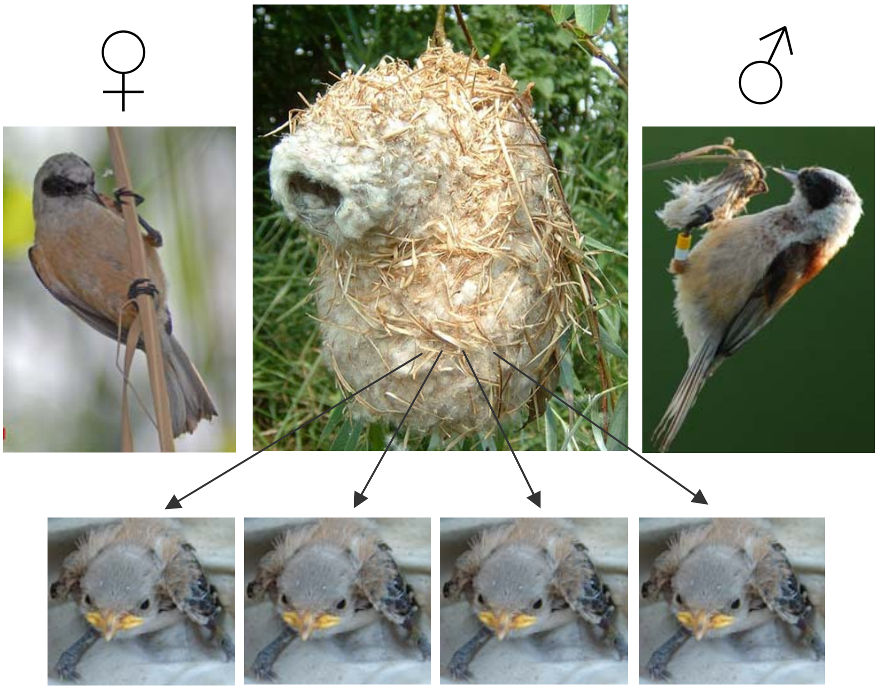
Breeding system and cuckoldry in the Eurasian penduline tit (*Remiz pendulinus*)

DOCTOR OF PHILOSOPHY DISSERTATION

Lídia Anna Mészáros



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Summary

In the unique breeding system of the Eurasian penduline tit (*Remiz pendulinus*) the male starts to build a tightly woven nest while he continuously tries to attract a female by calling and singing. If he is successful he will mate with a female and the couple finishes the nest together. After 4-5 days of mating, when the nest is nearly finished and the female already laid a few eggs, either one or both parents desert the clutch. For 45-65% of the cared clutches the female takes care, while 7-18% of the nests are male cared. 28-44% of the nests with the eggs inside are deserted by both parents. After desertion both sexes look for a new mate. A penduline tit can have up to six different mates during a single breeding season. What may be the meaning of this unique behaviour?

During my Ph.D. work I searched for an answer to the question by combining field observations with DNA analysis. The field data were collected at Fehér-tó, Hungary between 2002 and 2007. The area is used by 60-90 males and 45-50 females every year. Penduline tit nests were checked every other day, the recently arrived birds were caught, ringed, their standard biometrics were measured and a few drops of blood were taken from their wing vein. Ten days old young were also ringed and their blood samples were collected.

First we identified 11 penduline tit specific polymorphic microsatellite sequences. The microsatellites were amplified with fluorescently labelled PCR primers. Lengths of the PCR products were determined trough gel-electrophoresis. The identified 47 microsatellite variants were sufficient to get a good knowledge concerning the family relationships within the penduline tit clutches. Results of the parentage analyses are based on DNA fingerprint data of 123 males, 83 females and 443 young from a total of 118 nests. The most important findings of this study are as follows:

- We showed that, like in many other bird species, cuckoldry exists in the penduline tit. All three types of extra-pair young (extra-pair paternity, quasi-parasitism, egg dumping) occur within this species.
- Analysing the 365 offspring where either both social parents or only the social male was genotyped 62 were sired by an extra-pair male. Of the 78 young where we only have fingerprint data of the social female 12 were the result of either quasi-parasitism (extra-pair female) or egg dumping (both the male and the female are extra-pair).
- Analysis of the 166 young where both parents were known 50 were extra-pair: 39 originated from an extra-pair male, 7 were sired by an extra-pair female and 4 were the result of egg-dumping.

- The genotype of both parents could be identified in 55 nests. 33 of these nests contained at least one extra-pair young which derive either from an extra-pair male, from an extra-pair female or from egg dumping. In 29 nests at least one offspring were sired by an extra-pair male, in 9 nests at least one young was the result of quasi-parasitism and in 4 nests at least one chick had different parents then the social ones.
- We report that the number of extra-pair young within a nest do not predict whether the male/female will care or desert the clutch.

The main question what the meaning of this unusual mating system is we can not answer yet. The maintenance of high polymorphism might be one possibility. For a reliable answer further studies would be needed. Also for the identification of those factors that influence the rate of cuckoldry, act as cue during nest site selection we will need further studies.

In addition to the DNA-based data, we report that a properly chosen breeding site may highly influence the success of a breeding attempt. In two field experiments we found that a nest remnant acts as a cue for penduline tit males when choosing a nesting site. Furthermore penduline tit males tend to move to shorter distances than females between their consecutive nests; breeding site fidelity is lower for females than for males.

Introduction

For a successful breeding, the offspring of most bird species need parental care and a safe nesting site. There is a high variety in the strategies birds use to raise their young. Sometimes the male and the female share the work of caring and take care for the brood together [biparental care; white stork (*Ciconia ciconia*), barn swallow (*Hirundo rustica*), golden oriole (*Oriolus oriolus*)]. In other species only one parent (usually the female) provides care [uniparental care, pheasant (*Phasianus colchicus*), mallard (*Anas platyrhynchos*)]. A further possibility is not to provide any care and let others do the job [brood parasitism; cuckoo (*Cuculus canorus*)]. At the time of mating the decision which parent should take care for the young is not always clarified. In the Kentish plover (*Charadrius alexandrinus*) for example biparental and uniparental care occurs within the same population (Fraga and Amat 1996, Székely and Lessells 1993). Another bird species where the roles of the parents are not clear at the date of mating is a little migrating passerine, the Eurasian penduline tit (*Remiz pendulinus*). Once arrived to the breeding area at the beginning of April the penduline tit males start to build the tightly woven nest which usually hangs over water at a height ranging from 1-10 m, on branches of willows (*Salix sp.*), poplars (*Populus sp.*) and Russian olives (*Eleagnus angustifolia*, Gergely et al. 2008). Building a nest is time consuming and costs a lot of energy: males spend 17% of their daytime with building and it takes about 20 days to finish one nest (István Szentirmai and Tamás Székely unpublished data). During building, the male continuously tries to attract a female by calling and singing. If the male fails to attract a female over a longer time period, he deserts the nest and starts to build a new one at a different location. Like this one male can build up to nine nests during one breeding season (Figure 1, Szentirmai 2007). If the male is successful and he gets a mate the couple stays together for 4-5 days. They finish the nest together and the female starts to lay eggs. When the nest is ready and the female laid 1-3 eggs either the male, the female or both desert the brood. In 45-65% of the nests the male deserts and the female will incubate and raise the young after she increased the clutch size with 2-3 more eggs (female-cared nest; Persson and Öhrström 1989; van Dijk et al. 2007). 7-18% of the broods are deserted by the female and cared by the male (male-cared nest). In the remaining 28-44% both the male and the female abandon the nest with the eggs in it and look for a new mate (biparentally deserted nest; Persson and Öhrström 1989; Franz 1991; van Dijk 2009). The sex difference in desertion rates in penduline tits may partly be due to the risk for males to be cuckolded, so that the best interest of males is to secure and fertilize many females instead of investing into parental care (Trivers 1972; Queller 1997; Westneat and Stewart 2003; Kokko and Jennions 2008). During one breeding season both sexes can have up to six mates (Szentirmai et al. 2007, van Dijk 2009), whilst birds rarely mate multiply with the same mate (van Dijk et al. 2007, van Dijk 2009). Females often care for two broods while males only for one (rather towards the end of a breeding season) in a breeding season. Once deserted the birds do not return to the nest, one nest is used for only one breeding attempt. More attractive males (males with a bigger facial mask) are more likely to desert (van Dijk 2009) and the more attractive a male is the shorter the time period will be for him to find a mate (Kingma et al. 2008).

Stage 'A': the male fixes nest material at a crossing of two branches.

Stage 'B': the crossing of the two branches

Stage 'C-D': all sides of the running knot are tightly woven,

Stage 'E': there is only one opening at the place of the entrance, the opposite side is completely closed.

Stage 'F': a flue connects to the entrance.

Figure 1. Stages of nest building. Males only build their nest alone up to stage 'D'. If they are not successful in finding a mate till this stage they desert the nest and start to build a new one at a different location. (Drawing by Imre Bába, National Geographic Hungary.)

To find out more about the remarkably breeding system of the penduline tits we studied a breeding population over a six years period in Southern Hungary. We made a parentage analysis of the broods using the microsatellite based DNA-fingerprint analyses working on data of the whole six years time period. Furthermore we set up one field experiment to study the nest site selection behaviour and conducted a study about the breeding site fidelity of these birds.

Parentage analyses (rate of cuckoldry in a Hungarian penduline tit population)

Starting from the late 1980s, the assignment of parentage through new molecular tools (DNA-fingerprint analysis) lightened that having extra-pair offspring is a common phenomenon among various taxa: in fish about one-third of nests are cuckolded to some extent (DeWoody and Avise 2000) and extra-pair young (EPY) occurs in 90% of the studied bird species (Griffith et al. 2004). Extra-pair offspring are also relatively common in mammals with multi-male mating occurring in at least 133 species (Wolff and Macdonald 2004). Three different types of extra-pair young exist: EPP (the genetic and the social father of the young is different), quasi-parasitism (the genetic mother is different from the social mother) and egg dumping or intra-specific brood parasitism (both parents are different from the social ones, Wrege and Emlen 1987). In bird species quasi-parasitism and egg dumping are relatively rare phenomena occurring in only 12 out of 130 (Griffith et al. 2004) and in 20 out of 69 (Arnold and Owens 2002) species studied. All three types of extra-pair offspring were reported to be present in penduline tits (Schleicher et al. 1997).

The literature about whether parents should, or are able to adjust the amount of their parental care to the genetic share they hold in the offspring is divided (Sheldon 2002, Eliassen and Kokko 2008). Although some studies found a relationship between the amount of parental care and genetic share of the offspring (Dixon et al. 1994, Neff and Gross 2001, Alvergne et al. 2009) others did not (Bouwman et al. 2005). In birds the eggs and the young may not provide sufficient information to clearly obtain the real amount of EPY, which leads to the assumption that rather the risk of cuckoldry influences the decision of parents how much to investigate in their offspring (Davies 1992, Komdeur et al. 2007, Griffith et al. 2009). Furthermore the degree of cuckoldry is influenced by the mate guarding behaviour of the male (Parker 1974, Davies 1992, Osorio-Beristain and Drummond 2001): the risk that a male looses paternity in the eggs a female lay after he deserted increases if a male deserts too early and hence performs no mate guarding (Arnquist and Rowe 2005). Looking on EPY from a female's perspective we see, that a female would benefit indirectly from mating with multiply males and having young fathered by different males (Kempenaers 2007, Schmoll et al. 2009). Mating with genetically dissimilar males means an increased heterozygosity of the offspring, which leads to a better immune defence (MHC-complex, Richardson et al. 2005, Kempenaers 2007) and a higher success by finding a mate (genetic compatibility, Kempenaers 2007). Mating with an attractive male may lead to more attractive and/or better condition offspring and hence influences future mating success ('good genes' or 'sexy son' hypothesis, Weatherhead and Robertson 1979, Suter et al. 2007, Schmoll et al. 2009). Although various studies have found that EPY may grow faster, survive better and are more heterozygous than within-pair young (WPY, Ilmonen et al. 2009, Mulard et al. 2009), yet no consistent pattern exist in the literature (Kempenaers 2007, Magrath et al. 2009). As reviewed in Hansson and Westerberg (2002) the level of heterozygosity reflects the individual genetic diversity which is often in association with reproductive success. In the Hungarian penduline tit population we studied we expect a higher proportion of EPY in female-cared clutches compared to male-cared or biparentally deserted nests, since if a male deserts too early he will go a higher risk to be cuckolded. We predict the same if a male can somehow establish the risk of being cuckolded (either through mate guarding or by looking on potential extra-pair mates for the female): the higher the risk of cuckoldry during the mating period the less likely males will provide care, i.e. the rate of EPP should be higher in female-cared than in male-cared nests. Furthermore we analysed whether more attractive males (males with a bigger facial mask) are less cuckolded compared to males with a smaller mask, or not. We expect that if females seek extra-pair copulations to obtain indirect benefits a female mated with an attractive male will be more faithful than a female mated with a less attractive male.

In the last two decades many research papers were published on the heritability of different traits in birds. Depending on the subject of the research two main groups of studies exist: behavioural (Møller 2001, Dingemanse et al. 2002, Drent et al. 2003, MacColl and Hatchwell 2003, van Oers et al. 2004) and morphological (Smith 1993, Gosler and Harper 2000, Keller et al. 2001) studies. Part of these studies were conducted under laboratory conditions (Berthold and Pulido 1994, Drent et al. 2003, van Oers et al. 2004), while others analysed data collected from wild living populations (Gosler and Harper 2000, Møller 2001, Dingemanse et al. 2002). A heritable morphological trait was found by Smith (1993) who showed in a cross-fostering experiment that the tarsus length of starlings (*Sturnus vulgaris*) is heritable. In another study Gosler and Harper (2000) estimated the heritability of body condition in great tits (*Parus major*). They used a direct measure of condition (fat and pectoral muscle) and found a slight resemblance between parents and offspring in fat reserves when breeding and muscles in winter. Furthermore they showed that muscle sizes in the breeding season provide a stronger evidence for the inheritance of condition compared to the other measurements. In the other group of heritability studies which measure the heritability of different behavioural traits a high range of studies were conducted. Some of them found a heritable variation in the exploratory behaviour of great tits putting them in a novel environment (Dingemanse et al. 2002, Drent et al. 2003), others showed that individual great tits consistently differed in their risk-taking behaviour (van Oers et al. 2004). Latter furthermore found a tendency for risk-taking behaviour to be correlated with early exploratory behaviour, since birds with "fast' or "slow' exploratory behaviour also differ in their risk-taking behaviour. In a study on the long-tailed tit (*Aegithalos caudatus*) MacColl and Hatchwell (2003) showed that the amount of parental care (the rate at which parent(s) feed their offspring) has a significant heritable component. Another study on the savannah sparrow (*Passerculus sandwichensis*) found that the quantitative differences in feeding rates among males are attributed to the behavioural phenotypes of their fathers (Freeman-Gallant and Rothstein 1999). In penduline tits both sexes can take care for the brood. The decision about which parent will care for the offspring seems to be uncertain at the date of mating. A recent study of Pogány et al. (2008) showed that offspring desertion during one breeding season is repeatable in females but not in males. Females decision whether to care/desert varied little between subsequent nests, while if a male deserted one of his nests he was more likely to care for his next nest. Females either cared or deserted over the whole breeding season, while males deserted early in the breeding season and tended to care only late in the breeding season. Furthermore Bleeker et al. (2005) found in a previous study that a morphological trait, the body condition, influences the decision over parental care. They showed that nest building costs more energy than incubation and males in good condition desert their nest more often compared to males in poorer condition. However they did not find any relation between body condition and decision over parental care in females. It is likely that there is (are) a further trait(s) playing a role in the decision over parental care in penduline tits. One possible factor might be a fixed genetic effect. To prove the presence of a genetic component one has to make a heritability study between parents and offspring. If a heritable genetic factor is present we expect that young behave the same way as their parent(s) did; either care or desert at all their nests. Since the decision over care is only repeatable in females we only could include mothers and daughters in this analysis. Another trait that may also has a genetic component is the rate of cuckoldry in the nests of a given penduline tit. If the rate of cuckoldry stays constant in the subsequent nests of one individual, a genetic factor is likely to be included.

Nest site selection behaviour experiment

The survival of the progeny does depend on the nearby environment and also on the quality of the parents and the offspring. The outcome of a breeding attempt is influenced by several environmental factors, like: the rate of nest predation, the density of the vegetation, food and nest material availability. (Bart and Tornes 1989, Liker 1995, Clark et al. 1999, Martin et al. 2000, Jones 2001, Rounds et al. 2004, Houston et al. 2005, Warburton and Perrin 2005, Fontaine and Martin 2006, Morrison et al. 2007). Since selection of a suitable nest-site affects the survival, the breeding and migratory strategies, the traits involved in the selection of good nest-sites are likely to be favoured by natural selection (Alerstam and Högstedt 1982, Badyaev 1995). However, to decide whether a habitat patch is a good or rather a bad nest-site may be hard, since the environment may change, presence/absence of nest predators may not be apparent and it might be hard to predict food availability for the whole time period of breeding. An obvious cue that birds can use during selection of a good nest site is the presence of old nests. In cavity breeders using an old nest has both advantages and disadvantages. Birds save time and energy by excavating a new nest and by re-using the nest material, but they also go a higher risk since the old nests may be infected by parasites and pathogens (Davis et al. 1994, Mazgajski 2007).

Nest material seems to be a valuable source in penduline tits, since they like to steel nest material from other penduline tit nests built nearby. Another easy way of getting nest material is to destroy the deserted and not defended nest. Penduline tits building a nest nearby to an abandoned one destroy latter rapidly and use the nest material for they own nest. Also towards the end of the breeding season and during migration juvenile penduline tits, flying around in groups, like to destroy the deserted nests. However, after each breeding season a few nests remain and a part of these nests resist the harsh weather conditions during winter. Although nests that survived the winter are not useful as nest material for penduline tits any more, (their nest material got worn out and stiff) males arriving to the area and looking for a nesting site like to build their nest on trees with an old nest remnant (Gergely et al. 2008). To understand the nest site selection behaviour of the penduline tits we carried out two experiments. In our first experiment, carried out in 2006, we tested whether old nests act as cue during nest site selection. We predicted that males arriving to the breeding area rather choose a nesting site containing an old nest remnant than a nesting site without any nest remnant. Our second study, set up in 2007, was based on the results of the first experiment. Here we separated the cue hypothesis from the direct benefit one. The cue hypothesis predicts no preference between different types of old nests, while the direct benefit hypothesis predicts a preference for nesting sites containing one type of old nest.

Breeding site fidelity study

Movements between habitat patches (dispersal) may influence the structure and viability of a population. The dispersal between populations or subpopulations may enhance the viability of a population through changes in effective population size and by influencing genetic diversity (Gilpin 1991). Dispersal within a population on the other hand may affect local densities, the distribution of individuals of different quality in the population and the local sex ratio (Clobert et al. 2001). Both types of dispersal might be influenced by local ratios of sexually active males and females and thus affect mating systems (Székely et al. 2000, Pilastro et al. 2001). The special mating system of the penduline tit, especially the decision over parental care was suggested to be influenced by the local mating opportunities (Persson and Öhrström 1989). We expect if local mating opportunities are better for one sex this parent would gain more from re-mating and thus desert the nest more often. Also we suppose that the deserting female/male will build its next nest close to the previous one, i.e. breeding site fidelity is high when re-mating opportunities are good and stay constant within a breeding season. During a three years period (2002-2004) we studied the breeding site fidelity of a Hungarian penduline tit population. We compared breeding site fidelity of adult males and females within and between breeding seasons and looked whether site-fidelity of individual males and females is repeatable between consecutive nests (whether penduline tits build their nests at consistent distances from each other).

Materials and methods

Parentage analyses (rate of cuckoldry in a Hungarian penduline tit population)

Study site

Field data were collected between 2002 and 2007 at an extensive fishpond system (1321 ha) in Southern Hungary: Fehér-tó (46°19'N, 20°5'E). On the trees growing on the dykes separating the lakes 50-90 males and 40-50 females breed each year. (These are the numbers of the trapped and ringed individuals, biased towards males, because females are more difficult to catch; Szentirmai et al. 2007). The study area was searched for new nest building males and the already known nests were checked every second day for at least 15 min (this time period seems to be sufficient to confidentially detect the presence of a bird; van Dijk et al. 2007; van Dijk 2009). During these monitoring we determined the stage of nest building (Figure 1, Szentirmai 2007), the stage of the male (mated/unmated). In the case of mating we looked after the couple every day, recorded the mating date and checked which parent is present. If the nest owner(s) penduline tit(s) did not attend the nest at two consecutive nest checks (in this case the last check lasted for 30 min) this bird(s) was classified as 'deserted'. At nests with uniparental care we counted the number of eggs (approximately eight days after incubation started) and the number of ten days old chicks. Eggs from biparentally deserted nests were taken out and incubated artificially (see below). During the six years of our study none of the penduline tits classified as 'deserted' was re-sighted at the nest afterwards. When we found a new female/male we trapped the bird(s), measured standard biometrics (body mass, wing length, tarsus length) took a photo of the facial mask, marked the bird with an individual combination of one metal (from the Hungarian Ornithological and Nature protecting Association) and three colour rings (A. C. Hughes, UK) and finally took a blood sample of about 10 µl from the wing vein. Ten days old chicks were handles similarly, although they were ringed only with one metal ring, since the returning rate to the same area is very low in this species (Kiss and Szentirmai 2009). For a more detailed description of the field methods and how we took the photos see Kingma et al. 2008 and our Field Protocol at http://www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/PT_%20Field%20Guide_1_2.pdf 193.

Molecular methods

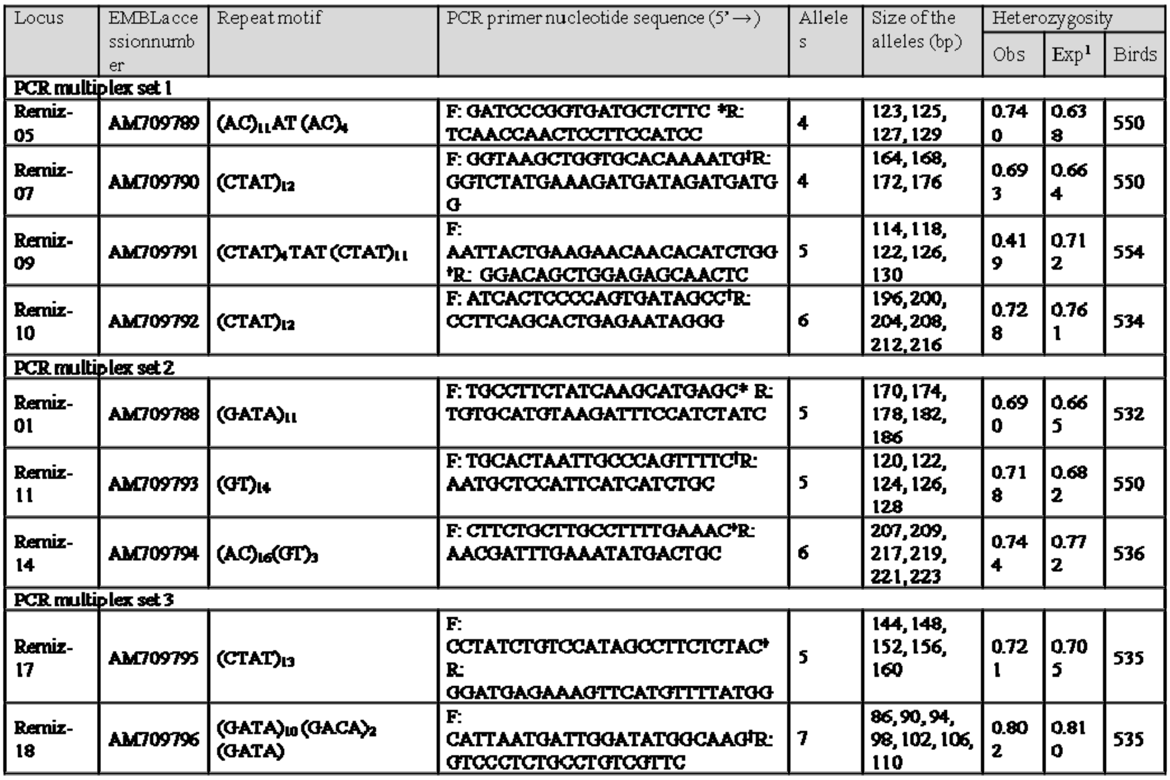
The blood samples taken were stored in an Eppendorf-tube containing 1ml of Queen's lysis buffer (Seutin et al. 1991) at 4-5°C. Eggs of the clutches that were deserted by both parents were collected and artificially incubated at 37.5°C for five days. If embryo development started we collected the embryos and stored them at -20°C. The DNA was extracted from the blood samples using the phenol-chloroform-isoamyl alcohol method (Krokene et al. 1996). To identify the penduline tits by their DNA, we needed species specific polymorphic DNA markers. The development of primers for DNA microsatellites loci was carried out by the Ecogenics GmbH, using a pool of 10 supposedly unrelated penduline tit DNA samples. For the parentage analyses we used nine polymorphic primer pairs (Remiz-01, Remiz-05, Remiz-07, Remiz-09, Remiz-10, Remiz-11, Remiz-14, Remiz-17, Remiz-18) where the forward primer was fluorescently labelled with either 6-FAM (blue), HEX (green) or NED (yellow). Amplifications (polymerase chain reaction, PCR) of microsatellites were performed in a Thermolyne amplitron II or in a Corbett Research thermocycler device. The nine microsatellite loci were amplified in three multiplex sets: (i) Remiz-05, Remiz-07, Remiz-09, Remiz-10, (ii) Remiz-01, Remiz-11, Remiz-14 and (iii) Remiz-17, Remiz-18. 1 µl of the PCR product was mixed with 1.2µl of a loading buffer, which contained a standard size marker (ROX350, Applied Biosystems). 1-1.2µl of each sample was loaded on a 10% denaturing polyacrylamid gel and ran on an Applied Biosystems (ABI) 377 XL DNA sequencer. Allele calling and sizing was carried out using the GENESCAN 3.1 (Applied Biosystems) and the GENOTYPER 2.5 software. The characteristics of the nine polymorphic microsatellite loci are summarized in Table 1. For a detailed description of PCR and gel electrophoresis conditions see Mészáros et al. 2008.

The DNA fingerprint data were analysed by the CERVUS 3.0 software (Kalinowski et al. 2007). The 47 alleles recorded during genotyping the penduline tits were used for the parentage analysis. The combined total exclusionary power of this marker set was 0.967 for the first parent (i.e. exclusion probability of the social father, given that he is not the father and the genotype of the mother is unknown) and 0.998 for the second parent (i.e. exclusion probability of the social father when the genotype of the mother is known). For subsequent analysis we only used those individuals where at least five loci were successfully genotyped (van Dijk et al. 2010). In 82% of the analyzed penduline tits all nine loci are known, only eight individuals (1.2%) having five loci were included. Assignment of paternity, maternity or both was carried out manually using the band sharing-coefficient (D; Lansman et al. 1981, Wetton et al. 1987) and the number of novel bands (Westneat 1993). As the lowest limit of the band sharing-coefficient we used the lowest value determined between a young and their parent(s) with no novel bands. For the number of novel bands we accepted an offspring as EPY if it had three or more novel bands. Those young which fit only one criterion were handled as within-pair young (WPY). For more information about the parentage analyses see van Dijk et al. 2010.

Statistics

We used binary logistic regression models to analyze if the proportion of EPP, predicted the parental care strategy (i.e. care or desert) by male, female or both. In addition, we repeated these analyses for the occurrence of EPY, but this did not qualitatively change our results (see Results). For the analyses with EPY, for males we pooled extra-pair paternity and egg-dumping, whereas female care was expected to be negatively affected by the occurrence of quasi-parasitism and egg-dumping and hence these were pooled in the analyses of female care strategy. In these models mating date was included to control for a change in parental care strategy over the course of the breeding season.

We used linear mixed models with restricted maximum likelihood (LMM) and 10000 iterations to test for differences in tarsus length, body mass and heterozygosity between offspring sired by extra-pair males and WPY. Year, parental care strategy, sex of the offspring and level of extra-pair paternity ('paternity') were included in these models as fixed effects and brood ID was included as a random effect. Including a seasonal covariate, mating date, resulted in that convergence could not be achieved and thus this term was not entered into the final LMMs (Table 3). However, brood ID also accounts for potential seasonal variation (e.g. food abundance). We kept year, parental care strategy and mating date in the final GLM (general linear model) (Table 4).



Locus	EMBLaccessionnumber	Repeat motif	PCR primer nucleotide sequence (5'→)	Alleles	Size of the alleles (bp)	Heterozygosity		
						Obs	Exp¹	Birds
PCR multiplex set 1								
Remiz-05	**AM709789**	**$(AC)_{11}AT(AC)_4$**	**F: GATCCCGGTGATGCTCTTC ♦R: TCAACCAACTCCTTCCATCC**	**4**	**123, 125, 127, 129**	**0.740**	**0.638**	**550**
Remiz-07	**AM709790**	**$(CTAT)_{12}$**	**F: GGTAAGCTGGTGCACAAAATG†R: GGTCTATGAAAGATGATAGATGATGG**	**4**	**164, 168, 172, 176**	**0.693**	**0.664**	**550**
Remiz-09	**AM709791**	**$(CTAT)_4TAT(CTAT)_{11}$**	**F: AATTACTGAAGAACAACACATCTGG ♦R: GGACAGCTGGAGAGCAACTC**	**5**	**114, 118, 122, 126, 130**	**0.419**	**0.712**	**554**
Remiz-10	**AM709792**	**$(CTAT)_{12}$**	**F: ATCACTCCCCAGTGATAGCC†R: CCTTCAGCACTGAGAATAGGG**	**6**	**196, 200, 204, 208, 212, 216**	**0.728**	**0.761**	**534**
PCR multiplex set 2								
Remiz-01	**AM709788**	**$(GATA)_{11}$**	**F: TGCCTTCTATCAAGCATGAGC* R: TGTGCATGTAAGATTTCCATCTATC**	**5**	**170, 174, 178, 182, 186**	**0.690**	**0.665**	**532**
Remiz-11	**AM709793**	**$(GT)_{14}$**	**F: TGCACTAATTGCCCAGTTTTC†R: AATGCTCCATTCATCATCTGC**	**5**	**120, 122, 124, 126, 128**	**0.718**	**0.682**	**550**
Remiz-14	**AM709794**	**$(AC)_{16}(GT)_3$**	**F: CTTCTGCTTGCCTTTTGAAAC♦R: AACGATTTGAAATATGACTGC**	**6**	**207, 209, 217, 219, 221, 223**	**0.744**	**0.772**	**536**
PCR multiplex set 3								
Remiz-17	**AM709795**	**$(CTAT)_{13}$**	**F: CCTATCTGTCCATAGCCTTCTCTAC♦ R: GGATGAGAAAGTTCATGTTTTATGG**	**5**	**144, 148, 152, 156, 160**	**0.721**	**0.705**	**535**
Remiz-18	**AM709796**	**$(GATA)_{10}(GACA)_2(GATA)$**	**F: CATTAATGATTGGATATGGCAAG†R: GTCCCTCTGCCTGTCGTTC**	**7**	**86, 90, 94, 98, 102, 106, 110**	**0.802**	**0.810**	**535**

Table 1. Characteristics of nine polymorphic microsatellite loci in the penduline tit *Remiz pendulinus*.
♦ 6-FAM-labeled; † HEX-labeled; * NED-labeled; [1] Based on Hardy-Weinberg-equilibrium analysis

We analyzed and present five different indices of heterozygosity, all of which have been previously related to measures of fitness in other species: (i) observed heterozygosity (H_o); (ii) mean heterozygosity of typed loci (mean H); (iii) standardized individual heterozygosity (SIH); (iv) mean d^2; (v) standardized mean d^2 (st mean d^2; see Table 5; Coulson et al. 1998; Coltman et al. 1999; Amos et al. 2001; Hansson et al. 2001; Foerster et al. 2003; Bouwman et al. 2007). To test for an association between the size of the mask of a male and heterozygosity, we used Pearson product moment correlation for mean H, st mean d^2, and square-root transformed mean d^2, whereas for H_o and SIH we used the Spearman rank correlation. All analyses of heterozygosity were restricted to mixed broods only.

All analyses were also run using a sub-sample of those nests where the genotype of both social parents was known. None of those results were qualitatively different from the ones we report here. All statistical analyses were performed in SPSS 16.0 (SPSS Inc., Chicago, USA). Non-significant terms were excluded from the models using stepwise elimination and we give statistics for excluded variables before their exclusion from the model. Two-tailed probabilities are given and we rejected the null-hypothesis at $P < 0.05$ level. For a more detailed description about statistics and their background see van Dijk et al. (2010).

Nest site selection behaviour experiment

The two experiments conducted to investigate the nest site selection behaviour of the penduline tits were performed at Csaj-tó fishpond system (958ha, 46°35'N, 20°5'E) in Southern Hungary, where 20-25 penduline tits breed every year (Gergely et al. 2008). During the breeding season in 2005, all nests GPS data build at Csaj-tó were recorded. After the breeding season in 2005 (October), all the penduline tit nests were removed from the study area and kept outdoors to be exposed to the winter conditions. Until spring the material of the nests got worn out and was of no use for the penduline tits any more ('worn out' nests). Before the next breeding season started (in April 2006), twenty suitable breeding habitat pairs were chosen. Each pair contained of one tree on which we hang a 'worn out' nest, while the other member of the pair remained empty. The habitats were checked weekly, GPS co-ordinates of new nests were recorded and if a new male/female was detected we trapped, measured and marked it the same way as birds captured at Fehér-tó. In the present study we only took the first nest of a male build in the area into account.

At the end of the breeding season in 2006, all the nests were removed from the fishpond system. This time fifteen nests were kept indoor to protect the nest material from weather conditions ('re-utilize' nests, the material of the nest is still usable for nest building), while other fifteen nests were kept again outdoors ('worn out' nests). Before the first penduline tit males arrived to the area, we selected 13 habitat triplets based on the data from 2005-2006. Each triplet contained three trees representing suitable habitats. It was randomly decided which habitat becomes a 'worn out', a 're-utilize' or no nest. Checking the area, capture of the birds and concerning of new nests followed the same protocol as in 2006. For a more detailed description of the two experiments see Gergely et al. 2008.

Breeding site fidelity study

For this study we used the GPS co-ordinates of nests built at Fehér-tó fishpond system in 2002-2004. Through the common area checks, the nests were spotted in early stage of nest building, thus we supposedly found all the nests. To describe the movements of the birds, we calculated one variable: the breeding distance (i.e. distance measured in meters between consecutive nests of males and females). This variable was calculated from the EOV (Unified National Projection) co-ordinates of nests using the Pythagorean Theorem. If a penduline tit had several nests in a breeding season only one randomly chosen breeding distance was included in the analyses. Also, if a bird was present for several years on the study area, only one randomly chosen year was concerned.

Since breeding distance was non-normally distributed, we used non-parametric statistical tests, expect the repeatability between subsequent nests (here we used the method of Harper 1994). All statistical tests were two-tailed and were performed using the R Project for Statistical Computing software (R Development Core Team 2004). For further information about the methods we used in the present (see also Mészáros et al. 2006).

Results

Parentage analyses (rate of cuckoldry in a Hungarian penduline tit population)

In the present study, 83 adult females, 123 adult males and 443 offspring were genotyped from 118 nests. In 166 young (38%) both social parents, in 199 (45%) only the social father and in 78 (18%) only the social mother of the offspring could be genotyped.

Frequency of extra-pair paternity, quasi-parasitism and egg-dumping

Of the 55 nests where both parents could be genotyped, 60.0% (n = 33) contained at least one EPY, either as a result of EPP, quasi-parasitism or egg dumping. 52.7% (n = 29) of these nests had at least one EPY sired by an extra-pair male, 16.4% (n = 9) contained at least one EPY from quasi-parasitism and 7.3% (n = 4) had at least one EPY as a result of egg dumping. Of the 166 offspring where both parents genotype was known, 30.1% (n = 50) were extra-pair: 23.5% (n = 39) of the young were sired by an extra-pair male, 4.2% (n = 7) of the offspring originated from a quasi-parasitic female. 2.4% (n =4) of the chicks were a result of egg dumping, since neither the social mother nor the social father did match the genotype of these young. Analyzing those 365 offspring where either both social parents were genotyped or we knew only the genotype of the social male, 17.0% (n = 62) were sired by an extra-pair male. Looking on those young where we only have fingerprint data of the social mother 15.4% (n = 12) were the result of either quasi-parasitism or egg dumping.

Extra-pair parentage and parental care

The parental care strategy was known at a total of 154 broods. 18.8% of these nests were male-cared, 70.8% were female-cared and 10.4% were biparentally deserted. The proportion of EPP in a brood did not predict whether the male would care or desert, the female would care or desert, nor whether either the male or the female would care or desert (Table 2, Figure 2). These results remained unchanged when we replaced EPP by EPY (i.e. EPP, quasi-parasitism or egg dumping). In the latter analyses we expected males to care less if the frequency of EPP and egg dumping increases (p = 0.746) and that females care is negatively affected by the occurrence of egg dumping and quasi-parasitism (p = 0.998).

Parental care		Model effect estimate ± SE	Wald	*P*
male behaviour	mating date	0.027 ± 0.015	3.245	0.072
care (22) v desert (92)	proportion EPP	0.004 ± 0.008	0.280	0.596
female behaviour	mating date	-0.006 ± 0.010	0.278	0.598
care (82) v desert (32)	proportion EPP	0.005 ± 0.008	0.397	0.529
parent's behaviour	mating date	0.024 ± 0.014	2.867	0.090
care (104) v desert (10)	proportion EPP	0.707 ± 154.719	0.000	0.996

Table 2. Binary logistic regression models of male and female care strategy in response to the proportion of extra-pair paternity (n = 114 nests, i.e. all nests where the parental care strategy and mating date were known, df = 1). Under 'parent's behaviour' the proportion EPP is compared between nests that are cared for by either the male or by the female and those that are biparentally deserted. Separate models were constructed for parental care behaviour (i.e. care or desert) of male, female and both parents. Sample size n (nests) is given between brackets for each care strategy. Predicted effect sizes and standard errors are provided.

a) b)

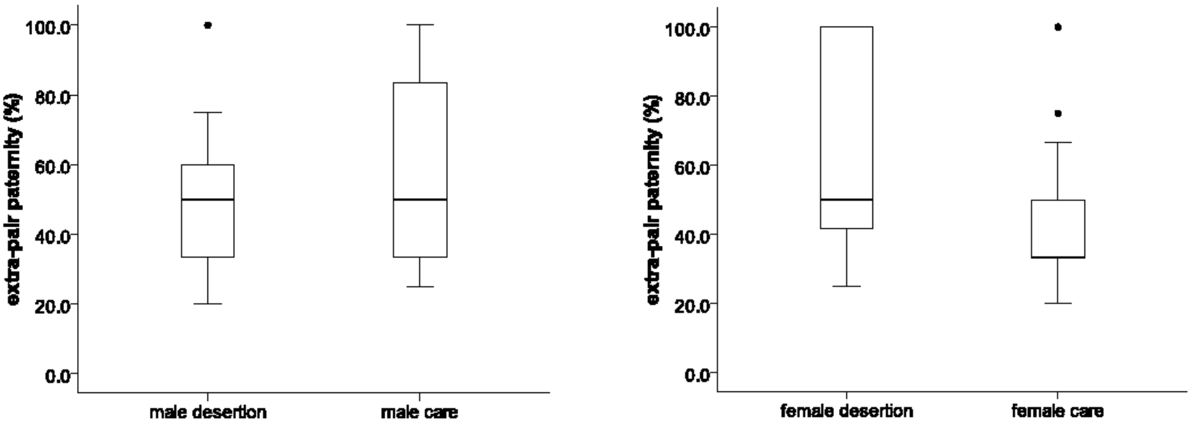


Figure 2. The association between (a) male care strategy and (b) female care strategy and level of extra-pair paternity (*n* = 47 broods). Nests containing 0% EPP are excluded from these figures for graphic purposes. See table 2 for statistics. Box plots show median, inter-quartile range, outliers and extreme cases.

Indirect benefits for the females

Tarsus length and body mass of the offspring were significantly different between years (Table 3). We found no difference in length of the tarsus or in the body mass between young sired by an extra-pair male [15.57mm ± 1.28mm; 8.17g ± 1.81g, (*n* = 63 offspring), respectively] and WPY [15.66mm ± 1.09mm (*n* = 342 offspring); 8.33g ± 1.50g, (*n* = 344 offspring), Table 3], i.e. we found no evidence that extra-pair young grow faster than their within-pair nest mates. Parental care strategy was significantly associated with tarsus length (Table 3), so that young cared for by females had a shorter tarsus than offspring rose by a male.

The number of ten day old nestlings in a nest (brood size) declined over the breeding season and differed significantly between breeding seasons. Parental care strategy predicted brood size, since female-cared nests were larger than the male-cared or biparentally deserted nests (Table 4). Brood size was not associated with the frequency of EPP in a nest (Table 4). Parental care strategy did not significantly contribute ($p > 0.381$) to our LMMs testing for a difference in heterozygosity between offspring sired by extra-pair males and WPY, and was thus excluded from the final models. SIH was significantly different between offspring sired by extra-pair males and WPY, so that WPY were more heterozygous than offspring sired by

a)

tarsus length	*df*	*F*	*P*
year	5	5.394	<0.001
parental care strategy	1	6.974	0.009

b)

paternity	1	0.048	0.826

body mass	*df*	*F*	*P*
year	5	5.725	<0.001
parental care strategy	1	4.529	0.035
paternity	1	0.121	0.728

Table 3. Linear mixed models comparing (a) tarsus length and (b) body mass of offspring sired by extra-pair males versus WPY (*n* = 135 broods).

number of nestlings	*df*	*F*	*P*
year	5	2.745	0.023
parental care strategy	2	33.121	<0.001
mating date	1	11.152	0.001
paternity	1	1.097	0.297

Table 4. General linear model testing for the association of the percentage of extra-pair paternity in a brood with brood size (*n* = 113 broods).

extra-pair males (Table 5). However, none of the other heterozygosity indices were significantly different between the offspring sired by extra-pair males and the WPY. The nine genotyped loci differed significantly in length of both alleles (Kruskal-Wallis; χ_2 = 773.09, $p < 0.001$, n = 5,731 differences in allele length). The standardized mean d^2 did not differ significantly between the offspring sired by extra-pair males and the WPY (Table 5). Male offspring were significantly more heterozygous than female offspring for standardized mean d^2, whereas female offspring had a higher mean and standardized individual heterozygosity (Table 5). None of the sex differences in heterozygosity was significant for the adults (Mann-Whitney U; $p > 0.076$, $n = 211$ adults). Although the sex of the offspring contributed significantly to some of the models in Table 5, it did not qualitatively change the results. None of the heterozygosity indices correlated with the size of the male's mask ($p > 0.235$, n = 55 males).

The mask size, and hence attractiveness, of a male was not associated with the risk of cuckoldry for a male, i.e. the percentage of EPP in its nest (GLM; $F = 0.044$, $p = 0.835$, n = 62 nests).

		df	*F*	*P*	*df*	*F*	*P*
H_o	year	2	1.084	0.350	4	1.342	0.272
	paternity	1	0.372	0.543	1	0.042	0.837
	sex	1	20836	0.095			

mean H	year	2	0.758	0.477	4	1.623	0.185
	paternity	1	1.217	0.272	1	20495	0.117
	sex	1	8.403	0.004			
SIH	year	2	0.162	0.851	4	0.420	0.793
	paternity	1	3.459	0.066	1	5.625	0.019
	sex	1	4.353	0.039			
mean d^2	year	2	0.495	0.614	4	1.289	0.288
	paternity	1	2.939	0.089	1	3.443	0.066
	sex	1	4.422	0.038			
st mean d^2	year	2	1.062	0.357	4	1.880	0.129
	paternity	1	1.142	0.288	1	1.191	0.277
	sex	1	6.894	0.010			

Table 5. Linear mixed models predicting five different indices of heterozygosity of offspring sired by extra-pair males versus WPY, restricted to mixed broods only, including and excluding sex of the offspring as a fixed effect [*n* = 35 broods (119 offspring) and *n* = 45 broods (151 offspring), respectively].
H_o, observed heterozygosity; mean H, mean heterozygosity; SIH, absolute SIH; mean d^2 mean squared difference in allele length per locus as a measure of the evolutionary similarity of alleles; st mean $d^{2,}$ standardized mean d^2 (i.e. the mean d^2 divided by the maximum d^2 for a given locus).

Nest site selection behaviour experiment

During the breeding season in 2005 a total of 42 nests were build in the study area. By the end of the breeding season, after the penduline tits left the area and migrated to their wintering sites, 11 nests remained intact, i.e. were not ripped down after desertion (Figure 3). From the 20 areas included in the experiment in 2006, the penduline tit males started to build a nest at twelve nesting sites (Figure 3). All of these nests were build on a tree containing a 'worn out' nest, making our result highly significant (binomial test, using p = 0.5, p = 0.004). Furthermore nine additional nests were build on trees which were not used as nesting sites in the 2005 breeding season. The experiment in 2007 included a total number of thirteen tree triplets. Ten new nests were built in the thirteen areas, eight on a tree with an old nest and two on trees without a nest. This result (i.e. 8/10) is marginally significant and goes into the direction of the result in 2006 (binomial test, p = 0.109). Of the eight nests built on trees with a nest remnant on it three were built on a tree with a 're-utilize' and five on a tree with a 'worn out' type of old nest. These two frequencies (3/8 and 5/8) are not significantly different (binomial test, $p = 0.727$), which result leads to the hypothesis that it is rather the presence and not the quality of an old nest that serves as cue during nest site selection in penduline tits.

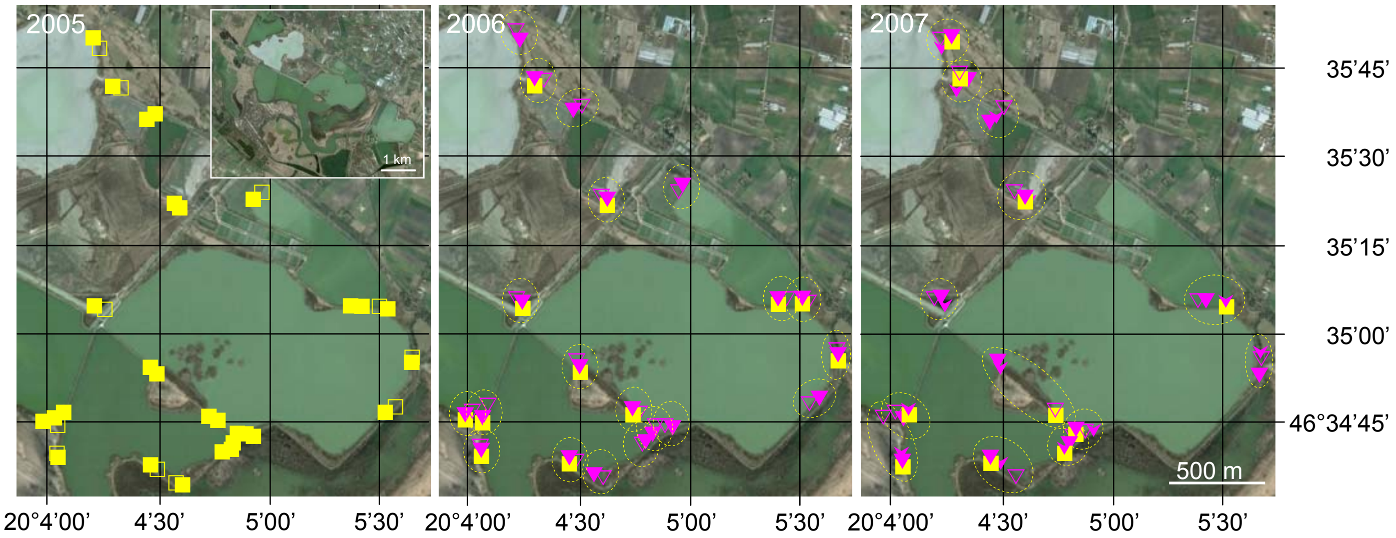


Figure 3. Google-Earth picture of the Csaj fishponds in southern Hungary at around 46°35'N and 20°5'E. The inlet in the 2005 picture shows a bird eye view of the fishponds. The symbols are as follows. ■ nest-sites; position of the nests built in 2005, 2006 and 2007. □ sites of intact nests at the end of the 2005 breeding season. ▼ and ⮟, trees onto which an old, 'worn out' and an old 're-utilize' nest was hung. ▽ tree without an old nest. The dotted lines represent the selected groups of two (in 2006) and three trees (in 2007).

Breeding site fidelity study

The breeding distance of 94 males and 19 females were included in this study. Median number of nests over one breeding season was two for males (range 1-8) and one for females (range 1-5). Since breeding distances were not different for males (median: 116 m, lower-upper quartile: 63-333 m) and females (median: 942 m, lower-upper quartile: 415-2382 m) between years (Kruskal-Wallis tests; $\chi^2 = 1.098$, $n = 94$ males, $p = 0.578$; $\chi^2 = 3.370$, $n = 19$ females, $p = 0.186$), we randomly chose one year for each penduline tit that we included in the analyses. Median breeding distances were skewed towards short distances (Figure 4) and they were significantly shorter for males than for females (Figure 5, Mann-Whitney *U* test, $U = 1313$, $n = 113$, $p < 0.001$). Breeding distances were moderately repeatable for males (Figure 6, $r = 0.563$, $F_{3.91} = 3.296$, $p = 0.024$) and not repeatable for females ($r = 1.095$, $F_{1.14} = 0.249$, $p = 0.626$): males tend to build their consecutive nests at consistent distances, while female's movements are highly variable between their nests.

a) b)

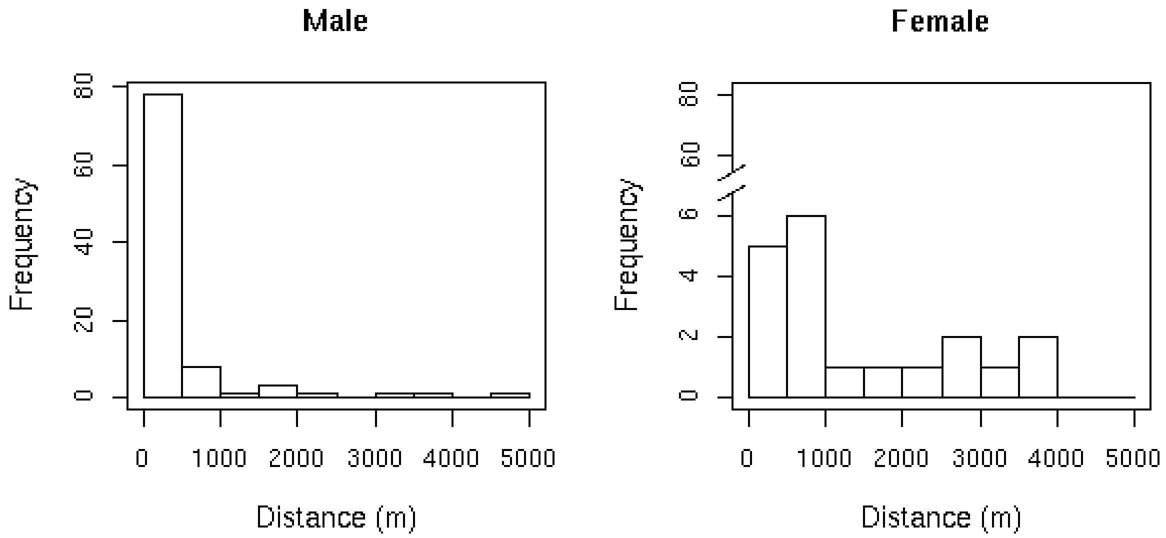


Figure 4. The distribution of median breeding distance of males (a; *n* = 94) and females (b; *n* = 19).

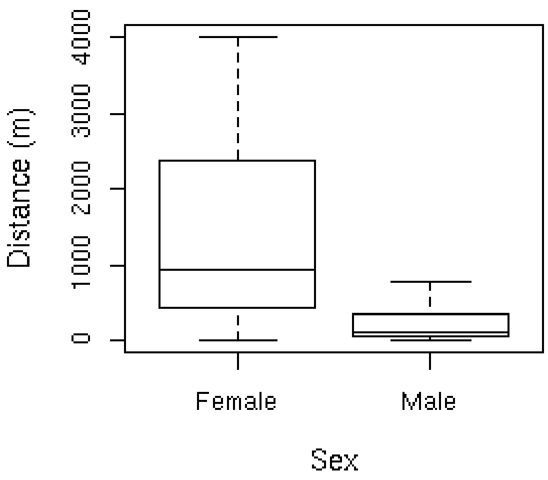


Figure 5. Box-plot of breeding distances randomly chosen for each male and female (*n* = 94 males and 19 females). The *boxes* present median, lower quartile – upper quartile, the *whiskers* stand for minimum and maximum.

a) b)

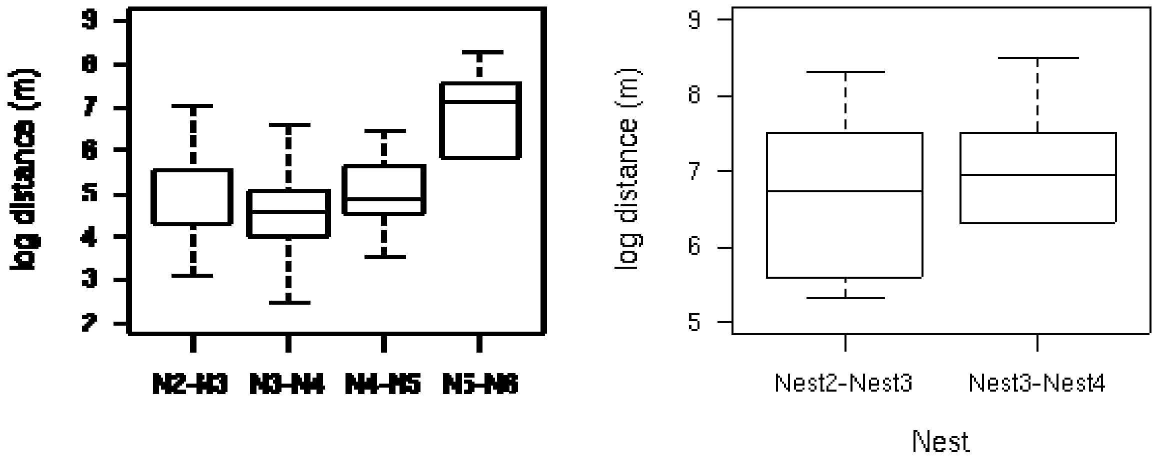


Figure 6. Breeding distances between consecutive nests of males (a) and females (b). N2-N3 refers to the distance between the second and the third nest, and similar logic applies for N3-N4, N4-N5, N5-N6 (*n* = 52 N2-N3 males and 11 females, N3-N4 24 males and 5 females, N4-N5 13 males, N5-N6 6 males).

Discussion

Parentage analyses (rate of cuckoldry in a Hungarian penduline tit population)

Making use of the microsatellite based DNA-fingerprint method we assigned parentage to 443 penduline tit offspring hatched in a Hungarian population over a six years period. As the first step of the microsatellite based DNA-fingerprint method we analyzed nine penduline tit specific polymorphic microsatellite loci. Analyzing the clutches we showed that EPP, quasi parasitism and egg dumping all occur in the Hungarian penduline tit population; latter two arising in low frequencies. In a previous study Schleicher et al. (1997) also found all three types of EPY occurring in penduline tits, although their results for EPP were substantially lower compared to our findings. In the population of Schleicher et al. (1997) 6.9% of the offspring were sired by an extra-male, while in our population the rate of EPP was 23.5%. A possible explanation for this discrepancy is the different genotyping method used in the two studies. Another alternative explanation is that the two populations vary in the frequency of extra-pair copulations, which might be influenced by mate availability, population density and density of vegetation (Davies 1992). Although other studies found little difference in EPP across population despite various densities, predation rates or in potential female benefits (Krokene and Lifjeld 2000, Conrad et al. 2001, Neff et al. 2008), for certainty we have to prove this hypothesis on these two populations.

The decision over parental care in birds is usually made during the egg-laying phase which makes the detection of EPY in the nest harder, may be even impossible. In penduline tits most eggs are without any scatter and the inside of the nest is rather dark (Mészáros LA unpublished observation) which possibly makes it hard for the female/male to recognize an EPY. Another factor, the assessed risk of being cuckolded, more likely influences the decision over parental care (Komdeur et al. 2007, Griffith et al. 2009). A nice example for latter provide inter-specific brood parasite species, like the cuckoo. If parasitic offspring can not be detected the host will care for the young of the cuckoo, but if parasitism has been recognized the host will reject the offspring more likely. Also if the risk of parasitism is too high the host is more likely to reject the offspring (Stokke et al. 2008, Langmore et al. 2009). Similarly, when mate guarding becomes more difficult and therefore the risk of cuckoldry increases male dunnocks (*Prunella modularis*) are less likely to provide paternal care (Davies 1992). In an experimental set up Osorio-Beristain and Drummond (2001) artificially increased the risk of cuckoldry at nests of blue-footed boobies (*Sula nebouxii*) with the result that the males expelled the eggs from their nest. In our study species mate-guarding behaviour is low (Schleicher et al. 1997, van Dijk 2009), so the ability to obtain the risk of being cuckolded might be also low. Nor does it seem likely that the decision of males to care/desert is influenced by the frequency of EPY in the nest since 80-95% of the males desert their offspring (Szentirmai et al. 2007, van Dijk et al., in press). Desertion by the male as a reaction on cuckoldry might be potentially adaptive since male cared clutches are smaller (containing three eggs on average) than female cared nests (females often increase the clutch size with 2-5 additional eggs after male desertion), so if only one EPY occurs in a male cared clutch this mainly influences his reproductive success. The behaviour that males desert females after she has lay about three eggs seems to underlie the theory that the risk to be cuckolded for a male due to too early desertion is low (after the first egg has been laid). A different explanation for male's behaviour could be that males might not be 'forced to' stay longer with their partner to assure paternity, since the amount of sperm a female can store to successfully fertilize the eggs is reached after she had laid the first eggs. Previous studies which suggest that frequent copulations only act effectively as a paternity assurance prior or in the early phases of clutch initiation (Birkhead et al. 1987, Møller et al. 1987, Crowe et al. 2009) give some support for our theory. However we also have to note that sperm rejection may influence the assurance in paternity to a great extent (Peretti and Eberhard 2010).

One more factor that often influences the decision about parental care is the phenotype of the mate. Attractive males may be more successful in gaining extra-pair copulations, thus they do better when investigating in mating rather then in parental effort (Bouwman et al. 2007, Albrecht et al. 2009), whereas less attractive males may gain more through caring (Magrath and Komdeur 2003, Mitchell et al. 2007). From the females perspective females mated with an attractive male may be more likely to care for the brood, whilst females mated with a less attractive male may only care if they can get extra-pair fertilizations. However, in our penduline tit population the risk for a male to be cuckolded was not associated with the attractiveness. We only found evidence that more attractive males are more likely to desert, whilst females are not more likely to care for the broods of these males (van Dijk 2009). Females mated with an attractive male do not gain indirect benefits through extra-pair mating with an attractive male, while females mated with a less attractive male may gain indirect benefits when copulating with a more attractive extra-pair male.

The indirect benefits that a female may gain from extra-pair copulations could not be proven unambiguously during this study. We found no strong evidence that EPY grow faster or survive better from egg to nestling than their WPY nest mates. Furthermore the proportion of EPY in a nest did not predict brood size. For non type of heterozygosity a difference between EPY and WPY existed. This finding also shows that current evidences for the indirect benefits a female might gain from obtaining EPP are not unambiguous. For example, Magrath et al. (2009) recently showed in blue tits that the apparent indirect benefits of extra-pair offspring over WPY disappeared when controlling for hatching order (see also: Arnqvist and Kirkpatrick 2005; Akcay and Roughgarden 2007).

The polygamous nature and the unusual breeding system of the penduline tit make a heritability study highly attractive. However after six years of research we still did not have sufficient data to conduct a reliable heritability analyses. Possible reasons for this lack are the very low returning rates to the same breeding area from one breeding season to the next of both adults and juveniles and that a majority of the birds only have one successful clutch during one breeding season.

On the whole we underlay the polygamous nature of the penduline tit showing with a microsatellite based DNA-fingerprint analyses that all three types of EPY occur in one population. Future studies conducted with more sensitive methods (increased number of microsatellites) and an increased sample size will make a heritability analyses and the search for the genetic father/mother of an EPY possible.

Nest site selection behaviour experiment

Similarly to several other bird species also penduline tits make use of a cue(s) when choosing a nesting site. The presence of an old nest often acts as a cue during nest site selection (Thompson and Neill 1991, Davis et al. 1994, Mazgajski 2007). The barn swallow (*Hirundo rustica*) for example reuses the old nests (Barclay 1988), while the rook (*Corvus frugilegus*) often builds a new nest upon the relict of a previous breeding season (Griffin 1999). Penduline tits never re-use a nest for a second breeding (they destroy abandoned nests and use its nest material for their own nest), but they like to choose nesting sites containing an old nest. To study the nest site selection behaviour of penduline tits we set up two field experiments. In the first year of the field experiment we showed that old nests that lasted throughout the winter seem to act as a cue during nest site selection for penduline tit males entering the study area. In the second years field experiment we aimed to separate the cue hypothesis from the direct benefit hypothesis, using "worn out' and "re-utilize' types of old nests. Our results were consistent with the cue hypothesis which predicts no preference between the two types of old nests. (The direct benefit hypothesis predicts that territories containing a 're-utilize' type of old nest have to be chosen significantly more frequent.) From this result we suggest that rather the presence than the availability of nest material act as a cue for penduline tit males during nest site selection. A possible explanation for our results might be that penduline tit males only have limited time to build a nest and attract mates they save time and increase their chance for a successful breeding by selecting a nesting site already used by someone in previous breeding season. Nesting sites containing a nest remnant might signal a save place against weather conditions (heavy rainfalls, stormy wind can easily destroy a nest) or predators; or they mark a place with a high food abundance. However, to find out more about the nest site selection behaviour of this bird species we would need further studies. A positive and useful outcome of this study is if we look on it from a conservation perspective; the results can be used during settlement of penduline tits on suitable, but so far not used habitats.

Breeding site fidelity study

Looking on a three years dataset we showed that in penduline tits both males and females move between breeding sites. Dispersal is typical during a breeding season and also between years. Latter did not vary between years for neither sex, but was still much higher compared to some closely related passerines, like the blue tit (*Parus caeruleus,* males: 40 m, females: 75 m, Winkel and Frantzen 1991) and the savannah sparrow (*Passerculus sandwichensis,* males: 16.9 m (median distance), females: 31.8 m (median distance), Wheelwright and Mauck 1998).

Within season dispersal of the penduline tit shows the typical avian pattern, i. e. female birds are less faithful to their breeding site than males (Cramp and Simmons 1983). Although males tend to move to shorter distances the penduline tit is still less faithful to its breeding sites than other closely related passerine bird species: the blue tit (median: 0 m) and the great tit (*Parus major,* median: 52 m, Könczey et al. 1997). Of course there are passerine species which show similar low breeding site fidelity like the penduline tit: the grey wagtail (*Motacilla cinerea,* Klemp 2003) and the song sparrow (*Melospiza melodia,* Weatherhead and Boak 1986). The low breeding site fidelity of the penduline tit we found in this study might be a reaction on the changes in the quality of a given breeding territory (food abundance, available nest material) over the breeding season. Another explanation might be that mating success or the quality of local mates varies over time. It is likely that local mating opportunities stay not constant in a given habitat over the whole breeding season. Thus it is more worth for the deserting parent to move further and start a new nest at a site with better re-mating opportunities. A third possibility could be that penduline tits have greater opportunities in choosing good nesting sites compared to closely related other tit species like great tits and blue tits. Latter species are both cavity breeders which influence their availability of nesting sites to a great extent, while penduline tits build a nest which hangs on branches; so good nesting sites are not so limited in this species.

In conclusion, we detected and successfully used nine polymorphic DNA microsatellite markers for genotyping penduline tits. By combining six years field data and DNA fingerprint data we performed a parentage analysis on 443 offspring penduline tits. The main results of the microsatellite work are: (i) the level of EPP in our penduline tit population is moderately high, (ii) the decision over parental care is not predicted by the level of EPY in the nest and (iii) we found no indirect benefits that females may gain from extra-pair copulations. Furthermore we showed in two other studies that penduline tits like to build their nest on nesting sites containing a nest remnant and that female penduline tits show a lower breeding site fidelity than males.

To further deepen our understanding which impacts influence the decision over parental care additional molecular and observational studies are needed. To observe how female behaviour is influenced by the occurrence of EPP during incubation and brood-rearing; and what costs a female might pay through reduced male care owing to EPP during nest building/mating period would bring us closer to what indirect benefits females gain from extra-pair copulations. On the molecular side we need improved methodology such as more loci with higher polymorphism and the introduction of new methods like sequencing techniques. Using an increased number of loci would furthermore give us the opportunity to identify the genetic father of an EPY. Comparing morphological and genetic traits of the genetic and the social father of an EPY would highly approve to our understanding of the unique mating system of the penduline tit.

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Összefoglalás

A függőcinege szaporodási rendszere és csapodársága

Az eurázsiai függőcinege (*Remiz pendulinus*) szaporodási rendszere egyedülálló a madárvilágban. A hím a költési időszak kezdetén kezdi fészkét építeni, miközben folyamatosan kelleti magát. Amennyiben erőfeszítései sikeresek, párba áll egy tojóval, és együtt fejezik be a fészket. Miután a fészek elkészült, és a tojó lerakta tojásait, vagy az egyik, vagy mindkét szülő elhagyja a fészket. A kész fészkek 28-44%-át, bennük a tojásokkal, mindkét szülő sorsára hagyja. A gondozott fészekaljak 45-65%-át a tojó, 7-18%-át a hím látja el. A fészket/párját hűtlenül elhagyó cinege új partner után néz. Egy költési időszakban a hímnek, és a tojónak is akár hat párja is lehet. Vajon mi a szokatlan viselkedés értelme?

A Ph.D. munkám során a DNS-ujjlenyomat egyik módszerével úgy kerestem a választ, hogy a terepi megfigyelések és a DNS szintű vizsgálatok eredményeit kombináltam. A függőcinegék szaporodási viselkedését a Fehér-tó területén végeztük 2002-2007 között. (Itt évente 60-90 hím, és 45-50 tojó él.) A költési időszak során minden másnap körbejártuk a fészkelőhelyeket, és tanulmányoztuk a fészkeket. A madarakat befogtuk, meggyűrűztük, megvizsgáltuk, a szárnyvénájukból vettünk néhány csepp vért, amelyből DNS-t izoláltunk. A fiókákat is meggyűrűztük, és vért is vettünk tőlük.

A DNS-el kapcsolatos munkáim első lépéseként 11 függőcinege-specifikus polimorf mikroszatellit szekvenciát azonosítottunk az Ecogenics GmbH (Zürich) közreműködésével. A polimorf mikroszatellitekkel 47 változatot lehet azonosítani, általuk rokonsági viszonyokat nagy biztonsággal megállapítani. A mikroszatelliteket fluoreszcensen jelölt PCR primerekkel amplifikáltuk. A PCR termékeket gél-elektroforézissel különítettük el, és határoztuk meg méretüket. Az adatok feldolgozása után rokonsági kapcsolatokat állapítottunk meg. Eredményeink 123 hímre, 83 tojóra és 443 fiókra vonatkoznak. Kutatásaink fontosabb eredményei a következők.

- A csapodárság, amint az ma már nagyon sok más faj esetében ismert, a függőcinegékre is jellemző. Megmutattuk, hogy a függőcinegénél a csapodárság mindhárom formája (páron-kívüli apaság, látszólagos parazitizmus, tojás-pottyantás) előfordul.

- Azon 365 fiókából ahol mindkét szülőtől vagy csak a hímtől volt mikroszatellit adatunk, 62-nek volt idegen hím az apja. Abból a 78 fiókából ahol csak a tojó volt ismert 12 fióka vagy látszólagos parazitizmus (idegen tojótól), vagy tojás-pottyantás (mindkét szülő idegen) eredménye volt.
- Azon 166 fiókából, amelynek mindkét szüleje ismert, 50 volt páron kívüli: 39 másik hímtől, 7 idegen tojótól, 4 pedig másik hímtől és tojótól származott.
- 55 fészek esetében tudtuk mindkét szülő genotípusát meghatározni. Az 55 fészek közül 33-ban nevelkedett legalább egy páron-kívüli fióka, mely vagy páron-kívüli apaságból, látszólagos parazitizmusból, vagy tojás-pottyantásból származott. 29 fészekben legalább egy fióka idegen hímtől, 9-ben legalább egy fióka másik tojótól, 4-ben pedig legalább egy tojás-pottyantásból származott.
- Arra a kérdésre, hogy a fészekben lévő páron-kívüli fiókák számából meg lehet-e mondani, hogy melyik szülő fogja gondját viselni az utódoknak, azt mondhatjuk, hogy nem.
- A körültekintően megválasztott költőhely nagyban befolyásolhatja a költés sikerességét. A függőcinegénél két kísérlettel bizonyítottuk, hogy a régi fészek jelenléte hatással van a hímek fészkelőhely választására. Egy további vizsgálattal pedig arra derült fény, hogy a hímek kisebb távolságra építik az egymást követő fészkeiket, mint a tojók.

Arra a kérdésre, hogy mi a függőcinege különös szaporodási rendszerének értelme, még nem tudunk határozott választ adni. Vélekedésünk szerint a nagyfokú polimorfizmus fenntartása. Ám a kérdés megbízható megválaszolásához további vizsgálatokra lenne szükség. Csakúgy, mint azon tényezők jellemzésére, amelyek a csapodárság mértékét, illetve a fészkelőhely megválasztását befolyásolhatják.