 toward 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- and extrapair nestlings. To avoid pseudoreplication, 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 extrapair 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 (Erdfelder et al., 1996). All data were tested for

Table 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
Incubation				
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)
Feeding rate				
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)

normality, and data that were not normally distributed were transformed to achieve normality, or nonparametric tests were used. Unless stated, means are expressed with standard errors, probability values are two tailed, and the level of significance was set at $p < .05$.

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 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 ± 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 ± 0.11 nestlings ($n = 79$ nests, range 2–6). Females incubated longer and provisioned nestlings at a higher rate than males (Table 2; Wilcoxon signed-rank test—incubation time: $Z = -4.86$, $n = 31$, $p < .001$; provisioning rate: $Z = -3.63$, $n = 31$, $p < .001$). Males assisted in incubating eggs in 35% of cases ($n = 31$ males). There was a significant negative correlation between male and female incubation time ($r_{Spearman} = -.82$, $n = 11$, $p = .002$). 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 $p > .5$, $n = 31$), on the time spent incubating by male or female (general linear model [GLM]— males [after log transformation]: all $p > .2$, $n = 11$; females: all $p > .2$, $n = 31$), or on the male share of incubation (GLM [after log transformation]: all $p > .8$, $n = 11$).

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 $F_{1,31} = 7.03$, $p = .01$, brood size $F_{1,31} = 4.03$, $p = .06$, rest $p > .4$; males: age $F_{1,31} = 12.03$, $p = .002$, rest $p > .3$). A male's

share of provisioning was not related to age of nestlings, time of day, day in season, or brood size (GLM: all $p > .2$, $n = 31$).

EPP

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 intraspecific brood parasitism were found; all nestlings had genotypes consistent with their being offspring of the female attending the nest, that is, 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 extrapair male (2001: 18/38 nestlings versus 2002: 128/256 nestlings; $\chi^2 = 0.017$, df $= 1$, $p = .90$). On average, 80.0% of nests contained at least one extrapair young (2001: 6/10 nests versus 2002: 54/65 nests; $\chi^2 = 1.62$, df $= 1$, $p = .20$). Males had no paternity in 22.7% of nests (2001: 4/10 nests, 2002: 13/65 nests), that is, all offspring were extrapair. 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 produced in the same year were genotyped, the proportion of extrapair young in a brood varied significantly between the 42 broods in the sample (deviance $= 81.89$, df $= 41$, p [randomization test] $= .003$). However, individual males did not differ in the proportion of extrapair young in their broods (change in deviance $= 40.04$, df $= 20$, p [randomization test] $= .82$). Neither did first broods differ systematically from second broods in the proportion of extrapair young (average percentage of EPP nest 1 and nest 2: 51% and 56%; change in deviance $= 0.70$, df $= 1$, p [randomization test] $= .48$). Nevertheless, there was a significant variation between broods within males (residual deviance $= 41.86$, df $= 21$, p [randomization test] $= .029$). This shows that the probability of a nestling having EPP differs between the broods produced by a single male in the same year.

Paternity and parental care

Care toward entire broods
Incubation observations and EPP levels were available for 12 pairs (consisting of the same partners remaining on the same territory) that produced two clutches in a single season (Table 1).
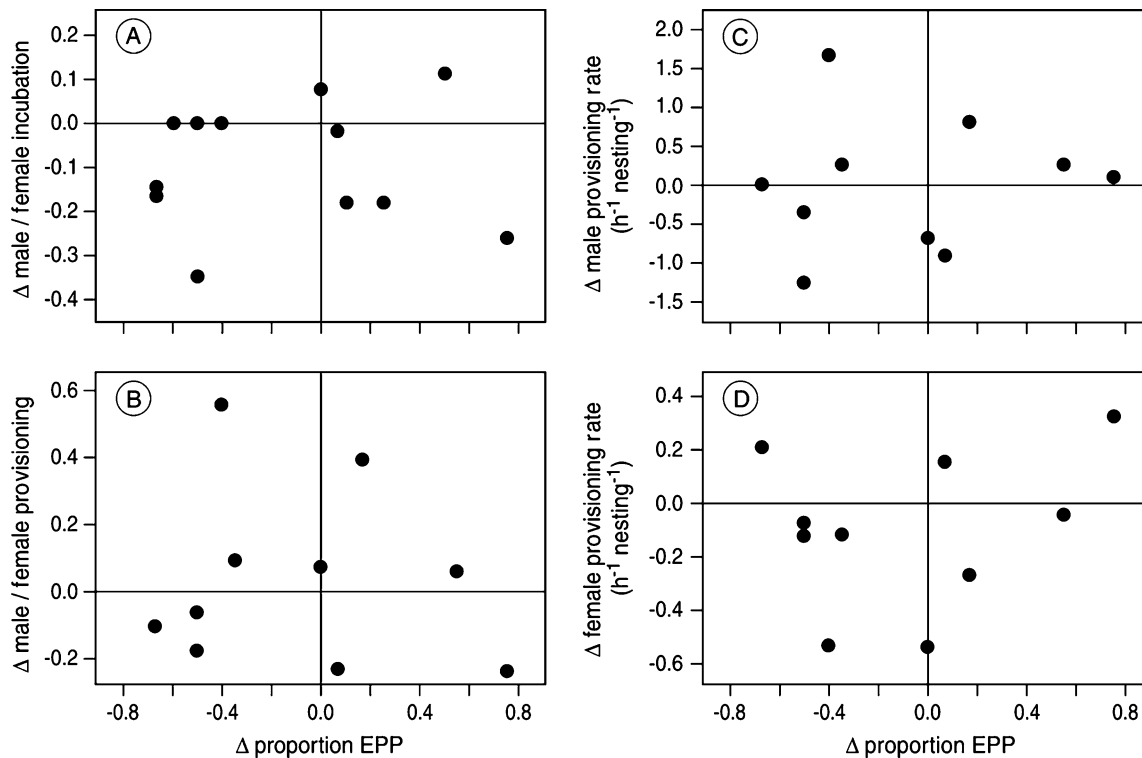
Figure 1
The relationship between the difference in the proportion of extrapair paternity (EPP) between two broods of the same pair and (a) the difference in male share of total incubation between two broods ($r = .03$, $p = .92$, $n = 12$); (b) the difference in male share of total provisioning between two broods ($r = -.11$, $p = .77$, $n = 10$); and the difference in provisioning rate per hour per nestling, corrected for nestling age (unstandardized residuals) for (c) males ($r = .09$, $p = .80$, $n = 10$) and (d) females ($r = .24$, $p = .51$, $n = 10$).

Eleven of these pairs had numerically different proportions of EPP in their first and second broods; overall, for the 12 pairs, there was a statistically significant variation between the two broods within-pairs (residual deviance = 25.95, df = 12, p [randomization test] = .040). We found no relationship between the difference in proportion of EPP between broods and the difference in male share of incubation (Figure 1a). Provisioning observations were available for 10 pairs that raised more than one brood in a season (Table 1). Proportions of EPP differed numerically between the first and second broods in nine of these pairs; overall, for the 10 pairs, there was a statistically significant variation between the two broods within the pairs (residual deviance = 22.61, df = 10, p [randomization test] = .013). No relationship was found between difference in proportion of EPP and difference in male share of provisioning (Figure 1b) or difference in maternal and paternal provisioning rates per hour per nestling (Figure 1c,d; 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 $p > .85$ for $\alpha = 0.05$ ($r = .75$, 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 of both incubation and provisioning between nests with no EPP and nests with at least one extrapair offspring (Mann-Whitney U test—incubation: $U = 81.0$, $n_{\text{no EPP}} = 8$, $n_{\text{EPP}} = 23$, $p = .59$; provisioning: $U = 72.5$, $n_{\text{no EPP}} = 6$, $n_{\text{EPP}} = 25$, $p = .90$). Furthermore, there was no relationship between male share of incubation and provisioning and the proportion of EPP in the brood among all males (incubation: $r_s = -.022$, $n = 31$, $p = .91$; provisioning: $r_s = .16$, $n = 31$, $p = .38$).

Costs and benefits of paternal care
Nestlings of 33 nests were weighed before fledging. In one nest, some nestlings died before 7 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 ($r = .15$, $n = 32$, $p = .40$). Furthermore, there was no correlation between male provisioning rate and average fledging mass (provisioning rate corrected for nestling age; $r = .04$, $n = 32$, $p = .83$). A negative trend was found between female provisioning rate and average fledging mass (provisioning rate corrected for nestling age; $r = -.32$, $n = 32$, $p = .07$). Total provisioning rate showed no relationship with average fledging mass (provisioning rate corrected for nestling age; $r = .17$, $n = 32$, $p = .36$).

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 ($U = 53.5$, $n_{\text{dead}} = 9$, $n_{\text{survive}} = 16$, $p = .30$).

Food provisioning to within- and extrapair 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: $Z = -0.412$, $p = .68$, $n = 30$; females: $Z = -0.738$, $p = .46$, $n = 30$). 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, extrapair young had similar mass as within-pair young when correcting for age (GLMM: $\chi^2 = 0.027$, $p = .87$, $n = 88$
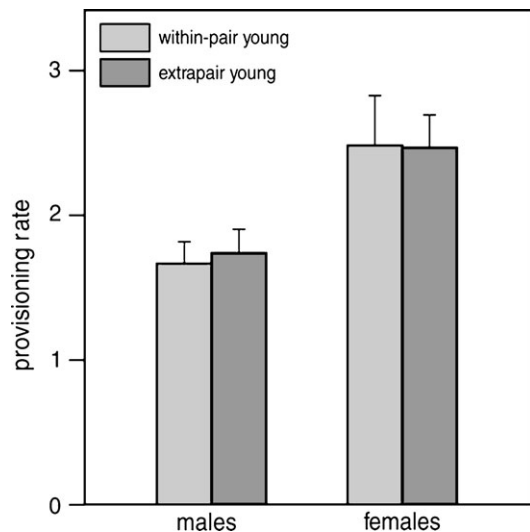
Figure 2
Average provisioning rate per hour per nestling (±SE) directed to either within- or extrapair young by males and females in broods of mixed paternity (Wilcoxon—males: $Z = -0.85$, $n = 20$; $p = .39$; females: $Z = -0.024$, $n = 20$; $p = .98$).

nestlings in 20 nests). Extrapair young received the same number of feeds as within-pair young (Figure 2).

DISCUSSION

Paternity and paternal care: care toward entire broods

For males to adjust their paternal care toward the entire brood in relation to their paternity, theoretical models require three assumptions to be met (Westneat and Sherman, 1993; Whittingham et al., 1992). First, levels of EPP 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 easily using molecular techniques; however, the certainty of paternity from a male point of view cannot be measured (Kempenaers and Sheldon, 1997). Different studies trying to experimentally decrease certainty of paternity generated different results, as some did (Sheldon and Ellegren, 1998) and others did not (Kempenaers 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 and 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 extrapair copulations (Ewen and 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 a 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, for example, 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 extrapair 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 extrapair copulations. Thus, possible cues may be (1) absence of the female during her fertile period, (2) the number of intruding males into the territory, or (3) 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 and Elgar, 1997; Smith, 1995; reviewed in Magrath and Komdeur, 2003). The costs of reducing parental care seem obvious in terms of decreased survival of offspring (Bart and 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 toward 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 extrapair 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 extrapair 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 and 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 that there was a high probability of detecting a relationship of comparable strength to the one found in the English population. Furthermore, the levels of EPP were similar for both populations (this study: 50% of nestlings versus Dixon et al.: 55% of nestlings: $\chi^2 = 1.04$, df = 1, $p = .31$), 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 0.8–1.8 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 ($p = .14$, $n = 13$; Buchanan, 2001).

Variability in paternal care in response to paternity between populations was also found in other species; for example, barn swallows, *Hirundo rustica* (Møller, 1988; Smith and 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 the 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 [$n = 26$] versus Poland: 2.3 [$n = 45$] versus 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 extrapair young

In the absence of a relationship between paternity and overall paternal care in the reed bunting, discrimination against nonkin 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 fol-

lowing 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 and 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 extrapair fertilization success) also need to be addressed to understand the trade-off between paternal care and other activities (such as self-maintenance or extrapair 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.

REFERENCES

Alatalo RV, Lundberg A, Stahlbrandt K, 1983. Do pied flycatcher males adopt broods of widow females? Oikos 41:91–93.

Bart J, Tornes A, 1989. Importance of monogamous male birds in determining reproductive success—evidence for house wrens and a review of male-removal studies. Behav Ecol Sociobiol 24:109–116.

Birkhead TR, Møller AP, 1992. Sperm competition in birds. Evolutionary causes and consequences. London: Academic Press.

Buchanan G, 2001. Mate choice in reed buntings (*Emberiza schoeniclus*) (PhD dissertation). Sunderlund, UK: University of Sunderland.

Burke T, Davies NB, Bruford MW, Hatchwell BJ, 1989. Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. Nature 338:249–251.

Cramp S, Perrins CM, 1994. Handbook of the birds of Europe, the Middle East, and North Africa. IX: birds of the western Palearctic: buntings and New World warblers. Oxford: Oxford University Press; 276-294.

Davies NB, Hatchwell B, Robson T, Burke T, 1992. Paternity and parental effort in dunnocks *Prunella modularis*: how good are male chick-feeding rules? Anim Behav 43:729–746.

Dickinson JL, 2003. Male share of provisioning is not influenced by actual or apparent loss of paternity in western bluebirds. Behav Ecol 14:360–366.

Dixon A, 1993. Parental investment and reproductive success in the reed bunting (*Emberiza schoeniclus*), investigated by DNA fingerprinting (PhD dissertation). Leicester, UK: University of Leicester.

Dixon A, Ross D, O'Malley SLC, Burke T, 1994. Paternal investment inversely related to degree of extra-pair paternity in the reed bunting. Nature 371:698–700.

Dunn PO, Whittingham LA, Lifjeld JT, Robertson RJ, Boag PT, 1994. Effects of breeding density, synchrony, and experience on extra-pair paternity in tree swallows. Behav Ecol 57:1071–1081.

Erdfelder E, Faul F, Buchner A, 1996. A general power of analysis program. Behav Res Methods 28:1–11.

Ewen JG, Armstrong DP, 2000. Male provisioning is negatively correlated with attempted extrapair copulation frequency in the stitchbird (or hihi). Anim Behav 60:429–433.

Grafen A, 1980. Opportunity cost, benefit and degree of relatedness. Anim Behav 28:967–968.

Griffith SC, Örnborg J, Russel AF, Andersson S, Sheldon BC, 2003. Correlations between ultraviolet coloration, overwinter survival and offspring sex ratio in the blue tit. J Evol Biol 16:1045–1054.

Griffith SC, Owens IPF, Thuman KA, 2002. Extra-pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol 11:2195–2212.

Griffith SC, Stewart IRK, Dawson DA, Owens IPF, Burke T, 1999. Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (Passer domesticus): is there an 'island effect'? Biol J Linn Soc Lond 68:303–316.

Griffiths R, Double MC, Orr K, Dawson RJG, 1998. A DNA test to sex most birds. Mol Ecol 7:1071–1075.

Hanotte O, Zanon C, Pugh A, Greig C, Dixon A, 1994. Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting Emberiza schoeniclus. Mol Ecol 3:529–530.

Johnstone RA, 1997. Recognition and the evolution of distinctive signatures: when does it pay to reveal identity? Proc R Soc Lond B 264:1547–1553.

Kempenaers B, Lanctot RB, Robertson RJ, 1998. Certainty of paternity and paternal investment in eastern bluebirds and tree swallows. Anim Behav 55:845–860.

Kempenaers B, Sheldon BC, 1996. Why do male birds not discriminate between their own and extra-pair offspring? Anim Behav 51:1165–1173.

Kempenaers B, Sheldon BC, 1997. Studying paternity and paternal care: pitfalls and problems. Anim Behav 53:423–427.

Lack D, 1968. Ecological adaptations for breeding in birds. London: Methuen.

Lessells K, 1994. Baby bunting in paternity probe. Nature 371:655–656.

Lifjeld JT, Slagsvold T, Ellegren H, 1998. Experimentally reduced paternity affects paternal effort and reproductive success in pied flycatchers. Anim Behav 55:319–329.

Magrath MJL, Elgar MA, 1997. Paternal care declines with increased opportunity for extra-pair matings in fairy martins. Proc R Soc Lond B 264:1731–1736.

Magrath MJL, Komdeur J, 2003. Is male care compromised by additional mating opportunity? Trends Ecol Evol 18:424–430.

Manly BFJ, 1997. Randomization, bootstrap and Monte Carlo methods in biology, 2nd ed. London: Chapman and Hall.

Marshall TC, Slate J, Kruuk L-EB, Pemberton JM, 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655.

Maynard Smith J, 1978. The evolution of sex. Cambridge: Cambridge University Press.

Møller AP, 1988. Paternity and paternal care in the swallow, Hirundo rustica. Anim Behav 36:996–1005.

Neff BD, 2003. Decisions about paternal care in response to perceived paternity. Nature 422:716–719.

Neff BD, Gross MR, 2001. Dynamic adjustment of parental care in response to perceived paternity. Proc R Soc Lond B 268:1559–1565.

Nur N, 1984. The consequences of brood-size for breeding blue tits I. Adult survival, weight change and the cost of reproduction. J Anim Ecol 53:479–496.

O'Malley SLC, 1993. Mating behaviour and reproductive success in the reed bunting Emberiza schoeniclus (PhD dissertation). Leicester, UK: University of Leicester.

Peterson KA, Thusius KJ, Whittingham LA, Dunn PO, 2001. Allocation of male parental care in relation to paternity within and among broods of the common yellowthroat (Geothlypis trichas). Ethology 107:573–586.

Richardson DS, Jury FL, Blaakmeer K, Komdeur J, Burke T, 2001. Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (Acrocephalus sechellensis). Mol Ecol 10:2263–2273.

Sheldon BC, Ellegren H, 1998. Paternal effort related to experimentally manipulated paternity of male collared flycatchers. Proc R Soc Lond B 265:1737–1742.

Sheldon BC, Rasanen K, Dias PC, 1997. Certainty of paternity and paternal effort in the collared flycatcher. Behav Ecol 8:421–428.

Silver R, Andrews H, Ball GW, 1985. Parental care in an ecological perspective: a quantitative analysis of avian subfamilies. Am Zool 823–840.

Smith HG, 1995. Experimental demonstration of a trade-off between mate attraction and paternal care. Proc R Soc Lond B 260:45–51.

Smith HG, Montgomerie R, 1992. Male incubation in barn swallows: the influence of nest temperature and sexual selection. Condor 94: 750–759.

Trivers RL, 1972. Parental investment and sexual selection. In: Sexual selection and the descent of man (Campbell B, ed). London: Heinemann.

Weatherhead PJ, Montgomerie R, Gibbs HL, Boag PT, 1994. The cost of extra-pair fertilizations to female red-winged blackbirds. Proc R Soc Lond B 258:315–320.

Westneat DF, 1995. Paternity and paternal behavior in the red-winged blackbird, Agelaius phoeniceus. Anim Behav 49:21–35.

Westneat DF, Clark AB, Rambo KC, 1995. Within-brood patterns of paternity and paternal behavior in red-winged blackbirds. Behav Ecol Sociobiol 37:349–356.

Westneat DF, Sherman PW, 1993. Parentage and the evolution of parental behavior. Behav Ecol 4:66–77.

Westneat DF, Sherman PW, Morton ML, 1990. The ecology and evolution of extrapair copulations in birds. In: Current ornithology, vol. 7 (Power DM, ed). New York: Plenum Press.

Whittingham LA, Dunn PO, 2001. Male parental care and paternity in birds. In: Current ornithology, vol. 16 (Nolan V Jr, Thompson CF, eds). New York: Kluwer Academic/Plenum Publishers.

Whittingham LA, Dunn PO, Robertson RJ, 1993. Confidence of paternity and male parental care: an experimental study in tree swallows. Anim Behav 46:139–147.

Whittingham LA, Lifjeld JT, 1995. High paternal investment in unrelated young: extra-pair paternity and male parental care in house martins. Behav Ecol Sociobiol 37:103–108.

Whittingham LA, Taylor PD, Robertson R, 1992. Confidence of paternity and male parental care. Am Nat 139:1115–1125.

Williams GC, 1966. Natural selection, the cost of reproduction, and a refinement of Lack's principle. Am Nat 100:687–690.

Wolf L, Ketterson ED, Nolan V, 1988. Paternal influence on growth and survival of dark-eyed junco young: do parental males benefit? Anim Behav 36:1601–1618.

Wright J, 1998. Paternity and paternal care. In: Sperm competition and sexual selection (Birkhead TR, Møller AP, eds). London: Academic Press.

Xia X, 1992. Uncertainty of paternity can select against paternal care. Am Nat 139:1126–1129.

Yezerinac S, Weatherhead PJ, Boag P, 1996. Cuckoldry and lack of parentage-dependent paternal care in yellow warblers: a cost–benefit approach. Anim Behav 52:821–832.