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Genetic relatedness in wintering groups of house sparrows (*Passer domesticus*)

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Abstract

Social behaviour of group-living animals is often influenced by the relatedness of individuals, thus understanding the genetic structure of groups is important for the interpretation of costs and benefits of social interactions. In this study, we investigated genetic relatedness in feeding aggregations of free-living house sparrows (*Passer domesticus*) during the nonbreeding season. This species is a frequent model system for studies of social behaviour (e.g. aggression, social foraging), but we lack adequate information on the kin structure of sparrow flocks. During two winters, we ringed and observed sparrows at feeding stations, and used resightings to identify stable flock-members and to calculate association indices between birds. We genotyped the birds using seven highly polymorphic microsatellite loci, and estimated pairwise relatedness coefficients and relatedness categories (close kin vs. unrelated) by maximum likelihood method. We found that most birds were unrelated to each other in the flocks (mean ± SE relatedness coefficient: 0.06 ± 0.002), although most individuals had at least a few close relatives in their home flock (14.3 ± 0.6% of flock-mates). Pairwise association between individuals was not significantly related to their genetic relatedness. Furthermore, there was no difference between within-flock vs. between-flock relatedness, and birds had similar proportions of close kin within and outside their home flock. Finally, relatedness among members of different flocks was unrelated to the distance between their flocks. Thus, sparrow flocks were not characterized by association of relatives, nevertheless the presence of some close kin may provide opportunity for kin-biased behaviours to evolve.

Keywords: association, dispersal, feeding groups, house sparrow, kinship

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Introduction

In group-living animals the genetic relatedness among group members often influences the costs and benefits of their social interactions, for example, via kin selection (Hamilton 1964; Wilson 1975; Krause & Ruxton 2002). Benefits gained through increased inclusive fitness of related group members may explain various kin-biased behaviours such as helping breeding relatives rearing offspring (Russell & Hatchwell 2001; Baglione *et al.* 2003), association between related males at display sites (Petrie *et al.* 1999; Shorey *et al.* 2000; Krakauer 2005), increased anti-predator behaviour in the presence of close kin (Dunford 1977) and differential exploitation of flock-mates during social foraging (Ha *et al.* 2003; Tóth *et al.* 2009). Thus, information about the fine-scale genetic structure of social groups is important when we are to get a realistic understanding of the pay-offs of social interactions between group-mates.

In birds, relatedness has been usually studied during breeding, typically in relation to helping behaviour, inbreeding and incest avoidance. In these contexts, a relatively high number of studies focused on cooperatively

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breeding groups (see examples above) and the genetic relationships of breeders within a population (e.g. Foerster *et al.* 2006; Lebigre *et al.* 2008; Szulkin & Sheldon 2008). Few studies investigated relatedness in nonbreeding social units, although knowledge on relatedness in such groups is as important as in breeding groups. First, group formation is widespread during migration and wintering, and individuals may aggregate during these periods even in solitarily breeding species (e.g. Ekman 1989). Second, social interactions are frequent in nonreproductive groups, including aggression for food and shelter, exploitation of others' food resources by scrounging, and alarming flock-mates upon predation risk (Elgar 1989; Giraldeau & Caraco 2000; Krause & Ruxton 2002). All of these interactions involve some conflict between group-mates' interests, so the presence of related individuals may provide opportunity for the evolution of kin-biased behaviour.

In this study, we investigated genetic relatedness in feeding aggregations of nonbreeding house sparrows (*Passer domesticus*). Sparrows are highly social, found in flocks at and around their feeding and roosting sites throughout the nonbreeding season (Anderson 2006). Their wintering flocks are frequently used model systems in studies of social behaviour, especially aggression and status signalling (Møller 1987; Liker & Barta 2001; Bókony *et al.* 2006; see Nakagawa *et al.* 2007 for a review), problem solving in groups (Liker & Bókony 2009) and social foraging (Liker & Barta 2002; Lendvai *et al.* 2004, 2006). In the latter context, Tóth *et al.* (2009) found that house sparrows spare their kin during aggressive scrounging: they exploit their close relatives less often and take less food away from them by this tactic than from unrelated flock-mates. That study suggests that kinship may be important in behavioural decisions during the nonbreeding season, at least when the benefits the birds may get by such kin-favouring behaviour are significant relative to its costs. However, despite the intense research on the social behaviour of the species, we essentially lack adequate information about the kinship structure of sparrow flocks.

House sparrows may aggregate at the same feeding and roosting sites throughout the whole nonbreeding period, or even year after year (e.g. we are aware of traditional daytime gathering and feeding sites that have been used since 5–10 years; A. Liker & V. Bókony, personal observations). Although the stability of flock composition has not been studied, it is likely to change dynamically to some extent because birds may use several feeding sites (Beer 1961). On the other hand, the highly sedentary nature of the house sparrows (Summers-Smith 1963; Anderson 2006) suggests that a substantial proportion of the birds in feeding aggregations may be resident around the sites and are present regularly at the same feeding sites, yielding some stability in the social structure. As dispersal is often limited in the species (Summers-Smith 1963; Altwegg *et al.* 2000; Anderson 2006; but see Fleischer *et al.* 1984), related individuals may regularly forage and roost as members of the same flock.

We used intense ringing and observation of wild birds, in combination with molecular genetic data on relatedness, to investigate whether feeding aggregations of house sparrows include aggregations of related individuals. Specifically, we tested (i) whether the degree of association among birds at feeding sites reflects their genetic relatedness; (ii) whether birds are more closely related to their flock-mates, i.e. to birds with whom they regularly feed together, than to members of other flocks that use different feeding sites; and (iii) whether the distance between flocks correlates with genetic relatedness between birds from these different flocks. As female house sparrows move to farther distances than males during natal dispersal (Altwegg *et al.* 2000), we also investigated the above questions separately for the sexes.

Materials and methods

Study sites

We investigated wintering sparrow flocks in Veszprém (47°05′ N, 17°54′ E), north-western Hungary. Four of our five study sites were situated in and around the Zoo of Veszprém (northern site, N; western site, W; central site, C; and southern site, S), whereas one additional site was on the nearby campus of the University of Pannonia (U), south-east from the zoo (Fig. 1). For a general description of the study area, see Bókony *et al.* (2008) and Liker *et al.* (2008; Appendix S1, supporting information). Each site had a group of shrubs where the sparrows regularly roosted during the day, and some adjacent open area where they could feed (see below). Distances between sites ranged between 229 and 1225 m (mean ± SE: 669 ± 111 m), and they were separated from each other by forest tracks (typically mature Austrian pine *Pinus nigra*) or built-up areas (Fig. 1).

Study subjects

Fieldwork was conducted in 2004–2006, which included ringing and blood-sampling of free-ranging sparrows and their subsequent observations at the study sites to collect data on group composition and associations between individuals. Sparrows were around the study sites all year-round. They bred in high numbers (>50 pairs) in the zoo, using both nest boxes and natural nesting sites, and for the whole zoo area (including sites N, W, C and S) the population size was estimated several hundreds in each autumn when young birds were

Fig. 1 Map of the study area. Study sites are indicated by circles, the diameter of the circles is proportional to core flock size (number of genotyped house sparrows observed exclusively at that site) in 2006. Lines connecting the circles indicate the movements of commuter birds, line width is proportional to the number of commuters between the respective sites in 2006.

present in large numbers (Bókony *et al.* 2008). Sparrows also bred in unknown (probably small) numbers on and around the buildings of the university campus, and they were present here in small flocks (10–50 birds) during autumn and winter. In 2006, we estimated the maximum number of birds present during the observations at each site, and the median of these flock sizes were 21, 32, 100, 65 and 12 individuals for site N, W, C, S and U respectively.

We ringed most adult birds during autumn and early winter (September–December) in both 2004 and 2005, before the start of subsequent observation periods (see below), with some additional ringing of breeding adults during spring and summer in 2005. Nonbreeding adults were captured with mist-nets at regular intervals (1 or 2 days weekly) near the study sites. In 2004, this regular ringing was restricted to two zoo sites (W and C), whereas in 2005 all sites were involved. Breeding birds were occasionally caught using a nest trap fitted on nest boxes during the nestling feeding period. In addition to adults, we ringed 9–11 days old nestlings (n = 241) both in nest boxes and at natural nest sites in the zoo during the 2005 breeding season. All birds (including nestlings) were ringed with a numbered metal ring and three colour rings for individual identification. We also took a small amount of blood from each bird from the brachial vein and stored it in Queen's lysis buffer (Dawson *et al.* 1998) until genetic analysis (see below). In total, 410 house sparrows were ringed and blood sampled before the start of the 2005 observation period, whereas the cumulative number of ringed individuals was 1244 by the start of the 2006 observation period.

Observations

We operated feeding stations at two sites (W and C) during the winter of 2004–2005, and at all five sites during the winter of 2005–2006. Seeds (mixture of wheat, sunflower, corn grit and millet) were provided on bird tables (one per site) erected on poles near the roosting bushes. In addition, the same food was provided on a feeding platform placed on the ground at the two sites used in 2004–2005 (Bókony *et al.* 2008). At site C, we also observed birds feeding in a nearby (70 m) outlet of the zoo's raccoons (*Procyon lotor*) where the raccoons' food was provided in three containers erected on separate poles (see Bókony *et al.* 2008), and this food was regularly consumed by the same groups of sparrows that visited the bird table (so we pooled observations at the bird table and at the raccoon feeders for site C). The provision of seeds on the bird tables started several weeks before the beginning of observations and was continued until the end of the study, while the raccoon feeders were operated continuously over the whole year.

We recorded the occurrence of ringed birds at the feeding stations during regular observations that were conducted between 5 January and 11 February in 2005, and between 11 January and 3 February in 2006. The weather during these periods was typically cold (below 0°C) with frequent snowfalls, so the sparrows readily used the feeders at all sites. In 2005, 20 and 40 observations were conducted respectively at site W and C (20 at the bird table and 20 at the raccoon feeders). In 2006, 13 observations were conducted at site N, 10 at W, 22 at C (12 at the bird table and 10 at the raccoon feeders), 14 at S and 10 at U. Observations were conducted from remote hidden locations (~50 m) using 20–60 × spotting scopes, and they usually lasted 60 min, although some of them had to be interrupted earlier because of zoo maintenance work or bad weather conditions. Most observations of a given site were carried out on different days, and usually all sites were observed on each day of the fieldwork. In some cases, two observations per day were conducted at the same site, and in these

cases there was at least a 1-h gap between subsequent observations. During each observation, we recorded the identity and sex of each colour-ringed sparrow that fed on the feeders or stayed nearby (e.g. near the platform or below the table on the ground) and was recognizable unambiguously by its colour rings. In 2005, additional recordings were collected from high resolution videotapes at the container feeders of site C (see Bókony *et al.* 2008), that were added to the records collected by the observers. Note that in some observations, we could not identify all colour-ringed birds present at the station, e.g. when a large number of birds used simultaneously the feeder but stayed there only for brief periods. In total, we collected 5220 records of resightings (including multiple records of the same individuals during a single observation) for 436 ringed birds during the 2 years of the study. Two (VB and AL) and three (VB, AK and AL) observers, working simultaneously at different sites, conducted the data collection in 2005 and 2006 respectively.

Selection of birds for the analyses

For the subsequent analyses, we selected those individuals that had been observed in at least three independent observations. Multiple recordings of the same individual within one observation were counted as one, and the above criterion was applied separately for 2005 and 2006. The mean ± SE number of observations for the selected birds was 11.5 ± 4.4 (n = 79 birds) and 4.9 ± 1.7 (n = 128 birds) in 2005 and 2006 respectively. All of these individuals (n = 188, because 19 birds were included in both years' analyses) were genotyped and used in the statistical analyses.

For some analyses, we categorized the selected birds into two groups, according to the number of sites they were observed at. First, birds were assigned to the 'core flock' of a specific site if they had been recorded exclusively at that site in all of their observations (n = 66 and 103 individuals in 2005 and 2006, respectively). Second, birds observed at more than one site within a year were categorized as 'commuters', i.e. these birds moved between sites at least once between the observations (n = 13 and 25 individuals; Fig. 1). Most of the commuters had a preferred site where they occurred in the majority (3–17) of their observations whereas usually in one or two observations at another site. This categorization was conducted for the two study years separately, and individuals present in both years were categorized for each year. The size of the core flocks correlated strongly with the estimated median flock sizes of the sites (see above; Pearson correlation: r = 0.94, P = 0.0195, n = 5 sites, data from 2006).

Genetic analyses and kinship estimation

DNA extraction from the stored blood samples was performed with standard phenol-chloroform procedure, or with Qiagen DNeasy Tissue Kit (QIAGEN), following the producer's instructions. Seven highly polymorphic microsatellite loci were used for genotyping (17.4 ± 0.7 alleles per locus; see electronic Appendix S1 for details about allele sizes and frequencies). Primers for four dinucleotide loci (*Pdo1*, *Pdo2* [Neumann & Wetton 1996;], *Pdo5* [Griffith *et al.* 1999;] and *Pdo8* [Griffith *et al.* 2007;]), one trinucleotide locus (*Pdo9*, Griffith *et al.* 2007) and one tetranucleotide locus (*Pdo3*, Neumann & Wetton 1996) were developed specifically for house sparrows. Another dinucleotide locus (*McyU4*) was originally isolated for the superb fairy-wren (*Malurus cyaneus*; Double *et al.* 1997), and was used successfully in genetic studies of sparrows (e.g. Jensen *et al.* 2003). In each primer pair (Sigma-Aldrich, Budapest, Hungary), forward primers were fluorescently labelled on the 5'-end with HEX, JOE or FAM-6 dyes (Applied Biosystems). PCR reactions consisted of approximately 100 ng of template DNA, 0.5 μM of each primer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1 unit of *Taq* DNA polymerase (Fermentas AB, Vilnius, Lithuania) and the 10X Taq buffer in a final volume of 25 μl. To resolve alleles, all amplified PCR products were analysed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) at the Biomi (Gödöllő, Hungary) using ROX-labelled ILS-600 internal standard (Promega). The data were analysed with the GeneScan software (Applied Biosystems).

We used the ML-Relate computer software (Kalinowski *et al.* 2006) to calculate maximum likelihood estimates of relatedness coefficients (R) and relationship categories between individuals from genotypical data. This method accommodates null alleles during the relatedness estimations which had high frequency at two loci (*Pdo2*: 0.27, *Pdo8*: 0.26; Appendix S1), and is considered to be more accurate than other estimators (Milligan 2003). Allele frequencies, pairwise genetic relatedness and kinship category estimations were performed by entering all individuals' genotype in the programme as if they were a single population, as no prior reference data were available about the studied sparrow population. We used ML-Relate to calculate the likelihood of four common relationships: U – unrelated, HS – half-siblings, FS – full-siblings, PO – parent–offspring (no other relationships are allowed by the software) and to determine the relationship that had the highest likelihood for each pair of individuals (Kalinowski *et al.* 2006). In the statistical analyses, birds with which a given individual had HS, FS and PO relationships were pooled and considered as 'close kin'. The 'unrelated' flock-mates were those birds with which a given

individual was estimated being unrelated (U). This categorization is likely to reflect real relationships with reasonable accuracy, because in our total sample of birds with known pedigree (including free-ranging sparrows of this study and individuals studied by Tóth *et al.* 2009), 85.4% and 89.9% of the assigned dyads (n = 1225) matched the real (pedigree-based) relationships in the close kin and the unrelated group respectively. Furthermore, for the same set of dyads with pedigree-based kinship information, pairwise relatedness coefficients were estimated 0.423 ± 0.032 for full-siblings and parent–offspring dyads (n = 48) and 0.042 ± 0.002 for unrelated birds (offspring from different broods of different parents; n = 1177 dyads).

Statistical analyses

In the first set of analyses, we used the records of all birds from the observations to calculate half-weighted association indices (A; Cairns & Schwager 1987) between each pair of individuals (i.e. including both core-flock and commuter birds). This calculation was performed using the SOCPROG 2.3 software (Whitehead 2007), with observations as sampling units (n = 60 and 69 units for 2005 and 2006 respectively). The half-weighted association index is a measure of the proportion of observations in which the two birds occurred together, taking the lowest value (0) for dyads that were never recorded in the same observation (e.g. they were observed exclusively at separate sites, or at the same sites but in different observations), whereas the highest value (1) is assigned to dyads that were exclusively recorded together (i.e. always occurred at the same site and in the same observations). This index was calculated separately for the 2 years. To test whether association in feeding groups was related to kinship between birds, we calculated correlations between R and A. Correlation of pairwise matrices was tested by the Mantel randomization tests with 10 000 permutations as implemented in SOCPROG 2.3. We analysed the 2 years separately, and run separate analyses for the pooled data set of all dyads and for sex-specific (male–male, female–female, and male–female) dyads. In a subset of analyses, we also investigated the core flock of each site separately (i.e. commuters were excluded).

In the second set of analyses, we focused on core-flock individuals, and tested whether relatedness of birds within a core flock (i.e. among individuals using exclusively the same feeding site) differed from the relatedness of these individuals to birds assigned to other core flocks (pooling individuals from all other sites). To do this, first we compared relatedness coefficients between the two dyad types (within vs. between core flocks) by randomization test (Mann–Whitney test with 10 000 permutations). Second, we calculated the proportion of close kin present within and outside the core flock for each individual (number of close kin in the flock per number of flock-mates; number of close kin outside the flock per number of nonflock-mates). Then we tested for differences between the two proportions by randomization test (paired Wilcoxon test with 10 000 permutations), using individuals as data points. We analysed the 2 years separately, and within each year a separate analysis was run for each core flock. These analyses were performed in the R statistical computing environment (R Development Core Team 2008).

Finally, we tested whether the spatial distances between core-flock individuals were correlated with their genetic relatedness. We assigned all individuals of a core flock to a single spatial location (feeder site). Within-flock distance was set to 0 m, whereas between-flock distances were calculated from the geographical coordinates of the sites. In this analysis, we only included data from 2006, yielding 10 pairwise distances for the five sites. The same distance was assigned to all dyads of each pair of core flocks. We analysed the relationship between pairwise spatial distances and relatedness coefficients by matrix correlations and Mantel randomization tests with 10 000 permutations using SOCPROG 2.3. Separate analyses were run for the pooled data set of all dyads and for sex-specific dyads. To augment these spatial analyses, we also tested for sex-biased dispersal based on the mean corrected assignment index (mAIc) described by Goudet *et al.* (2002), using FSTAT 2.9.3.2 software with 10 000 randomizations (Goudet 1995). A significantly lower mAIc value for one sex indicates that dispersal is biased towards that sex. This method was chosen because it is accepted as the most powerful tool for detecting sex-biased dispersal from genotypical data within a single population (Goudet *et al.* 2002).

Results

Association and relatedness

In 2005, there was no significant correlation between the relatedness of individuals and their association in the observations (Table 1, Fig. 2a). In 2006, relatedness coefficients and association indices correlated positively for the pooled (not sex-specific) data set (Table 1). However, this correlation was weak (r = 0.02; Fig. 2b) and became nonsignificant when significance levels were adjusted for multiple testing. We did not find any significant correlation between relatedness and association indices when either sex-specific dyad types or core flocks were analysed separately (Table 1).

Relatedness within and between core flocks

Average relatedness. The average relatedness within core flocks was low (Table 2), and in most cases it was not different from relatedness between birds belonging to different core flocks. Although we detected significant differences in some comparisons, these effects were small and inconsistent both among core flocks (e.g. N vs. C in 2005) and between the 2 years (core flock C), and did not remain statistically significant after sequential Bonferroni correction (Table 2). We obtained qualitatively identical results when sex-specific dyads were analysed separately for each core flock (electronic Appendix S2, supporting information). The variance in relatedness across dyads was also small, both in the pooled sample of all dyads in each year (0.008) and within each core flock (range: 0.003–0.013).

Proportion of close kin. Almost all sparrows had at least one close kin (FS, HS or PO) both within (96% of birds) and outside their flocks (97%). On average, the proportion of close kin was 0.143 ± 0.006 (range: 0–0.439) within core flocks and 0.149 ± 0.006 (range: 0–0.500) between different flocks (Fig. 3). Individuals did not differ in the proportion of their close kin within and outside their core flocks in any but one core flock, and this latter difference became nonsignificant after Bonferroni correction (Table 3). We obtained qualitatively the same results when the sexes were analysed separately for each core flock (electronic Appendix S3, supporting information).

Interflock distance and relatedness. Relatedness among birds was not correlated with the distance between their flocks in the sample of five flocks studied in 2006, either when we analysed the pooled sample of all dyads ($r = -0.009$, $P = 0.718$, $n = 103$ birds; Fig. 4) or sex-specific dyads separately (male–male: $r = 0.007$, $P = 0.427$, $n = 50$; female–female: $r = 0.023$, $P = 0.203$, $n = 53$; male–female: $r = -0.035$, $P = 0.748$, $n = 103$).

When we tested for sex-biased dispersal using the pooled sample of the 2 years, the mean corrected assignment index was lower for females than for males (−0.312 vs. 0.328, respectively), and this difference was marginally nonsignificant ($P = 0.068$, $n = 81$ females, 77 males). When we analysed the 2 years separately, females' tendency for having lower mAIc than males was not significant for the two sites in 2005 (−0.302 vs. 0.268, $P = 0.200$, $n = 31$ females, 35 males) but was significant for the five sites in 2006 (−0.392 vs. 0.416, $P = 0.041$, $n = 53$ females, 50 males).

Discussion

House sparrows are well known for their sedentary behaviour. After settling at a breeding site, adult birds may live within a small area throughout the year (Summers-Smith 1963; Perrins 1998; Anderson 2006). Natal dispersal is also limited in the species, although significant variation has been found among populations and the reported frequency of dispersal may depend on the spatial scale on which it was studied. For example in a metapopulation living in an archipelago, only 9.6% of female and 5.7% of male offspring dispersed from their natal island before their first breeding (Altwegg *et al.* 2000). On the other hand, Fleischer *et al.* (1984) reported much higher frequencies of dispersal (52% of females, 27% of males) among neighbouring (usually within

Table 1. Correlation between pairwise genetic relatedness (R) and association at feeding sites (A) among house sparrows. For the analyses of all, male–male, female–female and male–female dyads, we used all available individuals (core flocks plus commuters) from the studied year. In the separate analyses of core flocks, only within-flock dyads were included (core flocks N, S and U were studied only in 2006). Correlation coefficients (r) were calculated by matrix correlation, and the associated P values were calculated by Mantel randomization tests with 10 000 permutations; n = number of individuals and the number of dyads used in the tests are given in parentheses. Note that P values are one-tailed, as provided by the software (SOCPROG 2.3). Corrected significance levels (α) are given for $P < 0.05$ results (sequential Bonferroni correction for 15 comparisons, one-tailed)

	2005			2006		
Sample	n (dyads)	r	P	n (dyads)	r	P (corrected α)
All dyads	79 (6162)	−0.003	0.539	128 (16256)	0.022	0.034 (0.0017)
Male–male dyads	44 (1892)	0.018	0.270	63 (3906)	0.031	0.092
Female–female dyads	35 (1190)	−0.077	0.969	65 (4160)	0.037	0.051 (0.0018)
Male–female dyads	79 (1540)	0.018	0.167	128 (4095)	0.012	0.102
Core flock W	16 (240)	0.035	0.356	22 (462)	0.056	0.239
Core flock C	50 (2450)	−0.001	0.500	41 (1640)	0.032	0.189
Core flock N	—	—	—	11 (110)	0.035	0.395
Core flock S	—	—	—	23 (506)	−0.021	0.635
Core flock U	—	—	—	6 (30)	−0.495	0.972

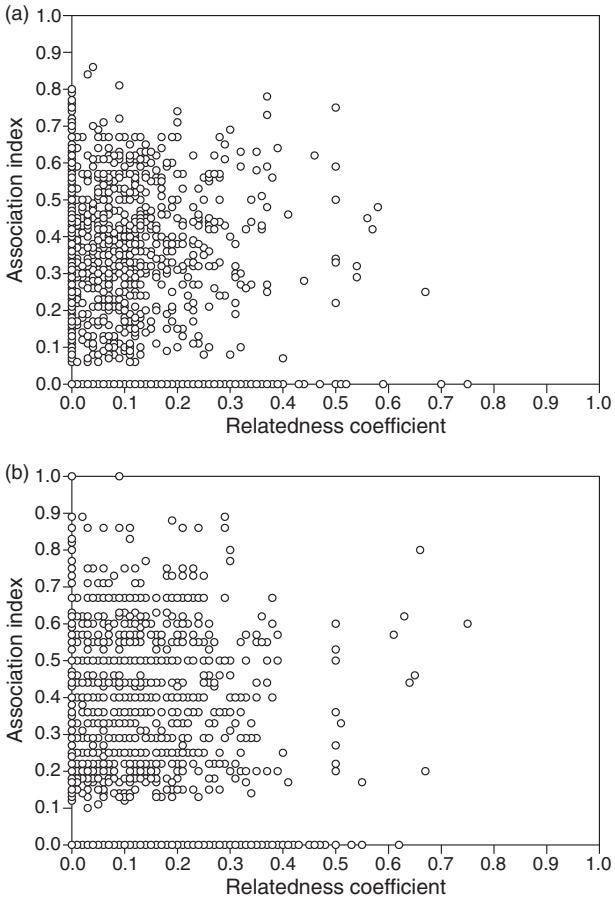

Fig. 2 Genetic relatedness (R) vs. association at feeding sites (A) between house sparrows in 2005 (a) and 2006 (b). Data points represent dyads. See Table 1 for statistical results.

1 km) colonies breeding in a farmland area. However, ringing data suggest that the majority of young sparrows settle close (usually < 2 km) to their natal area (Anderson 2006) and a significant proportion may remain in their natal colony. Such limited movements could result in most sparrows staying with their relatives (e.g. siblings or parents) in the nonbreeding flocks at feeding and roosting sites around the breeding colonies.

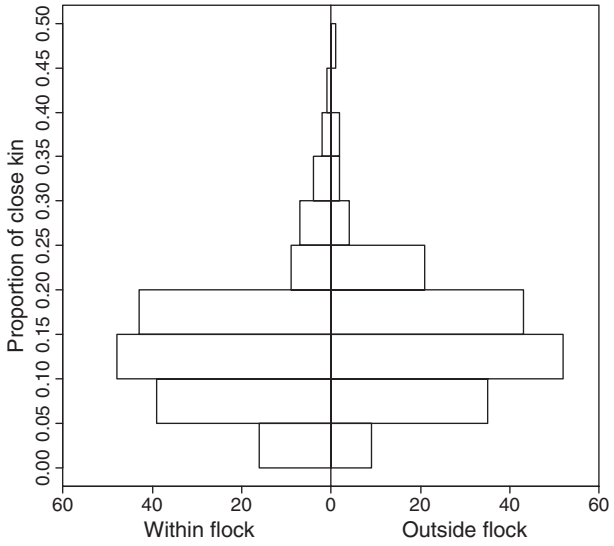

Fig. 3 Matched histograms for the proportion of close kin within and outside the individual's core flock (data pooled for all flocks and from both years). Close kin individuals were those who had full-sib, half-sib or parent–offspring relationships as assigned by maximum likelihood. The horizontal axis shows the number of individuals.

In contrast with this expectation, our results consistently showed that within nonbreeding groups the average relatedness was low and close kin flock-mates were rare. In most cases, relatedness within flocks was as low as between flocks, and the degree of relatedness was not correlated with the spatial distance between flocks. Thus, we found no aggregation of genetically related individuals in the studied flocks. One potential confounding factor that may reduce the chance of find-

Table 2. Comparison of pairwise genetic relatedness (R, mean ± SE) of dyads within vs. between core flocks. Core flocks were defined as groups of birds using exclusively a single feeding site during the study. Differences were tested by Mann–Whitney tests with two-tailed *P* values estimated by 10 000 permutations; *n* = number of individuals in the core flock, and the number of within-flock and between-flock dyads used in the tests are given in parentheses. Corrected significance levels (α) are given for $P < 0.05$ results (sequential Bonferroni correction for seven comparisons, two-tailed)

Sample	*n* (dyads)	R within the core flock	R with other core flocks	*P* (corrected α)
2005				
Core flock W	16 (120, 800)	0.066 ± 0.009	0.058 ± 0.003	0.220
Core flock C	50 (1225, 800)	0.050 ± 0.003	0.058 ± 0.003	0.029 (0.0085)
2006				
Core flock W	22 (231, 1782)	0.072 ± 0.007	0.053 ± 0.002	0.046 (0.0127)
Core flock C	41 (820, 2542)	0.060 ± 0.003	0.053 ± 0.002	0.020 (0.0073)
Core flock N	11 (55, 1012)	0.045 ± 0.012	0.062 ± 0.003	0.031 (0.0102)
Core flock S	23 (253, 1840)	0.053 ± 0.005	0.054 ± 0.002	0.396
Core flock U	6 (15, 582)	0.034 ± 0.014	0.057 ± 0.004	0.595

Table 3. Comparison of the proportion of close kin present within vs. outside core flocks (mean ± SE). Close kin individuals were those who were assigned full-sib, half-sib or parent–offspring relationships by maximum likelihood method using genotypical data, and their proportions (within and outside the core flock) were calculated for each bird. Differences were tested using paired Wilcoxon tests with two-tailed *P* values estimated by 10000 permutations; *n* = number of individuals in the core flock. The corrected significance level (α) is given for the $P < 0.05$ result (sequential Bonferroni correction for seven comparisons, two-tailed)

Sample	*n*	Proportion of close kin within the core flock	Proportion of close kin in other core flocks	*P* (corrected α)
2005				
Core flock W	16	0.141 ± 0.018	0.145 ± 0.012	0.811
Core flock C	50	0.126 ± 0.005	0.145 ± 0.015	0.477
2006				
Core flock W	22	0.194 ± 0.022	0.148 ± 0.010	0.081
Core flock C	41	0.164 ± 0.011	0.145 ± 0.011	0.116
Core flock N	11	0.116 ± 0.018	0.176 ± 0.013	0.011 (0.0073)
Core flock S	23	0.132 ± 0.016	0.157 ± 0.011	0.180
Core flock U	6	0.056 ± 0.035	0.155 ± 0.020	0.093

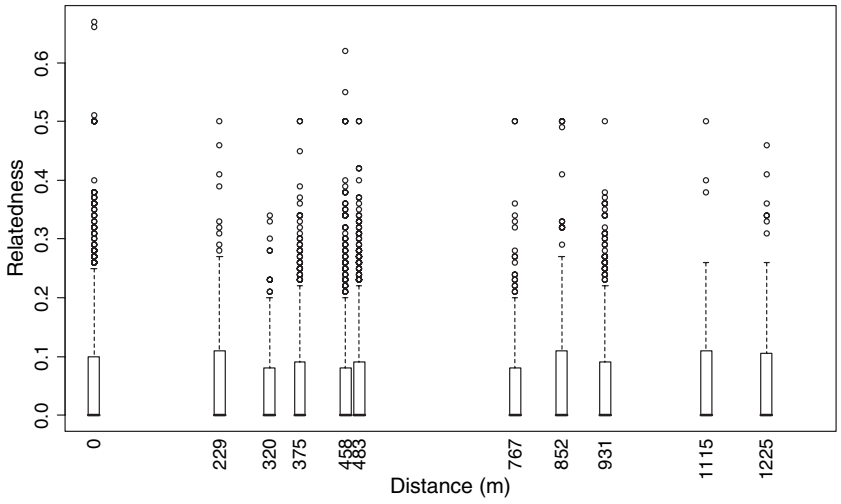

Fig. 4 Relatedness between birds (R) in relation to the spatial distance between their core flocks in 2006. Box plots show data for all flock-mates (distance = 0) and for each pair of flocks. Boxes represent the interquartile range, medians are marked by bold horizontal line, and the whiskers extend to the most extreme data point within 1.5 × interquartile range.

ing a significant kinship structure is the possibility that several subgroups, unrecognizable in our study, use the same feeding site. If these subgroups contain most of the related dyads and are mixed in the analyses, this could result in failure to detect the true kinship structure. However, we found that the association between individuals in the observations was not related to their genetic relatedness, even when we analysed each site separately. This latter result is inconsistent with the above scenario, because such subgroups, if they exist, are unlikely to visit the feeders at the same time in all cases.

Furthermore, we found that the variance in relatedness across all dyads (a measure of population relatedness composition) was small, closely resembling that of outbred birds and mammals which ranges between 0.0004 and 0.0106 (Csilléry *et al.* 2006). This result is in contrast with the significant inbreeding reported for island populations of the house sparrow (Jensen *et al.* 2008). One reason that may have contributed to this difference is the higher isolation of subpopulations in the island study.

We suggest three mutually nonexclusive reasons that may explain the low relatedness of individuals in nonbreeding house sparrow flocks. First, at the studied spatial scale, natal dispersal by juveniles may result in significant mixing of individuals between different parts of the study area (see above). In line with our results, Fleischer (1983) did not find significant genetic differentiation among neighbouring breeding colonies in a sparrow population with high dispersal rates. Second, there may be frequent movements between feeding sites during the nonbreeding season. During the less than 2 months observation periods we found that at least 16.5% of birds occurred at more than one site each year (13/79 and 25/128, see Methods), and this proportion is

probably higher for the whole (6–7 months) nonbreeding season. We believe that this rate of movements was not an artefact of our study setup, i.e. the use of artificial feeding stations relatively close to each other. Feeding and roosting sites of sparrows are often situated similarly closely in habitats with high population densities, for example, four of our five sites (with the exception of U) were regularly used by sparrow flocks before the start of the study. Finally, the mortality rate of house sparrows is high (around 50% annually for adults; Anderson 2006), thus there is a relatively low chance that both members of a closely related dyad staying in the same flock survive for a long period.

Our study failed to detect any consistent sex-difference in the genetic structure of sparrow groups. This result is again unexpected, given that dispersal frequencies are usually higher for female than male house sparrows (Fleischer *et al.* 1984; Altwegg *et al.* 2000), and our study population also showed a tendency for female-biased dispersal. We suggest that at the small spatial scale of our study (the maximum distance between flocks was ~1 km) the effect of sex-difference in dispersal may be masked by the frequent movements of birds between sites (see above). Furthermore, the power of our tests might have been low in some sex-specific analyses as a result of low sample sizes, especially when we analysed data separately for each core flock. Nevertheless, given the low overall relatedness we found within and among our flocks, it is unlikely that male and female sparrows differed considerably in their genetic relatedness to flock-mates.

Our results on sparrow flocks are similar to findings on feeding aggregations in some other birds. For example, Parker *et al.* (1994) reported low frequencies of closely related birds in foraging groups of ravens (*Corvus corax*). In a recent study of ocellated antbirds (*Phaenostictus mcleannani*), Chaves-Campos & DeWoody (2008) also reported low relatedness among birds aggregating at the same feeding sites and the relatedness among birds within feeding groups did not differ from between-group relatedness. These studies, together with our results, suggest that the nonbreeding feeding flocks of birds are often not aggregations of related individuals, in contrast with the reproductive groups of many cooperatively breeding species. One factor contributing to this difference may be that there is an increased opportunity to get indirect fitness benefits from interacting with breeding than nonbreeding relatives (e.g. by helping to feed their offspring), thus selection for staying with relatives may be stronger in breeding groups. Second, the size of the feeding groups are often larger than the size of breeding groups (e.g. feeding aggregations may be over a hundred in house sparrows), and within-group relatedness rapidly declines with group size in birds and mammals (Lukas *et al.* 2005).

Nevertheless, our study demonstrated that most house sparrows have at least a few close relatives in their feeding groups. In our core flocks, an average bird had 1–2 half-sib level (or closer) kin among every 10 flock-mates. The presence of at least some closely related birds in the flocks may provide opportunity for the evolution of kin-biased behaviours. Accordingly, we found that house sparrows recognize their close kin and spare them to some extent in their foraging flocks, using aggressive scrounging less frequently against them than against unrelated flock-mates (Tóth *et al.* 2009). This latter study suggests that kin selection may operate in nonbreeding groups when the benefits of kin-favouring behaviour exceed its costs, even when only a few kin are around in a crowd of unrelated individuals.

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AL and VB conduct research in behavioural and evolutionary ecology of birds, using the house sparrow as a model system, and also by applying phylogenetic comparative approaches. AK is a PhD student, investigating the effects of urbanization on house sparrows' behaviour. ZT is a graduating PhD student, interested in the evolution of social behaviour, kin selection and social network analysis. KS, BK and ZP are interested in population genetics and phylogenetics, focusing mainly on birds and insects.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Number of alleles, allele sizes and their estimated frequencies at the seven microsatellite loci used in this study.

Appendix S2 Comparison of pairwise genetic relatedness (R, mean ± SE) of sex-specific dyads within vs. between core flocks of house sparrows. Differences were tested by Mann–Whitney tests with two-tailed P values estimated by 10 000 permutations; n = number of within-flock and between-flock dyads used in the tests. Corrected significance levels (α) are given for $P < 0.05$ results (sequential Bonferroni correction for 21 comparisons, two-tailed).

Appendix S3 Sex-specific comparison of the proportion of close kin present within vs. outside core flocks (mean ± SE). Differences were tested by paired Wilcoxon tests with two-tailed P values estimated by 10 000 permutations; n = number of individuals of the respective sex in the core flock. Corrected significance levels (α) are given for $P < 0.05$ results (sequential Bonferroni correction for 14 comparisons, two-tailed).

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