

EXTRA-PAIR PATERNITY OF TREE SPARROW (*PASSER MONTANUS*) IN A SEMI-URBAN POPULATION

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Abstract. The level of extra-pair paternity (EPP) of birds is investigated in a great number of studies, revealing high variance in this trait among bird species. EPP in the House Sparrow, *Passer domesticus* has been also reported by many authors but there are much less data on the promiscuous behaviour of the closely related Tree Sparrow, *Passer montanus*. In this paper we tested whether microsatellite markers developed for House Sparrows are suitable for determining the level of EPP in a Tree Sparrow population. Three of seven candidate loci (Pdo3, Pdo5 and Pdo9) showed appropriate level of polymorphism and were used in the paternity analyses. We found that 9.2% of the chicks (76/77) were sired by extra-pair mates and 21.05% (19/4) of the broods contained at least one extra-pair young (EPY).

Keywords: *Tree Sparrow (Passer montanus), extra-pair paternity, microsatellite*

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Introduction

In the last 15 years it has become evident that social and genetic mating systems are different in many species, particularly in birds. It is now accepted that genetic mating systems cannot be predicted by simply observing the pattern of social bonds. The advances of the methods in paternity analysis have shown that promiscuous behaviour (the occurrence of extra-pair paternity, EPP) is common in many apparently socially monogamous species (Birkhead and Moller 1992, 1998). This means that the extra-pair youngs (EPY) are sired by a male other than the single putative father in socially monogamous species, whereas in polyandrous species, extra-pair young are those sired by a male outside of the social bond. EPP are common even among socially monogamous bird species: molecular genetic applications have revealed that, based on a review of 150 studies with near 130 Passerine species, true genetic monogamy (no EPP) has been

found in less than 25% of the studied socially monogamous species (Hasselquist and Sherman 2001). According to this dataset, average frequency of extra-pair youngs in socially monogamous species was 11.1 %

There is a remarkable variation among species and populations in the frequency of extra-pair copulations (EPC, Ligon 1999). It has been suggested that differences in individual quality or age (Moller and Ninni 1998), as well as ecological factors (e.g. breeding density, Wink and Dyrce 1999, Westneat and Sherman 1997), and behavioural responses to ecological factors (such as breeding synchrony, Stutchbury and Morton 1995) might have an influence on the level of extra-pair fertilizations (EPFs) within species.

To understand the variation in the frequency of EPCs we also have to consider the potential costs and benefits of this behaviour for both sexes. For males, it is obvious that they can easily enhance their reproductive success with the extra-pair copulations

(if it results in EPP). On the other hand, seeking of EPCs can be costly by reducing the male's effectivity in mate guarding, parental care, territorial defence or self-advertisement (e.g. Poston *et al.* 1998, Sherman and Morton 1988) or increasing his chances to get infected by parasites (Sheldon 1993). For females, EPCs may yield direct benefits, e.g. access to good territories or other kind of resources (Hunter and Davies 1998), additional help in parental care (Wagner 1992), insurance of fertile mating (Wetton and Parkin 1991). The female may gain indirect benefits from EPCs in form of "good genes" (Gowaty 1996, Hasselquist *et al.* 1994) or increased genetic diversity in the brood (Westneat *et al.* 1990). Costs for the females may include the increased risk of getting sexually transmitted diseases (STDs, Lombardo and Thorpe 2000, Westneat and Rambo 2000) aggression by the extra-pair male's mate (Mays and Hopper 2003) or the retaliation by their social mate. Namely, the social mate can respond to the cuckoldry by physical retaliation (Westneat *et al.* 1990), by reducing parental care (Chuang-Dobbs *et al.* 2001a, Moller and Cuervo 2000, Moller and Birkhead 1993a), or by deserting the female (Cézilly and Nager 1995). Finally, females sometimes do not resist unsolicited EPCs from unknown, nonpreferred males when resistance to this sexual harassment could result in physical injuries (e.g., McKinney and Everts 1998, Frederick 1987).

The promiscuous behaviour of the House Sparrow *Passer domesticus* has been widely investigated (e.g., Griffith *et al.* 1999, Cordero *et al.* 1999, Wetton *et al.* 1995), but to our best knowledge, only one study has examined the frequency of EPFs in the closely related Tree Sparrow *Passer montanus* (Cordero *et al.* 2002). In this paper, authors have investigated the frequency of EPFs in two separated Tree Sparrow populations. Using single-locus minisatellites developed for House Sparrow, as well as universal multilocus DNA-fingerprinting probes, they found that 8 and 10% of the nestlings were sired by extra-pair mates.

Despite of the fact that there are many microsatellite markers developed for House Sparrow (e.g. Neumann and Wetton 1996, Griffith *et al.* 1999), there are no such specific markers for Tree Sparrow. In this study, therefore, we had two main goals: first, our aim was to test whether microsatellite markers developed for House Sparrow could work on Tree Sparrow, showing sufficient level of polymorphism to use them in paternity analyses. Second, we examined the frequency of extra-pair fertilizations (EPFs) in a Tree Sparrow population.

Methods

Fieldwork

We studied a nestbox colony population of Tree Sparrows living in an urban park in Szeged, South-East Hungary. Nestboxes were arranged on the trunks of a platan (*Platanus hybrida*) avenue at a height of 5-6 meters and approximately 10-15 meters apart. Adult birds were captured using mist nets during the whole year, from November 2005 to August 2006. They were ringed and individually marked with a unique combination of three colour rings. Blood samples (ca. 20-50 ml) for parentage analyses were also taken by puncturing their ulnar vein. Samples were stored in Queen's lysis buffer (Seutin *et al.* 1991) at 4 °C until laboratory procedures.

In the breeding season, captured adults were sexed by the presence of the incubation patch (Summers-Smith 1995). When possible, sex was confirmed by the observation of sex-specific behavioural traits (displaying, mating, nest defence, provisioning or incubation).

In March, 2006 we started to monitor the nestbox occupation of the Tree Sparrows. For each nest, observed social parents were recorded (if they had been already ringed), standard reproductive biology traits (date of egg laying, clutch size, length of the incubation period, hatching date and success, length of the provisioning period, and number of the fledgelings leaving the nest) were registered during the whole breeding season. Blood samples were taken also from the nestlings (when they were 8-12 days old), with the same procedures as in the case of adults. Broods of altogether three breeding attempts were involved in this study.

All manipulation of the birds was carried out under the appropriate licenses and complied with the current laws of Hungary where the study was undertaken.

Molecular sexing

DNA extraction from blood samples was performed with standard proteinase-K/phenol-chloroform extraction (Kawasaki 1990). Since the Tree Sparrow is sexually monochromatic, male and female birds are almost indistinguishable in the hand. Sexing of adult birds by the presence of incubation patch is feasible only in the breeding season, therefore, sex of adult birds were confirmed by molecular sexing techniques. This was performed by the amplification of the CHD1-W and CHD1-Z genes (Ellegren and Sheldon 1997) using the primers

2550F and 2718R (Fridolfsson and Ellegren 1999). PCR products were separated by electrophoresis using 1% agarose gels containing ethidium bromide and visualized under UV light. Sexing of nestlings were performed with the same procedures as in the case of adults. The results of field and molecular sex determination were matching in almost all cases (34 of 35 adult birds captured in the breeding period).

Microsatellite optimisation and analysis

Seven candidate microsatellite loci were tested on our Tree Sparrow samples (Table 1). Six of these markers were developed for House Sparrow (Pdo1, Pdo2, Pdo3 [Neumann and Wetton 1996], Pdo5 [Griffith *et al.* 1999], Pdo8 [GenBank: AF354422], Pdo9, AF354423) and one for the Superb Fairy-wren *Malurus cyaneus* (McyU4, Double *et al.* 1997). The level of polymorphism was estimated on ten adults selected randomly. Loci with more than two alleles were considered to be polymorphic.

In each primer pair, forward primers were fluorescently labelled on the 5'-end with HEX, JOE or FAM-6 dyes (Applied Biosystems Inc.) for further visualization of the PCR products. PCR reactions consisted of approximately 100 ng of template DNA, 0.5 μ M of each primer, 0.2 mM dNTPs, 2 mM MgCl₂, 1 unit of Taq dna polymerase (Fermentas) and the 10X Taq buffer in a final volume of 25 μ l. At every examined locus, we gained the predicted size of PCR products, but only three of them showed appropriate level of polymorphism: Pdo3, Pdo5 and Pdo9 (Table 1). Further genotyping of the sparrow families were therefore performed using these three loci. To resolve alleles, amplified PCR products were analyzed on an Abi Prism 3100 Genetic Analyzer (Applied Biosystems Inc.) at the Biomi Ltd. (Gödöllő, Hungary) using ROX-labeled ILS-

600 internal standard (Promega). The data were analyzed with the GeneScan software (Applied Biosystems Inc.).

Data analysis

As there were cases where only one parent was known to us, we had several incomplete families to work with. In this cases we used the number of the different alleles in each brood to determine the presence of extra-pair paternity. In families where alleles of one parent were unknown to us we analysed only broods with at least three offsprings, because the detection of the extra pair alleles is impossible with fewer chicks. When we found five or more alleles in a brood we considered that a result of EPP.

Because of the low number of suitable microsatellite loci, chicks were regarded as extra-pair young (EPY) only when alleles were mismatching leastways at two different loci. Since microsatellite loci show a high mutation rate, we did not consider a young as an EPY when it had mismatching allele(s) at only one locus. We used this method to minimize the effects of genotyping errors and occurrent mismatching alleles between real descendants caused by the high mutational rate of microsatellites (Jarne and Lagoda 1996).

We assumed that two random individuals (potential fathers) matching in two or three examined loci is a very rare event in the population. Therefore, our assessment must be conservative.

To determine the parentage, we first examined whether the hypothetical (observed) female was the mother of all young in the brood. When we found nestlings with alleles different from the supposed mother's alleles, the brood was excluded (in the case of two broods).

Table. 1. Details of the microsatellite loci involved in the microsatellite optimization. The three loci typed bold were used for genotyping.

Locus name	GeneBank Acc. No.	Repeating motif	Originally described on	Size of the PCR products on the original species	Number of alleles in the original species	Size of PCR product in <i>P. montanus</i>	Number of alleles in <i>P. montanus</i>
pdo1	X93503	TG	<i>P. domesticus</i>	154-208 bp	14	154 bp	1
pdo2	X93504	TG	<i>P. domesticus</i>	170-208 bp	13	170-172 bp	2
pdo3	X93506	CCAT	<i>P. domesticus</i>	113-167 bp	14	117-145 bp	8
pdo5	Y15126	TG	<i>P. domesticus</i>	203-259 bp	16	222-261 bp	10
pdo8	AF354422	GA	<i>P. domesticus</i>	193-229 bp	15	179-191 bp	2
pdo9	AF354423	ATT	<i>P. domesticus</i>	375-426 bp	14	408-461 bp	9
mcyu4	U82388	GT	<i>M. cyaneus</i>	178-200 bp	11	170-172 bp	2

Table 2. Detailed data of the breeding biology and data collection

	1st attempt	2nd attempt	3rd attempt	Total
Number of broods investigated	14	14	11	43
Successful ¹ broods	13 (93%)	11 (78%)	7 (46%)	31 (72%)
Number of broods involved in genotyping	9	8	6	23
Number of broods involved in paternity analysis ²	8	7	4	19
Total number of eggs laid	83	90	70	243
Average egg number in broods	5.93	6.42	4.6	5.65
Number of eggs successfully hatched	57 (69%)	55 (61%)	26 (37%)	138 (57%)
Fledged chicks and fledging success ³	50 (60%)	41 (45%)	19 (27%)	117 (48%)
Number of chicks taken blood from	36	32	19	87
Number of chicks involved in paternity analysis ⁴	35	29	12	76

¹: At least one fledged young per brood.

^{2,4}: Some of the broods were excluded, in cases when we found nestlings with alleles different from the supposed mother's alleles.

³: Compared to the number of laid eggs.

Differential mortality within brood may also affect the estimation. No significant mortality had been detected during the period of the study. Because of the limited number of cases, this factor had not been analysed further.

Results and discussion

In this study we undertook to detect the EPP offsprings in a Tree Sparrow breeding colony living in an urban park in Szeged. Among seven candidate microsatellite markers developed for the House Sparrow, three loci (Pdo3, Pdo5, Pdo9) showed appropriate level of polymorphism in Tree Sparrows and were used successfully in the paternity analysis (Table 1).

During the three breeding attempts we totally recorded 43 broods in 21 occupied nestboxes. 31 of these can be considered successful (at least one fledged chick per brood): 14/13 (93%) successful attempts in the first breeding period, 14/11 (78%) in the second and 15/7 (46%) in the last, third period. Detailed data of the breeding biology is shown in Table 2.

Because of the insufficient data, 12 broods were excluded from the paternity analysis (parents were unknown, their bloodsample was lacking, offspring bloodsample was lacking or there were less than three offsprings with only one known parent). Finally, data of 19 broods and 76 chicks were involved in the paternity analyses.

Four of the total 19 nests (21,05%) contained at least one extra-pair young and 9,2% of the youngs (76/7) were sired by extra-pair mates. 10,5% of the broods contained only one EP offspring (19/2), 5,3% of them contained two EPYs (19/1) and 5,3% three (19/1) extra-pair youngs. We could not find more than three extra-pair young in any of the examined broods.

Our results suggest similar frequency of EPP to the level of EPP found in a Swiss and Spanish Tree Sparrow population (Cordero *et al.* 1999). They found that 25% (10/40) of Tree Sparrow broods in Spain and 23% (8/35) in Switzerland had at least one extra-pair offspring, and that 8% (12/151) of nestlings in Spain and 10% (12/114) in Switzerland were sired by extra-pair mates.

Because of the insufficient data, our results can be considered only preliminary. The number of incomplete families (one parent is unidentified, with no ring and blood sample) in the study was quite high, due to the low efficiency in catching and observing the parent birds. Identification of the adult Tree Sparrows (by observing them near to the nest) was sometimes problematical because of their confusing behaviour (nest boxes were close to each other and some of the adult birds were observed on several different nests in the same breeding attempt). Using nest traps, ratio of unidentified parents could have lowered, but adult birds showed considerable susceptibility to disturbance, so we determined not to apply this method. Additionally, accuracy of the parental analyses of the involved families could have been much greater if we could involve more than three microsatellite loci or loci especially developed for Tree Sparrow.

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References

- Birkhead, T.R. and Møller, A.P. (1998): Sperm Competition and Sexual Selection. London: Academic Press.
- Cézilly, F., Nager, R.G. (1995): Comparative evidence for a positive association between divorce and extra-pair paternity in birds. *Proc R Soc London B* 262, 7-12
- Chuang-Dobbs, H.C., Webster, M.S., Holmes, R.T. (2001a): Paternity and parental care in the black-throated blue warbler, *Dendroica caerulescens*. *Anim. Behav.* 62, 83-92
- Cordero J. P., Heeb P., Wetton J.H. and Parkin D.T., (2002): Extra-pair fertilizations in Tree Sparrow *Passer montanus*. Ibis, 144 (on-line), E67-E72
- Cordero, J.P., Wetton, J.H. and Parkin, D.T. (1999): Extra-pair paternity and male badge size in the House Sparrow. *J. Avian Biol.* 30, 97-102.
- Double, M.C., Dawson, D., Burke, T. and Cockburn, A. (1997): Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. *Mol. Ecol.* 6, 691-693.
- Frederick, P.C. (1987): Extra-pair copulations in the mating system of white ibis *Eudocimus albus*. *Behaviour* 100, 170-201
- Gowaty, P.A. (1996): Battles of the sexes and origins of monogamy. In: *Partnerships in birds: the study of monogamy* (Black JM, ed). Oxford: Oxford University Press; 21-52.
- Griffith, S.C., Owens, I.P.F., Thuman, K.A. (2002): Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol. Ecol.* 11, 2195-212
- Griffith, S.C., Stewart, I.R.K., Dawson, D.A., Owens, I.P.F. and Burke, T. (1999): Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow *Passer domesticus*: is there an 'island effect'? *Biol. J. Linn. Soc.* 68, 303-316.
- Hasselquist, D. (1994): Male attractiveness, mating tactics and realized fitness in the polygynous great reed warbler (thesis). Lund, sweden: Lund University.
- Hasselquist, D. and Sherman, P.W. (2001): Social mating systems and extra-pair fertilizations in passerine birds. *Behavioral Ecology*, Vol. 12, 4, 457-66.
- Hunter, F.M. and Davis, L.S. (1998): Female Adelide penguins acquire nest material from extra-pair males after engaging in extra-pair copulations. *Auk* 115, 526-528
- Jarne, P. and Lagoda, P.J.L. (1996): Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* 11, 424-429.
- Ligon, J. David (1999): *Evolution of Avian Breeding Systems*. Oxford University Press.
- Lombardo, M.P. and Thorpe, P.A. (2000): Microbes in tree swallow semen. *J. Wild. Dis.* 36, 460-68
- Mays, H.L. and Hopper, K.R. (2003): Differential responses of yellow-breasted chats (*Icteria virens*) to male and female conspecific model presentations. *Anim. Behav.* In press.
- McKinney, F. and Everts, S. (1998): Sexual coercion in waterfowl and other birds. See Parker and Burley 1998, 163-95
- Moller, A.P. and Birkhead, T.R. (1993a): Certainty of paternity covaries with parental care in birds. *Behav Ecol Sociobiol* 33, 261-268
- Moller, A.P. and Cuervo, J.J. (2000): The evolution of paternity and paternal care in birds *Behav Ecol* 11, 472-485
- Moller, A.P. and Ninni, P., (1998): Sperm competition and sexual selection: metaanalysis of paternity studies of birds. *Behavioral Ecology and Sociobiology*, 43, 345-58
- Neumann, K. and Wetton, K. (1996): Highly polymorphic microsatellites in the house sparrow *Passer domesticus*. *Mol. Ecol.* 5, 307-309.
- Poston, J.P., Wiley, R.H., Westneat, D.F. (1998): Male rank, female breeding synchrony, and patterns of paternity in the boat-tailed grackle. *Behav Ecol* 10, 444-451
- Seutin, G., White, B.N., Boag, P.T. (1991): Preservation of avian blood and tissue samples for DNA analyses. *Can J Zool* 69, 82-90.
- Sheldon, B.C. (1993): Venereal diseases in birds. *Phil Trans R Soc B* 339, 491-497
- Sherman, P.W. and Morton, M.I., (1988): Extra-pair fertilizations in mountain white-crowned sparrows. *Behav Ecol Sociobiol* 22:413-420
- Summers-Smith, J.D., (1995): *The Tree Sparrow*. The Bath Press, Bath.
- Stutchbury, B.J. and Morton, E.S. (1995): The effect of breeding synchrony on extra-pair mating systems in songbirds. *Behaviour*, 132, 675-690
- Wagner, R.H., Schug, M.D., Morton, E.S. (1996): Confidence of paternity and parental effort in razorbills. *Auk* 109, 556-562
- Westneat, D.F. (1990): Genetic parentage in the indigo bunting: a study using DNA fingerprinting. *Behavioral Ecology and Sociobiology*, 27, 67-76.
- Westneat, D.F. and Rambo, T.B. (2000): Copulation exposes female red-winged blackbirds to bacteria in male semen. *J. Avian Biol.* 31, 1-7
- Westneat, D.F., Sherman, P.W., (1997): Density and extra-pair fertilizations in birds: a comparative analysis. *Behavioral Ecology and Sociobiology*, 41, 205-15
- Westneat, D.F., Sherman, P.W. and Morton, M.L. (1990): The ecology and evolution of extra-pair copulations in birds. In: *Current Ornithology*, Vol. 7 (ed. Power, D.M.), 331-96. Plenum Press, New York
- Wetton, J.H., Burke, T., Parkin, D.T. and Cairns, E. (1995) Singlelocus DNA fingerprinting reveals that male reproductive success increases with age through extra-pair paternity in the house sparrow (*Passer domesticus*). *Proc. R. Soc. Lond. B* 260, 91-98.
- Wetton, J.H. and Parkin, D.T. (1991): An association between fertility and cuckoldry in the house sparrow, *Passer domesticus*. *Proc R Soc Lond B* 245, 227-233
- Wink, M., Dyrce, A., (1999): Mating systems in birds: a review of molecular studies. *Acta Ornithologica*, 34, 91-109