The illusion of monogamy

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Abstract

Extra-pair paternity is an important aspect of reproductive strategies in many species of birds. Given that in most species females control whether fertilisation occurs, they are expected to benefit in some way from the extra-pair matings. In this study we use patterns of extra-pair paternity in broods of individual reed buntings (*Emberiza schoeniclus*), both within and between seasons, to test three hypothesised female benefits: (1) assessing potential future partners, (2) seeking genetic diversity, and (3) seeking good genes. Reed buntings are socially monogamous, multi-brooded passerines with extremely high levels of extra-pair paternity. We studied a population of reed buntings in the Netherlands in 2002 and 2003; 51% of offspring in 74% of nests were extra-pair. We show that patterns of extra-pair paternity do not support the hypothesis that females assess future partners through extra-pair paternity. The genetic diversity hypothesis is not supported either, as (i) more broods than expected contained no extra-pair young, (ii) more broods than expected were sired by a single male, either the social male or an extra-pair male, and (iii) individual males differed in the proportion of extra-pair young in their broods. Instead, these patterns support the good genes hypothesis, which is in agreement with findings showing that older males are more successful. However, some patterns were consistent with the good genes hypothesis in one, but not in both years. We discuss whether females may seek other genetic benefits in addition to good genes.

PART I

Benefits of extra-pair paternity to females

CHAPTER 2

How female reed buntings benefit from extra-pair mating behaviour: testing hypotheses through patterns of paternity in sequential broods

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Introduction

Social monogamy, where a male and female form a pair and collaborate in raising offspring, is the most common mating system in birds (Lack 1968). However, recently molecular techniques have revealed that genetic monogamy is rare, as it is found in only 14% of social monogamous passerine species studied so far (Griffith *et al*. 2002). Males may gain direct benefits by engaging in extra-pair copulations (EPCs), as these can lead to extra-pair fertilisations (EPFs), increasing a male's reproductive output without additional paternal investment (Westneat *et al*. 1990; Birkhead & Møller 1992). Since the maximum reproductive success for females is limited by the number of eggs they can produce, the benefits to females of EPCs are less clear. Given that females have at least some control over whether insemination occurs and EPCs are likely to be costly (Birkhead & Møller 1992), females are only expected to engage in EPCs if there are potential benefits. Females may gain direct benefits, such as fertility insurance (Wetton & Parkin 1991), foraging rights on the extra-pair male's territory (Gray 1997) or the opportunity to assess males as potential future partners (Heg *et al*. 1993; Cézilly & Nager 1995). On the other hand, females may gain potential indirect benefits such as increasing the genetic diversity (Brooker *et al*. 1990) or the absolute quality ('good genes'; e.g. Kempenaers *et al*. 1992; Hasselquist *et al*. 1996; Sheldon *et al*. 1997; Kempenaers *et al*. 1997; Richardson & Burke 1999) of her offspring. Alternatively, if the quality of the offspring depends on the combination of maternal and paternal genotypes, females may seek to mate with males that are more genetically compatible (Johnsen *et al*. 2000; Tregenza & Wedell 2000; Foerster *et al*. 2003; reviewed in Jennions & Petrie 2000).

One way to investigate the validity of these hypotheses is to determine patterns of EPP in a series of broods produced by the same pair. These patterns enable us to test between predictions that are specific to three of the hypotheses mentioned above. Firstly, if females aim to increase the genetic diversity of their offspring, all females are expected to engage in EPCs; therefore most or all broods are expected to contain extra-pair young (EPY) that are sired by a number of different males (Westneat *et al*. 1990). Secondly, if females seek to increase the genetic quality of their offspring (good genes), only females paired with a low quality male (defined as a male with few extra-pair young (EPY)) should engage in EPCs; broods are expected to be sired by a single, preferred, male (Westneat *et al*. 1990) and paternity in sequential broods of the same pair is expected to be consistent (females being either faithful or unfaithful). As high quality males are expected to be preferred both by their own females as well as by extra-pair females, we expect a negative relationship between the number of EPY a male gains in a season, and the percentage of paternity he loses in his own nests (Kempenaers & Dhondt 1993). Females are expected to choose the same extra-pair male for the next

breeding attempt, if he is still alive (Weatherhead 1999). Thirdly, if females use EPCs to assess future mates, females with EPP in the first nest are expected to be more likely to change social mates before the next breeding attempt in the same or the following season than faithful females, and possibly select the former extra-pair mate as the next social partner (Heg *et al*. 1993). Only a few studies have examined variation in paternity for the same individuals between breeding seasons (Dunn *et al*. 1994; Yezerinac *et al*. 1996; Weatherhead 1999), and among broods within years (double-brooded species; Dixon *et al*. 1994; Yezerinac *et al*. 1996; Beheler & Rhodes 2003). However, none of these studies use the observed patterns of paternity to their full extent in distinguishing between these female benefit hypotheses.

We studied patterns of paternity in a population of reed buntings (*Emberiza schoeniclus*) in The Netherlands. The reed bunting is a small (18g), sexually dimorphic passerine. Social monogamy is the most common mating system, with high levels of EPP (50% of offspring in 80% of nests; Bouwman *et al*. 2005). Polygyny does occur occasionally (6% of males; Bouwman *et al*. 2005), often following the death of the neighbouring male. This species is capable of raising two successful broods in a single season. Adults show high site fidelity between breeding seasons (O'Malley 1993), thereby presenting an ideal opportunity to study patterns of EPP within individuals, both within and between seasons. In this study we investigate whether patterns of EPP in sequential nests of the same individuals match the predictions that arise from the three hypotheses to explain why females may benefit from engaging in EPCs. These hypotheses state that females may use EPCs to: (1) gain genetic diversity for their offspring, (2) gain good genes for their offspring, or alternatively (3) assess future mates.

Materials and Methods

Data collection

In 2002 and 2003 a population of reed buntings was studied in a 13-ha study site, on the island of Noorderplaat (45 ha) in the De Biesbosch National Park in the Netherlands (51º45´N, 4º45´E). The study site had an approximate density of 3 pairs per hectare. The vegetation consisted of a combination of reeds (*Phragmites australis*), soft rush (*Juncus effusus*), hard rush (*Juncus inflexus*) and various species of grasses. The height of the vegetation varied from 50 to 300 cm, with most of the vegetation below 150 cm. A grid with cells of approximately 20 x 40 metres was laid across the area for mapping territories and nests, using two-metre high bamboo poles (individually marked with coloured tape) placed at every intersection (figure 2.2).

Males arrived before females on the breeding site by the end of February, and occupied a territory. Pair formation occurred one to two months before the onset of breeding. Territories were mapped by plotting the location of the singing posts of males. No territorial conflicts were observed; therefore strict boundaries could not be drawn between neighbouring territories. In 2002 and 2003, respectively, 44 and 35 males held a territory in our study area. We did not see any floating males in our study site.

Within our study site, adult reed buntings were caught using mist nets, mainly during the spring arrival period (end of February to beginning of April) and during the incubation period. Birds were ringed with a numbered aluminium ring and a specific combination of three colour rings, one above the aluminium ring and two on the other leg, for individual recognition. A blood sample (20 μl) was taken from the brachial vein and stored in 96% ethanol at room temperature. The identities of the male and female belonging to a nest (territorial birds) were determined by direct or video observations of colour-ringed birds protecting the nest, incubating and feeding nestlings (for a description of the method using video recordings see chapter 4).

Nests are built on or just above the ground and are only used for a single nesting attempt. Clutches consisted of 2 to 6 eggs (4.16 ± 0.13, $n = 144$). Nests were located through systematic searches that flushed females off the nest, or through observing territorial birds for any nest-related activities. Nestlings were blood-sampled two days after hatching by taking a small blood sample (10 μl) from the leg vein. If eggs did not hatch, we inspected them for embryonic development, which, if present, was used as a source of DNA. To increase the number of DNA samples from sequential nests, we induced renesting by removing the first clutches of seventeen pairs after six to eleven days of incubation (2002: 6 pairs, 2003: 11 pairs; total incubation period: twelve to fourteen days; under licence of the Dutch ethical committee). The embryos of the first clutch were then used as a source of DNA, while the replacement clutch was blood-sampled after hatching.

Within our study site we found 97.4% of all nests that fledged young ($n = 78$); only in two cases did we see fledglings without locating the nest. However, due to high levels of predation (see below), we were unable to locate all nests in the study area. As there was no obvious difference in risk of predation across the site, we believe we obtained a random sample of individual reproductive success for all males in our site.

As was also found in a previous study of reed buntings (O'Malley 1993), there was a high probability of predation at both the egg and nestling stage (67%, $n = 46$ nests; sampled in 2001 without nest protection). The main predators were stoats (*Mustela erminea*) and polecats (*Mustela putorius*); no avian predators were seen in the study area. Therefore in the 2002 and 2003 seasons, after clutch

completion a nest was protected against predators using exclosures, of 30 cm height and a diameter of approximately 1 metre, made of wire netting and bamboo sticks and pinned down with tent pegs. Adults were accustomed to the exclosure by putting it around the nest but lowering it to the ground, initially enabling them to walk to their nest. After an hour we checked if the adults had returned to their eggs, indicating they had accepted the presence of the exclosure. If so, we increased the height of the exclosure in 4 steps (10 cm per 1 to 2 hours), allowing the adults to adjust their flyway into the nest. If the eggs were found to be cold, we removed the exclosure and repeated this procedure two days later. It is possible that the female abandoned her clutch in a maximum of 3 out of 83 nesting attempts due to the exclosure trials, but as none were resighted in the area subsequently these females may themselves have been predated. Three birds did not accept the exclosure after three attempts, after which we left these nests unprotected. To further minimise the risk of predation nest visits were kept to a minimum. Nest protection was removed when the nestlings were approximately 5 days of age. In 2002 the nest protection was found to be very effective, as 93% of broods were not predated when protected (n = 42). Apparently predators learned to circumvent the nest protection, as the three nests that were predated were among the last nests of the season. In 2003 broods were occasionally predated early in the season; after 37% of the protected nests had been predated (n = 27), we stopped protecting the nests (halfway through the breeding season).

Paternity analysis

DNA was extracted from blood and tissue samples using salt extraction (Richardson *et al*. 2001). The paternity of the nestlings was analysed using six fluorescently-labelled microsatellite markers: *Escμ*1, *Escμ*4, *Escμ*6 (Hanotte *et al*. 1994), *Pdoμ5* (Griffith *et al*. 1999), *Mcyμ*4 (Double *et al*. 1997) and *Ppi*2 (Martinez *et al*. 1999). PCR amplifications were performed using a Thermolyne amplitron II or a Corbett Research thermal cycler using an initial hot start for 3 minutes at 94°C, followed by 30 cycles of 1 minute at 94°C, 1 minute at annealing temperature and 1 minute at 72°C. Annealing temperatures were set at 55°C for *Escμ*1, *Escμ*4 and *Mcyμ*4, at 52°C for *Escμ*6, at 50°C for *Pdoμ5* and at 53°C for *Ppi*2. Each 10-μl mix contained 10-50 ng of DNA, 1.0 μM of each primer, 0.2 μM of each dNTP, 0.05 units of *Taq* polymerase (Advanced Biotechnologies) and 0.625 mM $MgCl_2$ in the 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 (except for *Ppi*2) were diluted by adding one volume of H_20. Diluted PCR-products were multiplexed in different combinations. *Escμ*1, *Escμ*4 and *Pdoμ5* were multiplexed in a ratio of 2:1:2 and *Escμ*6, *Mcyμ*4 and *Ppi*2 in a ratio of 1:1:2 for samples of 2002; *Escμ*1, *Mcyμ*4 and *Ppi*2 were multiplexed in a ratio of 1:2:2 and *Escμ*4, *Escμ*6 and *Pdoμ5* in a ratio of 2:2:1 for samples of 2003. One

microlitre of multiplex-mixture was mixed with 1.5 μl of a loading buffer containing 1.1 μl of deionised formamide, 0.18 μl of blue dextran loading dye and 0.22 ml of internal lane standard (ROX500, Applied Biosystems). These samples were denatured by heating at 94° for 2 minutes and then placing directly on ice. One microlitre of each sample was electrophoresed using a 10% denaturing polyacrylamide gel on an Applied Biotechnologies (ABI) 377 XL DNA sequencer. DNA fragments were analysed 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 (version 2.0) (Marshall *et al*. 1998). This program assesses the confidence of paternity assignment using criteria generated through a simulation taking into account allele frequencies in the population, the number of possible candidate parents, the proportion of candidate parents sampled, and the percentage of missing genetic data and genotyping errors. The simulation derives a criterion (the delta value) that estimates the critical difference between the LOD - the natural logarithm of the likelihood ratio - scores of the first and second most likely candidate parents at a level of >95% confidence and >80% confidence.

In the parentage analysis, we first assessed whether the female and male observed at the nest were the actual parents (using 'exclusion analysis'). Firstly, the maternity of the territorial female was assessed. Then the paternity of the territorial male was assessed using the mother as 'known parent' in the analysis. Using a 'known parent' increases the confidence level when determining the second parent (in this case the father). CERVUS was given the choice between two candidate parents: the territorial female or male and one potential, but unsampled, other female or male. The delta values for the exclusion analysis were calculated by entering the following simulation parameters in CERVUS: 10,000 cycles, 2 candidate parents present and 50% of candidate parents sampled. Genotypes were available for 99% of all loci and we assumed that 0.01% of loci were mistyped. The chosen level of typing error determines the number of mismatches CERVUS will allow when assigning paternity (figure 2.1A,B).

Next, paternity was assigned to offspring that were identified as not being sired by the territorial male. The genotype of every offspring was set against the genotypes of all ringed males present in the study site in that year (so called 'open analysis'), again using the mother as 'known parent'. The critical values were calculated by entering the following simulation parameters in CERVUS: 10,000 cycles, 68 (2002) or 62 (2003) candidate parents present, 90% of candidate parents sampled, 99% of loci typed, and 0.01% of loci mistyped. In 11 cases (2%) an assignment to a specific male was 'forced', when all the following requirements were fulfilled. If a specific male was not ranked as the best candidate by CERVUS, but that male was an extra-pair male which had already sired other offspring in that particular nest, and there were no mismatches between the genotype of the

offspring and that male, and the first ranked male did not father any other offspring in that nest, then we decided to accept the specific male as the genetic father (figure 2.1C).

None of the loci deviated significantly from Hardy-Weinberg equilibrium. Using the observed allele frequencies, CERVUS calculated a total exclusionary power for the six microsatellite loci; in both years the probability of exclusion was 0.993 for the first parent and 0.999 for the second parent.

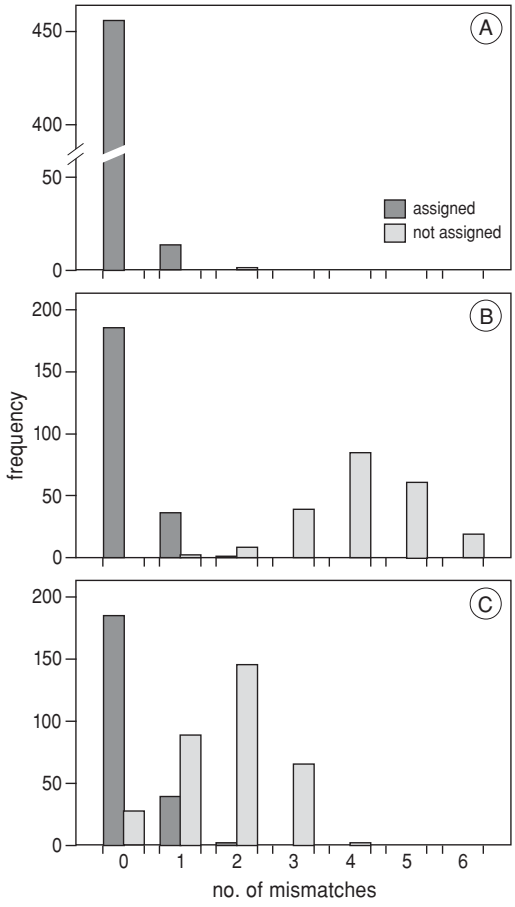

Figure 2.1. Frequency distribution of the number of mismatching alleles between (A) offspring and social mother, (B) offspring, known mother and social male, and (C) offspring, known mother and candidate male, when assigning paternity of extra-pair young (determined to be extra-pair in the exclusion analysis) to an extra-pair father.

Data analyses

Unless it is specifically stated that all nests were used, only nests from ringed males containing more than one offspring were included in the analyses, to avoid over-estimating the number of nests with no or all EPY. To avoid pseudo-replication, only one randomly selected nest for each pair was included where appropriate. Statistical analyses were performed using SPSS 11.0.1 (2001) and SAS. Non-parametric tests were used for data that were not normally distributed. Means are expressed with standard errors, probability values are two-tailed and the level of significance was set at $p < 0.05$.

If a brood contained offspring sired by more than one extra-pair male, then both males were included when analysing the mean distance between the cuckolded male's territory and the extra-pair male. When determining the total number of EPFs that a male gained, the analysis was performed both including all males in the study site, and excluding the males from territories on the edge of the study site (outer territories). This was done in order to avoid under-estimating the number of EPFs that the peripheral males gained, as we did not sample nests outside our study site.

We tested whether there was an excess of broods with no extra-pair young, or with no or all extra-pair young, by fitting binomial distributions to the number of extra-pair young per brood, and comparing the observed and expected distributions using a chi-square goodness-of-fit test. The number of broods expected to contain extra-pair young based on binomial distributions is calculated using the following formula: expected broods = ${}_nC_X \cdot p^X \cdot q^{n-X} \cdot N$, where ${}_nC_X$ (i.e. binomial coefficient) = $n! / ((n-X)!X!)$, p = proportion of EPP in the population, q = 1- p, n = brood size, X = number of EPY per brood, and N = number of broods of size n (Sokal & Rohlf 1994; Perreault *et al*. 1997). More generally, 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 in SAS. We 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. For a more detailed desription of the procedure see chapter 4).

Results

Paternity assignment

In two years, 501 offspring were typed from 129 nests (2002: 280 offspring from 71 nests, 2003: 221 offspring from 58 nests). In 88% of these cases both the territorial male and female were known (2002: 61/71 nests, 2003: 53/58 nests); only the female was known in 5% of nests (2002: 4/71 nests, 2003: 3/58 nests) and only the male was known in 6% of nests (2002: 6/71 nests, 2003: 2/58 nests). Within the nests with a sampled territorial female, 97% of offspring (2002: n = 254; 2003: n = 215) had genotypes consistent with their being offspring of the female attending the nest at a 95% confidence level, and almost 100% at an 80% confidence level. In 2002, for one offspring CERVUS excluded the territorial female to be the mother, which is expected to be the result of egg dumping (0.2%, n = 501 offspring). This nest was excluded from further analysis.

Paternity was assigned to 87% of offspring (n = 501) with 95% confidence, and to 88% of offspring with 80% confidence. Paternity could not be assigned to 49 young (10%). On average, 51% of all young were extra-pair (2002: 143/262, 2003: 98/211; χ^2 = 3.10, df = 1, p = 0.08) and 74% of all nests contained at least one EPY (2002: 56/66, 2003: 33/55; χ^2 = 9.52, df = 1, p = 0.002). As the frequency of nests containing EPP differed significantly between years, we addressed the two years both together and separately in subsequent analyses.

Distribution of EPP

On average, 81% of females produced at least one EPY in a year (2002: 35/39; 2003: 23/33; χ^2 = 4.59, df = 1, p = 0.03) and 70% of all EPFs occurred between males and females from neighbouring territories (figures 2.2 and 2.3A). In total, six exchanges in paternity were observed between two males (2002: n = 4; 2003: n = 2). EPY were not evenly distributed among broods (figure 2.3B); in both years significantly more broods without EPY were observed than expected from a binomial distribution (table 2.1), and the proportion of EPY varied significantly among broods (randomisaton test; 2002: deviance = 107.93, df = 43, $p < 0.001$; 2003: deviance = 112.62, df = 33, $p < 0.001$; 2002 + 2003: deviance = 171.12, df = 67, $p < 0.001$). Broods were sired by one to four different males (figure 2.3C). Out of 78 broods, 29 were sired by a single male, either the social or an extra-pair partner (2002: 16% by social male, 25% by extra-pair male, n = 44; 2003: 41% by social male, 21% by extra-pair male, n = 34). The number of broods sired by a single male was significantly larger than the combined number of broods expected to have either none or all EPY from a binomial distribution (2002: χ^2 = 17.68, df = 1, n = 44, p < 0.001; 2003: χ^2 = 46.51, df = 1, n = 34, $p < 0.001$; 2002 +2003: χ^2 = 67.38, df = 1, n = 68, $p < 0.001$; table 2.1).

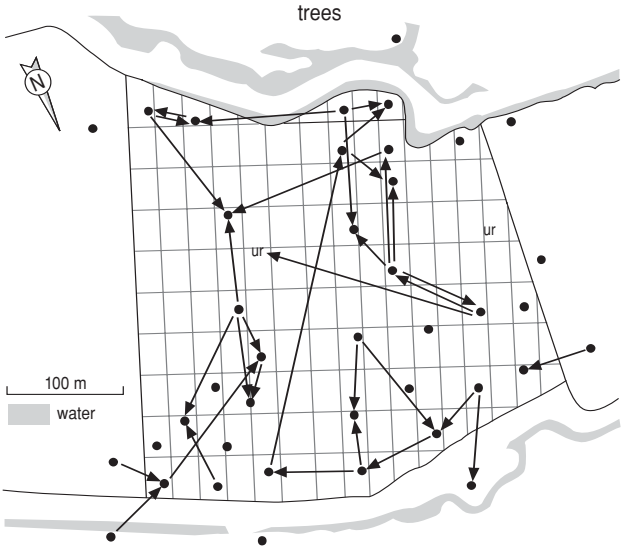

Figure 2.2. Map showing the extra-pair mating behaviour in 2003. The principal song-post of each ringed male, as an indicator of the centre of a territory, is identified by a spot. Unringed males are identified by "ur". The grid used for locating territories is shown; every section is approximately 20 by 40 metres. Arrows originate in the territory of the extra-pair male, and point at the territory of the cuckolded male.

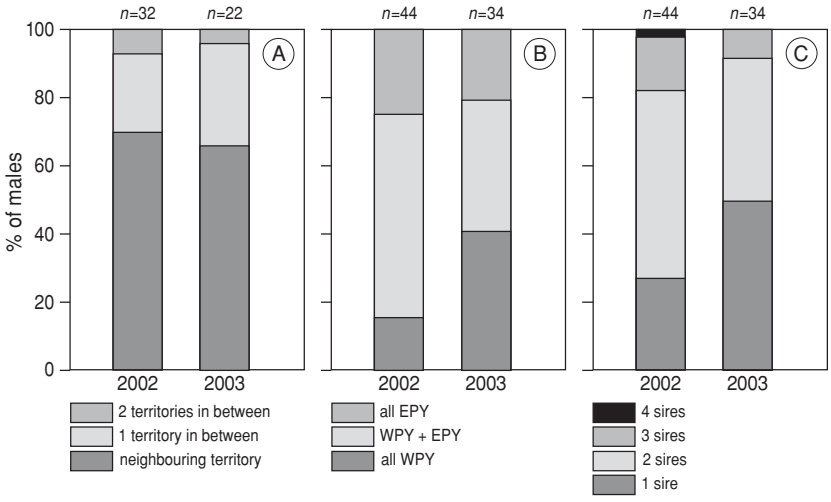

Figure 2.3. Percentage of reed bunting nests with (A) 0, 1 or 2 territories between the territories of the cuckolded male and the extra-pair male that gained a fertilization in the cuckolded male's nest, (B) only within-pair young (WPY), only extra-pair young (EPY), or both WPY and EPY, and (C) 1, 2, 3 or 4 males siring one or more offspring in that nest. Sample sizes are shown. Significant differences were found between the years for (B) ($\chi^2 = 6.38$, $df = 2$, $p = 0.04$), but not for (A) ($\chi^2 = 0.74$, $df = 2$, $p = 0.69$) and (C) ($\chi^2 = 4.89$, $df = 3$, $p = 0.18$).

Table 2.1. Distribution of EPP among nests. The observed and expected (in brackets) values of 2002 and 2003 combined are presented ($p = 0.51$). More broods than expected contain no EPY (2002 + 2003: $\chi^2 = 40.96$, $df = 1$, $n = 68$ nests, $p < 0.001$; 2002 ($p = 0.55$; data not shown): $\chi^2 = 5.57$, $df = 1$, $n = 44$ nests, $p = 0.02$; 2003 ($p = 0.46$; data not shown): $\chi^2 = 38.70$, $df = 1$, $n = 34$ nests, $p < 0.001$).

	No. of broods with the following no. of EPY						
Brood size	0	1	2	3	4	5	Total broods
2	2 (1.2)	2 (2.5)	1 (1.3)	-	-	-	5
3	6 (2.0)	3 (6.3)	2 (6.5)	6 (2.3)	-	-	17
4	4 (1.1)	2 (4.6)	4 (7.1)	6 (4.9)	3 (1.3)	-	19
5	7 (0.8)	1 (4.0)	3 (8.3)	6 (8.6)	1 (4.5)	9 (0.9)	29
total	19 (5.1)			49 (62.9)			68

Consistency of EPP within individuals

We found no general increase or decrease in EPP with time of season (2002: Spearman's rho (r_s) = -0.27, $n = 38$, $p = 0.10$; 2003: r_s = 0.12, $n = 31$, $p = 0.51$; 2002 + 2003: r_s = -0.10, $n = 50$, $p = 0.50$). Two successive broods were sampled in a single season for 32 pairs. Within these broods the proportion of EPY varied significantly (randomisation test; 2002: deviance = 77.9, $df = 39$, $p = 0.005$; 2003: deviance = 95.6, $df = 31$, $p < 0.001$; 2002+2003: deviance = 155.5, $df = 63$, $p < 0.001$). First broods did not differ systematically from second broods in the proportion of EPY (randomisation test; 2002: change in deviance = 0.3, $df = 1$, $p = 0.6$; 2003: change in deviance = 1.1, $df = 1$, $p = 0.5$; 2002+2003: change in deviance = 0.0, $df = 1$, $p = 0.9$). Individual males differed in the proportion of EPY in their double broods in 2003, but not in 2002 (randomisation test; 2002: change in deviance = 39.2, $df = 19$, $p = 0.3$; 2003: change in deviance = 68.6, $df = 15$, $p = 0.02$; 2002+2003: change in deviance = 102.4, $df = 31$, $p = 0.001$). There was no correlation in the frequency of EPP between the two broods when years were combined. However, when analysed separately, 2003 showed a significant positive correlation, indicating that in this year pairs that had fewer EPY in their first brood, also had fewer EPY in their second brood (figure 2.4).

While paired to the same social male, 24% (8/34) were 'faithful' to their social male (i.e. no EPY) in either their first or second brood, and only 9% (3/34) were 'faithful' to their social male in both broods (figure 2.5). The remaining 68% of females (23/34) were 'unfaithful' (i.e. at least one EPY) in both broods.

Social mate choice

In total, 44 females produced more than one clutch within a single season (including all nests found with known territorial male and female; 2002: $n = 21$, 2003: $n = 23$). In three of these cases (all in 2003), the social male disappeared (assumed dead) after the first clutch, and the female remated. Females did not change social partners within the same season when their original social partner was still present in the study site ($n = 41$).

On average, 56% of ringed males and 45% of ringed females returned the following year. There was no significant difference in return rates between the sexes (males: $n = 78$, females: $n = 72$; $\chi^2 = 1.83$, $df = 1$, $p = 0.18$), but between

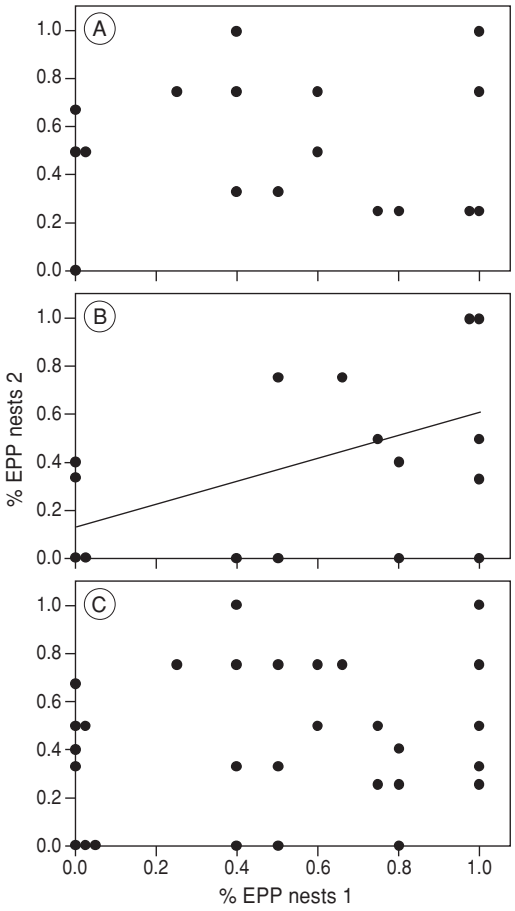

Figure 2.4. Consistency in the percentage of EPP of the same pair within a season (A) for 2002 (2002: $r_s = -0.06$, $n = 17$, $p = 0.82$), (B) for 2003 ($r_s = 0.54$, $n = 15$, $p = 0.04$), and (C) for 2002 and 2003 combined ($r_s = 0.22$, $n = 32$ pairs, $p = 0.22$).

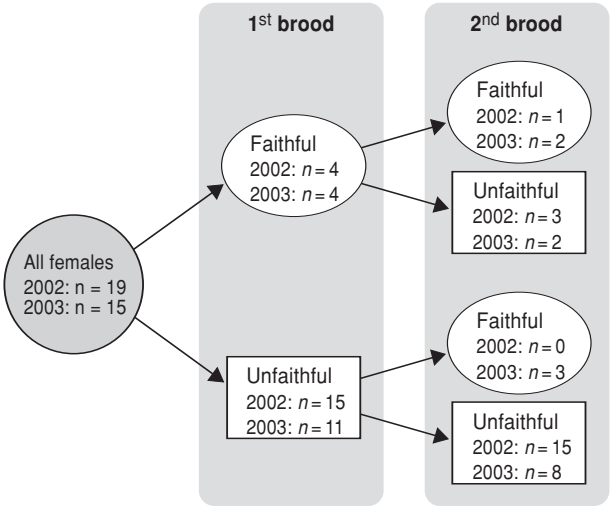

Figure 2.5. Consistency in the presence of EPY within subsequent broods of the same pairs (faithful = no EPY in brood; unfaithful = at least 1 EPY in brood). There was no difference between years ($\chi^2 = 1.43$, $df = 1$, $p = 0.23$).

years a near significant difference in return rates was found (both sexes combined; 2001/2002: 22/37 returned, 2002/2003: 47/113 returned; $\chi^2 = 3.58$, $df = 1$, $p = 0.06$). Males and females showed a high degree of site fidelity, with 92% of returning males ($n = 26$) and 69% of returning females ($n = 13$) occupying the same or adjacent territory as they held in 2002. Both the male and female of four pairs present in 2002 were also present in 2003; in three cases they changed social partners and in one case they remated with the same social partner. The three females that changed partner did not chose a previous extra-pair mate as their new social partner, and were equally unfaithful to their previous and new social partner (mean %EPP with previous partner (2002) vs. new partner (2003): 67% vs. 67%). In the single case where the pair remained together, the male lost paternity of a larger proportion of offspring in 2003 (0% in 2002 vs. 40% in 2003).

Genetic mate choice

Of the 21 females that produced EPY in both their broods within a single season, 65% produced extra-pair offspring sired by a different extra-pair male in the second brood than in the first brood, even though the extra-pair sires from the first brood were still alive (2002: 9/14; 2003: 5/7; $\chi^2 = 0.11$, $df = 1$, $p = 0.74$). Four out of six females that bred both in 2002 and 2003, and for which at least one extra-pair father from 2002 was still alive in 2003, produced extra-pair offspring sired by a different extra-pair mate in 2003.

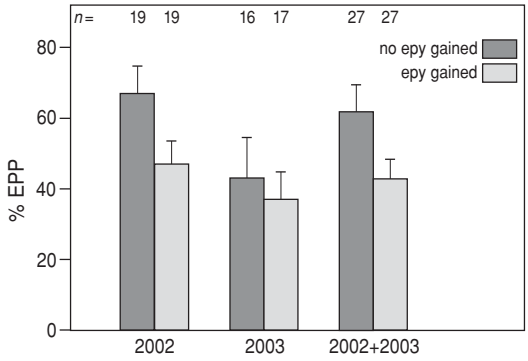

Figure 2.6. The percentage of EPP in broods of males that did and did not gain EPY in other broods. Sample sizes are indicated in the figure (Mann-Whitney *U* test; 2002: $U = 114.0$, $p = 0.050$; 2003: $U = 130.5$, $p = 0.84$; 2002+2003: $U = 260.0$, $p = 0.065$).

Males that sired EPY in other nests were less often cuckolded in their own nests than males that did not sire EPY in other nests in 2002, but not in 2003 (figure 2.6). Similar results were found when males from the outer territories were excluded (Mann-Whitney *U* test; 2002: $U = 26.0$, $n_{\text{no EPY}} = 9$, $n_{\text{EPY}} = 13$, $p = 0.03$; 2003: $U = 27.5$, $n_{\text{no EPY}} = 8$, $n_{\text{EPY}} = 9$, $p = 0.40$; 2002 + 2003: $U = 62.5$, $n_{\text{no EPY}} = 13$, $n_{\text{EPY}} = 15$, $p = 0.10$). However, we found no relationship between the proportion of EPP in a male's own nest and the number of EPY he sired in other nests (Spearman correlation; all males: $p > 0.25$; males from the outer territories excluded: $p > 0.4$).

Discussion

In our study population of reed buntings, 51% of offspring were sired by extra-pair males and 74% of broods contained at least one extra-pair young. This high frequency of EPP is comparable to that found in a British population (55% of offspring in 86% of broods; Dixon *et al*. 1994) and in a population in the north of the Netherlands (Lauwersmeer; 49% of offspring ($n = 70$) in 88% ($n = 17$) of broods; unpublished data); these frequencies of EPP are among the highest found in socially monogamous birds (Griffith *et al*. 2002). The occurrence of brood parasitism was very low (<1%). Extra-pair mating behaviour mainly occurred between neighbours, as shown in many other species (e.g. Yezerinac *et al*. 1995; Perreault *et al*. 1997; Webster *et al*. 2001). In this study three hypotheses for why females engage in extra-pair matings were investigated: to increase genetic diversity of offspring, to increase genetic quality of offspring, and to assess potential future partners.

Assessing potential future partners

High site fidelity and multiple breeding may provide females with the opportunity to select the best available partner based on information gained in the previous nesting attempt (Beletsky & Orians 1991; Weatherhead 1999). A multi-species comparison showed a positive association between EPP levels and divorce rate (Cézilly & Nager 1995). However, in our study no social mate switching was observed within a season, when both members of the pair were still present in the study area. Only few males and females belonging to the same pair survived to the subsequent season; of these the majority of individuals paired with a different social mate. However, when changing social partners, females did not form a pair with an extra-pair mate from the previous year. These results indicate that individuals do not engage in EPCs in order to test potential future partners, which is in agreement with findings in other species (yellow warbler (*Dendroica petechia*), Yezerinac *et al*. 1995; red-winged blackbird (*Agelaius phoeniceus*), Weatherhead 1999; black-capped chickadee (*Parus atricapillus*), Ramsay *et al*. 2000). In oystercatchers (*Haematopus ostralegus*), where life-long monogamy is the rule, there is some evidence that females use EPCs to test potential future mates; however these EPCs rarely result in EPFs (Heg *et al*. 1993).

Genetic diversity versus genetic quality

The genetic diversity hypothesis predicts that most or all broods should contain EPY, and that the brood should be sired by different males (Westneat *et al*. 1990). In contrast, the genetic quality hypothesis predicts that not all broods should contain EPY and broods should be sired by a single male (Westneat *et al*. 1990). Furthermore, if females are choosy and males signal honestly their genetic quality, paternity (both the frequency of EPP and the choice of father) should be consistent in subsequent broods (Weatherhead 1999). Although some authors have made clear predictions concerning the genetic diversity hypothesis (Westneat *et al*. 1990; Kempenaers & Dhondt 1993), others have discarded it as unlikely to be a reason for extra-pair mating behaviour (Birkhead & Møller 1992). Mating with just one male will produce considerable genetic diversity just through meiosis and recombination, and mating with multiple males will not increase this diversity to any great extent (Williams 1975).

The patterns of EPP observed in this study were inconsistent with the hypothesis that females seek genetic diversity for their offspring, and to a large extent support the hypothesis that females seek good genes for their offspring. First, less broods than expected contained EPY and the proportion of EPY in broods varied significantly. Secondly, more broods than expected from binomial probabilities were sired by a single male, either the social male or an extra-pair male. Thirdly, there was significant variation between individual males in the proportion of EPY in their broods in 2003 and both years combined, although not

in 2002 alone. Fourthly, females showed consistency in the proportion of EPP between their broods in 2003, although not in 2002. Fifthly, males that sired EPY in other nests were more successful at siring offspring in their own nests in 2002, although not in 2003.

Additional support for the good genes hypothesis comes from other recent findings in the reed bunting, showing that older males are more successful at gaining fertilisations both in their own brood and in other broods (chapter 3). Whether this results from female choice for older males, from enhanced mate guarding capability by older territorial males or from increased persistence by older extra-pair males is unclear. However, in a Norwegian population of reed buntings, where 30% of offspring in 54% of nests were extra-pair, there was no difference in mate guarding effort between new and old breeders (Marthinsen *et al*. 2005). Furthermore, we never witnessed aggression by extra-pair males against females; but as we also never witnessed EPCs, this may occur out of sight. These findings suggest that female choice is likely to be the main reason for the higher reproductive success of older males.

However, not all patterns observed in our study were consistent with the good genes hypothesis. First, there was no consistency in the proportion of EPP between nests of the same female within a season in 2002. Secondly, males that sired EPY in other nests were not more successful at siring offspring in their own nests in 2003. Thirdly, there was no relationship between the number of EPY a male gained in other nests and the percentage of paternity he gained in his own nests. The finding that there was no consistency in female choice of extra-pair male is not necessarily in conflict with the good genes hypothesis. If females seek genes from older males (chapter 3), they need not limit their choice of extra-pair partner to one male but include all males older than the social partner. This may lead to different males siring offspring in subsequent nests of the same female, which are all expected to be older than the social male.

Genetic compatibility

The genetic compatibility hypothesis is an alternative explanation for extra-pair matings, even though the actual source of compatibility may still be unknown (Kempenaers *et al*. 1999; Tregenza & Wedell 2000). This hypothesis states that the quality of the male is female-specific, as it depends on the combination of male and female genotypes (Tregenza & Wedell 2000). It comprises factors both from the genetic diversity and the good genes hypotheses: when seeking genetic compatibility, females try to increase the quality (for instance heterozygosity) of the offspring, but through choosing genetically dissimilar mates the genetic diversity is also likely to increase (Brown 1997). In contrast to the good genes hypothesis, the genetic compatibility hypothesis allows reciprocal extra-pair paternity between males and there is likely to be less variability in male mating

success (Kempenaers & Dhondt 1993). This is in agreement with patterns of paternity found in our study that did not fit the expectations of the good genes hypothesis, as we found some cases of reciprocal paternity and there was no relationship between a male's within-pair and extra-pair success.

The good genes and the genetic compatibility hypotheses need not be mutually exclusive, as females may choose high quality as well as compatible genes (Jennions & Petrie 2000). Female blue tits gained different benefits from different males, as copulations with local males produced offspring with good genes, while copulations with non-local males increased offspring heterozygosity (Foerster *et al*. 2003). Apart from pre-copulatory mechanisms (direct mate choice), post-copulatory mechanisms (either cryptic female choice or sperm competition; Jennions & Petrie 2000) could be used to gain genetic benefits. For instance, females may use pre-copulatory mate choice to choose males above a certain quality threshold, and after mating use post-copulatory mechanisms to identify genetically compatible sperm (Jennions & Petrie 2000). If multiple males are compatible, the identity of males siring offspring in subsequent nests may vary, as shown in this study.

In order to reveal underlying mechanisms, it is important to determine differences between maternal and paternal half-sibs (Griffith *et al*. 2002). If females seek genetic diversity, maternal half-sibs are not expected to be different from each other. If females seek good or compatible genes, EPY are expected to be 'fitter' than WPY when comparing maternal half-sibs (Griffith *et al*. 2002). In the case of compatible genes, EPY are also expected to be 'fitter' than their paternal half-sibs, raised in the father's own nest (Johnsen *et al*. 2000). These differences between WPY and EPY are presented in chapter 3.

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