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## The illusion of monogamy

Bouwman, Karen Marian

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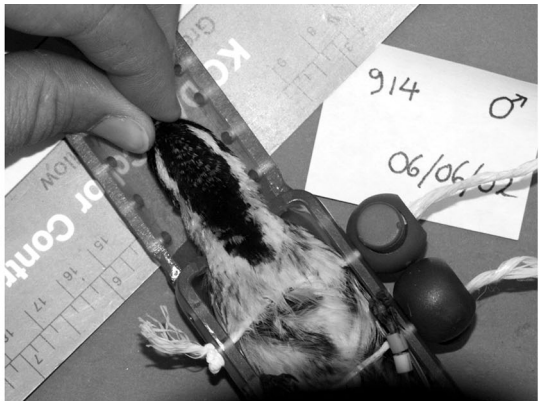
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## **Older male reed buntings are more successful at gaining within- and extra-pair fertilisations**

Karen M. Bouwman, René E. van Dijk, Jan J. Wilmenga & Jan Komdeur

## Abstract

The importance of extra-pair paternity (EPP) as an aspect of mixed reproductive strategies is currently the focus of many studies. Since females generally control the occurrence of inseminations, they are expected to engage in extra-pair copulations (EPCs) only if they benefit, for instance through gaining (i) high quality or (ii) compatible genes for their offspring, or (iii) insurance against infertility. To distinguish between these benefits, we studied characteristics related to inter-male variation in fertilisation success as well as differences in fitness between within- and extra-pair offspring in the reed bunting (*Emberiza schoeniclus*), a socially monogamous passerine with extremely high levels of EPP (i.e. 50% of young). We found that older males were more successful at siring offspring in their own broods as well as in other broods. We did not find differences between maternal or paternal half-siblings in biometrics, heterozygosity, hatching success or survival. Therefore the good genes or genetic compatibility hypotheses were not supported. However, more extra-pair young than expected hatched earlier than their maternal half-siblings, and thus were slightly older. As we found that male sperm storage capacity increases with age, we suggest that through EPCs with old males, females are insured against functional infertility of their young partner. As offspring that hatch early in the hatching-order often have a fitness advantage, extra-pair males may be selected to fertilise eggs early in a clutch, resulting in the observed bias in hatching order of extra-pair offspring.

## Introduction

With molecular techniques becoming widespread, the study of extra-pair mating behaviour in socially monogamous species, has become the main focus of many behavioural ecologists. Extra-pair paternity (EPP) has been found to occur in 86% of socially monogamous passerine species studied so far (Griffith *et al.* 2002). Males may increase their reproductive output without additional paternal investment by engaging in extra-pair copulations (EPCs), as these can lead to extra-pair fertilisations (EPFs; Westneat *et al.* 1990; Birkhead & Møller 1992). Since reproductive success for females is limited by the number of eggs produced, the benefits of EPCs to females are less clear. Given that EPCs are considered to be costly, that females have at least some control whether insemination occurs (Birkhead & Møller 1992), and that in some species females actively seek EPCs (reviewed in Westneat & Stewart 2003), females are only expected to engage in EPCs if there are potential benefits (Westneat *et al.* 1990; Birkhead & Møller 1992).

Females may obtain 'good genes' for their offspring through mating with a high quality extra-pair partner (Westneat *et al.* 1990; Birkhead & Møller 1992). Male characteristics that reflect male quality are expected to be related to fertilisation success: a positive relationship between male fertilisation success and certain male characteristics, such as age, size, plumage characteristics and song (reviewed in Griffith *et al.* 2002), is often interpreted as support for females obtaining genetic benefits for their offspring. However, a different explanation for this relationship is based on the possible association of functional fertility of a male (i.e. the success of ejaculates in fertilising eggs) with preferred male characteristics (Sheldon 1994b). Sheldon (1994b) argues that females may seek EPCs as a direct benefit to themselves, namely to insure against functional infertility of their partner, resulting in the same patterns of fertilisation success as would be found when females seek genetic benefits for their offspring. One way to distinguish between these two hypotheses, is to determine whether there are differences between offspring sired through EPCs and offspring that are sired through within-pair copulations (Sheldon 1994b).

If females gain genetic benefits for their offspring, then extra-pair young (EPY) are expected to be fitter than within-pair young (WPY) in the same brood (i.e. maternal half-siblings; Griffith *et al.* 2002). As fitness of offspring is often difficult to measure directly, factors have been examined which are expected to be related to fitness. EPY have previously been shown to have a higher survival to fledging (Kempnaers *et al.* 1997), a better condition at fledging (Sheldon *et al.* 1997), a higher post-fledging survival (Hasselquist *et al.* 1996), and a stronger immune response (Johnsen *et al.* 2000). However, several other studies showed no difference between WPY and EPY in survival (e.g. Strohbach *et al.* 1998; Krokene *et al.* 1998; Whittingham & Dunn 2001; Schmoll *et al.* 2003), reproductive

success (Schmoll *et al.* 2003), immune response (Kleven & Lifjeld 2004), or morphometrics (Krokene *et al.* 1998; Johnsen *et al.* 2000; Kraaijeveld *et al.* 2004; Kleven & Lifjeld 2004).

In addition to the intrinsic effects of paternal genes (i.e. good genes), the importance of the interaction between maternal and paternal genes (i.e. genetic compatibility; reviewed in Tregenza & Wedell 2000) have been assessed using extra-pair offspring. Heterozygosity reflects the individual level of genetic diversity and is often related to reproductive success (reviewed in Hansson & Westerberg 2002), therefore females may benefit through seeking copulations with genetically dissimilar mates (Blomqvist *et al.* 2002; Tregenza & Wedell 2002; Richardson *et al.* 2004, but see Hansson *et al.* 2004). In blue tit (*Parus caeruleus*) broods of mixed paternity, EPY were more heterozygous than their maternal half-siblings (Foerster *et al.* 2003). Extra-pair bluethroat (*Luscinia svecica*) offspring not only had a higher immunocompetence than their maternal half-siblings in the same brood, but also higher than their paternal half-siblings raised in the male's own nest (Johnsen *et al.* 2000). The authors therefore concluded that female bluethroats were seeking compatible genes for their offspring rather than just good genes.

We studied extra-pair mating behaviour and its fitness consequences in a population of reed buntings (*Emberiza schoeniclus*) in The Netherlands. The reed bunting is a small (18g), sexually dimorphic, socially monogamous passerine. Males have a black head and throat patch ('badge') with a contrasting white collar, and can often be seen singing high on reed stems or small bushes. Females lack these conspicuous traits, and are also more cryptic in their behaviour. The levels of extra-pair paternity found in different populations are among the highest found in socially monogamous passerines (55% in England, Dixon *et al.* 1994; 50% in The Netherlands, Bouwman *et al.* 2005). Three types of data are required to determine how females may benefit from EPP: (i) the distribution of EPP among broods, (ii) the distribution of EPP among males with different characteristics, and (iii) fitness differences between WPY and EPY in broods of mixed paternity (Griffith *et al.* 2002). Previously, when addressing the first type of question, we found that EPP was not evenly distributed among broods: more broods than expected contained no or all EPY (chapter 2). The aim of this study was to address the second and third type of question. First, we investigated whether male characteristics, such as age, biometrics, plumage characteristics, song quality and quantity and sperm storage capacity, were related to within- and extra-pair fertilisation success. Second, we investigated whether there were differences in biometrics, heterozygosity, hatching success, fledging success and recruitment rates between WPY and EPY. If females seek good paternal genes for their offspring, EPY are expected to be 'better' than WPY in broods of mixed paternity. If an additional effect of the maternal genes is important for the fitness of offspring, we expect a male's EPY to be 'better' than the WPY in his own brood.

## Methods

### *General*

From 2001 to 2003 we studied a population of reed buntings in a 13 ha study site, on the island of Noorderplaat (45 ha) in 'De Biesbosch' National Park in the Netherlands (51°45'N, 4°45'E). The study site had an approximate density of 3 pairs per hectare. For a full description of the study site and general methods see chapter 2. In brief, adult reed buntings were caught using mist nets and ringed with a numbered aluminium ring and a specific combination of three colour rings for individual recognition. Birds were measured and a blood sample was taken from the brachial vein for DNA analysis 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. In 2001 approximately 50% of adults were sampled, while in 2002 and 2003 nearly all adults were sampled (2002: 98%,  $n = 88$ ; 2003: 94%,  $n = 72$ ).

Nests are built on or just above the ground and located through systematic searches that flushed females off the nest, or through observing territorial birds for any nest-related activities. Nestlings were bloodsampled two days after hatching by taking a small blood sample 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. At seven days of age the nestlings were ringed and measured. Within our study site we found 97.4% of all nests that fledged young ( $n = 78$ ) in 2002 and 2003; only in two cases did we see fledglings without locating the nest. However, due to high levels of predation, we were unable to locate all nests in the study area. As there was no obvious gradient in risk of predation across the site, we believe we obtained a random sample of individual reproductive success for all males in our site.

### *Male morphological measurements*

We measured adult tarsus length with callipers (to the nearest 0.1mm), wing length with a stopruler (to the nearest 0.5mm) and body mass with a 30-g Pesola spring balance (to the nearest 0.1g). We took digital pictures of the badge, together with a ruler, while the male was positioned in a standardised way. The size of the badge was extracted from the picture using Adobe Photoshop 5.0.2., by comparing the number of pixels in 1 cm<sup>2</sup> to the number of pixels within the selected area covered by the badge. The repeatability of badge size within one capture event was high (Lessells & Boag 1987; table 3.1). The colour of the badge ranged from brown- due to brown fringes on the edge of the black badge feathers- (winter plumage), to completely black (full breeding plumage; Cramp & Perrins 1994). The colour of the badge was compared with 5 categories of pictures of reed



**Table 3.1.** Repeatabilities ( $r$ ) of male reed bunting characteristics

Characteristic	$F$	$df$ (between, within)	$r$
<b>Morphometrics</b>			
Mass (res)	6.37*	42, 43	0.73
Wing length	21.89*	45, 38	0.91
Tarsus length	38.03*	45, 38	0.95
Badge size (within capture)	11.47*	196, 197	0.57
Badge size (between captures)	3.69*	45, 37	0.84
<b>Song measures</b>			
Song length	3.70*	27, 127	0.33
Pause length	5.29*	27, 127	0.44
% performance	3.47*	27, 127	0.31
Syllable number	7.35*	27, 127	0.54

\*:  $p < 0.001$

bunting museum skins, and given a score on a scale of 1 (brown) to 5 (completely black). The badge colour was highly correlated with time of season ( $r_s = 0.72$ ,  $p < 0.001$ ,  $n = 92$ ), as was also found by Dixon (1993), and the time between measurements was highly correlated with the difference in colour score ( $r_s = 0.79$ ,  $p < 0.001$ ,  $n = 46$ ). We therefore restricted the measurements for badge colour to the peak of the breeding season, between 20 April and 30 June. There is no significant relationship between badge colour and day within this period ( $r_s = 0.21$ ,  $p = 0.26$ ,  $n = 30$ ). Neither was there a difference in return rates the following season between males in relation to their badge colour (score 1:  $n = 0$ ; score 2+3: 75% ( $n = 8$ ), score 4+5: 58% ( $n = 24$ );  $\chi^2_1 = 0.16$ ,  $p = 0.69$ ). We estimated the repeatability of measurements using 46 males that were captured on more than one occasion within a year (Lessells & Boag 1987; table 3.1). As there was a significant relationship between mass, day and time of day ( $F_{2,110} = 4.72$ ,  $p = 0.011$ ), we used the unstandardised residuals of the regression as a measure for mass.

For each male, we measured the cloacal protuberance (CP), an anatomical structure that results from the enlargement of the seminal glomerus (i.e. the site of sperm storage) during the reproductive season (Lake 1981), which reflects sperm storage capacity (Nakamura 1990; Birkhead *et al.* 1991; Birkhead *et al.* 1993). The CP approximates a barrel shape, therefore the height and width of the CP was measured using callipers (to the nearest 0.1mm) from 1 April onwards, and the volume of the CP ( $\text{mm}^3$ ) was calculated as the height \*  $\pi$  \*(width/2)<sup>2</sup> (Mulder & Cockburn 1993). As there was a significant curvilinear relationship between day of season and CP ( $F_{2,72} = 72.2$ ,  $p < 0.001$ ), we used the unstandardised residuals of

the regression as a measure for CP. As the CP was measured twice of only six individuals within one season, we did not calculate the repeatability for this characteristic.

#### *Age and survival*

In 2002 and 2003, on average 96% of adult individuals within the study area were ringed (2002:  $n = 88$ ; 2003:  $n = 72$ ) and approximately 80% of individuals from territories surrounding our study area were ringed ( $n = 20$ ). On average 42% of ringed adults breeding in 2002 returned to our study area the following breeding season and 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 and unringed adults to be 'young' breeders (following e.g. Veiga 1993; Yezerinac & Weatherhead 1997). This classification was supported by our measurements of wing length and badge colour, as winglength and scores for badge colour increased with age and 'old' males had longer wings and blacker badges than 'young' males (see results).

#### *Song recording and analysis*

Reed bunting males sing two types of song: a fast, pre-mating song and a slow, post-mating song (Nemeth 1996). The songpeak of the first is reached during territory settlement and the songpeak of the latter is reached during the incubation period of the social female (Nemeth 1996). Mated males sing throughout the day, but a peak in singing is reached approximately 1.5 hours before sunrise and a second peak at six to seven hours after sunrise (approximately 11:00 am; O'Malley 1993; pers.obs.). We recorded song of 29 different mated males in May and June 2003, using a Marantz portable cassette recorder (model PMD 222) and a Sennheiser directional microphone (model ME66/K6). Recordings were made throughout the day, whenever an identified male was singing. The mean total recording time for each male was 29.5 minutes ( $\pm$  SE 0.9; range: 17.4 – 39.8 minutes). On average 5.4 ( $\pm$  SE 0.4; range: 1 – 11) different song bouts were recorded per male; a bout was included when it consisted of a minimum of 20 songs. Tape-recordings were analysed using Avisoft SASLab Pro (version 4.23b for Windows), following Nemeth (1996).

We measured the length of each song (to the nearest 0.01 second), the length of the pause between songs (to the nearest 0.01 second), the percentage performance time (i.e. song length / (song length + pause length)), the number of syllables within a song and the total number of different syllables produced by a male (repertoire size). Song length, syllable number and percentage performance time were highly correlated (all:  $r > 0.54$ ,  $n = 29$ ,  $p < 0.003$ ) and pause length and percentage performance time were highly correlated ( $r = -0.75$ ,  $n = 29$ ,  $p < 0.001$ ). We

calculated the repeatability of these measures between different bouts of the same male (Lessells & Boag 1987; table 3.1). As syllable number and pause length showed the highest repeatability and are not significantly correlated with each other (table 3.2), we selected these variables for inclusion in further analyses. Although the total recording time differed between males, we found no relationship between the number of different syllables produced by a male and the total recording time ( $r = 0.23$ ,  $n = 29$ ,  $p = 0.24$ ). We therefore used the total number of different syllables produced by a male as an estimate for repertoire size. This measure showed no correlation with any of the other song quality measures (all:  $p > 0.17$ ).

#### *Nestling characteristics*

We weighed and measured 352 nestlings from 94 nests on several occasions between 0 (day of hatching) and 10 days after hatching. Final measurements were usually taken at day 7, as the risk of nestlings leaving the nest prematurely increased after this time (normal departure: day 10 to 12). Up to approximately day 3 after hatching, nestlings were only weighed (to the nearest 0.1g) using a Pesola spring balance of 10g or 30g. From day 4 to day 10, nestlings were weighed and tarsus length was measured using callipers (to the nearest 0.1mm).

Two indices of heterozygosity (i.e. individual heterozygosity and mean  $d^2$ ), as measures of degree of inbreeding, have been found to be related to measures of fitness in other species (Coulson *et al.* 1998; Hansson *et al.* 2001). Individual heterozygosity was defined as the proportion of scored loci that were heterozygous; mean  $d^2$  was calculated as the squared distance in repeat units between the two alleles, averaged for all scored loci (following Coulson *et al.* 1998). In total, 98% of sampled offspring ( $n = 501$ ) were scored at 6 microsatellite loci, while the remaining 2% were scored at 5 loci (see ‘nestling sex determination and paternity analysis’ below).

#### *Nestling sex determination and paternity analysis*

DNA was extracted from blood samples using salt extraction (Richardson *et al.* 2001). Nestlings were sexed using Griffith’s universal PCR method for the sexing of birds (Griffiths *et al.* 1998). Individuals were genotyped using six fluorescently labelled microsatellite markers and 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. For a detailed

Table 3.2. Correlations between characteristics

	Mass (res)	Wing length	Tarsus length	Badge size	Badge colour	% singing	Syllable no.	Pause length	Repertoire size
Wing length	0.39** (88)	-							
Tarsus length	0.40** (88)	0.22* (88)	-						
Badge size	0.17 (88)	0.41** (88)	0.03 (88)	-					
Badge colour	0.16 (44)	0.48** (47)	0.36* (47)	0.37* (47)	-				
% singing	0.25 (30)	0.35§ (30)	0.21 (30)	0.19 (30)	0.39§ (24)	-			
Syllable no.	0.21 (28)	-0.01 (28)	-0.22 (28)	-0.15 (28)	-0.33 (28)	-0.03 (26)	-		
Pause length	-0.14 (28)	-0.10 (28)	-0.12 (28)	-0.19 (28)	-0.16 (28)	-0.22 (28)	0.34§ (28)	-	
Repertoire size	-0.6 (28)	-0.12 (28)	-0.17 (28)	-0.18 (28)	-0.21 (28)	-0.1 (28)	0.05 (28)	-0.07 (28)	-
Cloacal protuberance	0.29* (49)	0.34* (49)	0.14 (49)	0.18 (49)	0.24 (39)	0.37 (18)	0.31 (16)	-0.17 (16)	0.35 (16)

The correlation coefficient, sample size (in brackets) and statistical significance is shown (§:  $p < 0.1$ ; \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ).

description of the methods chapter 2. In two years 501 offspring were genotyped from 129 nests. Paternity was assigned to 90% of offspring. Extra-pair males sired 54.6% of offspring in 2002 ( $n = 262$ ) and 46.4% of offspring in 2003 ( $n = 211$ ).

### Data analyses

#### MALE FERTILISATION SUCCESS

We investigated the relationship between male characteristics and male fertilisation success from three different points of view. First, we tested whether there was a relationship between the proportion of EPP in a male's own brood ('cuckoldry rate') and male characteristics. We measured characteristics of 52 different males of which at least one own brood (i.e. brood produced with the social female) was DNA-sampled. We used multilevel generalised linear mixed models to analyse the effect of male characteristics on the proportion of EPP in a male's own 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 binomial response data was built up with the male as the highest level and the different broods for each male as the lowest level. This approach

allowed us to make full use of all available data, while at the same time accounting for (i) having included individuals more than once, as males often had multiple broods (both within and between seasons), and (ii) the number of offspring in a brood, which varied between two and five offspring. The model was implemented using reweighted iterative generalised least squares (RIGLS) and 1<sup>st</sup> order marginal quasi-likelihood approximation (MQL; Rasbash *et al.* 2004). The significance of variables was tested using the Wald statistic, which follows a  $\chi^2$ -distribution. Non-significant variables were backwards eliminated.

Second, we tested whether there was a relationship between extra-pair fertilisation success and male characteristics. We defined extra-pair fertilisation success as the proportion of EPY sired out of the total number of EPY produced in that year ('EPP gained'), using males that were breeding within the study area. As males may have been included more than once if they bred in multiple years, we again used multilevel GLMM, as described above, but now using the total number of EPY as the denominator. The identity of the male was included as the highest level, and the different years of breeding as the lowest level. As males breeding on the edge of the study site (i.e. with at least one adjacent territory that we did not search for nests; 2002:  $n = 14$ , 2003:  $n = 20$ ) may have sired offspring in nests we did not sample for DNA, we also repeated this analysis excluding these edge males.

Third, we tested whether there was a relationship between total fertilisation success (i.e. the proportion of offspring sired out of the total number of offspring produced in that year) and male characteristics. The same approach was used as described for extra-pair fertilisation success (see above). Finally, we directly compared measurements of the cuckolded social male with the measurements of the extra-pair male, which both sired offspring in the same brood, in a paired test ('WPmale vs. EPmale';  $n = 48$ ). If more than one extra-pair male sired offspring in a brood, we used the average of their measurements in the analysis ( $n = 28$ ).

Data collected on male characteristics in 2001 to 2003 were used in determining age-related characteristics by comparing measurements of males caught in two subsequent years. Data from 2002 and 2003 were included in all other analyses. Morphological measurements, plumage characteristics and cloacal protuberance measures were collected in both 2002 and 2003, but in uneven quantities, while song characteristics and age classes were only available in 2003. Furthermore, several characteristics were intercorrelated (table 3.2). We therefore performed the analyses in four steps, to maximise the use of the available data. First, we analysed the relationship between the three levels of fertilisation success (as described above) and separate characteristics, to get an indication whether intercorrelated characteristics predicted similar responses of the dependent variable (table 3.3). Second, we analysed the relationship between the three levels of fertilisation success and morphological measurements and badge size for both 2002 and 2003 combined ('large dataset') in a multiple variable analysis (table

**Table 3.3.** Analysis of the relation between individual male characteristics and fertilisation success.

Variable	Cuckoldry rate	EPP gained	Total success	WPmale vs EPmale
Mass (res)	-0.53 (52/108)	+1.45 (54/73)	+3.14 § (54/73)	$t = +0.48$ (48)
<b>Wing length</b>	<b>-4.56 *</b> (52/108)	<b>+9.02 **</b> (54/73)	<b>+18.59 ***</b> (54/73)	$t = +2.35 *$ (48)
Tarsus length	+1.52 (52/108)	+0.97 (54/73)	-0.27 (54/73)	$t = -0.12$ (48)
Badge size	-0.89 (52/108)	+2.33 (54/73)	+1.97 (54/73)	$t = +1.09$ (48)
<b>Badge colour</b>	-0.86 (34/66)	<b>+9.02 **</b> (36/45)	<b>+6.79 **</b> (36/45)	$Z = +0.97$ (21)
<b>% singing</b>	-2.86 § (27/43)	<b>+4.02 *</b> (30/30)	<b>+4.17 *</b> (30/30)	$t = +1.52$ (14)
Syllable no.	-1.88 (24/39)	-1.51 (26/26)	-0.95 (26/26)	$t = +0.35$ (14)
Pause length	-0.40 (24/39)	-0.09 (26/26)	-0.22 (26/26)	$t = +0.70$ (14)
Repertoire size	+0.26 (24/39)	-0.57 (26/26)	-0.40 (26/26)	$t = +0.13$ (14)
<b>Age class</b>	-3.00 § (24/39)	<b>+11.71 ***</b> (26/26)	<b>+4.39 *</b> (26/26)	$Z = +2.11 *$ (17)
Cloacal protuberance	-1.34 (39/72)	+2.34 (41/49)	+2.23 (41/49)	$t = -1.8 §$ (25)

Fertilisation success is divided in (i) cuckoldry rate (i.e. proportion of EPP in male's own brood; sample size (n) indicates number of different males/number of broods in years combined), (ii) EPP gained (i.e. proportion of EPY gained in other broods; sample size indicates number of different males/number of males in years combined), (iii) total success (i.e. proportion of total number of offspring sired; sample size indicates number of different males/number of males in years combined), and (iv) direct comparison of cuckolded within-pair male (WPmale) and extra-pair male (EPmale; sample size indicates number of broods). Values of statistical tests (i.e. Wald statistics unless stated otherwise) and the directions of the relationships are shown, significance is indicated by §:  $p < 0.1$ , \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ . Significant variables are highlighted in bold.

3.4A). Third, as less data were available on cloacal protuberances, we analysed the relationship between the three levels of fertilisation success and morphological measurements, badge size and cloacal protuberance for both 2002 and 2003 combined ('medium dataset'; table 3.4B). Finally, we performed the same analysis but this time including song variables, age variables and those morphological measurements that were found to be significant in the large and medium dataset, using data from 2003 ('small dataset') in a multiple variable analysis (table 4C). The sample size of the small dataset was too small to include all variables in the multiple analysis.

**Table 3.4.** Multilevel variable analysis of relation between male characteristics and fertilisation success for data from 2002 and 2003: (A) 'large dataset', (B) 'medium dataset' and data from 2003: (C) 'small dataset'

(A) Large dataset (2002 + 2003)			
Variable	Cuckoldry rate ( <i>n</i> =52/108)	EPP gained ( <i>n</i> =54/73)	Total success ( <i>n</i> =54/73)
Mass (res)	+0.01	-0.01	+0.18
Wing length	<b>-7.57**</b>	<b>+9.02**</b>	<b>+18.59***</b>
Tarsus length	<b>+4.67*</b>	-0.02	-0.84
Badge size	+0.00	+0.57	+0.20
(B) Medium dataset (2002 + 2003)			
Variable	Cuckoldry rate ( <i>n</i> =39/72)	EPP gained ( <i>n</i> =41/49)	Total success ( <i>n</i> =41/49)
Mass (res)	+1.52	+0.01	-1.06
Wing length	<b>-3.06§</b>	<b>+8.51**</b>	<b>+16.76***</b>
Tarsus length	+0.37	-0.02	-0.19
Badge size	-0.01	+0.49	-0.07
Cloacal protuberance	-0.94	+0.38	+0.08
(C) Small dataset (2003)			
Variable	Cuckoldry rate ( <i>n</i> =24/39)	EPP gained ( <i>n</i> =26/26)	Total success ( <i>n</i> =26/26)
Mass (res)	-	-	-
Wing length	+0.01	-0.20	-0.04
Tarsus length	<b>+7.93**</b>	-	-
Badge size	-	-	-
Badge colour	+1.44	-0.70	-0.02
% singing	-1.80	+1.99	+0.14
Syllable no.	-1.38	-1.76	-0.51
Pause length	<b>-3.53§</b>	-0.59	-0.02
Repertoire size	+0.50	-0.03	-0.10
Age	<b>-7.52**</b>	<b>11.71***</b>	<b>+4.39*</b>

Fertilisation success is divided in (i) cuckoldry rate (i.e. proportion of EPP in male's own brood; sample size (*n*) indicates number of different males/number of broods in years combined), (ii) EPP gained (i.e. whether a male sired offspring in other broods; sample size indicates number of different males/number of males in years combined), and (iii) total success (i.e. proportion of total number of offspring sired; sample size indicates number of different males/number of males in years combined). Values of Wald statistics and directions of the relationships are shown. Significance is indicated by §:  $p < 0.1$ , \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$  and variables that were entered in the final model are highlighted in bold. Variables that were not entered in the model are indicated with '-'.

## WITHIN- VERSUS EXTRA-PAIR OFFSPRING CHARACTERISTICS

Offspring sex in relation to paternity was analysed using generalised multilevel linear model, assuming a binomial error distribution and transformed using the logit link function. The number of offspring in the brood was used as the denominator. We did not directly determine laying and hatching order of eggs; we therefore selected offspring that we weighed between 0 (day of hatching) and 3 days after hatching, assuming that the differences in mass between offspring in the same nest reflect differences in time of hatching (Slagsvold 1986; Amundsen & Slagsvold 1991; Eikenaar *et al.* 2003). We analysed the effects of hatching order by ranking the mass measurements within each nest and partitioned the ranks into two groups: 'early' and 'late' hatching offspring. We used the median to separate the ranks; in case of an odd number of offspring, the median mass measurement was omitted from the analysis.

We analysed morphological differences between within- and extra-pair offspring for both maternal and paternal half-siblings using the last measurements of a brood. These were usually taken at day 7 after hatching (i.e. when the nestlings were ringed; 71%,  $n = 80$ ), but if the last measurements were taken on day 5 or 6 after hatching (i.e. when a brood was predated before ringing; 13%,  $n = 80$ ) or after day 7 (i.e. when a nest was found with older nestlings; 16%,  $n = 80$ ) these were also included. For maternal half-siblings, comparisons were done within a brood, therefore correcting for age was not necessary. Paternal half-siblings were compared between broods, thus biometry measurements were corrected for age of the nestlings. Correcting for provisioning rates to WPY and EPY was not necessary, as we found no difference in provisioning rates to maternal half-siblings (chapter 4) nor to paternal half-siblings (provisioning rates corrected for age and number of nestlings;  $t_{21} = 1.27$ ,  $p = 0.22$ ).

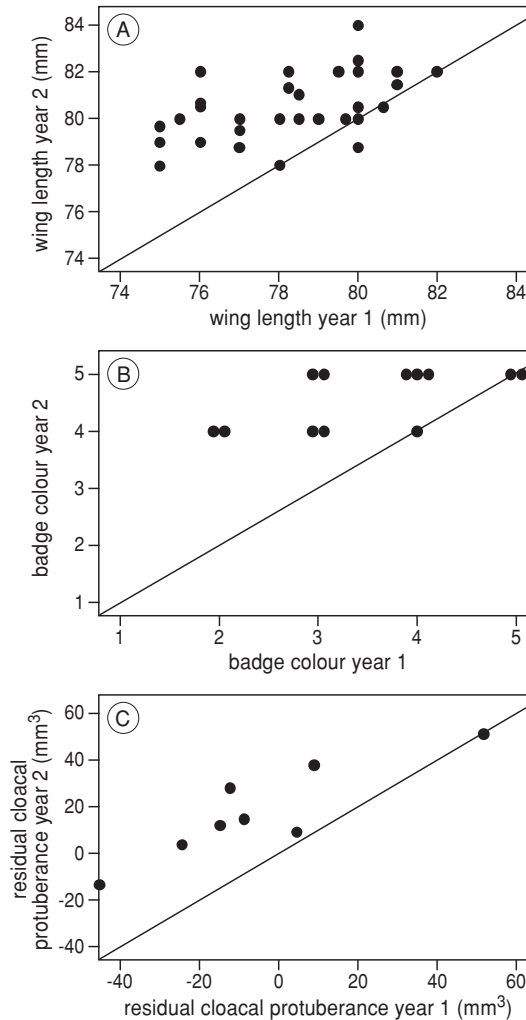
Statistical analyses were performed using SPSS 11.0.1 (2001) and MLwiN 2.0. Non-parametric tests were used in SPSS for data that were not normally distributed. Means are expressed with standard errors, probability values are two-tailed and we assumed significance if  $p < 0.05$ .



## Results

### Male age and characteristics

Repeat measurements of several male characteristics (mass (residuals), wing length, tarsus length, badge size, badge colour and cloacal protuberance) between years were available. Within males, there was a significant increase from one year to the next in wing length, badge score and cloacal protuberance (figure 3.1), but not in



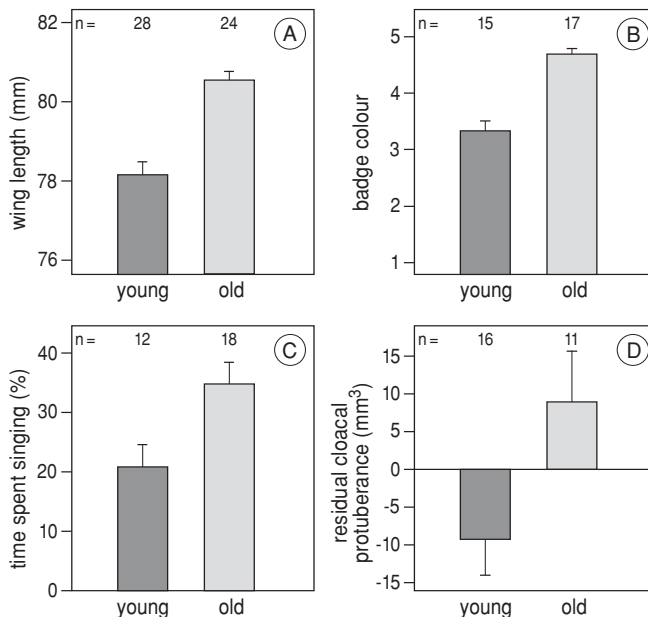
**Figure 3.1.** Repeat measurements in successive years for individual males of (A) wing length ( $t = -7.71$ ,  $n = 36$ ,  $p < 0.001$ ), (B) badge colour ( $Z = -2.88$ ,  $n = 12$ ,  $p = 0.004$ ), (C) cloacal protuberance (residuals over day;  $t = -4.63$ ,  $n = 8$ ,  $p < 0.002$ ). The relationship  $y = x$  is indicated by a line in the figure.

the other characteristics (all:  $p > 0.18$ ). The same result was found for wing length, badge colour and cloacal protuberance when comparing these characteristics in ‘young’ and ‘old’ males in 2003 (figure 3.2A,B,C). In addition, old males spent more time singing than young males (figure 3.2D). We found no difference between age classes in syllable number, pause length or repertoire size (all  $p > 0.31$ ).

### Male fertilisation success

#### CUCKOLDRY RATE

When analysing variables separately, we found a significant negative effect of wing length on cuckoldry rate (table 3.3). When combining the male characteristics in a multiple regression within the large dataset, we found that cuckoldry rate was significantly positively predicted by wing length and negatively by tarsus length (table 3.4A). In the medium dataset wing length tended to be related with cuckoldry rate, but no effect of tarsus was found (table 3.4B). Consistent with the large dataset, in the small dataset age class negatively predicted cuckoldry rate, and tarsus length positively predicted cuckoldry rate (table 3.4C). In addition, there was a trend for pause length between songs to negatively predict cuckoldry rate (table 3.4C).



**Figure 3.2.** Differences in characteristics between young and old males for (A) wing length ( $t_{50} = -5.22$ ,  $p < 0.001$ ), (B) badge colour ( $t_{30} = -5.92$ ,  $p < 0.001$ ), (C) percentage time spent singing ( $t_{28} = -2.61$ ,  $p = 0.014$ ), and (d) cloacal protuberance (residuals over day;  $t_{25} = -2.17$ ,  $p = 0.040$ ). Sample sizes are shown in the figure.

### EPP GAINED

In total, 148 offspring of the total 473 sampled with known paternity were a result of extra-pair fertilisations. Males that gained a large proportion of the total number of extra-pair fertilisations had longer wings, blacker badges, spent more time singing and were from a higher age class (table 3.3). All these are characteristics we identified to be strongly related to age (figure 3.2). We obtained similar results when excluding males from the edge of the study site (wing length, badge colour, % singing and age class:  $p < 0.05$ , all other variables: *ns*). After combining biometry characteristics from the large dataset in a multilevel model, wing length significantly predicted the proportion of EPP gained (table 3.4A), so that males with longer wings had more EPP. The same result was found within the medium dataset (table 3.4B). Within the small dataset we found that age class remained the single significant explanatory variable of the proportion of EPP gained (table 3.4C), thus older males had more EPP.

### TOTAL FERTILISATION SUCCESS

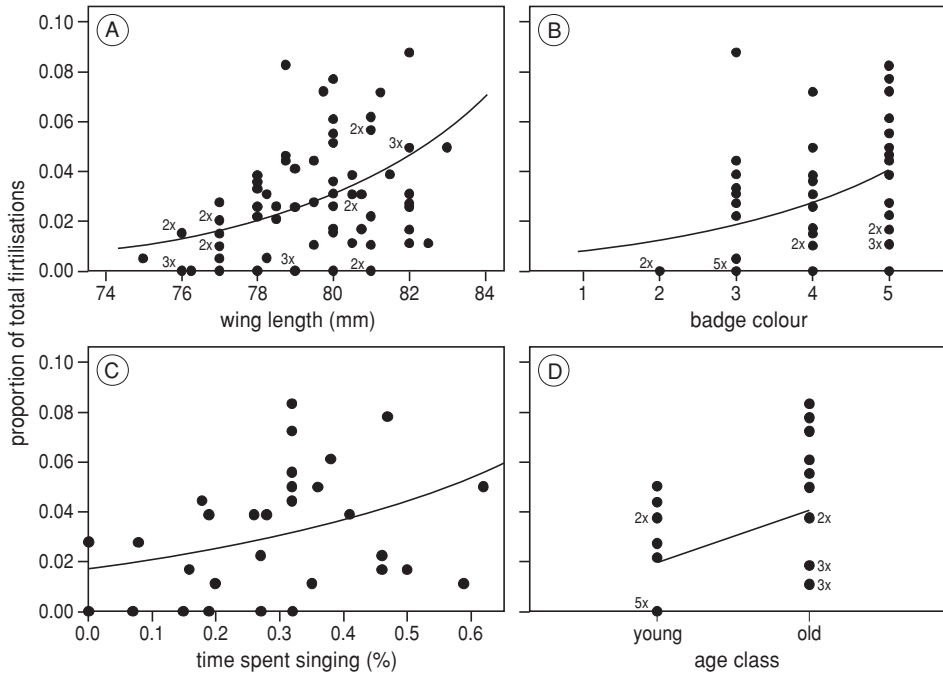
In total, 375 offspring were produced of which we had measured characteristics of the father (2002:  $n = 194$ , 2003:  $n = 181$ ). Similar to the results found for the proportion of EPP gained, males that gained a large proportion of the total number of fertilisations had longer wings, blacker badges, spent more time singing and were from an higher age class (table 3.3, figure 3.3). Furthermore, we found that the proportion of fertilisations gained was significantly predicted by wing length within the large and medium dataset (table 3.4A,B), or by age class within the small dataset (table 3.4C).

### CUCKOLDED MALE VS. EXTRA-PAIR MALE CHARACTERISTICS

Finally, in a direct comparison of characteristics of extra-pair males with the characteristics of social males they cuckolded, we found that wing length and age class were significantly different, so that extra-pair males had longer wings and were from a higher age class than the cuckolded social males (table 3.3; wing length social male vs. extra-pair male:  $79.2 \pm 0.3$  vs.  $80.0 \pm 0.2$  mm; age class social male vs. extra-pair male:  $1.53 \pm 0.12$  vs.  $1.88 \pm 0.07$ ). There was a tendency for extra-pair males to have larger cloacal protuberances than the cuckolded social males (table 3.3; residuals over day;  $6.0 \pm 3.8$  vs.  $-4.9 \pm 4.1$ ). There was no significant difference between cuckolded and extra-pair males in mass, tarsus length, badge size and colour, song quantity and quality measures (table 3.3).

### *Within-pair offspring vs. extra-pair offspring*

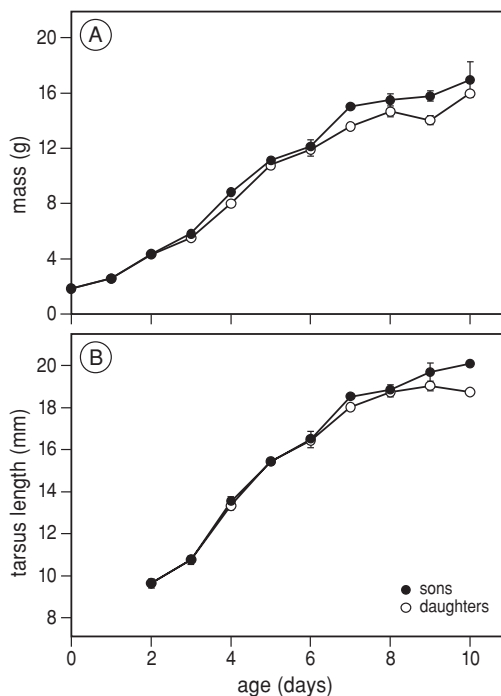
There was a significant effect of the interaction between sex and age on mass of the nestlings (figure 3.4A) and a near-significant effect of this interaction on tarsus



**Figure 3.3.** Relationship between total fertilisation success (i.e. the proportion of total number of offspring sired) and male characteristics: (A) wing length, (B) badge colour, (C) % time spent singing, and (D) age class. For details see table 3.3.

length (figure 3.4B) for nestlings between 0 and 10 days after hatching. We found no bias in sex ratio between early or late hatching offspring (early vs. late: 54% vs. 48%;  $\chi^2_1 = 0.49$ ,  $n = 65$  nests,  $p = 0.48$ ). However, we did find a bias in paternity, so that early hatching offspring were more likely to be extra-pair, while late hatching offspring were more likely to be within-pair (66% vs. 43% EPY;  $\chi^2_1 = 6.98$ ,  $n = 65$  nests,  $p = 0.008$ ). There is no general bias in sex ratio, as sons and daughters hatch (178 sons vs. 181 daughters:  $\chi^2_1 = 0.25$ ,  $p = 0.62$ ) and fledge (122 sons vs. 122 daughters:  $\chi^2_1 = 0.0$ ,  $p = 1.0$ ) in equal numbers. We found no relationship between the occurrence of EPP and unhatched eggs in a brood (% of broods with at least one unhatched egg for broods with no EPP vs. broods with EPP; 54% ( $n = 26$ ) vs. 47% ( $n = 70$ );  $\chi^2_1 = 0.34$ ,  $p = 0.56$ ).

We found no effect of paternity on sex ratio (including all offspring of known sex and paternity; sex ratio WPY vs. EPY: 49% ( $n = 194$ ) vs. 48% ( $n = 199$ );  $\chi^2_1 = 0.27$ ,  $p = 0.60$ ). There was no difference between within- and extra-pair offspring in pre-hatching mortality (WPY vs. EPY: 4.6% ( $N = 195$ ) vs. 5.0% ( $n = 199$ );  $\chi^2 = 0.36$ ,  $df = 1$ ,  $p = 0.85$ ) and pre-fledging mortality tended to be higher in EPY (10% ( $n = 137$ )) than in WPY (5% ( $n = 132$ );  $\chi^2_1 = 3.14$ ,  $p = 0.076$ ).



**Figure 3.4.** Nestling (A) mass and (B) tarsus length in relation to age for sons and daughters. There was a significant effect of the interaction between sex and age on mass of the nestlings ( $\chi^2_1 = 24.45$ ,  $p < 0.001$ ) and a near-significant effect of this interaction on tarsus length ( $\chi^2_1 = 3.69$ ,  $p = 0.055$ ).

Only three nests of mixed paternity had partial brood mortality; there was no difference in survival probability between WPY and EPY ( $\chi^2_1 = 0.74$ ,  $p = 0.39$ ). Recruitment rates were low: on average 5% ( $n = 173$ ) of offspring fledged in 2001 and 2002 was breeding in our study site the subsequent year. Daughters recruited at a significantly lower rate than sons (1% ( $n = 90$ ) vs. 8% ( $n = 91$ );  $\chi^2_1 = 4.64$ ,  $p = 0.031$ ). Only one (extra-pair) daughter and seven sons recruited in our population; of the latter four were extra-pair ( $\chi^2_1 = 0.03$ ,  $p = 0.86$ ). There was no tendency for recruited offspring to come from early ( $n = 3$  offspring) or late hatched eggs ( $n = 3$  offspring).

We compared mass, tarsus, percentage heterozygosity and mean  $d^2$  between maternal half-siblings in 34 broods of mixed paternity. As WPY and EPY did not differ in sex ratio, we included both sexes together in the analysis. EPY had longer tarsi than their within-pair half-siblings (table 3.5). However, this difference in tarsus length between maternal half-siblings disappeared when comparing within early hatched ( $t_{13} = -1.20$ ,  $p = 0.25$ ) or late hatched ( $t_{10} = 0.83$ ,  $p = 0.83$ ) offspring in a brood. There was no difference in mass, heterozygosity or mean  $d^2$

**Table 3.5.** Paired comparison of characteristics between maternal half-siblings in broods of mixed paternity ( $n = 34$ ), and paternal half-siblings between broods ( $n = 28$ ).

Variables	WPY	EPY	<i>t</i> -statistic	<i>p</i>
<b>Maternal half-siblings</b>				
Tarsus length (mm)	<b>17.6 ± 0.3</b>	<b>18.0 ± 0.3</b>	<b>-2.62</b>	<b>0.013</b>
Mass (g)	13.6 ± 0.5	13.8 ± 0.4	-0.89	0.38
Heterozygosity (%)	89 ± 2	85 ± 2	1.38	0.18
Mean $d^2$	861 ± 72	788 ± 75	0.97	0.34
<b>Paternal half-siblings</b>				
Residual tarsus length (mm)	0.12 ± 0.08	0.09 ± 0.09	0.23	0.82
Residual mass (g)	0.09 ± 0.15	0.06 ± 0.14	0.14	0.89
Heterozygosity (%)	86 ± 2	84 ± 2	0.78	0.45
Mean $d^2$	877 ± 86	885 ± 81	-0.08	0.94

Mean values ± SE for within-pair young (WPY) and extra-pair young (EPY) are presented. Significant variables are highlighted in bold.

between maternal half-siblings (table 3.5). There were 28 males of which measurements were available of both WPY in his own nest and EPY in other nests. We found no difference in mass, tarsus, heterozygosity or mean  $d^2$  when comparing these paternal half-siblings (mass and tarsus corrected for sex and age; table 3.5).

## Discussion

### *Male characteristics*

A male's fertilisation success can be determined at three different levels: (i) the proportion of paternity lost to other males in his own brood, (ii) the proportion of EPFs gained in other broods, and (iii) the proportion of total fertilisations gained, both in his own and in other broods. In this study we showed that male fertilisation success in reed buntings, at all three levels, is mainly predicted by characteristics related to male age. This is in agreement with the predictions of the good genes (Kempnaers & Dhondt 1993) and fertility insurance hypothesis (Sheldon 1994b), but not necessarily with the genetic compatibility hypothesis (Griffith *et al.* 2002). Older males will on average be of higher quality than younger males, as they have shown to be able to survive longer (Trivers 1972; Manning 1985; Kokko & Lindstrom 1996; Kokko 1998; but see Hansen & Price 1995). If females seek viability genes, they may benefit from selecting older males. However, if male functional fertility improves with male age, then females seeking EPFs to insure against infertility would generate a similar association between male

age and fertilisation success as females seeking viability genes for their offspring (Sheldon 1994b). The age-related increase in size of the cloacal protuberance supports Sheldon's suggestion (1994b) of a higher functional fertility of older males due to higher sperm storage capacities (Nakamura 1990; Birkhead *et al.* 1991; Birkhead *et al.* 1993). Females seeking compatible genes (Tregenza & Wedell 2000) are not expected to benefit from selecting older males, as the benefits of the combination of paternal and maternal genes does not change with age.

In many species, males show age-related differences in plumage (e.g. Sundberg & Dixon 1996; Richardson & Burke 1999), providing females with a cue to judge male age. In reed buntings, females may use badge colour and the proportion of time spent singing as an indication of male age, at least as being either young (1 year old) or old (>1 year old), as these variables increased with male age. In agreement, males with longer wings also spent a larger proportion of time singing in an English population of reed buntings (O'Malley 1993). Although wing length and cloacal protuberance size also increased with age, it is unlikely that they are used as cue to judge age. The direction of the relationships between age-related characteristics and fertilisation success were identical, but not all characteristics related to age predicted fertilisation success on each level with similar strength. Apparently these characteristics differ in importance when determining within- or extra-pair fertilisation success. Particularly the comparatively minor effect of cloacal protuberance on fertilisation success compared to the other age-related factors was surprising. This discrepancy may be due to the lower strength of the relationship between age and cloacal protuberance.

Males that were less cuckolded in their own broods appeared to be older and had shorter tarsi than males that were cuckolded more frequently. Older males sired more offspring in their own brood in several other species (Wagner *et al.* 1996; Perreault *et al.* 1997; Richardson & Burke 1999), but not in all (Wetton *et al.* 1995; Sundberg & Dixon 1996; Kempenaers *et al.* 1997; Johnsen *et al.* 2001). We do not have a good explanation for the negative relationship between tarsus length and cuckoldry rate (i.e. once the age of the male is taken into account). We would have expected larger males to be more successful, as tarsus length has been found to be positively related to success in other species (e.g. Kempenaers *et al.* 1997). Interestingly, there was a tendency for less cuckolded males to sing with longer pauses between songs. The pause length between songs may influence the 'vigilance' of males, i.e. time spent observing activity around him (O'Malley 1993), so that males singing with longer pauses are able to react more effectively to paternity threats, such as intruding extra-pair males. We are not aware of any other study reporting such a relationship.

Males that sired both more extra-pair offspring in other broods and more offspring in total were older, had longer wings, blacker badges and sang more. Due to intercorrelations between the age-related characteristics, we are only

confident to state an overall importance of age; to disentangle the relative importance of each of these different factors related to age, a larger sample size is required. Whether males are more successful because they are older, or for instance because they have blacker badges, remains to be determined. In other species, older males that were found to be more successful in siring offspring in their own broods, were often also found to be more successful in siring extra-pair offspring (Wagner *et al.* 1996; Perreault *et al.* 1997; Richardson & Burke 1999). However, being successful in siring within-pair offspring is not a prerequisite for being successful in siring extra-pair offspring (Wetton *et al.* 1995; Weatherhead & Boag 1995; Sundberg & Dixon 1996; Dunn & Cockburn 1999).

The strongest test of male success is a direct comparison of the social male and the extra-pair male he is cuckolded by. We found that extra-pair males had longer wings, were older and tended to have larger cloacal protuberances, confirming that age is the main predictor of male success. Although the difference was in the expected direction, badge colour and time spent singing did not significantly differ between the social and extra-pair male.

As reed buntings are highly sexually dimorphic, we were surprised that neither badge size nor our measures of song quality significantly affected male fertilisation success. Plumage ornaments and song quality significantly predict fertilisation success in many species (reviewed in Griffith *et al.* 2002). Possibly these characteristics do not play an important role in intersexual signalling in reed buntings, but perhaps more in intra-sexual signalling (e.g. Møller 1987).

An interesting question which remains, is which sex initiates extra-pair mating behaviour in the reed bunting. Older males may be more successful, both when they are actively selected by females, as is suggested to occur in Bullock's orioles (Richardson & Burke 1999), and when older males are more persistent or better able to exploit EPC opportunities, as suggested for red-winged blackbirds (Weatherhead & Boag 1995). Male reed buntings were often seen intruding in neighbouring territories, especially during the fertile period of the female, whereas females were not seen making extra-territorial forays or actively soliciting EPCs (Marthinsen *et al.* 2005; pers.obs.). Buchanan (2001) however described seeing two female reed buntings actively solicit EPCs on neighbouring territories. Due to the secretive nature of females, we cannot exclude this occurs in our population, or that females solicit copulations from intruding males. The occurrence of EPP is the result of an interaction between social male, social female and extra-pair male, and the fitness of all three depends on the behaviour of the others (reviewed in Westneat & Stewart 2003). In support of this view, we previously found that the age combination of the pair members affected the rate of cuckoldry, so that broods of young males paired to old females contained a higher proportion of EPP (chapter 5). More behavioural observations are needed on interactions between the participants in extra-pair mating behaviour in the reed bunting.



*Within-pair young versus extra-pair young*

One way to distinguish between the three female benefit hypotheses is to determine differences between within- and extra-pair young (Sheldon 1994b; Griffith *et al.* 2002). If females seek insurance against infertility through EPCs, WPY and EPY are not expected to differ in fitness, whereas if genetic benefits are driving extra-pair mating behaviour, then EPY are expected to be of higher fitness than WPY. To support the good genes hypothesis, EPY are expected to be fitter than their maternal half-siblings, and to support the genetic compatibility hypothesis, this should be the case within both maternal and paternal half-siblings.

In a Norwegian population of reed buntings, no differences were found in immunocompetence, growth rate or condition between within- and extra-pair nestlings (Kleven & Lifjeld 2004). In our study, we found no differences in survival, recruitment (although the sample size was very low), heterozygosity, mean  $d^2$  and fledgling mass. However, we did find that EPY had significantly longer tarsi than their maternal half-siblings, a trend also found in a reed bunting population in Switzerland (Kaiser & Suter, unpubl.data). This difference in tarsus length may be a result of (i) paternal genes, (ii) differential provisioning behaviour to WPY or EPY, or (iii) EPY hatching earlier than WPY.

In blue tits and red bishops (*Euplectes orix*), successful males have longer tarsi than unsuccessful males (Kempnaers *et al.* 1997; Friedl & Klump 2002). In several species, including blue tits, tarsus length has been found to be heritable (e.g. Dhondt 1982; Alatalo *et al.* 1989; Smith 1993). However, although extra-pair blue tit nestlings had on average longer tarsi than their within-pair half-siblings, this difference was not significant (Kempnaers *et al.* 1997). We showed that male reed buntings with longer tarsi were not more successful; on the contrary, males with shorter tarsi were more successful in siring offspring in their own brood. A direct genetic link between tarsus length of father and offspring is therefore not likely.

Tarsus growth has been shown to be influenced by the provisioning behaviour of the parents (e.g. Alatalo & Lundberg 1986; Moreno *et al.* 1997); possibly females provide more food to extra-pair nestlings, thereby enhancing the growth of tarsi. Previously we studied provisioning rates to individual reed bunting nestlings in broods of mixed paternity and showed that males and females provisioned to within- and extra-pair offspring at similar rates (chapter 4).

An alternative explanation for the larger tarsi of EPY compared to WPY is based on the finding that at an early age EPY are heavier than WPY, suggesting that EPY either (i) hatch from larger eggs (Cunningham & Russell 2000), (ii) develop faster (Neff 2004), or (iii) hatch earlier. Although we do not know directly from which eggs offspring hatched, our data suggest that EPY hatch earlier than WPY, resulting in longer tarsi around day 7, just because EPY are slightly older. In agreement, the difference in tarsus length between maternal half-siblings

disappeared when comparing within early hatched or late hatched offspring in a brood. Earlier hatching may be caused by faster development in the egg, either due to superior genes or maternal investment (Sheldon 2000), or, as we suggest, due to hatching from eggs produced early in the laying sequence. Previous studies on the relation between EPP and laying or hatching order have generated mixed results. Significantly more EPY were found early in the laying sequence in house sparrows (Cordero *et al.* 1999). In contrast, extra-pair offspring were biased towards eggs laid later in a clutch in house martins (*Delichon urbica*; Riley *et al.* 1995) and snow geese (*Chen caerulescens*; Dunn *et al.* 1999), and no relation was found in red-winged blackbirds (*Agelaius phoeniceus*; Westneat *et al.* 1995) or tree swallows (*Tachycineta bicolor*; Whittingham *et al.* 2003).

The relation between hatching order and EPP in the reed bunting may be a consequence of extra-pair male intrusion behaviour. In an English population, intrusion rates of extra-pair males are highest during the three days before the first egg is laid (O'Malley 1993), although this was not the case in a Norwegian population (Marthinsen *et al.* 2005). In addition, male reed buntings guard their females by close following during the fertile period before egg-laying, but reduce mate guarding abruptly on the day the first egg is laid (Marthinsen *et al.* 2005; O'Malley 1993). Possibly males perceive that the risk of losing paternity is higher early in the clutch, and adjust their mate guarding behaviour accordingly. If young males have a lower functional fertility than old males, as was suggested by the lower sperm storage capacity (cloacal protuberance), females may seek insurance against infertility of all her eggs by gaining EPFs from older males. We suggest that this young male disadvantage in functional fertility combined with the timing of intrusions by extra-pair males is a likely explanation for the observed bias of EPY early in the hatching order. However, unlike Wetton & Parkin (1991) and Gray (1997), we did not find a relationship between the hatching success of a brood and the occurrence of EPP in that brood, which would have been in support of the fertility insurance hypothesis. Neither was there a difference in hatching success between nests of young or old males ( $p = 0.98$ ).

It may be adaptive for extra-pair males to maximise the probability of fertilising eggs early in the clutch, to increase the survival chances of their offspring. The last eggs of a clutch are more likely not to hatch due to parental neglect when care is directed to the earlier hatched offspring, or late hatched nestlings are more likely to die at an early age, due to a lower competitive ability to gain food compared to the earlier hatched offspring (Krebs 1999 and references therein). In reed buntings, the last and sometimes fore last laid egg often hatched a day later than the earlier laid eggs (pers.obs.). Thus EPCs fertilising the first eggs of a clutch may result in offspring with a higher chance of surviving. However, we did not find an indication that recruiting offspring hatched from eggs laid early in the clutch, although the sample size was very small ( $n = 6$ ). Neither did we find a significant

difference between within- and extra-pair offspring in pre-fledging mortality, but high offspring mortality may be limited to years with low food conditions that we may not have encountered during our study period.

If early hatching extra-pair offspring have a fitness benefit, females would also gain genetic benefits for their offspring through extra-pair matings, although these may not have been apparent in our short-term measures of fitness. Similar patterns in peaks of EPCs have been found in other species, but these appeared to be a result of active female choice. Female chaffinches (*Fringilla coelebs*) and red-winged blackbirds solicited EPCs closer to egg-laying than they did within-pair copulations (Sheldon 1994a; Gray 1997). However, whether this resulted in a bias of EPY towards early in the laying order is unknown.

We found no support for the genetic compatibility hypothesis, as we found no effect of the combination of maternal and paternal genes on offspring characteristics. There was no significant difference in heterozygosity or mean  $d^2$  between maternal half-siblings from the same brood. Furthermore, we found no indication that there was a difference in measurements between paternal half-siblings, although in this case we could not account for any maternal or environmental differences, besides provisioning rates, between within- and extra-pair broods of the same male. In addition, in a Norwegian population of reed buntings no difference was found in immunocompetence between paternal half-siblings (Kleven & Lifjeld 2004). However, as data on long-term survival and reproductive success are still lacking in reed buntings and our sample size was relatively small when compared to the study by Foerster *et al.* (2003;  $n = 101$  broods of mixed paternity), we cannot exclude the possibility that females do gain genetic benefits through extra-pair matings.

### *Conclusion*

Older males are more successful in gaining fertilisations both within their own brood and in other broods. Females engaging in EPCs may thus gain either viability genes for their offspring or insurance against functional infertility of their partner. When investigating differences between WPY and EPY, we found that more EPY than expected hatched from eggs laid early in a clutch, resulting in EPY being slightly older than WPY. Apart from this difference in age, we found no differences between maternal and paternal half-siblings in several fitness components. Therefore our data do not support either of the two genetic benefit hypotheses. As we found a significant difference in sperm storage capacity between young and old males, we suggest that through EPCs with old males, female reed buntings are insured against functional infertility of their young social partner. As offspring hatching early in a brood may have a fitness advantage, extra-pair males may be selected to attempt to fertilise eggs early in a clutch, leading to the observed bias in hatching order of EPY. This suggestion is supported by the

pattern of intrusion behaviour of extra-pair males in an English population, as intrusions peak just before the first egg of a clutch is laid (O'Malley 1993).

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