

University of Groningen

## The illusion of monogamy

Bouwman, Karen Marian

**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

*Publication date:*  
2005

[Link to publication in University of Groningen/UMCG research database](#)

### *Citation for published version (APA):*

Bouwman, K. M. (2005). *The illusion of monogamy: Patterns of extra-pair paternity in the reed bunting (Emberiza schoeniclus)*. s.n.

### **Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### **Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

## **Constraints on extra-pair mating behaviour**



**Old female reed buntings  
(*Emberiza schoeniclus*)  
increase extra-pair paternity in their broods  
when mated to young males**

Karen M. Bouwman & Jan Komdeur

## Abstract

In birds, females are generally assumed to determine whether extra-pair copulations occur, and thus most studies on extra-pair paternity (EPP) have focussed on female preference for male traits, whereas female traits have been largely overlooked. However, the occurrence of EPP is likely to be a result of behavioural interactions (e.g. mate guarding by the social male and escaping mate guarding by the female), and may be related to individual experience, which is expected to increase with age. We investigated the effect of age on levels of EPP in reed buntings (*Emberiza schoeniclus*) a socially monogamous passerine with extremely high levels of extra-pair young (more than 50% of offspring). In broods of older males the rate of cuckoldry declined, which is in agreement with our previous finding that older males are more successful, either through female choice or through male experience. In contrast, older females tended to increase the level of extra-pair paternity in their broods. When including the age of the partner, we found that young males, but not old males, were cuckolded more by old females than by young females. The increase in EPP with female age is not likely to be due to disassortative mating with respect to age, an increased capacity of older females to raise a brood without male help, nor a male preference for older females. We suggest that with increase in age, females become more choosy when selecting their mating partner, or alternatively, become more experienced at circumventing paternity assurance tactics of young males.

## Introduction

Extra-pair paternity (EPP) has been the focus of many studies of socially monogamous birds (Griffith *et al.* 2002). By gaining extra-pair fertilisations (EPFs), males may increase their reproductive success, while the expected benefits to females are more controversial (Birkhead & Møller 1992). Given that EPCs are considered to be costly, females are assumed to determine whether insemination occurs (Birkhead & Møller 1992), and evidence has been found for females actively seeking extra-pair copulations (EPCs) in several species (e.g. Smith 1988; Kempenaers *et al.* 1992; Double & Cockburn 2000), females are only expected to engage in EPCs if there are potential benefits (Westneat *et al.* 1990; Birkhead & Møller 1992). Most widespread support has been found for females gaining genetic benefits for their offspring through extra-pair matings, but the results are not consistent in all studied species (reviewed by Griffith *et al.* 2002).

Many studies have found that male characteristics, such as plumage coloration, song, but most commonly age, are related to fertilisation success (reviewed by Griffith *et al.* 2002). This non-random distribution may be a result of two, not mutually exclusive, mechanisms: (i) females may actively choose good viability genes of older males for their offspring (Richardson & Burke 1999), or (ii) older males increase their experience or investment in protecting paternity in their own brood and gaining paternity in other broods (Weatherhead & Boag 1995). Due to the increased risk of cuckoldry, younger males have been found to mate guard more intensively than older males in several species (e.g. Wagner *et al.* 1996; Johnsen *et al.* 2003). However, this does not necessarily translate into higher levels of paternity, indicating that females can circumvent these paternity guards.

As individuals are often found to enjoy increased breeding success with greater experience (Clutton-Brock 1988; Saether 1990), possibly females also increase successful extra-pair mating behaviour with greater experience and thus age. However, the relation between female characteristics and EPP has received far less attention so far than the relationship between male characteristics and EPP (but see Westneat 1992; Dunn *et al.* 1994; Wagner *et al.* 1996; Stutchbury *et al.* 1997; Cordero *et al.* 1999; Kempenaers *et al.* 1999; Weatherhead 1999; Li & Brown 2000; Veiga & Boto 2000; Dickinson 2001). Only three studies have reported female age to be correlated with the proportion of EPP in their broods. Young female hooded warblers (*Wilsonia citrina*) had higher levels of EPP in their broods; they arrived later on the breeding site than older females, possibly restricting them in their choice of social mates (Stutchbury *et al.* 1997). Females mated to low quality social males may seek more EPFs than females mated to high quality males (Westneat *et al.* 1990). In contrast, old female tree swallows (*Tachycineta bicolor*) and coal tits (*Parus ater*) were more likely to have higher levels of EPP in their broods compared to young females (Kempenaers *et al.* 1999;

Dietrich *et al.* 2004). However, in coal tits, this appeared only to be the case when females were paired to young males, but not when paired to old males, which suggests an interaction between the ability of males to mateguard on the one hand and of females to escape mateguarding on the other (Dietrich *et al.* 2004). The occurrence of EPP is thus not likely to be a result of only the female deciding whether or not to engage in extra-pair copulations, but of the interaction between the female, social male and extra-pair male and this interaction deserves more attention in behavioural studies than received so far (Westneat & Stewart 2003).

We investigated levels of within brood EPP in relation to age of the social parents in the reed bunting (*Emberiza schoeniclus*), a socially monogamous passerine with very high rates of EPP (55% of offspring (Dixon *et al.* 1994) and 50% of offspring (Bouwman *et al.* 2005)). We previously found that older males were more successful in siring offspring both within and outside their own brood (chapter 3). Assuming that females actively seek EPCs to gain viability genes for their offspring, and gain experience in extra-pair mating behaviour with age, we expected the proportion of EPP in the broods of individual females to increase from one year to the next. More specifically, when including the age of the partner, we expected to find a pattern similar to the one observed in coal tits (Dietrich *et al.* 2004). As old males are most successful in siring offspring, we expected young and old females paired to old males to have low levels of EPP in their broods. Young males may increase their investment in mateguarding as a result of the increased risk of cuckoldry; thus we expected young females paired to young males also to have lower levels of EPP in their broods. However, old females should be more experienced at escaping mateguarding, and thus we predicted old females paired to young males to have higher levels of EPP in their broods. Finally, we assessed whether the quality of reed buntings increased with age, by using adult mass, onset of breeding, clutch size of the first brood, hatching and fledging success and share of nestling food provisioning rates as measures of quality.

## Methods

### *General*

We studied a population of reed buntings in 2002 and 2003 on the island of Noorderplaat in 'De Biesbosch' National Park in the Netherlands (51°45'N, 4°45'E). Adults were caught using mistnets, individually colour-ringed, blood sampled and weighed. Forty-four and 35 pairs bred in our study area in 2002 and 2003 respectively. In 2002, we ringed 97% of all individuals ( $n = 88$ ) within the study area and 80% of individuals ( $n = 20$ ) from surrounding territories. On average 42% of ringed adults breeding in 2002 returned to our study area the following breeding season and all reoccupied the same or a nearby territory. As

recruitment rates of fledglings are very low and adult reed buntings cannot be reliably aged using plumage characteristics, few adults were of exact known age. We assumed ringed adults returning in 2003 to be 'old' breeders ( $n = 37$ ) and unringed adults to be 'young' breeders ( $n = 41$ ; Veiga 1993; Yezerinac & Weatherhead 1997). In support of our assumption, we found that wing length increased for most individuals from one year to the next ( $p < 0.001$ ) and, in agreement, 'young breeders' had significantly shorter wings than 'old' breeders ( $p < 0.001$ ; chapter 3).

Nests were located through systematic searches that flushed females off the nest, or by checking territories for any nest-related activities. The identities of the male and female belonging to a nest were determined by direct observations of colour-ringed birds protecting the nest, or video recordings of birds incubating and feeding nestlings. Blood samples of nestlings were taken two days after hatching. Unhatched eggs were removed and inspected for embryonic development, which was used as a source of DNA. Video recordings (3 hours) were made of adults feeding nestlings when the oldest nestling was between 4 and 6 days of age.

#### *Paternity analysis*

DNA was extracted from blood samples using salt extraction (Richardson *et al.* 2001). Individuals were genotyped using six fluorescently labelled microsatellite markers: *Escu1*, *Escu4*, *Escu6* (Hanotte *et al.* 1994), *Pdoμ5* (Griffith *et al.* 1999), *Mcyμ4* (Double *et al.* 1997) and *Ppi2* (Martinez *et al.* 1999). For a detailed description of the methods see chapter 2. 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 criteria (delta criteria) estimate the critical difference between the LOD scores -the natural logarithm of the likelihood ratio- of the first and second most likely candidate at a level of >95% confidence and >80% confidence.

#### *Data analysis*

First, we determined the proportion of EPP from one year to the next within individuals. We used 15 males and 9 females that produced at least one brood with known paternity in both 2002 and 2003. Of these only one pair remained mated to the same partner in both 2002 and 2003. The pair that remained together was included in the analysis and highlighted in the figure. If within a year more than one brood was sampled per pair, we averaged the level of EPP. The level of EPP was arcsin transformed to reach a normal distribution, after which a paired t-test was used.



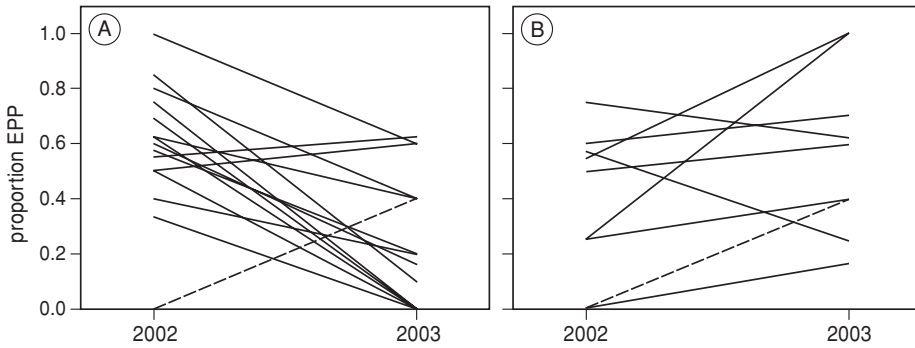
Second, we determined the proportion of EPP in relation to age across individuals. As we could only distinguish between ‘young’ (unringed) and ‘old’ (colour-ringed in 2002) breeders in 2003, we used 30 pairs breeding in 2003 to determine the effect of age (i.e. young vs. old) and the interaction between male and female age on EPP. We used multilevel generalized linear mixed models to analyse the effect of age on the proportion of EPP in a brood, assuming a binomial error distribution with logit-link function and using the number of offspring in a brood as the denominator. The two-level hierarchical structure of the proportional response data was built up with the identity of the pair as the highest level and the different broods for each pair as the lowest level (i.e. one or two broods). This approach allowed us to make full use of all available broods, while accounting for including more than one brood per pair. The model was estimated using reweighted iterative generalised least squares (RIGLS) and 2<sup>nd</sup> order penalised quasi-likelihood approximation (PQL; Rasbash *et al.* 2004). The significance of variables was tested using the Wald statistic, which follows a  $\chi^2$ -distribution.

We investigated the relationship between the proportion of EPP in broods of individuals in 2002 and their probability of returning to our study site in 2003 by using multilevel generalized linear mixed models, again assuming a binomial error distribution with logit-link function and using the number of offspring in a brood as the denominator. The two-level hierarchical structure of the proportion of EPP was built up with the identity of the male or female as the highest level and the different broods for each individual as the lowest level.

The first egg was laid six days earlier in 2002 than in 2003. The first egg day for an individual was corrected for year-effect by subtracting the first egg day for that year. Fledging success was not comparable within individuals between years, as predation rates differed between years. Statistical analyses were performed using SPSS 11.0.1 (SPSS Inc.) and MLwiN 2.0. Non-parametric tests were used if data were not normally distributed after transformation. Probability values are two-tailed and we assumed significance if  $p < 0.05$ .

## Results

Extra-pair males sired 54.6% of offspring in 2002 ( $n = 262$ ) and 46.4% of offspring in 2003 ( $n = 211$ ; chapter 2). There is no statistically significant difference in level of EPP between these years ( $\chi^2 = 3.10$ ,  $df = 1$ ,  $p = 0.08$ ). The proportion of EPP in a male’s brood declined as he aged one year (figure 5.1A), while females tended to have a higher proportion of EPP in their brood when they grew one year older (figure 5.1B). After combining male and female age and their interaction in one model, we found a significant negative effect of the interaction between male and female age on the proportion of EPP in a brood (table 5.1). When comparing the



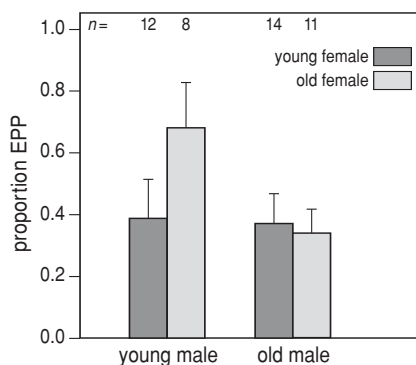
**Figure 5.1.** Proportion of EPP in broods produced in 2002 and 2003 by individual (A) male and (B) female reed buntings. Only one pair remained together in both years; the male and female of this pair are indicated by a dashed line. The proportion of EPP decreased for individual males (paired t-test;  $t = 3.65$ ,  $n = 15$ ,  $p = 0.003$ ) and tended to increase for individual females ( $t = -2.08$ ,  $n = 9$ ,  $p = 0.072$ ).

**Table 5.1.** Multilevel GLM showing the effect of male and female age on the proportion of EPP in broods ( $n = 45$  broods of 30 different pairs). The parameter estimates ( $\pm$  se), Wald test statistic and level of significance are shown.

Model terms	Estimate (se)	Wald statistic	df	p
Male age	0.12 (1.09)	0.012	1	0.91
Female age	3.32 (1.23)	7.28	1	0.007
Male age * female age	-3.58 (1.65)	4.70	1	0.03

proportion of EPP for each age combination of the social parents, it appears that the combination of young male and old female is more likely to have a higher proportion of EPP in their broods than the other three age combinations (figure 5.2).

There was no assortative mating with respect to age, as we found no difference between the number of males or females paired with a younger, older or similar aged partner in 2003 ( $\chi^2 = 0.25$ ,  $df = 1$ ,  $n = 38$  pairs,  $p = 0.62$ ). Males significantly increased in mass from one year to the next, while females did not (table 5.2). Both males and females advanced the day the first egg was laid in their nest with increasing age (table 5.2). Neither males nor females showed a change in clutch size, hatching success or their share of total provisioning to nestlings from 2002 to 2003 (table 5.2). When comparing mass, first egg date, clutch size, hatching success, share of provisioning and fledging success between young and old individuals in 2003, we found no differences between the two age groups (all:  $p > 0.17$ ).



**Figure 5.2.** Proportion of EPP in reed bunting broods in relation to age of the pair members in 2003. Bars indicate mean + se and sample sizes are shown above the bars.

**Table 5.2.** Comparison of quality measures ( $\pm$  se) for individual male and female reed buntings between 2002 and 2003. Mass, first egg date (corrected for year effect), clutch size of first brood, hatching success (proportion of eggs hatched) and share of total provisioning within individuals from 2002 to 2003. Statistics for Wilcoxon signed rank tests are presented.

	<i>n</i>	2002	2003	<i>Z</i>	<i>p</i>
<b>Males:</b>					
Mass (grams)	15	19.2 $\pm$ 0.2	19.5 $\pm$ 0.2	-2.45	0.014
1st egg date	15	15.0 $\pm$ 2.3	6.1 $\pm$ 1.6	-2.33	0.020
Clutch size	15	4.9 $\pm$ 0.1	4.9 $\pm$ 0.1	0.0	1.0
Hatching success	15	0.91 $\pm$ 0.04	0.85 $\pm$ 0.05	-0.95	0.34
M/(M+F) provisioning	13	0.37 $\pm$ 0.04	0.32 $\pm$ 0.04	-0.94	0.35
<b>Females:</b>					
Mass (grams)	8	18.0 $\pm$ 0.4	18.6 $\pm$ 0.3	-1.54	0.12
1st egg date	9	22.3 $\pm$ 5.4	6.4 $\pm$ 2.1	-2.31	0.021
Clutch size	9	5.0 $\pm$ 0.2	5.0 $\pm$ 0.0	0.0	1.0
Hatching success	9	0.90 $\pm$ 0.04	0.96 $\pm$ 0.3	-1.30	0.19
F/(M+F) provisioning	5	0.61 $\pm$ 0.07	0.59 $\pm$ 0.03	-0.67	0.50

The probability of males returning to our study site in 2003 was not affected by the proportion of EPP in their broods in 2002 (average proportion of EPP for resighted vs. non-resighted males: 0.52 vs. 0.60;  $n = 66$  broods of 38 males;  $\chi^2 = 1.13$ ,  $df = 1$ ,  $p = 0.29$ ). In contrast, females with a higher proportion of EPP in their broods in 2002, were less likely to return to our study site in 2003 (average proportion of EPP for resighted vs. non-resighted females: 0.40 vs. 0.66;  $n = 60$  broods of 39 females;  $\chi^2 = 6.73$ ,  $df = 1$ ,  $p = 0.01$ ).

## Discussion

As expected following the higher total fertilisation success of older males (chapter 3), the proportion of EPP in their broods decreased with age for individual male reed buntings. This pattern was not due to higher return rates for high quality (i.e. less cuckolded) males than for low quality (i.e. more cuckolded) males, as we found no relation between the degree of cuckoldry in 2002 and the probability of a male's resighting in 2003. In contrast to males, the level of EPP in the broods of females tended to increase with age. The finding that females with high levels of EPP in their broods were less likely to return to our site, may be due to older females (with higher levels of EPP) being more likely to die as a consequence of ageing. This suggestion was supported to some extent, as females that returned tended to have shorter wings (i.e. were younger;  $72.2 \pm 0.65$  mm,  $n = 10$ ) than females that did not return ( $73.4 \pm 0.38$  mm,  $n = 26$ ;  $t = 1.68$ ,  $df = 35$ ,  $p = 0.10$ ). Alternatively, engaging in extra-pair mating behaviour may be costly to females (e.g. through male harassment, disease transmittance or reduction in paternal care (Birkhead & Møller 1992). This could lead to lower return rates for females with high levels of EPP, either through lower survival or by moving to other breeding sites.

Similarly, comparing across individuals in 2003 also revealed that older females and younger males had higher proportions of EPP in their broods. However, the pattern was also dependent on the age of their social partner. When comparing the level of EPP for each age combination of the social parents, we found that pairs consisting of young males and old females had higher levels of EPP in their broods compared to the other pair combinations. This pattern is similar to that found in coal tits (Dietrich *et al.* 2004), but to our knowledge has not been reported in any other species.

There are several possible explanations why older females have higher proportions of EPP in their brood than younger females. Firstly, extra-pair mating behaviour may be constrained by the need for paternal care (Westneat *et al.* 1990; Gowaty 1996). If males reduce paternal care when the rate of cuckoldry increases, then only females that are capable of raising a brood alone or with reduced male assistance, for instance older females (Saether 1990), can afford producing a high proportion of EPP in their brood. Several studies have reported a decline in paternal care with increasing cuckoldry (Dixon *et al.* 1994; Sheldon & Ellegren 1998; Lifjeld *et al.* 1998; Chuang-Dobbs *et al.* 2001), but others showed no relationship (e.g. Kempenaers *et al.* 1998; Peterson *et al.* 2001; Dickinson 2003). However, this is unlikely to explain our results because female reed buntings that produced a high proportion of extra-pair young did not suffer the cost of decreased paternal care in our population (chapter 4). Furthermore, older females did not feed offspring more in relation to their partner than younger females.

A second explanation for increased EPP with female age may be that males prefer attempting EPCs with older females, as older females may be of higher quality. We found that females start breeding earlier when they were older and early breeding has been found to increase reproductive success in other species (e.g. Perrins 1970). However, extra-pair fertilisations were not biased towards early clutches (chapter 2 and 4). Furthermore, mass, clutch size and hatching success within individual female reed buntings did not change between years and none of the measured variables, including fledging success, differed between age groups in 2003.

A third explanation for increased EPP with female age may be that older females become more choosy when selecting their mating partner, and may be more inclined to seek EPCs when paired to a young male. Female mating preferences have been shown to change with age in satin bowerbirds (*Ptilonorhynchus violaceus*; Coleman *et al.* 2004) and guppies (*Poecilia reticulata*; Kodric-Brown & Nicoletto 2001). Alternatively, older females may become more experienced at escaping mate guarding behaviour of their partner, leading to more EPFs when a young male is paired to an old female than to a young female. A study on a Norwegian population of reed buntings showed mate guarding by the social male to be effective, as it was negatively related to cuckoldry rate (Marthinsen *et al.* 2005). However, our expectation that young males invest more in mateguarding was not supported in this Norwegian population, as there was no difference in the time that old and young males spent mateguarding (Marthinsen *et al.* 2005). Unfortunately, we did not measure mate guarding behaviour in our population, so we could not determine if mate guarding intensity changed with age. Older females having fewer extra-pair young in their brood when paired with old males may be explained either by females choosing not to mate outside the pairbond or by older males being more successful at guarding their females. Detailed behavioural observations would be required to distinguish between these two possibilities.

Our results emphasize that not only male age, but also female age and their interaction is potentially a very important aspect to understanding extra-pair mating behaviour. This pattern of partner age and proportions of EPP has now been revealed in two species (Dietrich *et al.* 2004; this study), and may be quite general across avian species. Furthermore, as suggested by Westneat and Stewart (2003), it is clear that detailed behavioural observations are needed to understand how interactions in extra-pair mating behaviour occur.

## Acknowledgements

We thank Jan Wijmenga, René van Dijk, Cas Eikenaar, Suzette Flantua and Leonie Raijmakers for assistance in the field and with video analysis. Staatsbosbeheer kindly allowed us to work and live in “De Biesbosch” and we thank Dirk Feij and Bart Weel for arranging practicalities. Martijn van de Pol provided valuable statistical advice. Michael Magrath, Mathew Berg, Rudi Drent, Simon Verhulst and two anonymous referees made useful comments on the manuscript. This study was financially supported by NWO to JK (809-34-005) and Schure-Beijerinck-Popping Fund of KNAW to KMB (SBP/JK/2003-30).