Articles

Malaria infection status predicts extra-pair paternity in the blue tit

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Extra-pair matings comprise a common reproductive strategy among socially monogamous bird species. However, it remains unclear why females decide to mate with extra-pair males. Indirect benefits in terms of improving offspring genetic quality are usually invoked to explain this phenomenon. Parasite resistance genes are often considered as a female target of seeking extra-pair matings, but the direct test of this hypothesis is generally lacking.

Here, we report on a relationship between the status of infection with malaria parasites (*Plasmodium* and *Haemoproteus*) and occurrence of extra-pair paternity in a wild population of the blue tit *Cyanistes caeruleus* inhabiting Gotland (Sweden). We found that the probability of extra-pair paternity is significantly related to the infection status of social parents. Infected males showed higher probability of being cuckolded than uninfected ones. However, this was observed only among males mated to uninfected females. Thus, avian malaria may potentially contribute to explanation of extra-pair mating behaviour.

Extra-pair copulations (EPCs) are widespread among monogamous species. They are observed in many mammals (Cohas and Allainé 2009), but majority of the data comes from studies performed on birds (Schmoll 2011). Extra-pair matings have raised a considerable research interest, but the evolutionary explanations of this behaviour still remain unclear. Male benefits from extra-pair matings are usually obvious and well documented (Vedder et al. 2011), while female benefits seem to be subtle or unclear (Forstmeier et al. 2014). Numerous empirical data show that multiple matings may incur costs to females, such as the reduction in parental care of a social partner (Dunn and Whittingham 2007). Also the risk of pathogen transmission may rise with increasing number of sexual partners (Keller and Reeve 1995). Still, there is evidence that, at least in some species, females seem to actively seek matings with multiple partners (Kempenaers et al. 1997, Richardson and Burke 1999).

Improvement of the genetic quality of the offspring is often invoked to explain extra-pair mating behaviour in birds (Schmoll 2011). In contrast to polygynous species, among monogamous species some females must be mated to low quality males. In such a case, the female may seek compensatory genetic benefits via EPCs (Møller and Alatalo 1999). Yet, this appealing hypothesis has not received satisfactory support in empirical studies. Parasite resistance genes have been often considered to be a target interest for the female seeking extra-pair matings. In fact, individual quality may be strongly affected by parasites (Merino et al. 2000, Marzal et al. 2005, Martínez-De La Puente et al. 2010),

so the resistance genes may constitute an important fitness component of female's progeny. However, this may work only if a female is able to recognize infection status of a potential mate, e.g. based on the secondary sexual characters (Hamilton and Zuk 1982, del Cerro et al. 2010). Thus, assuming female ability to recognize parasitic infection one may expect that females should avoid matings with a parasitized male (reviewed by Beltran-Bech and Richard 2014) and if already mated to such a male she should seek resistance genes for her offspring by mating with extra-pair males. However, this hypothesis has not been often considered in the empirical studies and only few studies looked into the relationship between the occurrence of parasitic infections and extra-pair matings (Weatherhead and Boag 1995, Wagner et al. 1997, Kempenaers et al. 2001, MacDougall-Shackleton et al. 2002, Durrant and Hughes 2006, Lessard et al. 2014).

Here, we aimed at studying whether the probability of occurrence of extra-pair offspring (EPO) is related to the status of infection with malaria parasites (i.e. species from the genus *Plasmodium* and *Haemoproteus*, sensu Pérez-Tris et al. 2005) of the social partner, in a small passerine, the blue tit *Cyanistes caeruleus*. We expected that females paired with a partner carrying a parasitic infection should be more willing to produce extra-pair offspring, than females paired to uninfected males. We also expected that female infection status may play an important role in mating decisions, as infected females should be more inclined to search resistance genes for her offspring if her own genes do not provide effective resistance.

Material and methods

The study was conducted from 2009 to 2011 on the island of Gotland, Sweden (57°03′N, 18°17′E) in the population of blue tits breeding in nest boxes (see Przybylo et al. 2000 for a more detailed description of the study area). In this population females lay on average 11 eggs, chicks hatch after two weeks of incubation and fledge after 18–22 d. Nest-boxes were regularly inspected from the end of April to determine the date of egg laying, the number of eggs and the date of hatching. Nestlings were uniquely marked by nail clipping two days after hatching, and blood-sampled. Blood was drawn from the leg vein to the capillary and stored at room temperature in 96% ethanol. Adult birds were captured, either in the nest box with self-releasing traps installed inside the box or by mist-netting, while feeding 10–14 d old nestlings. Sex and age of adults were assessed in accordance with standard techniques based on plumage characteristics and the presence of a brood patch (Svensson 1994). Adults were bled from the brachial vein using a capillary and samples were stored in room temperature in 96% ethanol. DNA was extracted using Chelex (Bio-Rad, Munich, Germany).

The occurrence of infection with *Plasmodium* and *Haemoproteus* was determined using nested polymerase chain reaction (nested PCR), which amplifies the gene encoding the cytochrome *b* of these parasites (Waldenström et al. 2004). PCR products from the second reaction were run on 2% agarose gels stained with GelRed (Biotium, Hayward, CA, USA) and visualized under ultraviolet light. The PCR products of positive samples were purified and sequenced directly using BigDye terminator ver. 3.1 (Applied Biosystems) to assess parasite genus. For more details see Podmokła et al. (2014).

Paternity was identified on the basis of microsatellite loci polymorphism analysis, using PCR primers designed for blue tits (in 2009 and 2010: five loci and in 2011 six loci, Dawson et al. 2000, Olano-Marin et al. 2010, for more details see Arct et al. 2013). PCR products were separated on ABI 3031 DNA analyser (Applied Biosystems) and the obtained data were analysed using GeneMapper 6.0 software. The paternity was assessed by comparing the genotypes of each nestling with its social father (Jones et al. 2010). Mismatches between social paternal and offspring genotypes at a single locus were regarded as mutations, and mismatches at two or more loci were regarded as extra-pair paternity.

In total, occurrence of extra-pair offspring and infection status of both parents were known for 68 broods. However, in this data set there were two females and two males breeding in two successive years and one male recorded in three years. Even though in each case these individuals were paired with a different partner we decided to use data only from the first occurrence of each individual. Thus, our analyses were based on the subset of 62 broods. The dataset comprised 10 broods in which both parents were uninfected, 10 in which only male was infected, 11 in which only female was infected and 31 in which both parents were infected. We performed generalized linear mixed model (Proc GLIMMIX in SAS ver. 9.3) assuming binomial error distribution and a logit link to model the probability of occurrence of extra-pair paternity in a brood. Infection status (infected vs non-infected) of a male and a female and individual age (one year old vs two or more years old) were defined as fixed categorical variables, and body condition (residuals of a regression of body mass on tarsus) as a covariate. The model included two way interactions between the variables and also higher level random factors: year of the study and territory identity (nest ID) to account for the fact that males and females shared the same territory/ same brood. Firstly we tested the full model (Supplementary material Appendix 1, Table A1), but then we reduced it by sequentially removing non-significant interactions. Separate models for birds infected either with *Plasmodium* or *Haemoproteus* were not run because of the low sample size, which did not allow to test the interaction effects.

Results

In the dataset considered in this study the prevalence of infection with malaria parasites was 66.9% (83 individuals carrying parasitic infection out of 124). Most infections were caused by parasites from the genus *Plasmodium* (73.5 vs 26.5% infected with parasites from the genus *Haemoproteus*). There was no difference in the prevalence of blood parasites neither between sexes (67.7% infected females vs 66.1% males, $\chi^2_1 = 0.04$, p = 0.85), nor between age classes (59.7% infected yearlings vs 71.4% older birds, $\chi^2_1 = 1.79$, p = 0.18).

We found that the probability of occurrence of extra-pair offspring in the brood was significantly explained by an interaction of male and female infection status ($F_{1,44} = 5.66$, p = 0.022, Table 1). Infected males were cuckolded more often than uninfected if mated to uninfected females (post-hoc analysis; $t_{44} = 2.26$, p = 0.029, Fig. 1) while the effect of male infection status appeared non-significant among infected females ($t_{42.9} = 0.98$, p = 0.332). We did not find any effect of individuals age nor body condition on the probability of occurrence of extra-pair offspring in the brood (Table 1).

Discussion

Our data suggests that infection with malaria parasites may constitute an important determinant of extra-pair matings. Male infection status seems to play a crucial role, but it is not independent of female infection status. Infected males are more likely to lose paternity only if mated to uninfected female. To our knowledge this is the first study reporting the link between the infection status and the incidence of extra-pair paternity. In the previous studies that examined

Table 1. Probability of extra-pair paternity in relation to parental infection status, age and body condition. The results of the generalized linear mixed model (Proc GLIMMIX in SAS) using binomial errors and a logit link. The model included random higher order factors: year of the study and territory identity (results not shown).

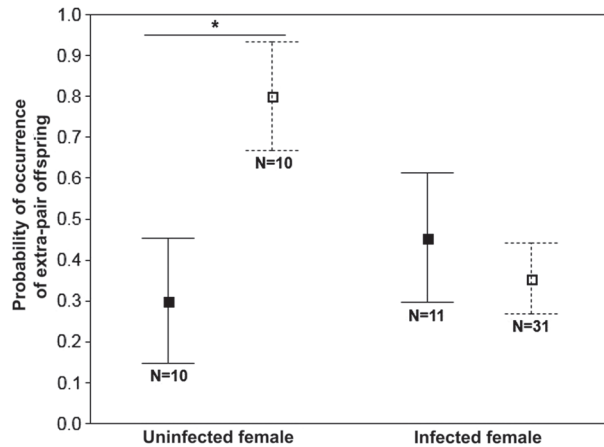| Predictors	DF	Den DF	F-value	Pr > F
Female infection status	1	44	0.00	0.9762
Male infection status	1	44	1.50	0.2272
Female age	1	44	0.69	0.4121
Male age	1	44	0.20	0.6559
Female condition	1	44	0.28	0.5977
Male condition	1	44	0.12	0.7255
Female infection status × male infection status	1	44	5.66	**0.0217**

Figure 1. Effect of parental infection status on the probability of occurrence of extra-pair offspring. Filled squares denote uninfected males and open squares – infected males. Points represent means and whiskers – standard errors from the raw data. Line with an asterisk connects significantly different groups (p < 0.05).

the relationship between parasitic infection with avian malaria and incidence of EPCs, such link was not confirmed (Weatherhead and Boag 1995, Wagner et al. 1997, Durrant and Hughes 2006) or it was not direct (MacDougall-Shackleton et al. 2002).

Our results may be interpreted in terms of the 'good genes' hypothesis assuming that female benefits from extra-pair paternity by providing superior alleles of certain genes to their offspring. However, a direct test of this hypothesis is not possible using our dataset. Firstly, one should expect that extra-pair males should be characterized by superior health (in this case resistant to avian malaria). Unfortunately, in case of our study identifying true fathers is almost impossible since only a part of the population is monitored. Therefore, we do not know if extra-pair males are indeed uninfected. Our data only suggest that uninfected female may be more willing to seek extra-pair mating if her social partner is caring parasitic infection. Secondly, the good genes hypothesis predicts that offspring of extra-pair males should be of superior quality (Griffith et al. 2002, Akçay and Roughgarden 2007), by inheriting parasite resistance genes and thus should be less prone to parasite infections than their half-siblings. Unfortunately, it is very difficult to verify this prediction since the detection of infection with malaria parasites in nestlings is rather impossible, because the period required for developing infection is longer than the nestling period of most bird species (Merino and Potti 1995). This period depends on host and parasite species and for *Haemoproteus* varies between 11 d and 3 weeks, and for *Plasmodium* between 1week and even several months (Valkiūnas 2005). Cosgrove et al. (2006) reported that none of 195 14-d-old blue tit nestlings had malaria infection, while infected individuals were detected in 30–33-d-old great tit fledglings (Krams et al. 2013). Those findings suggest that young should be screened for the presence of malaria parasites during the post-fledging period, whereas we followed their performance only up to fledging (ca 18 d post-hatching). Comparison of immunocompetence of extra-pair and within-pair offspring may provide some approximation of the potential differences between within- and extra-pair offspring in parasitic resistance. Such

differences were in fact previously found in our population (Arct et al. 2013), but higher immune response to phytohemagglutinin of the extra-pair than within-pair offspring was observed only among experimentally enlarged broods.

It is very intriguing why the higher frequency of extra-pair offspring is observed only among uninfected females mated to infected males, while it might be expected that rather infected females should be more interested in acquiring immunity genes for their offspring than uninfected ones. It is possible that infected females may be less choosy due to potential costs of seeking for an extra mate and possibly higher costs of being abandoned by a social male. In fact, some previous studies showed that the parasitic infection may disturb female mate preferences making them less choosy (Aguilar et al. 2008, Beckers and Wagner 2013). On the other hand, we cannot exclude a scenario in which the occurrence of EPO results not only from female decision but also from mate guarding provided by a social male (Kempenaers et al. 1995). In such case infected male may be unable to guard its paternity effectively when paired with an uninfected female. The situation may change with female's infection and makes mate guarding more effective. However, additional behavioral observations would be required to confirm such hypothesis.

In our study we assumed that the blue tit female can recognize the parasitic infection of her social mate. In fact, female may assess parasite resistance of her partner based on the secondary sexual traits (Hamilton and Zuk 1982). Blue tit female may use the carotenoid-based plumage coloration as a cue to assess parasitic resistance (del Cerro et al. 2010). To assess mate's quality birds may also use ultraviolet coloration (Limbourg et al. 2013), song characteristics (Kagawa and Soma 2013), personality traits (van Oers et al. 2008) or even odours (Whittaker et al. 2013).

In conclusion, our analyses suggest that infection status of both partners may contribute to explaining the occurrence of extra pair-paternity. However, our study does not strictly allow to infer the causality. Moreover, our conclusions rely on some assumptions, like the one concerning potential ability of individuals to recognize infection status of a potential mate. Future studies are needed to replicate our finding, but more importantly some experimental manipulations of infection status followed by identification of extra-pair mates would provide a causal link between infection status and the probability of occurrence of extra-pair matings.

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Supplementary material (Appendix JAV-00599 at <www.avianbiology.org/readers/appendix>). Appendix 1.