



# Individual Quality and Extra-Pair Paternity in the Blue Tit: Sexy Males Bear the Costs

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Adaptive explanations for the evolution of extra-pair paternity (EPP) suggest that females seek extra-pair copulations with high quality males. Still, the link between ornamentation, individual quality, and paternity remains unclear. Moreover, honest signaling is essential when explaining EPP because it is needed for sexual selection to occur; yet, it is understudied in multiple ornaments. Because blue tits (*Cyanistes caeruleus*) show variable color expression in several plumage patches, we tested: (i) over two seasons, whether males in better condition, more ornamented and less infected by blood parasites gain EPP and have higher reproductive success, and (ii) over three seasons, whether mating patterns affect color change. Males with more saturated yellow feathers, brighter tails, and in better condition had higher reproductive success in one of the seasons. Contrary to expectation, in another season, males that gained EPP were parasitized by blood parasites, suggesting increased vector exposure during extra-pair copulations. Our results for two seasons show that males siring more extra-pair young were older and grew brighter cheek or tail feathers for the following season. Despite the increased mating costs, in socially monogamous avian systems, high quality males incur in EPP without compromising traits that may be under sexual selection.

**KEY WORDS:** carry-over effects, feather coloration, fecundity, mating systems, parasitism, sexual selection.

Among socially monogamous bird species, matings outside the social pair are a relatively common strategy that result in 11% of offspring being extra-pair on average (Griffith et al. 2002; Westneat and Stewart 2003). Both polygyny and extra-pair paternity increase the variance in reproductive success in males (Vedder et al. 2011), and although the benefits for females are less clear (Forstmeier et al. 2014), in some passerines, females are known to actively seek extra-pair copulations (Kempnaers et al. 1992; Birkhead and Møller 1993). Thus, avian mating systems serve as an excellent model to study the evolution of extra-pair paternity.

Adaptive explanations behind the evolution of promiscuity traditionally assume that extra-pair matings involve high quality males (reviewed in Forstmeier et al. 2014). However, conflicting

results have been reported with respect to male quality and paternity gain. In passerines, for example, an experimental reduction in the males' dominance rank did not result in paternity loss (Johannessen et al. 2005). Still, adaptive explanations for this mating behavior are supported by the immunogenetic benefits of extra-pair copulations (Johnsen et al. 2000; Fossøy et al. 2008). The performance of the immune system is an important trait reflecting individual quality. Indeed, genetic diversity in immune genes, that is, the major histocompatibility complex (MHC), has been related to higher individual quality (Woelfing et al. 2009), and thus, it is an expected target for female mate choice because extra-pair copulations could increase genetic diversity in the offspring. Some studies suggest a female preference for maximal MHC-dissimilar

mates (Griggio et al. 2011a), while others indicate that females may prefer mates with intermediate MHC-dissimilarity (Forsberg et al. 2007). The relationship between extra-pair paternity and multiple parasitic infections could shed light on this conundrum because how individuals cope with infections could be used as a proxy for performance of the immune system. Only a few studies have suggested that males infected with avian malaria tend to lose paternity (Jacobs et al. 2015) or are more likely to be cuckolded (Podmokła et al. 2015). However, these studies combined more than one parasite species and related “avian malarial infection” to paternity, and thus, the relationship between multiple infections and paternity remains unclear.

The avian malaria system provides a unique opportunity to explore parasite-mediated sexual selection and thus the interplay between paternity, infection, and ornamentation. According to Hamilton and Zuk’s seminal paper, more ornamented individuals are expected to be of higher quality because they signal resistance to parasites (Hamilton and Zuk 1982). The relationship between parasitic infections and ornamentation has long been studied (for a review, see Hill 2006), but the link between ornamentation and paternity is far from clear. Some studies have found that females prefer males that are ornamented in their carotenoid-based plumage patches for extra-pair matings (Kappes et al. 2009), while others have found experimental evidence that more ornamented males are less cuckolded (Helfenstein et al. 2008). Regarding structural feather coloration, similar confusion arises, even in the same species. Delhey et al. (2003) found that more UV-ornamentation in blue tits was associated with increased within-pair success instead of fathering more extra-pair offspring. However, experimentally manipulated males with higher UV reflectance in the blue crown sired more extra-pair offspring (Delhey et al. 2006a). A study in male yellow warblers (*Stetophaga petechia*) suggests that mating patterns in relation to feather coloration may be more complex than expected because different ornaments may be associated with within-pair or extra-pair success (Grunst and Grunst 2014). Thus far, the relationship between ornamentation and paternity gain requires further investigation in species that show multiple ornamentation under sexual selection (Peters et al. 2007; Vedder et al. 2011).

Moreover, the male’s performance during the breeding season may be mirrored in plumage characteristics obtained after the molt (Griggio et al. 2009). For example, low-quality individuals molt faster and develop duller and less saturated blue crown feathers (Griggio et al. 2009). In many passerines breeding in temperate regions, the molt is constrained to the time immediately after the reproductive event (Holmgren and Hedenström 1995). Thus, how individuals cope with a single reproductive event may have important consequences for the following season, because the plumage color achieved after reproduction is maintained throughout the subsequent reproductive period (Nils-

son and Svensson 1996). Increasing reproductive effort reveals a trade-off between the resources allocated to reproduction against those allocated to ornamentation in males of several bird species (Gustafsson et al. 1995; Griffith 2000; Siefferman and Hill 2007). One hypothesis could be that males engaging in extra-pair copulations pay the costs of this behavior in terms of reduced plumage ornamentation for the following season. An alternative hypothesis could be that low-quality males invest in mate guarding and maximize within-pair success but they develop less ornamented feathers because guarding activities are costly (Birkhead and Møller 1992). High-quality males on the contrary, maximize reproductive success through extra-pair paternity while they are still able to allocate sufficient resources to ornamentation during the molt. The relationship between paternity during a single reproductive event and the feather coloration achieved after the molt remains unknown.

In this study, we aimed at elucidating the relationship between paternity and individual quality in a socially monogamous avian model system, the blue tit. Previous research has shown that older blue tits (Kempnaers et al. 1997) or yearlings that were more UV-ornamented in the blue crown (Peters et al. 2006) gained paternity, but other studies have failed to find support for the predicted relationship (Delhey et al. 2006b). Male blue tits that are more ornamented in their yellow breast feathers are commonly high quality foragers (Senar et al. 2002; Galván 2011; García-Navas et al. 2012), but studies on the relationship between carotenoid coloration and paternity are missing. Furthermore, the link between paternity gain and multiple male ornaments is understudied, when this could provide important insights on the evolution of sexual selection in a species in which both sexes show similar ornamentation (Kraaijeveld et al. 2007).

Similar to carotenoid ornamentation, physiological parameters act as an indicator of an individual’s body condition, and thus they may be related to extra-pair paternity. For example, hemoglobin levels in blood have been positively related to survival (Bañura et al. 2007) and health (Słomczyński et al. 2006) in nestling blue tits; validating the use of this parameter as a proxy for condition (for a review, Minias 2015). Infection status has been recently related to paternity in the blue tit (Podmokła et al. 2015) but studies combining a comprehensive panel of quality indicators are absent in the literature.

To unravel the link between paternity and individual quality in the blue tit, we explored paternity gain, paternity loss, and number of extra-pair young sired over two consecutive breeding seasons and color change over three consecutive seasons. Reproductive success and individual quality were also explored in the second season (in the first season it was affected by a different experimental treatment, see Badás et al. 2015). As explained above, individual quality in this species may be indicated by condition (body mass), age, phenological variables from the males’ social

nest (clutch size and hatching date), feather coloration in multiple ornaments (crown, cheek, breast, and tail feathers), physiological parameters (total hemoglobin in blood), and intensity of the infections by several blood parasites. Thus, we predict that higher quality males are more ornamented, in better physiological and body condition and harbor less blood parasites, siring extra-pair offspring and having higher seasonal reproductive success. Similarly, poorer quality males, as indicated by less ornamentation, poorer physiological condition and intense parasitic infections may lose paternity in their social nest. Between seasons, higher quality males (1) may have their ornamental coloration compromised after engaging in a costly mating strategy (Birkhead and Møller 1992), or (2), they may still develop similar feather color after the molt, if their coloration is less affected by the costs of a reproductive event (Doutrelant et al. 2012). Our findings are discussed in the context of mating strategies, sexual selection, multiple ornamentation, and host–parasite interactions.

## Methods

### STUDY SITE AND SAMPLING

Our study was carried out during the springs of 2012, 2013, and 2014 (hereafter seasons 1, 2, and 3) in a Pyrenean Oak (*Quercus pyrenaica*) forest located in central Spain (Segovia, 40°54'N, 4°01'W, 1200 m. above sea level). Long-term studies of breeding activities have been on-going in the present blue tit population since 1991 (Sanz et al. 2003).

During the springs of seasons 1 and 2, adult female and male birds were captured at their nest box while provisioning 3-day-old nestlings (hatching date = 0), weighed to nearest 0.01 g with a digital balance, and tarsus length and time of the day were recorded in order to calculate the corrected body mass index (following Senar 2002). Age was assigned based on standard plumage characteristics: birds were classified as first-years identified by possession of distinctive, non-adult greater wing coverts, or as second-years or older (Svensson 1992). We also took a drop of blood from the brachial vein in all birds, which was collected in heparinized microcapillaries and later used for paternity analyses and parasite quantification (only in adult birds). We detected and quantified several parasite species using molecular-based methods: *Haemoproteus majoris*, *Plasmodium* sp. haplotype cyan1, *Leucocytozoon majoris* haplotypes leuA, leuA1, and leuB, and *Lankesterella valsainiensis* (see Badás et al. 2015 for details on the relative quantitative PCR and primers used). Following previous studies in the population, the variable *Leucocytozoon A* includes haplotypes A and A1. Another drop of blood was used to determine hemoglobin concentration in the field using a portable HemoCue Hb 201<sup>+</sup> photometer (HemoCue AB, Ängelholm, Sweden), following Burness et al. (2001). The hemoglobinometer is a reliable method that has high sensitivity and specificity (in hu-

mans, 0.85 and 0.94, respectively, Mills and Meadows 1989). Nestlings were banded with an aluminum ring for identification, weighed, measured for tarsus and wing length, and bled for paternity analyses when they were 15 days old. In the spring of season 3, following the same procedure, we captured adult birds at their nest box at nestling age 3 days old but blood for paternity analyses was not available this season because adult birds were subjected to a different experimental design.

During the springs of seasons 1, 2, and 3, data on feather coloration was collected using a portable spectrophotometer (Ocean Optics Inc., Dunedin, FL, USA) connected to a Pulsed Xenon Light Source (Jaz-PX lamp; see Badás et al. 2017 for more details on how measurements were taken). Feather color reflectance was measured in adult breeding birds in the following plumage patches: in breast and cheek feathers in season 1 (other plumage patches could not be measured this season due to technical difficulties, that is, feather bristling, see Badás et al. 2017) and in crown, breast, cheek, and tail feathers in seasons 2 and 3 (the handle used for the spectrophotometer avoided feather bristling). For all seasons, relative quantum photon catches were obtained to build models of blue tit vision (Endler and Mielke 2005; Stevens et al. 2009). From this, three variables describing color were obtained for each color patch (hue, saturation, and luminance), because this is the most common approach used to model avian color vision and coloration in recent ecological studies (Stoddard and Prum 2008; Kemp et al. 2015; see Badás et al. 2018 for details on the formulas used).

### PATERNITY ANALYSES

Parents and nestlings were genotyped for eight microsatellite loci in seasons 1 and 2; information on microsatellites, primers, and polymerase chain reaction (PCR) conditions are detailed in Badás et al. (2017). Allele lengths were determined with the Genemapper 4.0 software. The offspring was assigned as extra-pair if there were at least two mismatches between the genotype of the social father and offspring. Extra-pair paternity (EPP) for a male different than the social male was assigned when one of the sampled males matched all of the offspring's paternal alleles. Other studies in the same focal species have used a likelihood-based approach to assign paternity when a higher number of microsatellite loci were genotyped (11–14 in García-Navas et al. 2013 and García-Navas et al. 2014), but in this study we used a more conservative approach when genotyping for eight microsatellite loci, which has successfully been used before in our population (Badás et al. 2017). Results on paternity in season 1 are detailed in Badás et al. (2017). Paternity in season 2 was assigned for 73% of all identified extra-pair fledglings ( $N = 98$ ) in the 2013-breeding season using Cervus 3.0 (Kalinowski et al. 2007). Maternity of the social female was confirmed for all nestlings. The mean exclusion probability of the eight markers was calculated to be 0.99968 for the first

(female) parent and 0.99999 for the second (male) parent (given the genotype of the first parent).

## STATISTICAL ANALYSES

All analyses were performed in R version 3.6.1 (R Foundation for Statistical Computing, Vienna). Although extra-pair copulations occur before the nestling provisioning phase and therefore are unlikely to be affected by an experimental treatment to adult birds during this phase, we confirmed that the experiments in 2012 (Badás et al. 2015) and 2013 (unpubl. data) were not related to extra-pair paternity (2012:  $\chi^2_3 = 2.64$ ,  $P$ -value = 0.45,  $N = 78$ ; 2013:  $\chi^2_2 = 2.29$ ,  $P$ -value = 0.32,  $N = 63$ ).

First, in the seasons for which paternity data was available (seasons 1 and 2), we explored whether the male's gain or loss of paternity was related to age, prevalence of infection, and coinfection by parasites (four parasite species, see sampling section above) using Chi-square test ( $\chi^2$ ) and Fisher exact tests, which is more robust when sample sizes are small (Agresti 1992) (in season 1  $N = 72$ , and in season 2  $N = 63$ , but due to missing data for parasite analyses sample sizes are indicated for each analyses in the results section). The alternative hypothesis in Fisher exact tests is that the odds ratio between two categorical variables is not equal to 1, therefore, exact  $P$ -values, odds ratio, and 95% confidence intervals are reported in the Results section along with Chi-square statistics.

Second, we explored whether (1) the male's gain of extra-pair paternity, (2) the male's loss of paternity, or (3) the number of extra-pair young were related to feather color, blood parasite infections, body and physiological condition, and breeding parameters. In season 2, we also explored whether (4) total reproductive success (number of fledglings including extra-pair offspring) was related to the above-mentioned variables. In season 1, reproductive success could not be evaluated because this was affected by an experimental treatment (Badás et al. 2015). For each season and response variable, to avoid multicollinearity or sample size problems when a large number of explanatory variables are evaluated, we designed partial least squares regression models (PLSR; Carrascal et al. 2009). The PLSR allowed us to include highly correlated color variables and extract the most relevant variables explaining variation in the dependent variable. This method is becoming increasingly popular in ecological studies because it is extremely robust when the number of observations is similar to the number of variables included in the model (Galván et al. 2014; Badás et al. 2017). We used 14 explanatory variables for season 1 (cheek and breast color variables, infection intensity by five parasite species, hatching date and clutch size in the male's social nest, age, and condition, see Results section) and 20 explanatory variables for season 2 (cheek, crown, tail, and breast color variables, infection intensity by five parasite species, hatching date and clutch size in the male's social nest, and condition, see Results

section). In season 2, age was not included as predictor variable in the models because all males that gained paternity were second-years or older. We built three and four PLSR models for seasons 1 and 2, respectively, using: (1) a binary (yes/no) response variable that recorded whether the male had extra-pair young, (2) a binary (yes/no) response variable that recorded whether the male was cuckolded, (3) a negative-binomial distributed response variable that coded the number of extra-pair young (ranging from 0 to 8), and (4) a Poisson distributed response variable that coded seasonal reproductive success (but in this case only for season 2 as explained above). The models were fitted using the R package *plsRglm* version 1.2.5 (Bertrand and Maumy-Bertrand 2019) for binomial/Poisson PLSR, and data on all variables were available for 46 males in season 1 and for 44 males in season 2. To elucidate whether the most parsimonious PLSR contained one or more latent components describing variance in the response variable, we used the difference in AIC (Akaike Information Criterion, Akaike 1973). Models with one latent component were selected over models that contained two components because  $\Delta AIC < 10$  (Bolker et al. 2009). Finally, the weights for each variable in the PLSR models were averaged after bootstrapping with 5000 samples in order to obtain robust parameter estimates and 95% confidence intervals.

PLSR does not allow for missing data. However, complete data on feather coloration was available for 51 males in season 1 and 63 males in season 2. Thus, in order to confirm our results on paternity and coloration, we performed additional models that included saturation and luminance for the cheek, breast, and crown (season 2 only) plumage patches, and the most important condition and parasite variables extracted from the PLSR. Hue variables were not included in the models because they were highly correlated to saturation variables in each patch (all  $P$ -value  $< 0.0001$ ,  $r > 0.85$ ). In season 2, tail saturation was highly correlated to crown saturation, and it was dropped from the models ( $t = 12.06$ ,  $df = 59$ ,  $P$ -value  $< 0.0001$ ,  $r = 0.84$ ,  $N = 63$ ). Tail luminance gave high variance inflation factor ( $VIF > 9$ ) when included in the season 2 models so it was also dropped. To obtain robust parameter estimates for reduced sample size we fitted Generalized Linear Models (GLMs) using robust regression (package *robustbase* version 0.93.5) with binomial, negative binomial, or Poisson error distribution. Models were checked for collinearity (all  $VIF < 3$ ) and overdispersion (overdispersion parameter  $\Phi < 1$ ).

Third, we described color change from season 1 to season 2 and season 2 to season 3 as the ratio of color change between seasons for each available plumage patch following Badás et al. (2018). We then evaluated whether extra-pair paternity had an effect on color change using Welch two-sample  $t$ -tests (when exploring gain of paternity and color change), negative binomial GLMs (when exploring number of extra-pair young and color change), or Poisson GLMs (when exploring reproductive success

and color change). Effect sizes are shown as Cohen's *D* (Cohen 1998) or the nonparametric version when the differences between group variances were high (Cliff's *Delta*, R package *effsize* version 0.7.4, Torchiano 2016) for *t*-tests. Traditionally,  $d < 0.2$  is considered a "small" effect size and  $d > 0.5$  a "large" effect size. For GLMs, effect sizes are shown as beta-standardized estimates along with standard errors.

Finally, we extracted JND scores, or Just Noticeable Differences (Siddiqi et al. 2004), to describe perceptibility of color change between seasons 1 and 2 through chromatic (color) or achromatic (luminance) contrasts. JND scores between seasons 2 and 3 are available in Badás et al. (2018).

## Results

### PATERNITY, AGE, PREVALENCE OF INFECTION, AND COINFECTION

Paternity data for season 1 is available in Badás et al. (2017). In season 1, neither paternity gain nor paternity loss was related to age, prevalence of infection by blood parasites, or coinfection (all *P*-values  $> 0.05$ ). Paternity gain was also not related to paternity loss (*P*-value  $> 0.05$ ).

In season 2, a total of 164 adults from 89 breeding pairs and 522 nestlings from 78 nests were genotyped for paternity. Nestlings from 11 nests could not be genotyped because of nest desertion, and thus, these adult males were not included in the analyses (although they were included as candidate fathers for genotyping). Nest desertion was probably due to extreme weather conditions during the 2013-breeding season (as seen in other blue tit populations throughout Europe, Gładalski et al. 2014). Overall, more than half of the nests (55%, 43/78 nests) contained at least one extra-pair young and 19% (98/522 nestlings) of all offspring genotyped were sired by a male other than the social father. We successfully assigned paternity to 70% (69/98 nestlings) of all extra-pair offspring.

Paternity gain was significantly different with age in season 2 ( $\chi^2_1 = 12.58$ , *P*-value = 0.0004, *N* = 63); in fact, no yearling males had extra-pair nestlings. Paternity gain was not related to parasite prevalence of infection by *Haemoproteus*, *Plasmodium*, or *Leucocytozoon* (all *P*-value  $> 0.05$ ), but it was marginally positively non-significantly related to prevalence of infection by *Lankesterella* ( $\chi^2_1 = 3.16$ , *P*-value = 0.061, odds ratio = 0.33, upper CI = 1.1, lower CI = 0.1, *N* = 58). Regarding coinfection and paternity gain, male blue tits that harbored infections by *Lankesterella* and *Haemoproteus* ( $\chi^2_1 = 4.16$ , *P*-value = 0.029, odds ratio = 0.28, upper CI = 0.95, lower CI = 0.07, *N* = 58), or *Lankesterella* and *Leucocytozoon A* ( $\chi^2_1 = 4.17$ , *P*-value = 0.031, odds ratio = 0.28, upper CI = 0.95, lower CI = 0.08, *N* = 58) were more likely to sire extra-pair young. Paternity loss was not related to age, prevalence of infection by blood parasites, or

coinfection (all *P*-values  $> 0.05$ ). However, there was a trend that cuckolded males in season 2 were less likely to harbor coinfections by *Lankesterella* and *Plasmodium* (*P*-value = 0.09, odds ratio = 3.84, upper CI = 25.34, lower CI = 0.79, *N* = 58). Extra-pair paternity gain was independent of cuckoldry (*P*-value  $> 0.05$ ).

### PATERNITY, CONDITION, FEATHER COLOR, AND INTENSITY OF INFECTION

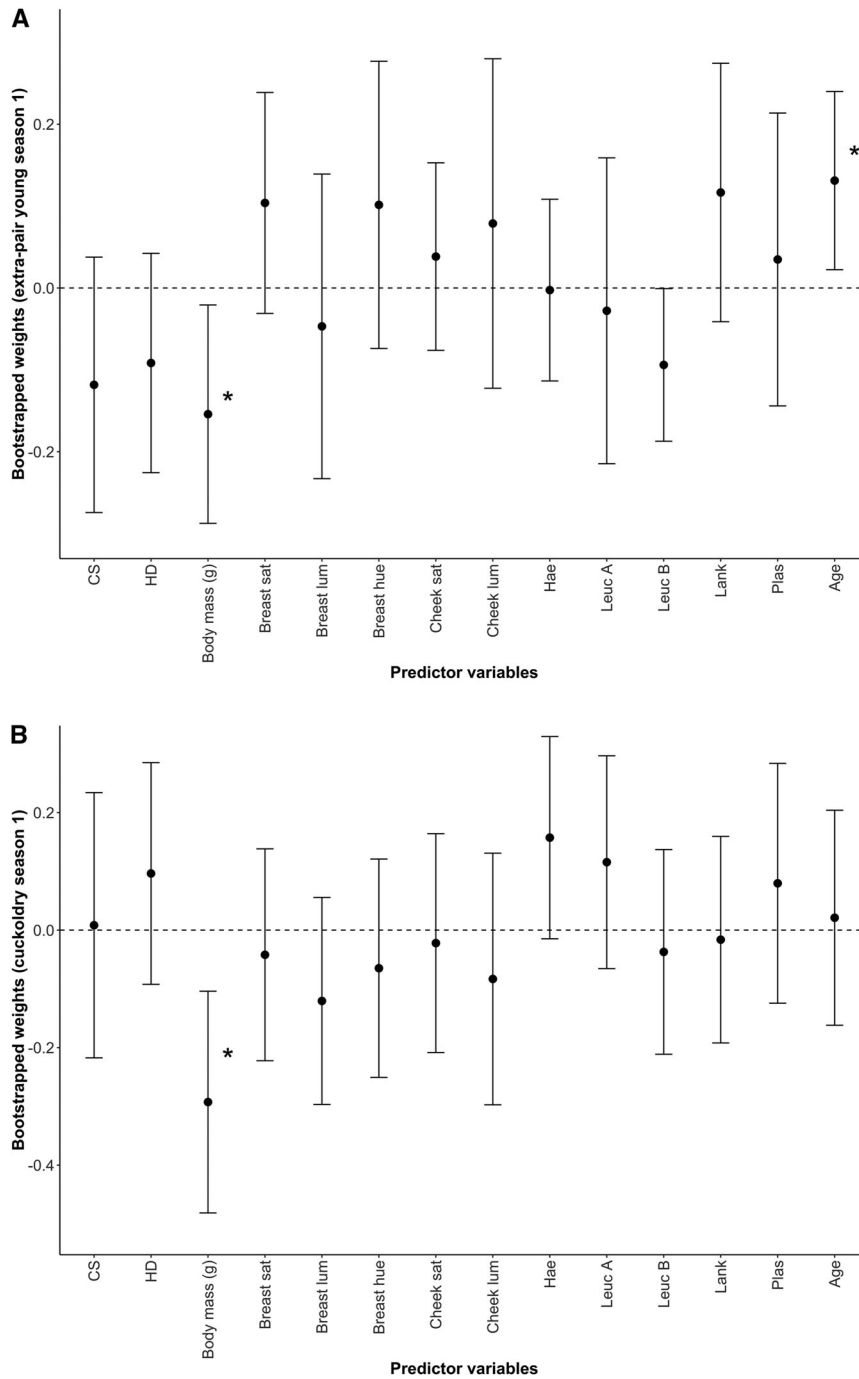
In season 1, paternity gain was explained by a single PLSR component that accounted for 17.96% of the variance in extra-pair paternity (Table 1, *N* = 46). No feather color, intensity of infection, or condition variables were significant after bootstrapping, but males that gained paternity tended to have more saturated yellow breast feathers (*P*-value = 0.09, Fig. S1). The number of extra-pair young were explained by a single PLSR component that accounted for 50.94% of the variance (Table 1, *N* = 46). Males that sired more extra-pair young were older, lighter, and tended to harbor less *Leucocytozoon B* parasites (*P*-value = 0.06, Fig. 1A). Loss of paternity in season 1 was explained by a single PLSR component that accounted for 48.77% of the variance in cuckoldry (Table 1, *N* = 46). Lighter males and males that tended to be more intensely infected by the blood parasite *Haemoproteus* (*P*-value = 0.07) were more likely to be cuckolded (Fig. 1B). An additional model with feather color variables but bigger sample size confirmed that males were more likely to be cuckolded when they were lighter (binomial GLM, explained deviance = 0.2%,  $\beta$  estimate =  $-1.74$ , SE = 0.60,  $z = -1.74$ , *P*-value  $< 0.01$ , *N* = 51) and when they had duller white cheek feathers ( $\beta$  estimate =  $-1.54$ , SE = 0.68,  $z = -1.54$ , *P*-value = 0.02, *N* = 51).

In season 2, paternity gain was explained by a single PLSR component that accounted for 53.08% of the variance in extra-pair paternity (Table 1, *N* = 44). Males that had nestlings outside the social nest were: (1) more saturated and had higher values of hue in their yellow breast feathers, (2) more intensely infected by the blood parasite *Lankesterella valsainiensis*, and (3) had higher hemoglobin levels in blood (Table 1, Fig. 2A). A binomial GLM model with feather color variables, intensity of infection by *Lankesterella*, and hemoglobin concentration explained 47% of the deviance compared to a null model. This model confirmed that males with extra-pair offspring had higher hemoglobin concentration ( $\beta$  estimate = 2.03, SE = 1.02,  $z = 1.97$ , *P*-value = 0.04, *N* = 61, Fig. 3A), higher parasitic loads ( $\beta$  estimate = 2.94, SE = 1.24,  $z = 2.37$ , *P*-value = 0.02, *N* = 61, Fig. 3B) and higher breast saturation ( $\beta$  estimate = 1.87, SE = 0.93,  $z = 2.01$ , *P*-value = 0.04, *N* = 61, Fig. 3C). We also found that males with extra-pair nestlings tended to have higher cheek luminance ( $\beta$  estimate = 1.39, SE = 0.82,  $z = 1.69$ , *P*-value = 0.09, *N* = 61).

The number of extra-pair young sired in season 2 was explained by a single PLSR component that accounted for 32.32%

**Table 1.** Results for the partial least squares regression (PLSR) on extra-pair paternity, number of extra-pair young, and reproductive success of male blue tits. All predictor variables describing the single latent component for each model and their weights are shown. Variables that were significant after bootstrapping are in bold type. Note that in season 2 age was not included in the models because all males that gained paternity were second-years or older. Codes: EPP, extra-pair paternity; EPY, extra-pair young.

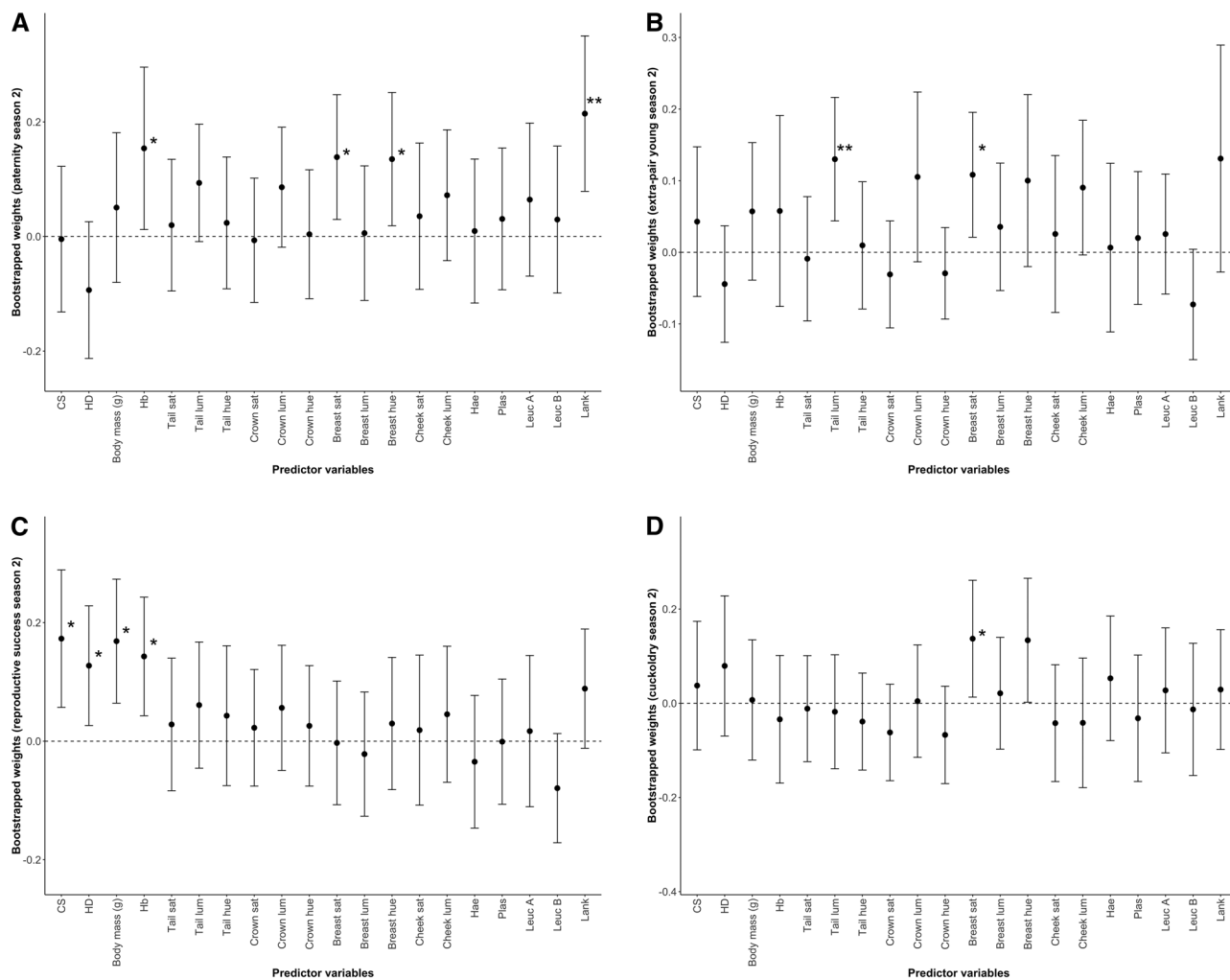
Predictors	Season 1				Season 2				Reproductive success					
	EPP gain (Yes/no)		EPP loss (Yes/no)		Number of EPY		EPP gain (Yes/no)		EPP loss (Yes/no)		Number of EPY		Reproductive success	
	Weight	R <sup>2</sup> for variables (in %)	Weight	R <sup>2</sup> for variables (in %)	Weight	R <sup>2</sup> for variables (in %)	Weight	R <sup>2</sup> for variables (in %)	Weight	R <sup>2</sup> for variables (in %)	Weight	R <sup>2</sup> for variables (in %)	Weight	R <sup>2</sup> for variables (in %)
Phenology	-	2.85	-	1.62	-	7.88	-	2.34	-	1.57	-	1.40	-	15.31
<b>Clutch size</b>	-0.300	1.62	-0.001	0.00	-0.239	2.91	-0.013	0.01	0.127	0.29	0.147	0.70	<b>0.490</b>	<b>10.23</b>
<b>Hatching date</b>	-0.262	1.23	0.182	1.62	-0.312	4.97	-0.210	2.33	0.266	1.28	-0.147	0.70	<b>0.345</b>	<b>5.08</b>
Age	0.213	0.82	0.050	0.12	<b>0.393</b>	<b>7.85</b>	-	-	-	-	-	-	-	-
Condition	-	-	-	-	-	-	7.86	-	0.35	-	1.7	-	-	17.09
<b>Body mass</b>	-0.247	1.10	<b>-0.768</b>	<b>28.74</b>	<b>-0.373</b>	<b>7.08</b>	0.105	0.59	0.020	0.01	0.181	1.06	<b>0.467</b>	<b>9.30</b>
<b>Hemoglobin</b>	-	-	-	-	-	-	<b>0.370</b>	<b>7.27</b>	-0.138	0.34	0.141	0.64	<b>0.428</b>	<b>7.79</b>
Plumage color	-	7.74	-	6.45	-	11.08	-	17.93	-	14.94	-	21.4	-	4.94
<b>Breast</b>	-	6.96	-	5.09	-	8.35	-	11.38	-	10.77	-	8.36	-	0.49
<b>Saturation</b>	0.387	2.7	-0.097	0.46	0.294	4.41	<b>0.332</b>	<b>5.84</b>	<b>0.545</b>	<b>5.38</b>	<b>0.385</b>	<b>4.79</b>	-0.014	0.01
Luminance	-0.431	3.34	-0.277	3.74	-0.055	0.15	0.029	0.04	0.109	0.21	0.147	0.70	-0.064	0.18
<b>Hue</b>	0.226	0.92	-0.135	0.89	0.273	3.79	<b>0.322</b>	<b>5.5</b>	0.535	5.18	0.298	2.87	0.084	0.30
Cheek	-	0.78	-	1.36	-	2.73	-	1.65	-	0.74	-	3.11	-	0.83
Saturation	0.183	0.6	-0.042	0.09	0.065	0.22	0.071	0.27	-0.173	0.54	0.050	0.08	0.059	0.15
Luminance	-0.101	0.18	-0.161	1.27	0.222	2.51	0.161	1.38	-0.106	0.20	0.306	3.03	0.126	0.68
Crown	-	-	-	-	-	-	-	2.30	-	2.57	-	4.02	-	1.55
Saturation	-	-	-	-	-	-	-0.031	0.05	-0.254	1.16	-0.142	0.65	0.064	0.18
Luminance	-	-	-	-	-	-	0.206	2.25	0.056	0.06	0.290	2.71	0.164	1.15
Hue	-	-	-	-	-	-	-0.08	0.00	-0.273	1.35	-0.143	0.66	0.071	0.22
<b>Tail</b>	-	-	-	-	-	-	-	2.60	-	0.86	-	5.91	-	2.07
Saturation	-	-	-	-	-	-	0.022	0.03	-0.092	0.15	-0.070	0.16	0.077	0.25
<b>Luminance</b>	-	-	-	-	-	-	0.218	2.51	-0.019	0.01	<b>0.422</b>	<b>5.75</b>	0.171	1.24
Hue	-	-	-	-	-	-	0.035	0.06	-0.196	0.70	-0.002	0.00	0.116	0.58
Blood parasites	-	5.46	-	11.84	-	17.07	-	24.94	-	1.23	-	7.87	-	5.27
<i>Hemoproteus</i>	-0.120	0.26	0.365	6.49	0.012	0.01	0.024	0.03	0.185	0.62	0.023	0.02	-0.100	0.43
<i>Plasmodium</i>	-0.009	0.00	0.188	1.73	0.065	0.22	0.072	0.27	-0.090	0.15	0.065	0.14	-0.008	0.00
<i>Leucocytozoon A</i>	-0.087	0.14	0.244	2.90	-0.092	0.43	0.123	0.84	0.088	0.14	0.060	0.14	0.045	0.09
<b><i>Leucocytozoon B</i></b>	-0.466	3.91	-0.108	0.57	<b>-0.533</b>	<b>14.49</b>	0.078	0.32	-0.053	0.05	-0.369	4.40	-0.237	2.40
<i>Lankesterella</i>	0.253	1.15	-0.056	0.15	0.194	1.92	<b>0.665</b>	<b>23.48</b>	0.122	0.27	0.313	3.17	0.235	2.35
<b>Total R<sup>2</sup> (in %)</b>	-	<b>18.0</b>	-	<b>49.0</b>	-	<b>50.9</b>	-	<b>53.1</b>	-	<b>18.1</b>	-	<b>32.3</b>	-	<b>42.6</b>



**Figure 1.** PLS regression on variables explaining (A) extra-pair paternity and (B) cuckoldry in male blue tits in season 1. Shown are bootstrapped weights for each predictor variable included in the model. Negative weight values indicate a negative relationship with the response variable. Significant coefficients are indicated with an asterisk: \* $P < 0.05$ . Bars denote 95% confidence intervals. Codes: CS, clutch size; HD, hatching date; sat, saturation; lum, luminance; Hae, *Haemoproteus majoris*; Plas, *Plasmodium* spp.; Leuc, *Leucocytozoon* spp.; Lank, *Lankesterella valsainiensis*.

of the variance (Table 1,  $N = 44$ ). Males that sired more extra-pair young had brighter tails and more saturated breast feathers (Table 1, Fig. 2B), and marginally brighter white cheeks (Fig. 2B,  $P$ -value = 0.06) and lower parasitic loads of *Leucocytozoon* B (Fig. 2B,  $P$ -value = 0.06).

Males that sired extra-pair offspring in season 2 had higher reproductive success ( $t = 4.32$ ,  $df = 60.34$ ,  $P$ -value < 0.0001, effect size  $ES = 0.56$ ,  $N = 63$ ). Total reproductive success was also explained by a single PLSR component that accounted for 42.6% of the variance. Males that had higher reproductive success had:



**Figure 2.** PLS regression on variables explaining (A) extra-pair paternity, (B) number of extra-pair young, (C) reproductive success, and (d) cuckoldry in male blue tits in season 2. Shown are bootstrapped weights for each predictor variable included in the model. Negative weight values indicate a negative relationship with the response variable. Significant coefficients are indicated with an asterisk: \* $P < 0.05$ , \*\* $P < 0.01$ . Bars denote 95% confidence intervals. Codes: CS, clutch size; HD, hatching date; Hb, hemoglobin concentration; sat, saturation; lum, luminance; Hae, *Haemoproteus majoris*; Plas, *Plasmodium* spp.; Leuc, *Leucocytozoon* spp.; Lank, *Lankesterella valsainiensis*.

(1) larger clutches, (2) clutches that hatched later in the season, (3) higher body mass, and (4) higher hemoglobin levels in blood (Table 1, Fig. 2C,  $N = 44$ ). Males that had higher reproductive success also tended to be more infected by *Lankesterella* parasites (2C,  $P$ -value = 0.09), confirming our results from the paternity PLSR. The model including coloration variables, intensity of infection by *Lankesterella* and hemoglobin concentration, and bigger sample size confirmed that total male reproductive success was explained by hemoglobin concentration (Poisson GLM, explained deviance = 18.5%,  $\beta$  estimate =  $-0.17$ , SE = 0.07,  $z = 2.52$ ,  $P$ -value = 0.01,  $N = 61$ ).

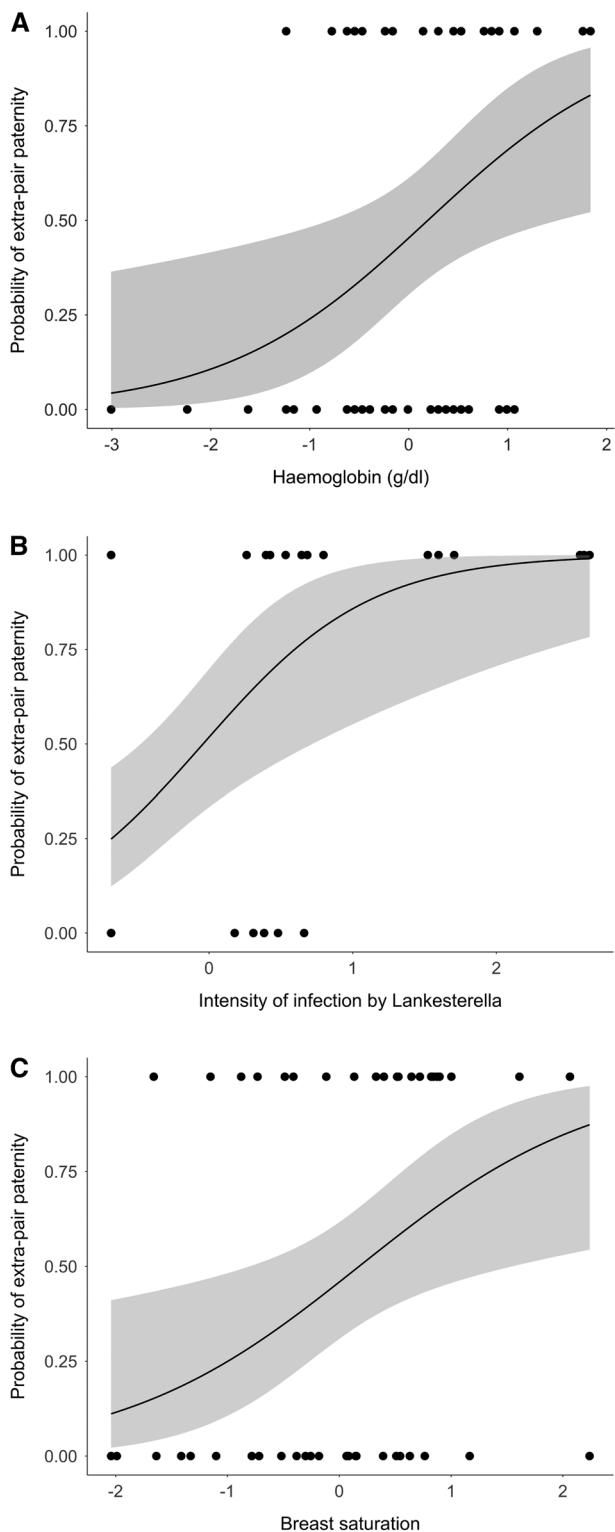
Loss of paternity in season 2 was explained by a single PLSR component that accounted for 18.09% of the variance in cuckoldry. Males that had more saturated yellow breast feathers (Table 1, Fig. 2D) and marginally higher hue values in that same

plumage patch (Fig. 2D,  $P$ -value = 0.05) were more likely to be cuckolded.

## PATERNITY AND COLOR CHANGE BETWEEN SEASONS

Color change between seasons 1 and 2 in the white cheek feathers was related to the number of extra-pair young sired in season 1. The general trend was that males increased luminance in their white cheek feathers from season 1 to season 2 (mean ratio of color change = 0.40, upper CI = 0.81, lower CI =  $-0.27$ ,  $N = 34$ ). When males sired more extra-pair young, their white feathers' brightness increased more between seasons (binomial GLM, explained deviance = 20.93%,  $\beta$  estimate = 0.62, SE = 0.19,  $z = 3.35$ ,  $P$ -value < 0.001,  $N = 26$ ). Achromatic contrasts confirmed that this color change between seasons was of enough





**Figure 3.** The relationship between paternity gain (yes/no) in season 2 and (A) hemoglobin concentration, (B) intensity of infection by *Lankesterella*, and (C) yellow breast saturation. All dependent variables were standardized in the model. Shown are raw values and predicted regression line from the binomial GLM. Shaded areas denote 95% confidence intervals.

magnitude to be perceived by avian vision (JND scores, Table S1).

Color change between seasons 2 and 3 in the tail feathers was related to the number of extra-pair young sired in season 2. The general trend was that males decreased their luminance in their tail feathers from season 2 to season 3 (mean ratio of color change =  $-0.07$ , upper CI =  $0.21$ , lower CI =  $-0.18$ ,  $N = 25$ ). When males sired more extra-pair young, their tail feathers' luminance changed less between seasons (binomial GLM, explained deviance =  $15.62\%$ , bootstrapped  $\beta$  estimate =  $-0.88$ , upper CI =  $-0.08$ , lower CI =  $-2.60$ ,  $N = 25$ ). Achromatic contrasts confirmed that this color change between seasons could be perceived by avian vision (JND scores, available in Badás et al. 2018).

### Discussion

Here, we investigated the relationship between paternity and male quality over two consecutive breeding seasons, and between paternity and feather color change over three seasons. Our results support our initial prediction that higher quality males gain paternity and sire more extra-pair young. We found that age was strongly related to extra-pair paternity, with older male blue tits either siring more extra-pair young or gaining paternity. We found no evidence that the costs of engaging in extra-pair paternity affected color change. Instead, over two seasons, males siring more extra-pair young were able to maintain high quality ornaments for the following season (i.e. brighter cheek or brighter tail feathers). Males that lost paternity were lighter and tended to be more parasitized and less ornamented, but these results were only confirmed in season 1.

Older males were more likely to sire extra-pair offspring in two consecutive breeding seasons. In fact, in season 2, only males second year or older gained extra-pair paternity. Similar results have been found in other avian mating systems (Delhey et al. 2006a; Vedder et al. 2011; Girndt et al. 2018; Micháľková et al. 2019). However, the opposite was found in another blue tit population, with extra-pair paternity being almost absent in older males (Johannessen et al. 2005). These findings suggest that the link between male age and extra-pair mating behaviors might vary among populations and breeding seasons. Future studies that include the male's partner age, female ornaments or species-specific mating behaviors will continue to shed light on the relationship between age and paternity (Mahr et al. 2012; Girndt et al. 2018; Micháľková et al. 2019).

The relationship between age and paternity has been explained by increased crown coloration in older blue tit males (Delhey et al. 2006a; Vedder et al. 2011). Indeed, feather coloration explained extra-pair paternity in this study in season 2, but in different ornaments. Older male blue tits with more

saturated yellow breast feathers and brighter tails were more likely to sire extra-pair young and had more extra-pair young. In season 1, the relationship between breast saturation and gain of paternity was only marginal (note, however, that this model explained less variance in paternity: 18% in season 1 vs. 53% in season 2). Males bearing costly ornaments may be preferred by females for extra-pair matings because they were higher-quality males. Yellow saturation is a good indicator of the amount of carotenoids obtained through diet (Saks et al. 2003; Senar et al. 2008), and more ornamented carotenoid-based plumage has been related to better foraging abilities (García-Navas et al. 2012; Pagani-Núñez and Senar 2014) and less blood parasites (del Cerro et al. 2010; Badás et al. 2017).

Contrary to our prediction, in season 2, males that gained paternity were more intensely infected by the coccidian parasite *Lankesterella valsaininesis* and more likely to harbor coinfections by the coccidian and other malaria-like parasites (*Haemoproteus* spp. or *Leucocytozoon* A). Coccidian parasites can disrupt carotenoid absorption in the bird's intestine, which results in loss of feather color in carotenoid-based ornaments (Brawner et al. 2000; Hōrak et al. 2004). However, the parasites quantified here were extra-intestinal stages of *Lankesterella* that infect lymphocytes in the blue tit's peripheral blood (Merino et al. 2006), so these stages may not correlate to those found in fecal samples (intestinal stages). In this study, the lack of negative effects of the infection on the host's feather coloration and physiological variables could be explained by two non-alternative hypotheses: (1) low virulence in infections by lankesterellids (albeit further information on the host's tolerance is lacking); and (2) individuals with stronger immune defenses might minimize virulence without necessarily reducing parasite loads (Little et al. 2010). Males that gain paternity could be more parasitized if they are more exposed to vectors that transmit *Lankesterella*. The blood-sucking mite *Dermanyssus gallinae* is a vector for this blood parasite (Lainson 1960), and this mite is common in blue tits nests in our population (Castaño-Vázquez et al. 2018). Adult blue tits could then become infected with *Lankesterella* (1) by direct contact between infected individuals or (2) by visiting nest boxes at the start of nest building. To sire extra-pair offspring, males often engage in multiple extra-pair copulations with one or more females (Kempnaers et al. 1992), and thus, they are more likely to be in contact with infected individuals. Additionally, empty nest cavities may act as reservoirs for vectors that transmit *Lankesterella* parasites (Cantarero et al. 2013). At the beginning of the breeding season, exploration of potential nest cavities is common and extra-pair matings are likely to occur even before egg laying (Kempnaers et al. 1995).

Despite being more parasitized by several blood parasite species, evidence that males gaining paternity are of higher quality was also supported by physiological parameters. Males that sired

extra-pair young and had higher reproductive success had higher hemoglobin concentration in blood (only available in season 2), probably as a result of higher activity when searching for additional matings (fights and chases with the resident male may occur in some species, Stutchbury 1998). Whole-blood hemoglobin levels have been related to adult performance since more oxygen throughout the body is needed during demanding activities (Scott and Milsom 2006). Moreover, higher hemoglobin levels have been related to better condition and better nutrition status in the same species, albeit in nestlings (Gładalski et al. 2016).

Another parameter of breeding performance is hatching date. Hatching date and clutch size are often influenced by food availability (Smith et al. 2013) and habitat quality (Amininasab et al. 2016). Here, increased reproductive success in male blue tits was related to later clutches in season 2, which may reflect that high quality males mated with females that bred later to maximize reproductive performance in a particularly extreme breeding season (Gładalski et al. 2014).

Additional evidence supporting the relationship between paternity gain and higher quality is seen in color change in both seasons. Previous results in the present blue tit population showed that the conditions experienced during a single reproductive event can have an effect on feather coloration obtained during the subsequent late summer molt (Badás et al. 2018). Here, we found that after season 1, males with more extra-pair offspring increased their white cheek brightness, which has already been related to higher individual quality (Badás et al. 2018). The following season (season 2), males with brighter cheek feathers tended to gain paternity and sire more extra-pair young. The same pattern was found in another plumage patch after the molt in season 2. Males with more extra-pair offspring in season 2 changed their tail brightness significantly less for the following season (season 3). Because the general trend was a decrease in tail feather brightness from season 2 to season 3, this result indicates that higher quality males retained brighter tail feathers for the consecutive breeding event despite harboring higher parasite loads and coinfections. These findings confirm our hypothesis that higher quality males bear the costs of extra-pair mating strategies because feather coloration for the following reproductive event is not compromised (as seen in seasons 1 and 2 in this study).

Another study in blue tits suggests that the change in carotenoid-based coloration between seasons may depend on quality in both sexes (Doutrelant et al. 2012). Our results suggest that feather coloration in the yellow breast, in the white cheek, and in the blue-green tail of male blue tits may contain relevant information regarding quality. Females may use a combination of signals to assess male quality, which might explain why similar patterns are found across plumage patches, studies, and populations. Behavioral and morphological components can signal different aspects of male quality or individual condition at

different time scales, according to multiple ornamentation theory (Marchetti 1998). Different ornaments may (1) act as amplifiers by offering redundant information, (2) have an additive effect (when the information of several traits is complementary), or (3) provide different information about different qualities of the bearer (Møller and Pomiankowski 1993). Moreover, our results provide evidence for two feather patches acting as honest signals of individual quality, since high quality males were able to maintain bright white cheek or tail feathers for two consecutive reproductive events. The white plumage patch has been suggested to act as an ornament in the blue tit and other passerines (Griggio et al. 2011b; Zanollo et al. 2012; Ruiz-De-Castañeda et al. 2015; Badás et al. 2018), but whether tail brightness is a sexually selected ornament in the blue tit awaits further confirmation.

Lower quality males, on the contrary, may be more cuckolded. Our results from season 1 support this premise, since we found that males that were lighter, and that tended to be more intensely infected by *Haemoproteus* and duller in their white cheek feathers, were more likely to be cuckolded. Complex interactions in bird blood parasite systems that include avian malaria and malaria-like parasites have been previously reported (Marzal et al. 2008; del Cerro et al. 2010; Badás et al. 2017), with some parasite species having clearer negative effects than others depending on varying environmental conditions (Møller et al. 2013). *Haemoproteus*, for example, may be more virulent than coccidian parasites, since previous studies have shown that birds infected with this blood parasite had reduced survival (Martinez-de la Puente et al. 2010; Bielański et al. 2017). In season 2, we found that cuckolded males had more saturated breast feathers. The reason for this pattern could be that high quality males (males with more saturated carotenoid-based ornaments) may lose paternity if they disregard mate-guarding (Garcia-Navas et al. 2014). However, gain of paternity was not related to cuckoldry, and thus, we cannot confirm this hypothesis. Still, the relationship between increased yellow saturation and cuckoldry in season 2 should be taken with caution because, in fact, these model explain little variance when compared to the season 1 cuckoldry model (18% vs. 49%).

In conclusion, we showed that individuals bearing high-quality ornaments gain extra-pair paternity while coping with the costs of this mating behavior. These costs may be apparent in males that sire more extra-pair young, for example, as a reduction in body mass (i.e., season 1 in this study), or as infections by blood parasites (i.e., season 2 in this study). We also provide evidence that feather color changes between seasons may increase the male's fitness during the following reproductive event, and that the direction of the color changes depends on individual quality, enhancing the opportunity for sexual selection in this species. Our results indicate that even after a costly reproductive event, high quality males can access the necessary resources to maintain honest signaling for the following season: fewer changes in plumage

patches that are already more ornamented or increased changes in other ornaments to attain brighter ornaments. In socially monogamous mating systems, complex relationships between parasitic infections, physiological condition and feather color change between seasons explain paternity and reproductive success, with important implications for the evolution of sexual signaling and mating behavior.

#### AUTHOR CONTRIBUTIONS

The study was conceived by E.P.B., J.M., and S.M. Fieldwork was conducted by E.P.B., A.A., J.M., J.R.A., and S.M.; paternity analyses were developed by E.P.B. and A.A.; molecular parasite analyses were carried out by J.M. Statistical analyses and manuscript writing were conducted by E.P.B. with input from all authors. All authors gave final approval for publication.

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#### DATA ARCHIVING

The doi for our data is: 10.5061/dryad.pzgmsbcgf. Code will be made available using Github: <https://github.com/elisa-P-badas>.

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### Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Chromatic and achromatic color contrasts describing differences in male feather color.

**Figure S1.** PLS regression on variables explaining extra-pair paternity in male blue tits in the spring of 2012.