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 Przybyło 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 cytomegalo 6 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 Podmokł a 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 3103 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 tar-sus) 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, *χ²*₁ = 0.04, *p* = 0.85), nor between age classes (59.7% infected yearlings vs 71.4% older birds, *χ²*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*₁₄ = 2.26, *p* = 0.029, Fig. 1) while the effect of male infection status appeared non-significant among infected females (*t*₂₂ = 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

| 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 | 1  | 44     | 5.66    | 0.0217 |
within- and extra-pair offspring in parasitic resistance. Such
tence of extra-pair and within-pair offspring may provide
(ca 18 d post-hatching). Comparison of immunocompe-
ence of malaria parasites during the post-fl edging period,
Those findings suggest that young should be screened for the
in 30 – 33-d-old great tit fl edglings (Krams et   al. 2013).
reported that none of 195 14-d-old blue tit nestlings had
several months (Valki ū nas 2005). Cosgrove et   al. (2006)
and parasite species and for
quality (Griffi  th et   al. 2002, Ak ç ay and Roughgarden 2007),
dictates that off spring of extra-pair males should be of superior
parasitic infection. Secondly, the good genes hypothesis pre-
willing to seek extra-pair mating if her social partner is caring
infection is longer than the nestling period of most bird
This period depends on host
Laboratory studies showed that the parasitic infection may disturb female mate
infection status followed by identifi cation of extra-pair mates
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4.1.1. Framework Programme 6. The study conforms to the legal
requirements of Sweden.
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research ethics standards and relevant laws. All experiments were performed
in accordance with the ethical standards of the relevant institutional
body and with the approved guidelines (Law No. 633/1997). However,
additional behavioral observations would be required to confirm such hypothesis.

Figure 1. Eff ect of parental infection status on the probability of
currence 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 signifi cantly different groups (p < 0.05).

the relationship between parasitic infection with avian malaria and incidence of EPCs, such link was not confi rmed
(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 benefi ts 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

References

Aguilar, T. M., Maia, R., Santos, E. S. A. and Macedo, R. H.
2008. Parasite levels in blue-black grassquits correlate with
male displays but not female mate preference. – Behav. Ecol.
19: 292–301.
Akcay, E. and Roughgarden, J. 2007. Extra-pair paternity in birds:
review of the genetic benefi ts. – Evol. Ecol. Res. 9: 855–868.
Arct, A., Drobníak, S. M., Podmoklá, E., Gustafson, L. and Cichoń, M. 2013. Benefits of extra-pair mating may depend on environmental conditions – an experimental study in the blue tit (Cyanistes caeruleus). – Behav. Ecol. Sociobiol. 67: 1809–1815.

Beckers, O. M. and Wagner, W. E. 2013. Parasitoid infestation changes female mating preferences. – Anim. Behav. 85: 791–796.

Beltran-Bech, S. and Richard, F.-J. 2014. Impact of infection on mate choice. – Anim. Behav. 90: 159–170.

Cohas, A. and Allainé, D. 2009. Social structure influences extra-pair paternity in socially monogamous mammals. – Biol. Lett. 5: 313–316.

Cosgrove, C. L., Knowles, S. C. L., Day, K. P. and Sheldon, B. C. 2006. No evidence for avian malaria infection during the nestling phase in a passerine bird. – J. Parasitol. 92: 1302–1304.

Dawson, D. A., Hanotte, O., Greig, C., Stewart, I. R. K. and Burke, T. 2000. Polymorphic microsatellites in the blue tit (Parus caeruleus) and their cross-species utility in twenty songbird families. – Mol. Ecol. 9: 1941–1944.

del Cerro, S., Merino, S., Martínez-de la Puente, J., Lobato, E., Ruiz-de-Castañeda, R., Rivero-de Aguilar, J., Martínez, J., Morales, J., Tomás, G. and Moreno, J. 2010. Carotenoid-based plumage colouration is associated with blood parasite richness and stress protein levels in blue tits (Cyanistes caeruleus). – Oecologia 162: 825–835.

Dunn, P. O. and Whittingham, L. A. 2007. Search costs influence the spatial distribution, but not the level, of extra-pair mating in tree swallows. – Behav. Ecol. Sociobiol. 61: 449–454.

Durrant, K. L. and Hughes, J. M. 2006. Are there correlates of male Australian magpie Gymnorhina tibicen reproductive success in a population with high rates of extra-group paternity? – Ibis 148: 313–320.

Forstmeier, W., Nakagawa, S., Griffith, S. C. and Kempenaers, B. 2014. Female extra-pair mating: adaptation or genetic constraint? – Trends Ecol. Evol. 29: 456–464.

Griffith, S. C., Owens, I. P. F. and Thuman, K. A. 2002. Extra pair paternity in birds: a review of intraspecific variation and adaptive function. – Mol. Ecol. 11: 2195–2212.

Hamilton, W. D. and Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? – Science 218: 384–387.

Jones, A. G., Small, C. M., Paczolt, K. A. and Ratterman, N. L. 2010. A practical guide to methods of parentage analysis. – Mol. Ecol. Res. 10: 6–30.

Kagawa, H. and Soma, M. 2013. Song performance and elaboration as potential indicators of male quality in Java sparrows. – Behav. Process. 99: 138–144.

Keller, L. and Reeve, H. K. 1995. Why do females mate with multiple males? The sexually selected sperm hypothesis. – Adv. Stud. Behav. 24: 291–315.

Kempenaers, B., Verheyen, G. R. and Dhondt, A. A. 1995. Mate guarding and copulation behaviour in monogamous and polygynous blue tits: do males follow a best-of-a-bad-job strategy? – Behav. Ecol. Sociobiol. 36: 33–42.

Kempenaers, B., Verheyen, G. R. and Dhondt, A. A. 1997. Extra-pair paternity in the blue tit (Parus caeruleus): male choice, male characteristics, and offspring quality. – Behav. Ecol. 8: 481–492.

Kempenaers, B., Everding, S., Bishop, C., Boag, P. and Robertson, R. J. 2001. Extra-pair paternity and the reproductive role of male floaters in the tree swallow (Tachycineta bicolor). – Behav. Ecol. Sociobiol. 49: 251–259.

Krams, I. A., Suraka, V., Rantala, M. J., Sepp, T., Mierauskas, P., Vrublevskas, J. and Krama, T. 2013. Acute infection of avian malaria impairs concentration of haemoglobin and survival in juvenile altricial birds. – J. Zool. 291: 34–41.

Lessard, A., Boursier, A., Bélisle, M., Pelletier, F. and Garant, D. 2014. Individual and environmental determinants of reproductive success in male tree swallow (Tachycineta bicolor). – Behav. Ecol. Sociobiol. 68: 733–742.

Limbourg, T., Mateeman, A. C. and Lessells, C. M. 2013. Parental care and UV coloration in blue tits: opposite correlations in males and females between provisioning rate and mate’s coloration. – J. Avian Biol. 44: 17–26.

MacDougall-Shackleton, E. A., Derryberry, E. P. and Hahn, T. P. 2002. Nonlocal male mountain white-crowned sparrows have lower paternity and higher parasite loads than males singing local dialects. – Behav. Ecol. 13: 682–689.

Martínez-De La Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E., García-Fraile, S. and Belda, E. J. 2010. The blood parasite Haemoproteus reduces survival in a wild bird: a medication experiment. – Biol. Lett. 6: 663–665.

Marzal, A., de Lope, F., Navarro, C. and Möller, A. P. 2005. Malaria parasites decrease reproductive success: experimental study in a passerine bird. – Oecologia 142: 541–545.

Merino, S. and Porti, J. 1995. High prevalence of hematozoa in nestlings of a passerine species, the pied flycatcher (Ficedula hypoleuca). – Auk 112: 1041–1043.

Merino, S., Moreno, J., Sanz, J. J. and Arriero, E. 2000. Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (Parus caeruleus). – Proc. R. Soc. B 267: 2507–2510.

Møller, A. P. and Alatalo, R. V. 1999. Good-genes effects in sexual selection. – Proc. R. Soc. B 266: 85–91.

Olano-Marín, J., Dawson, D. A., Girg, A., Hansson, B., Ljungqvist, M., Kempenaers, B. and Mueller, J. C. 2010. A genome-wide set of 106 microsatellite markers for the blue tit (Cyanistes caeruleus). – Mol. Ecol. Res. 10: 516–532.

Pérez-Tris, J., Hasselquist, D., Hellgren, O., Krizanauskiené, A., Waldenström, J. and Bensch, S. 2005. What are malaria parasites? – Trends Parasitol. 21: 209–211.

Podmoklá, E., Dubiec, A., Drobníak, S. M., Arct, A., Gustafson, L. and Cichoń, M. 2014. Avian malaria is associated with increased reproductive investment in the blue tit. – J. Avian Biol. 45: 219–224.

Przybylo, R., Sheldon, B. C. and Merilä, J. 2000. Climatic effects on breeding and morphology: evidence for phenotypic plasticity. – J. Anim. Ecol. 69: 395–403.

Richardson, D. S. and Burke, T. 1999. Extra-pair paternity in relation to male age in Bullock’s orioles. – Mol. Ecol. 8: 2115–2126.

Schmoll, T. 2011. A review and perspective on context-dependent genetic effects of extra-pair mating in birds. – J. Ornithol. 152: 265–277.

Svensson, L. 1994. Identification guide to European passerines. – Svensson, Stockholm.

Valkuunas, G. 2005. Avian malaria parasites and other Haemosporidia. – CRC Press.

van Oers, K., Drent, P. J., Dingemanse, N. J. and Kempenaers, B. 2008. Personality is associated with extrapair paternity in great tits, Parus major. – Anim. Behav. 76: 555–563.

Vedder, O., Komdeur, J., van der Velde, M., Schut, E. and Magrath, M. J. L. 2011. Polygyny and extra-pair paternity enhance the opportunity for sexual selection in blue tits. – Behav. Ecol. Sociobiol. 65: 741–752.

Wagner, R. H., Davidar, P., Schug, M. D. and Morton, E. S. 1997. Do blood parasites affect paternity, provisioning and mate-guarding in purple martins? – Condor 99: 520–523.

Waldenström, J., Bensch, S., Hasselquist, D. and Östman, Ö. 2004. A new nested PCR method very efficient in detecting Plasmodium and Haemoproteus infections from avian blood. – J. Parasitol. 90: 191–194.

Weatherhead, P. J. and Boag, P. T. 1995. Pair and extra-pair mating success relative to male quality in red-winged blackbirds. – Behav. Ecol. Sociobiol. 37: 81–91.

Whittaker, D. J., Gerlach, N. M., Soini, H. A., Novorny, M. V. and Ketterson, E. D. 2013. Bird odour predicts reproductive success. – Anim. Behav. 86: 697–703.