

# MALE CHARACTERISTICS AND FERTILISATION SUCCESS IN BLUETHROATS

by

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## Summary

Extra-pair copulations (EPCs) can create or intensify sexual selection and, provided that fertilisation success is related to phenotypic traits, help explain sexual dimorphism in socially monogamous species. Previous experimental manipulations of the ornamental coloration in male bluethroats, *Luscinia s. svecica*, have shown effects on their social mating success, mate-guarding behaviour, and within-pair- and extra-pair paternity. This study investigates the relationship between male characteristics (reflectance of the blue throat feathers, width of the chestnut breast band, wing length, body condition and age) and fertilisation success under natural, non-experimental conditions. Combining three breeding seasons, 29% of 720 offspring were sired by extra-pair males and 54% of 136 nests contained one or more extra-pair offspring. The chroma (spectral purity) of the blue throat feathers and the width of the chestnut band were positively related to paternity in own nest, and for blue chroma this translated into a significantly positive relation with total fertilisation success. This suggests that differential within-pair paternity success exerts directional selection on the colour signal. None of the throat colour measures or morphological traits were significantly related to

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overall extra-pair fertilisation (EPF) success. However, restricting the analysis to males with one or more EPFs, there was a positive relation between amount of extra-pair paternity and blue chroma. Old males were more successful than young ones in achieving EPFs. Pair-wise comparisons showed no plumage differences between cuckolded males and the males that cuckolded them. The absence of phenotypic correlates of male EPC-success agrees with our recent finding that females improve offspring quality through individual choice of EPC partners with 'compatible genes' rather than 'good genes' in an absolute sense. Our results indicate that experiments where traits are manipulated outside the natural range should be interpreted with caution, and illustrate the importance of a dual approach (experimental and correlative) in studies of sexual selection in the wild.

## Introduction

Extra-pair copulations (EPCs) may affect the opportunity for sexual selection by increasing the variance in reproductive success among males (Birkhead & Møller, 1998). Male secondary sexual characters might thus evolve if females prefer particular male traits in extra-pair mate choice or if certain traits are advantageous in male contest competition for fertilisations. In both cases, male secondary sexual characters should reflect some aspect of male quality (Andersson, 1994). Since, in birds, extra-pair males normally do not contribute other resources than sperm to the female, any benefits from extra-pair mate choice are likely to be genetic, *e.g.* in the form of increased viability of extra-pair offspring (Birkhead & Møller, 1992; Williams, 1966).

Several studies of birds have found extra-pair fertilisation (EPF) success to correlate with some male phenotypic trait (*e.g.* Hasselquist *et al.*, 1996; Kempenaers *et al.*, 1992; Sheldon *et al.*, 1997), supporting the idea that females choose high quality males as EPC partners (the 'good genes hypothesis'). However, quite a few studies have failed to find such correlates (*e.g.* Hill *et al.*, 1994; Krokene *et al.*, 1998; Reyer *et al.*, 1997), suggesting that even if EPCs do lead to sperm competition, they need not necessarily invoke directional sexual selection on male secondary sexual traits. An explanation of this might be that females have individual preferences for maximally compatible copulation partners (Trivers, 1972). According to the 'compatible genes hypothesis', a favourable combination of genes from the two mates should give rise to offspring of higher viability, *i.e.* similar to the good genes hypothesis. However, the two hypotheses differ with respect to whether females have similar or different mating preferences. These processes (directionality/compatibility) need not be mutually exclusive, for

instance if females select for compatible males only among those exceeding some minimum level of ornamentation.

The bluethroat, *Luscinia s. svecica*, is well suited for a study of the relation between male traits and fertilisation success for several reasons. First, the species is highly sexually dichromatic, with males displaying a complex throat ornament towards both females and males (Peiponen, 1960). The ornament shows considerable individual variation in colour and pattern. In particular, the blue feathers, which have a strong ultraviolet (UV) component, vary between males in several respects (Andersson & Amundsen, 1997, this study), and the lower chestnut breast band varies in width (T. Amundsen, unpubl. results; this study). Second, sperm competition is intense in the species, with 19-32% of the chicks being sired by extra-pair males (Krokene *et al.*, 1996, this study). Third, there is experimental evidence to suggest that male phenotypic appearance influences female choice of both social and genetic partner (Andersson & Amundsen, 1997; Fiske & Amundsen, 1997; Johnsen & Lifjeld, 1995; Johnsen *et al.*, 1998a, b). For example, blackening of the male throat patch led to a decreased paternity compared to controls, despite the fact that blackened males guarded their mates more intensely and experienced the same frequency of intrusion by neighbouring males (Johnsen *et al.*, 1998b). Similarly, experimental reduction of the UV reflectance of the throat patch resulted in decreased EPF-success (Johnsen *et al.*, 1998a). These results suggest female choice for copulation partners based on the throat ornament. However, since the manipulations extended far outside the natural range of variation in male plumage coloration, we can not infer from these experiments that there is female extra-pair mate choice based on plumage ornamentation under normal conditions. It is thus essential to explore the fitness consequences of natural trait variation, which is the main objective of this study.

Further motivation to investigate the natural variation in fertilisation success comes from our recent study (Johnsen *et al.*, 2000) that suggests that there are individual mating preferences that do not create directional selection on male phenotype. We tested the immune response of maternal half-siblings within broods and paternal half-siblings in different broods. We found that extra-pair offspring had a significantly higher T-cell mediated immune response than their half siblings in both cases, thereby demonstrating a genetic benefit of EPCs to both sexes in the bluethroat, possibly arising from a genetic compatibility effect. At first sight, results from the plumage

manipulation experiments and the offspring immunocompetence study may seem contradictory, the former suggesting consistent female preferences for specific traits and the latter individual preferences for compatible mates. In the present study, we investigate this problem further, by correlating natural variation in male characteristics with measures of fertilisation success in a large sample including the broods from the offspring immunocompetence study (Johnsen *et al.*, 2000).

## Methods

### *Field procedures*

The study was conducted at Øvre Heimdalen field station, east of the Jotunheimen mountains, Norway (61°25'N, 8°52'E), during the breeding seasons of 1992, 1998 and 1999. Adult birds were caught in mist nets, measured (mass, wing length, tarsus length), aged (second year (SY) or after second year (ASY) according to Svensson (1992)), and a blood sample of about 50  $\mu$ L was taken by puncturing the brachial vein. Nestlings were blood sampled (maximum 25  $\mu$ L) between days 2-10 post-hatch.

### *Reflectance and colorimetrics*

The blue throat plumage reflects primarily in the ultraviolet waveband (320-400 nm; see Fig. 1 in Andersson & Amundsen, 1997), continuing with a steep decline above 400 nm that produces the brilliant blue colour perceived by human eyes. Previous demonstrations of bluethroat colour vision and mate choice criteria (Andersson & Amundsen, 1997; Johnsen *et al.*, 1998a) have specifically addressed and manipulated the UV signal component. Although this is interesting because of the earlier neglect of UV communication in avian ecology (Bennett *et al.*, 1994), the 400 nm limit of human vision has no relevance for the wider spectral range of birds.

In 1998, we therefore measured the natural reflectance variation in the bluethroat population, and computed objective colour measures in relation to male reproductive success. We used a PS 1000 diode-array spectrometer system (Ocean Optics Inc., Dunedin, USA), a DH 2000 deuterium-halogen light source and a fiberoptic probe measuring direct perpendicular reflectance from a 4 mm wide plumage spot and in relation to WS-2 white standard (Avantes Ltd., Eerbeek, Netherlands). Five scans, removing the probe between each, were taken from the same spot of the male blue chin plumage.

Objective indices of the three main dimensions of colour perception (Hailman, 1977) were computed as follows: 'Brightness' (spectral intensity) was estimated by  $R_{320-700}$ , the sum of reflectance from 320-700 nm, corresponding to the spectral range of most birds studied to date (Hart *et al.*, 2000). 'Hue' (spectral location) was estimated as  $\lambda_{\max}$ , the wavelength of maximum (peak) reflectance, which has been used as a hue measure in several studies of avian short-wave colours (*e.g.*, Hunt *et al.*, 1998; Keyser & Hill, 1999; Sheldon *et al.*, 1999). There are several potential ways to objectively estimate 'Chroma' (spectral purity), such as the ratio or difference between  $R_{\max}$  and  $R_{\min}$ , or the maximum slope. The difference

between two spectral segments, divided by the total reflectance (see Endler, 1990) should be particularly relevant. We use  $\lambda(R50)$ , the wavelength at which reflectance is halfway between its minimum ( $R_{\min}$ ) and its maximum ( $R_{\max}$ ), as segment divider, and compute  $C[R50]$  as  $R_{[\lambda(R50)-700]} - R_{[\lambda(R50)-320]} / R_{320-700}$ . We term this 'Shortwave chroma' to separate it from 'UV chroma' (below). The main reason for using the individual-specific  $\lambda(R50)$  as divider is to obtain an objective (receiver-independent) measure of chroma that is as independent from hue as possible. If segments are separated by a fixed wavelength (as in 'UV chroma' — see below), variation in the spectral location (*i.e.* hue) of a reflectance peak or a cut-off will influence the chroma measure also when reflectance shape is constant. A dynamic, individual-specific divider, on the other hand, allows the slope and height aspects of the reflectance curve to be isolated, without confounding it with peak location. Since the mechanisms behind peak location (hue) and slope (chroma) often are likely to be different (for example type and amount of pigment), it is useful to separate these aspects of the signal variation (Hailman, 1977). In a recent study of the relationship between male coloration and paternal care in the bluethroat, Smiseth *et al.* (2001) used a chroma measure that was based on a fixed segment division located between the blue and green cone functions in starlings, *Sturnus vulgaris*. This has the merit of potentially being closer to avian colour perception (assuming similar cone functions in bluethroats and neural opponency between the two shortwave and the two longwave cone types). However, after further discussion and analyses of reflectance variation, we prefer the present  $C(R50)$  chroma measure. As explained above, it is more objective in the sense that it makes fewer assumptions about the as yet unknown visual physiology and behavioural colour vision of the receiver. For comparison, we also computed 'UV chroma' ( $R_{320-400} / R_{320-700}$ ), since this reflectance component has been used previously in descriptions and experiments addressing the specific importance of UV (Andersson & Amundsen, 1997; Andersson *et al.*, 1998; Sheldon *et al.*, 1999).

The chestnut band below the blue chest is another potentially important plumage trait. It is based on reddish melanin and varies minimally in reflectance (S. Andersson, unpubl. results), but differs considerably in width (*i.e.* how far it extends down the belly) between males. We measured the width of the chestnut band with a slide calliper in 1992 and 1999. The two measured components of the male throat ornament (the reflectance of the blue chin feathers and the width of the chestnut band) were chosen because they show relatively high individual variation compared with other throat components, and because they can be measured reliably (A. Johnsen, J.T. Lifjeld, S. Andersson & T. Amundsen, unpubl. results).

### Parentage analysis

The collected blood samples and some tissue (dead chicks, embryos from unhatched eggs) were subject to multilocus DNA fingerprinting (1992 samples) or microsatellite typing (1998 and 1999 samples). The samples from 1992 were extracted with a proteinase K-phenol:chloroform protocol (see Krokene *et al.* (1996) for details), whereas the samples from 1998 and 1999 were extracted with the QIAamp blood/tissue kits (Qiagen). Details of the multilocus DNA fingerprinting protocol and criteria for exclusion can be found in Krokene *et al.* (1996), and details of the microsatellite analysis can be found in Johnsen *et al.* (1998b). The following six heterologous microsatellite markers were used: *FhU2* (Ellegren, 1992), *FhU3* (Primmer *et al.*, 1996), *HrU7* (Primmer *et al.*, 1995), *Phr2* (Fridolfsson *et al.*, 1997), *Pocc5* (Bensch *et al.*, 1996) and *Mcyu4* (Double *et al.*, 1997). The combined

probability of exclusion (Jamieson, 1994) for this marker set was 0.995 and 0.996, and the probability that two individuals would have identical genotypes  $3.5 \times 10^{-7}$  and  $2.7 \times 10^{-7}$ , for the two years respectively.

We determined parentage for a total of 136 nests (29 in 1992, 55 in 1998, 52 in 1999), totalling 721 nestlings (143 in 1992, 297 in 1998, 281 in 1999). This represented 99.9% of the collected samples; only one collected egg proved impossible to analyse, probably due to DNA degradation. Most of the families were complete with both putative parents, but for six nests we only had DNA from the social father. In addition, we genotyped 52 territorial males from 1998 and 1999 whose nests we were not able to find.

### *Parentage assignment*

#### 1992

Details of parentage for chicks in 16 of the 29 nests from 1992 have been published previously (Krokene *et al.*, 1996). For the remaining 13 nests in that year, the mean ( $\pm$ SD) band-sharing coefficient between putatively unrelated pair mates was  $0.20 \pm 0.06$ . Forty-one of the 60 offspring in these nests had 0–2 novel fragments and high band-sharing coefficients with both parents (mean  $\pm$  SD band sharing: mother:  $0.53 \pm 0.14$ , father:  $0.55 \pm 0.08$ ). These offspring were thus sired by both of their social parents. Nineteen offspring had three or more novel fragments, a high band sharing with the putative mother ( $0.55 \pm 0.08$ ) and a low band sharing with the putative father ( $0.10 \pm 0.06$ ). We consider these offspring to have been sired by extra-pair males.

#### 1998 and 1999

The present sample encompasses the broods in our recently published paper on offspring immunocompetence (Johnsen *et al.*, 2000), but as it consists of 21 more nests we present the entire paternity analysis here. Combining the two years, 390 offspring showed a complete match with the genotypes of the parents, with an average probability of chance inclusion (Jeffreys *et al.*, 1992) of  $0.0056 \pm 0.0070$  SD (range  $1.5 \times 10^{-6} - 0.04$ ). Eight offspring had one mismatch with either parent (three female, two male, three unclear because the parents had similar alleles). For the male- and unclear mismatches, the probability of false inclusion was relatively low when excluding the mismatched locus (mean  $0.0059 \pm 0.0076$  SD, range  $7 \times 10^{-5} - 0.016$ ). We consider all of these 398 offspring to be descendants of their putative parents, the single mismatches being due to mutations. Of the remaining 180 offspring, 174 had two or more mismatches and five had one mismatch with the social father, whereas the mother matched an allele at all loci. The five offspring with single mismatches all fulfilled one or both of the following conditions: (1) a relatively high probability of chance inclusion for the social father when excluding the mismatched locus from the calculations (mean  $0.088 \pm 0.084$  SD, range  $0.012 - 0.23$ ), (2) a neighbouring male showing a complete match with the paternal genotype of the chick (see below). In conclusion, we regard all of these 179 offspring to have been sired through extra-pair paternity. The last offspring showed mismatch with both parents at two loci and was thus the result of egg dumping.

For 120 of the extra-pair offspring (EPO), we found one ( $N = 111$ ) or two ( $N = 9$ ) males that matched the paternal genotype completely. For 101 of the 111 unequivocally assigned EPO, we knew the nests of both males. In 100 of these, the genetic father resided within three

territories of the nest with the EPO, and in the last case there were four territories between the respective nests. The present data thus supports the conclusion from previous work on the same population that cuckolders are found predominantly among close neighbours (Johnsen *et al.*, 1998a, b).

In the nine cases where several males matched the paternal genotype, one of the potential fathers was always a close neighbour (nearest neighbour: 7 cases, second nearest neighbour: 2 cases) whereas the alternative male(s) had territories further away (third nearest neighbour: 2 cases, five or more territories away: 7 cases). Furthermore, in six cases (including the ones where an alternative male resided within three territories) this particular male was also the unequivocal sire of at least one other EPO in the same nest. We therefore assigned paternity to these males giving a mean probability of chance inclusion of  $0.0031 \pm 0.0038$  SD (range  $4.1 \times 10^{-7} - 0.020$ ) for the 120 EPO with complete match. In addition, we assigned paternity to three males that showed one mismatch with the respective EPO (likely due to mutation), but a low probability of chance inclusion (mean  $0.0077 \pm 0.0089$  SD, range  $0.0005 - 0.020$ ). All of these males were the nearest neighbours and two of them also were the sole potential sire of another offspring in the same nest.

We calculated four measures of male success, based on clutch size and paternity of offspring (including embryos from unhatched eggs). We used paternity data from both nestlings and unhatched eggs in the analyses because (1) we did not have data on brood fate, and (2) there was no tendency for unhatched eggs to be biased towards either within-pair or extra-pair offspring (A. Johnsen & J.T. Lifjeld, unpubl. results). Apparent success is the number of eggs in the clutch. Extra-pair fertilisation success is expressed both as a binomial variable (no EPFs/one or more EPFs) and as the number of offspring sired through EPC. Within-pair paternity is expressed as the proportion of offspring in the nest sired by the attending male (see Statistical procedure). Total fertilisation success is the number of offspring sired in the social nest plus the number of offspring sired in other nests through EPC.

### *Statistical procedure*

There was significant annual variation in wing length ( $F = 18.6$ ,  $p < 0.0001$ ) and condition (residuals from a regression of body mass on tarsus length;  $F = 13.1$ ,  $p < 0.0001$ ). However, we pooled the data from different years in the presented analyses, since controlling for the year effect had no impact on any of the relationships between these male characteristics and measures of male success.

The data from 1992 were only included in tests of relations between within-pair paternity and male characters, as we were unable to assign paternity to EPO using DNA fingerprinting. A few males bred in both 1998 and 1999. In order to avoid pseudo-replication, the data from these males were treated in the following way: (1) In analyses of within-pair paternity, extra-pair fertilisation success and total fertilisation success, we only included their first recorded breeding (1998). (2) In the pair-wise analyses of the traits of cuckolded males *versus* cuckolders, we included all pairs of males because the combination of males was never the same. Six males were polygynous. Only their primary nest was included in analyses of within-pair paternity, whereas both nests were included in the analyses of total fertilisation success.

The relationships between male characteristics and within-pair paternity were analysed with generalised linear models (GLMStat 5.2), with the number of offspring sired by the attending male as dependent variable, brood size as binomial denominator and binomial error

distribution. For the remaining analyses, parametric tests were used when the assumptions for such tests were met. Multiple tests of the same prediction were sequentially Bonferroni adjusted (Chandler, 1995), in order to keep the prediction-wide threshold for significant  $p$ -values at  $\alpha = 0.05$ . All  $p$ -values are two-tailed.

## Results

### *Pattern of extra-pair paternity*

In total, extra-pair males sired 29% of the offspring, and 54% of nests had one or more EPO. There were no significant differences between the years in proportion of EPO within nests (Kruskal-Wallis  $H_2 = 1.24$ ,  $p = 0.54$ ; Table 1), or in proportion of nests that contained one or more EPO ( $\chi^2 = 3.4$ ,  $df = 2$ ,  $p = 0.19$ ; Table 1).

### *Opportunity for sexual selection*

The distribution of EPO within nests was significantly different from random ( $\chi^2 = 52.2$ ,  $df = 4$ ,  $p < 0.0001$ ), showing a bimodal distribution with overrepresentation of nests having none or many ( $\leq 4$ ) EPO (Fig. 1).

The variance was considerably higher in male total fertilisation success ( $s^2 = 8.71$ ) than in apparent success ( $s^2 = 2.51$ ; variance ratio test,  $F_{92,92} = 3.48$ ,  $p < 0.0001$ ). Calculating the index for the opportunity for sexual selection ( $I_s$ : the variance in male success divided by the square of mean success), as proposed by Wade & Arnold (1980), we found the opportunity for selection to be about 4.5 times higher based on actual fertilisation success ( $I_s = 0.37$ ) than what would be expected from apparent success ( $I_s = 0.08$ ).

### *Relationships among male traits*

The two components of the throat ornament (the reflectance of the blue chin feathers and the width of the chestnut band) could not be related

TABLE 1. *Percentage of extra-pair offspring and nests with one or more extra-pair offspring, in the three years of study*

Year	Percentage of offspring	Percentage of nests
1992	25% (36/143)	45% (13/29)
1998	32% (95/297)	64% (35/55)
1999	28% (78/280)	50% (26/52)

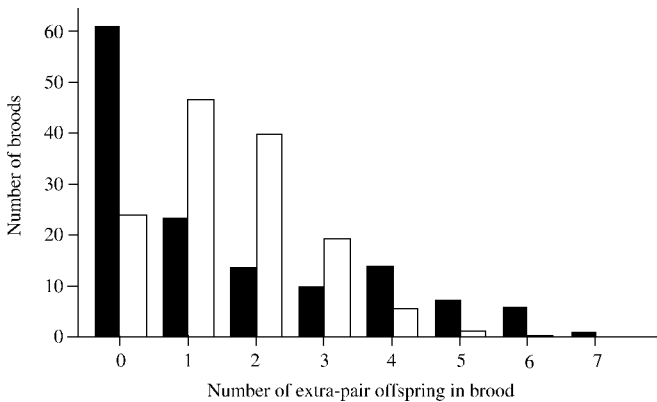


Fig. 1. Distribution of extra-pair offspring within broods. Black bars represent observed frequencies and white bars expected frequencies calculated from a permutation test with 1000 randomisations.

to each other, since they were measured in different years. There were positive relationships between shortwave chroma and UV chroma (Pearson's  $r = 0.44$ ,  $p = 0.0004$ ) and between shortwave chroma and brightness ( $r = 0.51$ ,  $p = <0.0001$ ), and a negative relationship between UV chroma and hue ( $r = -0.39$ ,  $p = 0.002$ ), illustrating how a fixed segment division confounds hue and chroma (see methods). None of the other correlations among reflectance components were significant (all  $r < 0.18$ , all  $p > 0.16$ ). Male condition was not significantly related to any of the ornament measurements (all  $r < 0.21$ , all  $p > 0.14$ ). Old males were in significantly better condition than young ones (mean  $\pm$  SE condition for ASY males:  $0.09 \pm 0.09$ ,  $N = 102$ , SY males:  $-0.23 \pm 0.14$ ,  $N = 44$ ,  $t = 1.99$ ,  $p = 0.048$ ). The two age-classes also differed in the width of the chestnut band (ASY males:  $6.95 \pm 0.26$  mm,  $N = 78$ , SY males:  $5.74 \pm 0.34$ ,  $N = 34$ ,  $t = 2.66$ ,  $p = 0.009$ ), in shortwave chroma (ASY males:  $0.27 \pm 0.01$ ,  $N = 37$ , SY males:  $0.22 \pm 0.01$ ,  $N = 22$ ,  $t = 3.38$ ,  $p = 0.001$ ), and in UV chroma (ASY males:  $0.361 \pm 0.003$ ,  $N = 37$ , SY males:  $0.345 \pm 0.004$ ,  $N = 22$ ,  $t = 3.28$ ,  $p = 0.002$ ). Hence, old males were in somewhat better condition, had a more chromatic UV/blue coloration and a wider chestnut band.

#### *Within-pair paternity*

Both shortwave chroma and the width of the chestnut band were significantly related to within-pair paternity (Table 2). Males with highly chromatic blue

feathers had higher paternity than males with less chromatic feathers (Fig. 2). Likewise, males with wide chestnut bands gained a higher paternity than males with narrow borders. However, a generalised linear model with year, chestnut band and year  $\times$  chestnut band as predictor variables, revealed a significant interaction between year and chestnut band ( $p < 0.0001$ ). Splitting the data by year, chestnut band was strongly positively related

TABLE 2. Generalised linear model tests of relationships between male characteristics and within-pair paternity (proportion of offspring sired by the attending male)

Trait	<i>N</i>	$\chi^2$	<i>p</i>	Direction
Shortwave chroma	42	9.74	0.0018 <sup>a</sup>	+
UV chroma	42	3.68	0.055	+
Brightness	42	0.053	0.82	÷
Hue	42	0.054	0.82	+
Chestnut band	67	7.95	0.0048 <sup>a</sup>	+
Wing length	118	2.09	0.15	+
Condition <sup>b</sup>	118	3.28	0.070	÷
Age (ASY/SY)	121	0.020	0.96	÷

See methods for explanation of colour measurement calculations.

<sup>a</sup> Significant after sequential Bonferroni adjustment of the five *p*-values testing the prediction that male coloration is associated with paternity.

<sup>b</sup> Residuals from a regression of body mass on tarsus length.

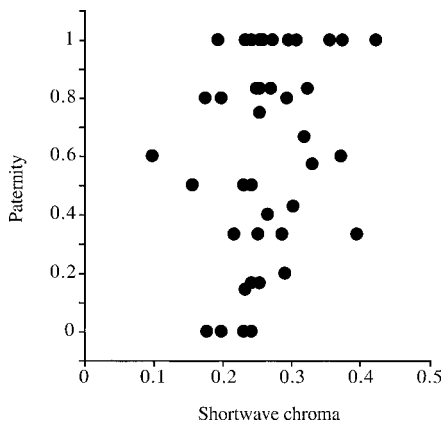


Fig. 2. Relationship between chroma of the blue feathers (shortwave chroma) and within-pair paternity. See methods for explanation of chroma calculations.

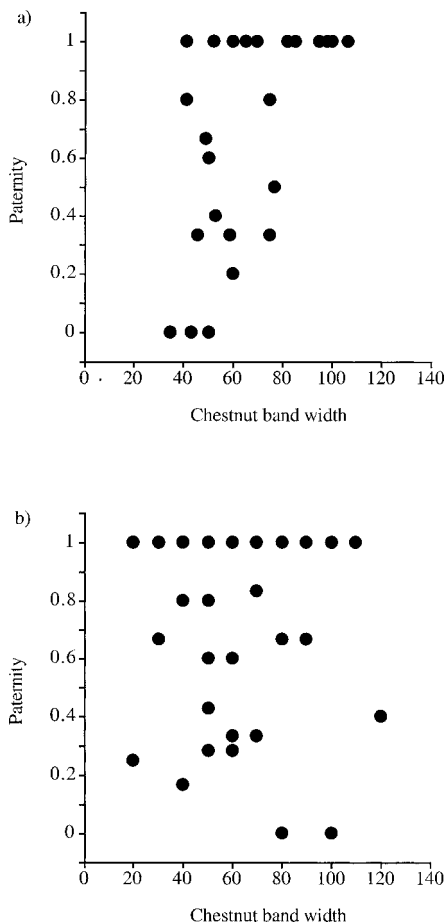


Fig. 3. Relationship between the width of the chestnut band and within-pair paternity in (a) 1992, and (b) 1999.

to within-pair paternity in 1992 ( $\chi^2 = 26.9$ ,  $N = 28$ ,  $p < 0.0001$ ; Fig. 3a), but there was no such relationship in 1999 ( $\chi^2 = 0.012$ ,  $N = 39$ ,  $p = 0.91$ ; Fig. 3b). None of the other measures (remaining blue colour components, wing length, condition or age) were significantly related to within-pair paternity (Table 2).

There was no significant difference in within-pair paternity between males that had EPO in other nests (mean  $0.71 \pm 0.06$ ) and males that did not sire EPO (mean  $0.71 \pm 0.04$ ; Mann-Whitney test,  $N_1 = 29$ ,  $N_2 = 64$ ,  $z = 0.28$ ,  $p = 0.78$ ). Finally, there was no significant correlation between the number

of EPO sired in other nests and within-pair paternity (Spearman correlation,  $N = 93$ ,  $r_s = 0.11$ ,  $p = 0.31$ ).

### *Extra-pair fertilisation success*

Male age was strongly positively related to EPF-success. Thirty-four of the 38 identified extra-pair fathers were ASY males (Table 3). None of the morphological variables were significantly related to EPF-success (Table 3). Similar results were obtained when using the number of EPO as response variable; only male age was significantly related to success (mean  $\pm$  SE number of EPO for ASY males:  $1.07 \pm 0.20$ ,  $N = 91$ , SY males:  $0.31 \pm 0.21$ ,  $N = 35$ ,  $z = -2.83$ ,  $p = 0.0046$ ; all other  $p > 0.44$ ). Old males with breeding experience from the study area from the previous year ( $N = 19$ ), were not significantly more likely to obtain EPFs than old males that were new to the area ( $N = 82$ ; Fisher's exact test,  $p = 0.19$ ).

The above tests included a number of males that had no EPFs, a situation that may arise for many different reasons (*e.g.* that the males were not seeking EPCs or that they were unsuccessful because they were unattractive or lost in sperm competition). We therefore performed the tests also within the potentially more behaviourally uniform group of males that had one or more EPO, using the proportion of EPO (number of EPO divided by total clutch size of all extra-pair nests involved) as response variable. Interestingly, there was a significantly positive correlation between shortwave chroma and

TABLE 3. *Relationships between male traits and EPF success in the bluethroat*

Trait	$\geq 1$ EPF	No EPF	Test statistic	$p$
Shortwave chroma	$0.27 \pm 0.01$ (19)	$0.25 \pm 0.01$ (32)	$t = 0.71$	0.48
UV chroma	$0.36 \pm 0.004$ (19)	$0.36 \pm 0.003$ (32)	$t = 0.42$	0.67
Brightness	$96.9 \pm 2.3$ (19)	$96.1 \pm 1.4$ (32)	$t = 0.33$	0.74
Hue	$365.4 \pm 1.7$ (19)	$365.7 \pm 1.6$ (32)	$t = -0.12$	0.91
Chestnut band (mm)	$6.4 \pm 0.66$ (14)	$6.7 \pm 0.38$ (43)	$t = -0.44$	0.66
Wing length (mm)	$76.5 \pm 0.32$ (35)	$76.2 \pm 0.20$ (83)	$z = 0.51$	0.61
Condition <sup>b</sup>	$0.21 \pm 0.17$ (35)	$0.08 \pm 0.09$ (83)	$t = 0.72$	0.47
Age (ASY/SY)	34/4	57/31	$\chi^2 = 8.5$	0.004 <sup>a</sup>

Sample sizes are shown in brackets. Values are mean  $\pm$  SE. See methods for explanation of colour measurement calculations.

<sup>a</sup>Significant after sequential Bonferroni adjustment.

<sup>b</sup>Residuals from a regression of body mass on tarsus length.

proportion of EPO ( $N = 19$ ,  $r_s = 0.52$ ,  $p = 0.027$ ), whereas none of the other male traits were significantly related to this measure (all  $r < 0.42$ , all  $p > 0.08$ ). Furthermore, within this subset of males, there was a significantly positive correlation between within-pair paternity and proportion of EPO ( $N = 32$ ,  $r_s = 0.41$ ,  $p = 0.022$ ).

Pair-wise comparisons of the traits of cuckolded males with those of their cuckolders showed no significant differences in blue colour measures ( $N = 26$  pairs), width of the chestnut band ( $N = 17$  pairs), wing length or condition ( $N = 49$ -55 pairs; paired  $t$ -tests, all  $p > 0.45$ ). In 46 cases both males were ASY, in eight cases the cuckolder was ASY and the cuckolded SY, in three cases both males were SY, and in two cases the cuckolder was SY and the cuckolded ASY. These figures are significantly different from what would be expected if mating was random with respect to male age (Fisher's exact test,  $p = 0.04$ ), showing that cuckolders tended to be older or of the same age-class as the males they cuckolded.

#### *Total fertilisation success*

Shortwave chroma was positively correlated with total fertilisation success (Table 4, Fig. 4), whereas none of the other male traits were significantly related to this measure of male success (Table 4).

TABLE 4. *Relationships between male traits and total fertilisation success in the bluethroat*

Trait	<i>N</i>	Test statistic	<i>p</i>
Shortwave chroma	42	$r_s = 0.40$	0.01 <sup>b</sup>
UV chroma	42	$r_s = 0.15$	0.33
Brightness	42	$r_s = 0.13$	0.40
Hue	42	$r_s = 0.04$	0.80
Chestnut band	39	$r_s = -0.06$	0.73
Wing length	90	$r_s = 0.07$	0.54
Condition <sup>c</sup>	90	$r_s = -0.13$	0.21
Age (ASY/SY)	69, 24	$z = -0.24^a$	0.81

See methods for explanation of colour measurement calculations.

<sup>a</sup>Mann-Whitney test. Mean total fertilisation success  $\pm$  SE: ASY males:  $5.0 \pm 0.4$  offspring, SY males:  $4.5 \pm 0.5$  offspring.

<sup>b</sup>Significant after sequential Bonferroni adjustment of the five  $p$ -values testing the prediction that male coloration is associated with total fertilisation success.

<sup>c</sup>Residuals from a regression of body mass on tarsus length.

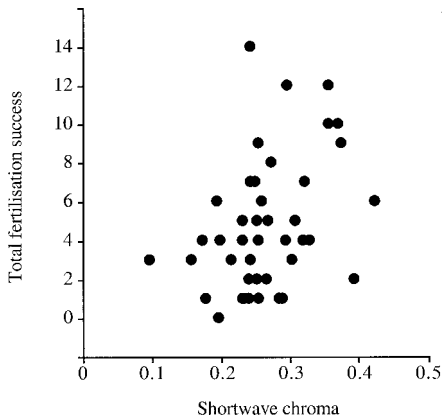


Fig. 4. Relationship between chroma of the blue feathers (shortwave chroma) and total fertilisation success. See methods for explanation of chroma calculations.

## Discussion

This study shows that the variance in male fertilisation success and thus the opportunity for sexual selection increases due to EPCs in the bluethroat. We found that shortwave chroma correlated positively with male total fertilisation success, which suggests that there is sexual selection for at least one element of male throat coloration. Both shortwave chroma and width of the chestnut band correlated positively with within-pair paternity, but neither of them was related to male extra-pair fertilisation success. These results are unexpected if male coloration reflects overall genetic quality, and if females have preference for these traits. Females would then be expected to exert their preferences with respect to both within-pair and extra-pair paternity. Likewise, old males had a higher success in EPCs than young males, whereas there was no difference between the age classes in paternity in own nest or in total fertilisation success. In the following, we will discuss possible explanations for these apparently inconsistent results.

Male coloration was positively related to within-pair paternity, but largely unrelated to extra-pair paternity (see below). Accordingly, the positive correlation between total fertilisation success and shortwave chroma was mainly a result of chromatic males achieving higher paternity in their own nest. For the chestnut band, we only found a significant relationship with within-pair paternity in one of two years, which may help explain the absence of a relationship with total fertilisation success for this throat component.

Taken together, these results suggest that male throat coloration is subject to directional selection via differential within-pair paternity success in the bluethroat. Possible explanations for this pattern include that colourful males were better at protecting paternity, had sperm that competed better with rival sperm, and/or had more faithful mates. Colourful males tend to guard their mates less intensely than drabber males (A. Johnsen & J.T. Lifjeld, unpubl. results), which speaks against a mate-guarding explanation. However, colourful males might still be better at timing copulations or at protecting paternity in other ways than through mate guarding. The sperm quality explanation might also have some merit, as suggested by the positive relationship between shortwave chroma and proportion of EPO among males that achieved at least one EPO. Finally, a tentative scenario is that females prefer colourful males as social mates, and hence are more faithful to such males. Possibly, they gain some direct or indirect benefit from the social pairing that is unrelated to the compatibility criteria (Johnsen *et al.*, 2000) employed in the (active or passive) selection of extra-pair sires. One possibility could be that colourful males were better parents and that females were faithful in order to achieve full investment from such males. However, a recent experimental bluethroat study documented no relation between male coloration and paternal investment (Smiseth *et al.*, 2001). Clearly, further research is needed to determine how colourful males are able to secure higher within-pair paternity than drabber males.

Several lines of evidence indicated that female bluethroats do not have concordant preferences in extra-pair mate choice based on male phenotypic appearance. First, there were no relationships between measures of coloration and EPF-success when including all males in the analyses. Second, comparisons between the traits of cuckolded males with the traits of their cuckolders revealed no significant differences. Third, if some males were consistently preferred by females as genetic mates, one would predict that males that were successful in obtaining EPFs would also achieve high paternity in their own nest, irrespective of what specific trait females prefer. This was not the case: males that achieved one or more EPFs did not have different level of paternity loss from males without EPFs. The only indication that coloration was related to male EPF-success came from the analyses within the restricted sample of males achieving one or more EPO. Within this sample, there was a positive correlation between shortwave chroma and

the relative amount of EPOs achieved. In light of the overall lack of relationships between colour and EPF-success, we speculate whether this positive relation between coloration and proportion of EPO may reflect differential sperm competitiveness (via ejaculate size or sperm quality) rather than female preferences (via copulation frequency or cryptic female choice).

Consistent female preferences have been indicated in our earlier experimental studies of the bluethroat (Johnsen *et al.*, 1998a, b). These experiments dramatically reduced male throat coloration (complete blackening and UV deprivation, respectively), and resulted in reduced fertilisation success for experimental males. The experimental effects, unconfirmed in the present correlational study, might be explained by the fact that the manipulations extended far outside the natural phenotypic variation, and that females discriminated against such atypical males using some threshold criterion. Above this threshold, females might not actively discriminate with respect to our measures of male phenotypic variation. The present results pinpoint the potential pitfalls of performing experiments that extend beyond natural phenotypic variation. Unless such studies are accompanied by studies of the natural variation and fitness consequences of the trait in question, little can be deduced about the selection pressures acting on the trait in natural populations.

In a recent study, we presented evidence that female bluethroats gain genetic benefits from engaging in EPCs (Johnsen *et al.*, 2000). Testing the T-cell mediated immune response of nestlings by injection of phytohaemagglutinin, we found extra-pair offspring to have a higher immune response than their maternal half-siblings (in the same nest) and paternal half-siblings (in different nests). These results suggest that extra-pair mates have more compatible genotypes than social mates, possibly due to constraints on social mate choice (Møller, 1992). The apparent lack of general (as opposed to relative) EPC preferences in this study is in agreement with female choice of compatible male genes. Although there might be external identification markers of 'compatibility', the choice process may also occur within the female genital tract, *i.e.* by cryptic female choice (Eberhard, 1996; Birkhead, 1998).

Old males were substantially more successful in getting EPFs than young ones. Two non-exclusive scenarios may explain this pattern. First, the age-differences may have been the result of differences in activity pattern between the age classes. There is a distinct behavioural age-difference during the peak of the fertile period, with young males spending more time guarding

their mates than old males do (A. Johnsen & J.T. Lifjeld, unpubl. results). This indicates that old males may have been over-represented among extra-pair fathers simply because they spent more time searching for EPCs. The reason for this difference in behaviour may at least partly be that the ability to pursue EPCs increases with age. This might in turn be connected to previous knowledge of the habitat, but old males with previous breeding experience from the area did not have higher success in EPCs than old males that were new to the area. However, other types of experience, such as ability to locate and court fertile females, can be important. Second, it is possible that the age-difference in EPF-success was caused by directional female preferences for old males, targeting for example their proven survival abilities (Brooks & Kemp, 2001; Kokko & Lindström, 1996, but see Hansen & Price, 1995). The higher mate-guarding intensity of young males could then be a consequence of a higher perceived threat to paternity in addition to lower perceived prospect of obtaining EPCs. This scenario agrees well with our earlier experimental results (Johnsen *et al.*, 1998a, b). Furthermore, the finding that old males are more colourful and in better condition indicates that such males may be of higher phenotypic and/or genotypic quality. If old males were consistently preferred as genetic mates they should also have higher fertilisation success in their own nest compared with young males. However, despite the fact that old males on average were more colourful than young ones and that there was an overall positive relation between coloration and within-pair paternity, there was no difference between the age groups in within-pair paternity. It is important to note that these seemingly inconsistent results arise from tests on somewhat differing samples, and that none of the correlations were particularly high. In addition, the crude classification of males as young or old might possibly mask trends related to the actual age of males. Nevertheless, the fact that old males did not have consistently higher success than young ones, suggests that female choice for old copulation partners cannot fully explain the age-related pattern of EPF-success in this study.

In conclusion, this study has demonstrated positive relationships between two aspects of the male's throat patch and within-pair paternity, and for one of them (the chroma of the blue throat feathers) this translated into a positive relationship with total fertilisation success. Our results therefore suggest directional sexual selection on the male's plumage, mediated through differential within-pair paternity success. However, selection resulting from

differential EPC-success seems to be weak in this bluethroat population, at least with regard to the phenotypic traits measured in this study. Absence of consistent phenotypic cues for female choice of EPC-partners is consistent with the idea (Trivers, 1972; Johnsen *et al.*, 2000) that females make individual (rather than concordant) choices of genetically compatible males. We suggest that preferences for genetically compatible males constrain the opportunity for directional sexual selection in bluethroats, and perhaps also in other species. Future research should address the relative importance of directional and compatibility selection, and how these mechanisms may interact, in shaping animal mating patterns.

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