Behav Ecol Sociobiol (2007) 61:1725–1733
DOI 10.1007/s00265-007-0404-4

ORIGINAL PAPER

Female tree swallows (*Tachycineta bicolor*) increase offspring heterozygosity through extrapair mating

Mary K. Stapleton · Oddmund Kleven · Jan T. Lifjeld · Raleigh J. Robertson

Received: 17 September 2006 / Revised: 18 March 2007 / Accepted: 24 April 2007 / Published online: 15 May 2007

Abstract Recent attention has focused on genetic compatibility as an adaptive function for why females engage in extrapair mating. We tested the genetic compatibility hypothesis in tree swallows (*Tachycineta bicolor*) over five breeding seasons using data from ten microsatellite loci. Tree swallows are socially monogamous passerines exhibiting high levels of extrapair paternity. Overall, we found that 47% of offspring were the result of extrapair fertilizations, and 83% of females produced at least one extrapair offspring. Consistently for all years, extrapair offspring were more heterozygous than their maternal half-siblings, which is in accordance with the genetic compatibility hypothesis. The difference was largely caused by the high heterozygosity of extrapair offspring sired by unknown males, suggesting that females are engaging in extrapair copulations with geographically distant males to increase the likelihood of being inseminated by a more compatible mate. Our findings support the idea that postcopulatory mechanisms are important for females when assessing potential sires for their offspring.

Keywords Tree swallows · Genetic similarity · Extrapair mating · Extrapair paternity · *Tachycineta bicolor* · Heterozygosity · Genetic compatibility · Genetic diversity · Microsatellites

Communicated by I. Hartley

M. K. Stapleton (✉) · R. J. Robertson
Department of Biology, Queen's University,
Kingston, ON K7L 3N6, Canada
e-mail: marykstapleton@gmail.com

O. Kleven · J. T. Lifjeld
Department of Zoology, Natural History Museum,
University of Oslo,
P.O. Box 1172 Blindern, 0318 Oslo, Norway

Introduction

Extrapair mating (EPM) is a common reproductive tactic in many species and has been intensively studied in passerine birds. Typically, most passerines are socially monogamous, yet the majority of species are genetically promiscuous (reviewed in Griffith et al. 2002). The adaptive function of EPM for males is clear (e.g., the potential to increase the number of young produced in a single breeding season), while the benefit(s) to females are less clear and debated (Trivers 1972; Birkhead 1998; Jennions and Petrie 2000; Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005).

Females in socially monogamous species are often constrained in their choice of social mates (e.g., late arriving females find a smaller pool of unmated males than early arriving females), and EPM has been suggested as a way females can modify their initial mate choice (Møller 1992). One potential benefit of EPM for females is increased offspring viability or fitness through mating with males whose genome best complements their own (reviewed in Tregenza and Wedell 2000). An important element of the genetic compatibility hypothesis (GCH) is that there is no single best male in the population; a male's quality is relative to each female.

Brown (1997, 1998) suggested that genetic compatibility results from increased offspring fitness due to increased heterozygosity, an idea he referred to as the "heterozygosity theory." Several authors refer to this idea as the GCH, a term we will use throughout this paper (reviewed in Tregenza and Wedell 2000). Avoidance of mating with more related individuals can increase the chances of producing more heterozygous offspring, and evidence for increased fitness by choosing dissimilar mates has been found in many taxa, including invertebrates (Zeh 1997;

Tregenza and Wedell 1998; Newcomer et al. 1999), reptiles (Madsen et al. 1992; Olsson et al. 1994), mammals (Coltman et al. 1999; Coulson et al. 1999; Slate et al. 2000; Amos et al. 2001), and birds (Bensch et al. 1994; Tregenza and Wedell 2000; Foerster et al. 2003). For example, Bensch et al. (1994) found that in great reed warblers (*Acrocephalus arundinaceus*) hatching failure was positively correlated with genetic similarity of mates. Genetic similarity between social mates has also been shown to be positively correlated with the presence of extrapair offspring (EPO) in a few bird species (Blomqvist et al. 2002; Freeman-Gallant et al. 2003; Tarvin et al. 2005). Yet, the latter type of studies showing an association between genetic similarity and presence of EPO provides only weak, if any, support to the GCH as long as the extrapair sires are unidentified or no information about potential fitness effects on offspring are presented. A comparison between maternal half siblings provides a powerful tool for examining potential indirect genetic benefits for females through multiple mating. A recent study applying a maternal half sibling comparison on blue tits (*Parus caeruleus*) found that EPO were more heterozygous than withinpair offspring (WPO; Foerster et al. 2003). As heterozygosity is correlated with survival and several measures of reproductive success in this species (Foerster et al. 2003), the findings suggest that female blue tits engage in EPM with genetically more dissimilar mates to increase offspring heterozygosity and thus fitness.

The choice of a compatible mate can occur either before copulation, based on phenotypic cues, or after copulation, based on interactions between sperm and the female reproductive tract (Birkhead and Brillard 2007). Increasingly, evidence is being found in many taxa for the latter mechanism, often referred to as cryptic female choice (reviewed in Eberhard 1996).

Tree swallows (*Tachycineta bicolor*) are, for several reasons, ideal for examining the potential role of genetic compatibility through female mate choice. First, they are socially monogamous and show high levels of extrapair paternity with up to 84% of broods containing at least one EPO, and as many as 69% of all young resulting from extrapair fertilizations (Lifjeld et al. 1993; Dunn et al. 1994a, b; Barber et al. 1996; Conrad et al. 2001; Whittingham and Dunn 2001). Second, female tree swallows have been found to actively select and reject copulations, thus indicating that females of this species are in control of copulations (Lifjeld and Robertson 1992). Third, behavioral evidence indicates that tree swallow populations are outbred (Butler 1988), facilitating an examination of the effects of parental genetic similarity in a species not experiencing high levels of inbreeding.

In this study, we examined mate choice by comparing genetic similarity (a measure of genetic relatedness generated using microsatellite loci) of a female's genetic and social partner. We also investigated the outcome of female mating preferences by comparing heterozygosity of maternal half-siblings.

Materials and methods

Data for this study were collected over five breeding seasons (1997 and 2000–2003) at the Queen's University Biological Station (44°34′N, 76°19′W), Chaffey's Locks, Ontario Canada between April and July each year. Data were collected from tree swallows breeding in nestboxes located on grids and solitary boxes located on fence posts along a road (see Kempenaers et al. 1998 for details of nestbox arrangements). Field methods for 1997 are detailed in Kempenaers et al. (2001). Similar methods were used in 2000–2003.

Field methods

Adult birds were caught in their boxes or using mistnets, usually during the nestling period. They were banded with a uniquely numbered Canadian Wildlife Service (CWS) metal band as well as a single-color band (blue for males and red for females). Adults were sexed by presence of brown plumage (second year female), the presence (female) or absence (male) of a brood patch during incubation and feeding, or wing length (Hussell 1983). Nestlings were fitted with a metal CWS band to enable individual identification. We collected any unhatched eggs or dead nestlings. Blood samples were taken from the brachial vein (~25 to 50 μl) for genetic analyses and stored in Queen's lysis buffer (Seutin et al. 1991).

Genetic analyses

DNA was extracted from blood using a standard salt extraction method (Miller et al. 1988) or GFX Genomic Blood DNA purification Kit (Amersham Biosciences). DNA from other tissue (e.g., brain or liver) was extracted using a standard phenol chloroform method (Sambrook et al. 1989) or DNeasy Tissue Kit (Qiagen).

DNA from individual samples was amplified at 11 microsatellite loci (Table 1). The majority of individuals (81%) were typed at all 11 loci. Genotypes for the 1997 samples at HrU7 ($n=145$ birds) were taken from a previous study (see Kempenaers et al. 2001, for details). Due to different genotyping methods at this locus (manual genotyping using radioactively-labeled primers in 1997 versus automated genotyping on ABI 3100 using fluorescently labeled primers in 2000–2003), sizes at HrU7 were calibrated between the data sets. A conversion factor was

Table 1 PCR protocols used to amplify 11 microsatellite loci in tree swallows (*Tachycineta bicolor*)

Locus	PCR conditions	*A*	No. of adults	H_O	H_E	E_{Pej}	N_e	H-W
HrU7 (Primmer et al. 1995)	94(3 min)-57(60 s)-72(60 s), [94(30 s)-57(30 s)-72(40 s)]×23, 72(5 min)	8	213	0.74	0.74	0.52	0.000	NS
Tbi81 (Stenzler 2001)	94(3 min), [94(30 s)-60-(30 s)-72(45 s)]×28, 72(5 min)	14	226	0.90	0.89	0.78	0.000	NA
Pdoμ5 (Griffith et al. 1999)	94(3 min), [94(30 s)-*x*(45 s)-72(45 s)]×20, [94(30 s)-45(45 s),72(45 s)]×15, 72(5 min)[a]	7	225	0.81	0.79	0.60	0.000	NS
Pocc6 (Bensch et al. 1997)	94(5 min), [94(45 s)-55-(45 s)-72(60 s)]×27, 72(10 min)	6	222	0.69	0.71	0.46	0.011	NS
Pca3 (Dawson et al. 2000)	94(3 min), [94(30 s)-*x*(45 s)-72(45 s)]×8, [94(30 s)-48(45 s)-72(45 s)]×72(5 min)[b]	13	225	0.67	0.69	0.51	0.019	NS
Tbi104 (Stenzler 2001)	94(5 min), [94(30 s)-60(30 s)-72(45 s)]×32, 72(5 min)	13	224	0.73	0.75	0.58	0.019	NS
IbiMP5-29 (Crossman 1996)	94(5 min), [94(30 s)-60(30 s)-72°C(40 s)]×27, 72(5 min)	6	220	0.67	0.65	0.37	0.000	NS
Pat MP 43 (Otter et al. 1998)	94(3 min), [94(30 s)-50(30 s)-72(40 s)]×32, 72(5 min)	3	229	0.10	0.10	0.05	0.000	NA
Ase29 (Richardson et al. 2000)	94(3 min), [94(30 s)-*x*(45 s)-72(45 s)]×20, [94(30 s)-45(45 s),72(45 s)]×15, 72(5 min)[a]	15	216	0.81	0.81	0.64	0.002	NS
Ase55 (Richardson et al. 2000)	94(3 min), [94(30 s)-*x*(45 s)-72(45 s)]×20, [94(30 s)-45(45 s),72(45 s)]×17, 72(5 min)[a]	3	228	0.29	0.30	0.13	0.011	NS
Ltmr6 (McDonald and Potts 1994)	94(5 min), [94(45 s)-56(45 s)-72(60 s)]×28, 72(10 min)	13	203	0.70	0.76	0.56	0.041	*

Data on the number of alleles (*A*), number of adults genotyped, observed heterozygosity (H_O), expected heterozygosity (H_E), probability of exclusion assuming one parent known (P_{ej}), and estimated frequency of null-alleles (N_e). Deviations from Hardy Weinberg equilibrium (*H-W*) were tested, and *NS* denotes not significant and *NA* not applicable. * denotes a significant deviation

[a] x=55–45°C; decrease 1°C/2 cycles

[b] x=60–51°C; decrease 3°C/2 cycles

determined by comparing the allelic distributions and frequencies between the two data sets. All samples from 2000–2003 were genotyped using the following conditions. Polymerase chain reactions (PCR) were performed in 10 μl volumes using 50–100 ng of genomic DNA, 0.5 mM forward and reverse primers, 10 mM Tris–HCl, 1.5 mM $MgCl_2$, 0.6 mM deoxyribonucleotide triphosphate (dNTP) and 0.6 units polymerase. Details on PCR cycling protocols are listed in Table 1.

All individuals typed at five or fewer loci (3.4%) were excluded from analyses (except a single female who was genotyped at only three loci that was included in our data set for purposes of assigning parentage). Probability of exclusion (the probability of correctly excluding a false sire) for all 11 loci was 0.999 with one parent (mother) known (Jamieson 1994).

Parentage analysis

We had DNA samples from both the social male and female, and a minimum of one offspring (mean 5.1±1.2 SD offspring per brood) for 99 first nests in 5 years combined. Nests from 1997 (n=19) were all from a single grid (HU) and had been used in a previous study where the extrapair sires had been identified (see Kempenaers et al. 2001). All males caught in each year were genotyped in an attempt to assign paternity to EPO (n=143 unique males in all years combined). When all years were combined, we had family genotypic data on 14 females that bred in two or more years, as well as a single female for which we had genotypic data for her original brood, plus her replacement brood after being depredated. Twelve of the fourteen females that bred in repeated years chose a new social mate every season; therefore, each breeding attempt by these 12 females was included in all the analyses except for analyses between female heterozygosity and hatching and fledging success of nestlings. In this case, only the female's first breeding record was included to avoid pseudoreplication. Similarly, for the two cases where a female remained with the same male in repeated years, as well as for the female where we had her original clutch as well as her replacement clutch, only data from the first nest were included in analyses to avoid pseudoreplication.

Nestling genotypes were first compared with the social mother (attendant female at nest). Previous studies on this population (Dunn and Robertson 1993; Lifjeld et al. 1993; Dunn et al. 1994a; Kempenaers et al. 1999, 2001) have revealed extremely low levels (0 to 0.01%) of intraspecific brood parasitism. As expected, the majority of nestlings in this study shared an allele at all loci genotyped with their social mother. There were four nestlings that mismatched their social mother at a single locus but were considered to be the biological offspring of the social female as the mismatches were assumed to be the result of mutations or genotyping errors (see Fernando et al. 2001). Two other nestlings were considered the result of intraspecific brood parasitism, i.e., a single nestling mismatched the mother at two loci and a second nestling in the same nest mismatched the mother at one locus. The nestling that only mismatched at one locus was genotyped at an additional four loci and found it mismatched at a total of 4 out of 15 loci (see Kempenaers et al. 2001). Both nestlings were excluded from further analyses.

Genotypes of the remaining nestlings ($n=502$) were then compared to that of their social father. Nestlings that matched the social male at all loci typed were considered WPO. If a nestling mismatched the social male at a single locus, they were also considered WPO, and the mismatches were assumed to be due to mutations or typing errors (see Fernando et al. 2001). The probability of chance inclusion (the probability that a randomly chosen male would share the same genotype of the nestling) for nestlings that mismatched at a single locus was calculated as the product of $\left(2p_i - p_i^2\right)$ where p_i is the frequency of the paternally transmitted allele at locus i (Jeffreys et al. 1992). These values ranged from 2.1×10^{-11} to 5.0×10^{-5} (mean±SD= $7.99\times10^{-6}\pm1.76\times10^{-5}$, $n=11$ nestlings).

A nestling was considered to be an EPO if it mismatched the social male at two or more loci that could not be explained by the presence of null alleles [i.e., the nestling mismatched at greater than two loci at which either the social male or offspring (or both) were heterozygous]. Extrapair sires were assigned by comparing the paternal genotypes of all EPO to the genotypes of all males sampled using NEWPAT (available at http://www.zoo.cam.ac.uk/zoostaff/amos/newpat.htm) and described in Worthington Wilmer et al. (1999). Because not all individuals were genotyped at all loci, we were conservative in our assignment of extrapair sires, and we required there be no more than four untyped loci in either candidate male, candidate female, or nestling. A male was assigned as a sire if he was the only male that matched the nestling at all loci typed ($n=109$ nestlings). There were eight cases in which NEWPAT returned two potential sires. We examined these on a case by case basis to assign a sire. We assigned paternity to one of the candidate males if he sired other young in the nest ($n=4$ nestlings) or if one of the candidate males was captured in the year the nestling hatched and the other candidate male was not captured that year ($n=3$ nestlings). For one nestling, there were two candidate sires, but one was scored at ten loci and one was scored at seven loci, and in this case, we used the assignment with the most scored loci. There were two cases in which NEWPAT returned 4 and 12 candidate males. In both of these cases, the nestlings were genotyped at only seven loci, thus reducing our ability to discriminate amongst the candidate males. Therefore, we did not assign sires to these two young. The probability of chance inclusion for nestlings that had been assigned an extrapair sire ranged from 9.9×10^{-9} to 9.2×10^{-3} (mean±SD=$2.8\times10^{-5}\pm0.001$, $n=117$ nestlings).

Heterozygosity and genetic similarity

A standardized measure of heterozygosity (hereafter referred to as heterozygosity) was used as not all individuals were typed at all loci. Standardized heterozygosity takes into account the population level of heterozygosity and is calculated as the proportion of heterozygous loci in an individual divided by the observed mean heterozygosity of typed loci (Coltman et al. 1999). Relatedness (r), hereafter referred to as genetic similarity, was calculated using the software program Relatedness 5.0.8 (Queller and Goodnight 1989, http://www.gsoftnet.us/gsoft.html). Standard errors were obtained by jackknifing over loci for average genetic similarity values. Average genetic similarity among all breeding adults in the population was −0.0045±0.0001 (expected value is 0).

A single locus (Ltmr6) was excluded from the heterozygosity and genetic similarity analyses due to an estimated high frequency (0.041) of nonamplifying (null) alleles (Table 1). When paternal genotypes are unknown (i.e., EPO with no assigned sire), null alleles are less likely to be detected, and hence might bias estimates of both heterozygosity and genetic similarity. A paired comparison of offspring heterozygosity in relation to paternal assignment (i.e., offspring with known and unknown sires) with and without Ltmr6 revealed a significant change in heterozygosity for young with unknown sires, while not for young with known sires (data not shown). The change in heterozygosity for extrapair young with an unknown sire was in the predicted direction, with an increase in heterozygosity when excluding Ltmr6.

Statistics

Statistical analyses were performed in JMP IN 4.0.3 (SAS Institute 2000), SPSS 12 (SPSS 2003), and STATISTICA 6.1 (StatSoft 2003). All probabilities reported for t tests and paired t tests are two-tailed, and the assumed level of significance for all tests was $p<0.05$. Confidence intervals (95%) are given for the main statistical tests (Colgrave and Ruxton 2003). ‘Mate combination’ is defined as each unique pair of male and female birds that produced young and is used when examining the correlation between offspring heterozygosity and pairwise genetic similarity estimates of genetic parents. Average values of extrapair males were obtained in broods with more than a single identified extrapair sire. Heterozygosity of WPO and EPO was analyzed with a linear mixed model [restricted maximum likelihood (REML) estimation], and brood was used as a random effect to control for maternal genotype. We used residuals of the regressions of Julian first egg date on hatching and fledging success to control for potential seasonal effects when examining correlations between female heterozygosity and offspring survival.

Results

In total, 47% (237/502) of offspring were the result of extrapair copulations and 83% (82/99) of broods contained at least one EPO. We assigned paternity to 49% (117/237) of the EPO, and at least one extrapair mate was identified for 68% (56/82) of females that produced EPO. In the latter cases, we identified a single extrapair sire in 79% (44/56) of the broods, two extrapair sires in 20% (11/56) of the broods, and three extrapair sires in 2% of the broods (1/56).

Presence of EPO was not predicted by the genetic similarity of social pair mates (GLM; $\chi=0.3$, $p=0.59$, $n=98$ pairs). Social mates did not differ from extrapair mates in genetic similarity with females (paired *t* test; $t_{1,55}=0.79$, $p=0.43$, $n=56$ dyads). Pairwise genetic similarity estimates of genetic parents was negatively correlated with offspring heterozygosity (calculated as the mean heterozygosity of all nestlings produced for each mate combination; Pearson correlation; $r=-0.53$, $p<0.001$, $n=156$ mate combinations). EPO were, however, significantly more heterozygous than WPO in mixed paternity broods (REML estimation; $F_{1,352}=6.5$, $p=0.013$ $n=70$ broods; Fig. 1). The average difference in heterozygosity between maternal half-siblings (EPO minus WPO) was consistent and positive for all 5 years (Fig. 2). Hence, in the following analyses of offspring heterozygosity, we have excluded 'year' as a factor. In broods that contained at least one identified extrapair sire, there was no significant difference in levels of heterozygosity between EPO (sired by identified males) and WPO (REML estimation; $F_{1,213}=1.5$, $p=0.23$, $n=47$ dyads; Fig. 3). In broods with at least one unknown extrapair sire, EPO sired by unknown males were significantly more heterozygous than WPO (REML estimation; $F_{1,172}=5.9$, $p=0.017$, $n=40$ dyads; Fig. 3). In broods that contained EPO sired by both known and unknown extrapair sires, the EPO with unknown extrapair sires were significantly more heterozygous than their maternal half siblings with known extrapair sires (REML; $F_{1,82}=4.8$, $p=0.026$, $n=24$ dyads).

A potential benefit of increased offspring heterozygosity is based on the assumption that heterozygosity is somehow correlated with fitness. Female levels of heterozygosity were not correlated with hatching or fledging success (Spearman's rho; $r_s=-0.03$, $p>0.79$, $n=79$; $r_s=-0.12$, $p>0.38$, $n=57$, respectively). In nests with mixed fledging success, mean offspring heterozygosity did not differ between nestlings that fledged and those that did not fledge (paired *t* test, $t_{1,23}=-1.61$, $p=0.12$, $n=24$ broods).

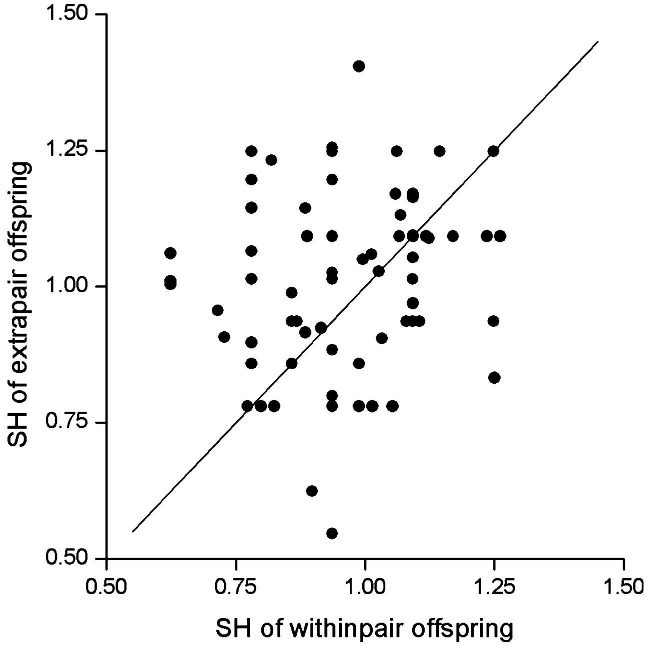

Fig. 1 Pairwise comparison of heterozygosity (SH) between maternal half-siblings. The *dots* represent mean values of withinpair (WPO) and extrapair offspring (EPO) in mixed paternity broods ($n=70$ dyads). An *isoline* indicates identical heterozygosity values

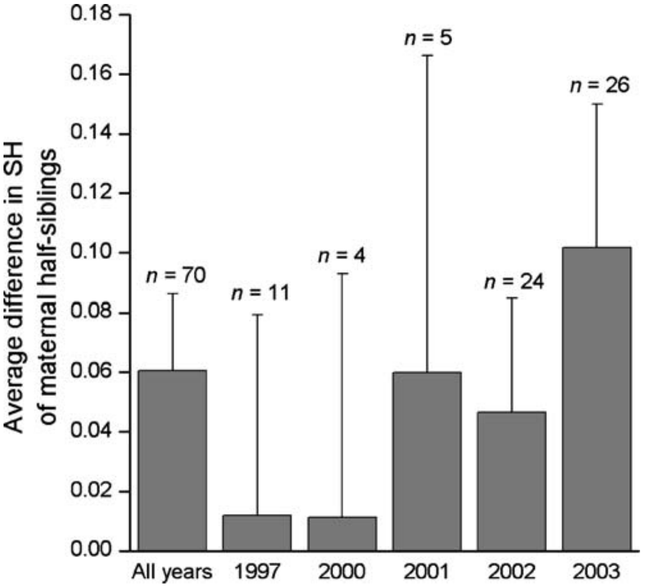

Fig. 2 Average difference in heterozygosity (SH) of maternal half-siblings (SH for brood means of extrapair offspring minus SH for brood means of withinpair offspring) among years. *Vertical lines* indicate +1 standard error and *figures* denote number of broods analysed for each year

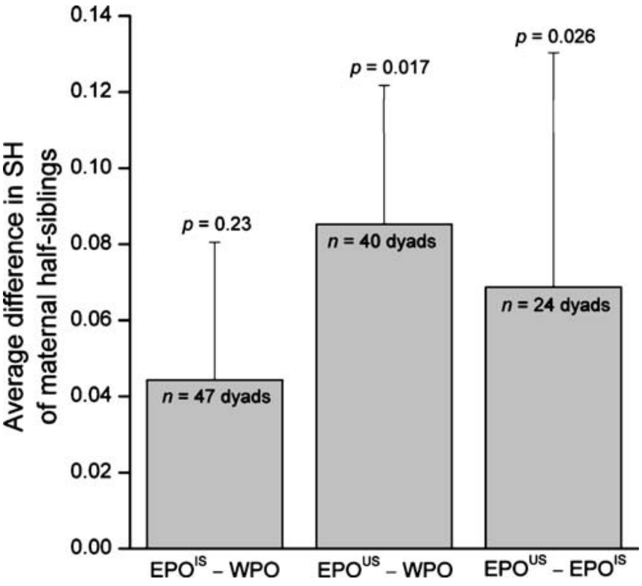

Fig. 3 The effects of extrapair sire identity on offspring heterozygosity. Shown are average differences (+1 standard error) in heterozygosity (SH) between maternal half siblings in mixed paternity broods in relation to identity of extrapair sire(s). EPO^{IS} denotes extrapair offspring with identified sires, EPO^{US} denotes extrapair offspring with unidentified sires, and *WPO* denotes withinpair offspring

Discussion

In our population of tree swallows EPO had higher levels of heterozygosity than their withinpair maternal half-siblings, suggesting a genetic benefit to females engaging in EPM in this species. This result is particularly exciting as no clear benefit to female tree swallows engaging in EPM has previously been found, despite intensive study (Dunn et al. 1994a; Kempenaers et al. 1999, 2001; Whittingham and Dunn 2001; Barber et al. 2005; Whittingham et al. 2006).

A more detailed analysis of our main finding suggests that the higher levels of heterozygosity in EPO are being driven by fertilizations with unidentified sires. The unknown extrapair sires are likely to be birds either breeding in natural cavities or floaters (Kempenaers et al. 2001). These males are presumably more geographically distant (i.e., nonlocal) individuals as we were able to sample the majority of the males breeding in our box nesting population. Genetic structuring (i.e., if females are less related to geographically more distant breeding males) could explain why EPO from unknown (presumably more distant) males are more heterozygous in our population. For example, in a study of blue tits, Foerster et al. (2003) found that females were less related to males that were breeding further away geographically, and their finding of increased heterozygosity of EPO was mainly due to higher heterozygosity of offspring from these nonneighboring males. Unfortunately, our data were unsuitable to test for a potential genetic structuring, as we only have genetic data from local individuals.

Presently, it is unclear why females mate with neighboring males if EPO from unknown (presumably more geographically distant) males have greater levels of heterozygosity. One explanation might be that some of these local males are able to coerce females to copulate (Westneat and Stewart 2003). In tree swallows, however, this seems unlikely as available evidence suggests females control copulations (Lifjeld and Robertson 1992; Venier et al. 1993; Dunn and Whittingham 2007). A recent study by Dunn and Whittingham (2007) found that females that had been artificially handicapped through the clipping of flight feathers had the same level of extrapair fertilization as control females. Handicapped females, however, were more likely to have EPO sired by nearby males than were control females. The authors suggested that the change in the spatial distribution, but not the level of EPM, was due to female choice and not male coercion. Several authors have suggested both traditional 'good genes' mechanisms and 'genetic compatibility' mechanisms could be operating within a population simultaneously (Foerster et al. 2003; Mays and Hill 2004; Neff and Pitcher 2005; Tarvin et al. 2005). However, there is presently no evidence suggesting that females are obtaining 'good genes' benefits in tree swallows (Lifjeld et al. 1993; Dunn et al. 1994a; Kempenaers et al. 1999; Whittingham and Dunn 2001). Competition among sperm from different males within the female's reproductive tract must also be considered (Birkhead and Møller 1998). However, it is difficult to differentiate between female (cryptic female choice) and male (sperm competition) influence of sperm utilization (Simmons et al. 1996). Alternatively, some female tree swallows may be more restricted in their EPM opportunities than others, as recent telemetry studies in this species have revealed variation in individual female movement patterns before egg laying (Dunn and Whittingham 2005; Stapleton and Robertson 2006). Presently, very little is known about actual mating behavior away from the immediate nest site area in tree swallows, and this aspect of tree swallow breeding ecology warrants further attention.

Our finding of support for the GCH in tree swallows is in contrast to a recent study by Barber et al. (2005) in this same population of tree swallows (albeit in different years and based on different molecular methods, i.e., microsatellite loci versus DNA fingerprinting), which found no evidence for the GCH. Both studies failed to find a relationship between genetic similarity of social pair mates and presence of EPO. Why, then, do our conclusions about the role of the GCH in this population of tree swallows differ? The main reason is that in our study, in addition to examining correlations between pairwise genetic similarity values and presence of EPO, we also examined the effect of

EPM on offspring heterozygosity. This is a more direct test of the GCH, and in our study, EPO were significantly more heterozygous than WPO. Most importantly, this significant result was mainly driven by offspring sired by unidentified males, a category of sires not dealt with in the study by Barber et al. (2005).

While the fitness benefits of increased heterozygosity have been demonstrated in other species (Hansson and Westerberg 2002; Keller and Waller 2002; Foerster et al. 2003), we found no significant heterozygosity–fitness correlations in our study population. However, we had few fitness-related variables available for testing. Benefits of increased heterozygosity may well manifest postfledging in the form of increased recruitment (Hansson et al. 2001), for which we have no data, or in other parameters of reproductive success not studied by us.

Some recent papers (e.g., Slate et al. 2004 and Balloux et al. 2004) have shown that the correlation between marker heterozygosity and the inbreeding coefficient is not very strong when few markers are used in outbred populations. We are therefore not able to tell whether our results reflect general inbreeding avoidance in EPM or whether there is a preference for extrapair mates that are dissimilar at one or a few target loci being in linkage disequilibrium with our microsatellite markers.

How is mate compatibility assessed? Premating compatibility is likely best assessed using chemical signals (reviewed in Penn 2002). Birds, however, are generally thought to have weak olfaction abilities (Jones and Roper 1997), and tree swallows in particular seem to have poorer olfaction abilities than other passerines (Clark 1991). Zeh and Zeh (1996, 1997) have suggested that interactions between a female's immune system and a male's sperm could provide an indicator of postfertilization complimentarity. A recent review by Mays and Hill (2004) points out that for some species, premating genotype assessment may be constrained and acknowledges that cryptic female choice may be the only mechanism through which compatibility can be assessed. It is possible that female tree swallows mate with several extrapair males to increase the likelihood of obtaining fertilizations with a genetically more compatible sire, and a postcopulatory sperm selection mechanism (i.e., cryptic choice) could be involved (Eberhard 1996). Many female tree swallows have multiple extrapair sires per brood (Lifjeld et al. 1993; Kempenaers et al. 1999; Whittingham et al. 2006) indicating they have chosen multiple extrapair partners. However, while it has been shown that the number of sires and proportion of EPO are repeatable for female tree swallows, female choice of particular extrapair sires is not (Whittingham et al. 2006).

In this study, we have documented a potential female genetic benefit of EPM in the highly promiscuous tree swallow, as EPO were more heterozygous compared with their maternal half-siblings. This finding, combined with the observation of multiple sires per brood and a lack of evidence for phenotypic cues in extrapair mate preferences in previous studies of this species (Dunn et al. 1994a; Kempenaers et al. 1999, 2001), lends support to the idea that postcopulatory mechanisms may play an important role in female extrapair mate choice in tree swallows.

Acknowledgement We thank T. Burg, B. Campbell, F. Fossøy, R. Vallender, F. Jacobsen, A. Johnsen, L.E. Johannessen, R. Montgomerie, and L. Ratcliffe for helpful comments on this manuscript. Bill Amos kindly provided modified macros for our use in calculation of standardized heterozygosity, and L. Stenzler provided unpublished microsatellite primer sequences (Tbi81 and Tbi104). C. Scott provided helpful advice in the laboratory, and C. Dale and S. Harper provided excellent assistance with fieldwork. The facilities and logistical support of the Queen's University Biological Station contributed to this study. Banding was conducted under Canadian Wildlife Service permit 10302, and all methods in this study were approved by the Queen's University Animal Care Committee under permit no. RobertsRJ-040. Funding was provided through grants from the American Ornithologists' Union (MKS), the Society for Canadian Ornithologists (MKS), Queen's University (MKS), and an NSERC Discovery grant (RJR) and an NSERC Major Facilities Grant (to QUBS). The experiments comply with the current laws of the country in which they were performed.

References

Amos W, Wilmer JW, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T (2001) The influence of parental relatedness on reproductive success. Proc Biol Sci 268:2021–2027

Arnqvist G, Kirkpatrick M (2005) The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. Am Nat 165:S26–S37

Balloux F, Amos W, Coulson T (2004) Does heterozygosity estimate inbreeding in real populations? Mol Ecol 13:3021–3031

Barber CA, Robertson RJ, Boag PT (1996) The high frequency of extra-pair paternity in tree swallows is not an artifact of nestboxes. Behav Ecol Sociobiol 38:425–430

Barber CA, Edwards MJ, Robertson RJ (2005) A test of the genetic compatibility hypothesis with tree swallows, *Tachycineta bicolor*. Can J Zool 83:955–961

Bensch S, Hasselquist D, Von Schantz T (1994) Genetic similarity between parents predicts hatching failure: nonincestuous inbreeding in the great reed warbler? Evolution 48:317–326

Bensch S, Price T, Kohn J (1997) Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. Mol Ecol 6:91–92

Birkhead TR (1998) Sperm competition in birds: mechanisms and function. In: Birkhead TR, Møller AP (eds) Sperm competition and sexual selection. Cambridge University Press, Cambridge

Birkhead TR, Brillard JP (2007) Reproductive isolation in birds: postcopulatory prezygotic barriers. Trends Ecol Evol 22:266–272

Birkhead TR, Møller AP (1998) Sperm competition and sexual selection. Academic, San Diego, California

Blomqvist D, Andersson M, Kuepper C, Cuthill IC, Kis J, Lanctot RB, Sandercock BK, Szekely T, Wallander J, Kempenaers B (2002) Genetic similarity between mates and extra-pair parentage in three species of shorebirds. Nature (Lond) 419:613–615

Brown JL (1997) A theory of mate choice based on heterozygosity. Behav Ecol 8:60–65

Brown JL (1998) The new heterozygosity theory of mate choice and the MHC. Genetica (Dordr) 104:215–221

Butler RW (1988) Population dynamics and migration routes of tree swallows *Tachycineta bicolor* in North America. J Field Ornithol 59:395–402

Clark L (1991) Odor detection thresholds in tree swallows and cedar waxwings. Auk 108:177–180

Colgrave N, Ruxton GD (2003) Confidence intervals are a more useful complement to nonsignificant tests than are power calculations. Behav Ecol 14:446–447

Coltman DW, Pilkington JG, Smith JA, Pemberton JM (1999) Parasite-mediated selection against inbred Soay sheep in a free-living, island population. Evolution 53:1259–1267

Conrad KF, Johnston PV, Crossman C, Kempenaers B, Robertson RJ, Wheelwright NT, Boag PT (2001) High levels of extra-pair paternity in an isolated, low-density, island population of tree swallows (*Tachycineta bicolor*). Mol Ecol 10:1301–1308

Coulson T, Albon S, Slate J, Pemberton J (1999) Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. Evolution 53:1951–1960

Crossman C (1996) Single-locus DNA profiling in the tree swallow, *Tachycineta bicolor*: a comparison of methods. MSc, Queen's University, Kingston

Dawson DA, Hanotte O, Greig C, Stewart IRK, Burke T (2000) Polymorphic microsatellites in the blue tit *Parus caeruleus* and their cross-species utility in 20 songbird families. Mol Ecol 9:1941–1944

Dunn PO, Robertson RJ (1993) Extra-pair paternity in polygynous tree swallows. Anim Behav 45:231–239

Dunn PO, Whittingham LA (2005) Radio-tracking of female tree swallows prior to egg-laying. J Field Ornithol 76:259–263

Dunn PO, Whittingham LA (2007) Search costs influence the spatial distribution, but not the level, of extra-pair mating in tree swallows. Behav Ecol Sociobiol 61:449–454

Dunn PO, Robertson RJ, Michaud-Freeman D, Boag PT (1994a) Extra-pair paternity in tree swallows: why do females mate with more than one male? Behav Ecol Sociobiol 35:273–281

Dunn PO, Whittingham LA, Lifjeld JT, Robertson RJ, Boag PT (1994b) Effects of breeding density, synchrony, and experience on extrapair paternity in tree swallows. Behav Ecol 5:123–129

Eberhard WG (1996) Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, New Jersey

Fernando P, Evans BJ, Morales JC, Melnick DJ (2001) Electrophoresis artifacts—a previously unrecognized cause of error in microsatellite analysis. Mol Ecol Notes 1:325–328

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. Nature (Lond) 425:714–717

Freeman-Gallant CR, Meguerdichian M, Wheelwright NT, Sollecito SV (2003) Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. Mol Ecol 12:3077–3083

Griffith SC, Stewart IRK, Dawson DA, Owens IPF, 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

Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol 11:2195–2212

Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness in natural populations. Mol Ecol 11:2467–2474

Hansson B, Bensch S, Hasselquist D, Akesson M (2001) Microsatellite diversity predicts recruitment of sibling great reed warblers. Proc Biol Sci 268:1287–1291

Hussell DJT (1983) Age and plumage color in female tree swallows. J Field Ornithol 54:312–318

Jamieson A (1994) The effectiveness of using co-dominant polymorphic allelic series for (1) checking pedigrees and (2) distinguishing full-sib pair members. Anim Genet 25:37–44

Jeffreys AJ, Allen MJ, Hagelberg E, Sonnberg A (1992) Identification of the skeletal remains of Josef Mengele by DNA analysis. Forensic Sci Int 56:65–76

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol Rev (Camb) 75:21–64

Jones RB, Roper TJ (1997) Olfaction in the domestic fowl: a critical review. Phys and Behav 62:1009–1018

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230–241

Kempenaers B, Lanctot RB, Robertson RJ (1998) Certainty of paternity and paternal investment in eastern bluebirds and tree swallows. Anim Behav 55:845–860

Kempenaers B, Congdon B, Boag P, Robertson RJ (1999) Extrapair paternity and egg hatchability in tree swallows: evidence for the genetic compatibility hypothesis? Behav Ecol 10:304–311

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

Lifjeld JT, Robertson RJ (1992) Female control of extra-pair fertilization in tree swallows. Behav Ecol Sociobiol 31:89–96

Lifjeld JT, Dunn PO, Robertson RJ, Boag PT (1993) Extra-pair paternity in monogamous tree swallows. Anim Behav 45:213–229

Madsen T, Shine R, Loman J, Hakansson T (1992) Why do female adders copulate so frequently? Nature (Lond) 355:440–441

Mays HL Jr, Hill G (2004) Choosing mates: good genes versus genes that are a good fit. Trends Ecol Evol 19:554–559

McDonald DB, Potts WK (1994) Cooperative display and relatedness among males in a lek-mating bird. Science (Wash DC) 266:1030–1032

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16

Møller AP (1992) Frequency of female copulations with multiple males and sexual selection. Am Nat 139:1089–1101

Neff BD, Pitcher TE (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Mol Ecol 14:19–38

Newcomer SD, Zeh JA, Zeh DW (1999) Genetic benefits enhance the reproductive success of polyandrous females. Proc Natl Acad Sci USA 96:10236–10241

Olsson M, Gullberg A, Tegelstrom H (1994) Sperm competition in the sand lizard, *Lacerta agilis*. Anim Behav 48:193–200

Otter K, Ratcliffe L, Michaud D, Boag PT (1998) Do female black-capped chickadees prefer high-ranking males as extra-pair partners? Behav Ecol Sociobiol 43:25–36

Penn DJ (2002) The scent of genetic compatibility: sexual selection and the major histocompatibility complex. Ethology 108:1–21

Primmer CR, Møller AP, Ellegren H (1995) Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica*. Mol Ecol 4:493–498

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

Richardson DS, Jury FL, Dawson DA, Salgueiro P, Komdeur J, Burke T (2000) Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. Mol Ecol 9:2225–2229

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

SAS Institute (2000), JMPIN, 4.0.3. SAS Institute, Minneapolis, Minnesota

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Can J Zool 69:82–90

Simmons LW, Stockley P, Jackson RL, Parker GA (1996) Sperm competition or sperm selection: no evidence for female influence over paternity in yellow dung flies *Scatophaga stercoraria*. Behav Ecol Sociobiol 38:199–206

Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH (2000) Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). Proc R Soc B 267:1657–1662

Slate J, David P, Dodds KG, Veenvliet BA, Glass BC, Broad TE, McEwan JC (2004) Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. Heredity 93:255–265

SPSS (2003), SPSS for Windows, Release 12. SPSS, Chicago

Stapleton M, Robertson RJ (2006) Female tree swallow home range movements during their fertile period revealed by radio-tracking. Wilson J Ornithol 118:502–507

StatSoft (2003), STATISTICA, 6th edition, StatSoft

Stenzler LM (2001) Genetic population structure in two bird species with contrasting dispersal behavior: the tree swallow (*Tachycineta bicolor*) and the Florida scrub-jab (*Aphelocoma coerulescens*). MSc Thesis, Cornell University, Ithaca, New York

Tarvin KA, Webster MS, Tuttle EM, Pruett-Jones S (2005) Genetic similarity of social mates predicts the level of extrapair paternity in splendid fairy-wrens. Anim Behav 70:945–955

Tregenza T, Wedell N (1998) Benefits of multiple mates in the cricket, *Gryllus bimaculatus*. Evolution 52:1726–1730

Tregenza T, Wedell N (2000) Genetic compatibility, mate choice and patterns of parentage: invited review. Mol Ecol 9:1013–1027

Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) Sexual selection and the descent of man 1871–1971. Aldine, Chicago, pp 136–179

Venier LA, Dunn PO, Lifjeld JT, Robertson RJ (1993) Behavioural patterns of extra-pair copulation in tree swallows. Anim Behav 45:412–415

Westneat DF, Stewart IRK (2003) Extra-pair paternity in birds: causes, correlates, and conflict. Annu Rev Ecol Evol Syst 34:365–396

Whittingham LA, Dunn PO (2001) Survival of extrapair and within-pair young in tree swallows. Behav Ecol 12:496–500

Whittingham LA, Dunn PO, Stapleton MK (2006) Repeatability of extra-pair mating in tree swallows. Mol Ecol 15:841–849

Worthington Wilmer J, Allen PJ, Pomeroy PP, Twiss SD, Amos W (1999) Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (*Halichoerus grypus*). Mol Ecol 8:1417–1429

Zeh JA (1997) Polyandry and enhanced reproductive success in the harlequin-beetle-riding pseudoscorpion. Behav Ecol Sociobiol 40:111–118

Zeh JA, Zeh DW (1996) The evolution of polyandry I: intragenomic conflict and genetic incompatibility. Proc R Soc Lond B Biol Sci 263:1711–1717

Zeh JA, Zeh DW (1997) The evolution of polyandry II: post-copulatory defences against genetic incompatibility. Proc R Soc Lond B Biol Sci 264:69–75