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# Social network structure in wintering golden-crowned sparrows is not correlated with kinship

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## Abstract

**Stable social organization in a wide variety of organisms has been linked to kinship, which can minimize conflict due to the indirect fitness benefits from cooperating with relatives. In birds, kin selection has been mostly studied in the context of reproduction or in species that are social year round. Many birds however are migratory, and the role of kinship in the winter societies of these species is virtually unexplored. In a previous study, we discovered striking social complexity and stability in a wintering population of migratory golden-crowned sparrows (*Zonotrichia atricapilla*) – individuals repeatedly form close associations with the same social partners, including across multiple winters. Here, we test the possibility that kinship might be involved in these close and stable social affiliations. We examine the relationship between kinship and social structure for two of the consecutive wintering seasons from the previous study. We found no evidence that social structure was influenced by kinship. Relatedness between most pairs of individuals was at most that of first cousins (and mostly far lower). Genetic networks based on relatedness do not correspond to the social networks, and Mantel tests revealed no relationship between kinship and pairwise interaction frequency. Kinship also failed to predict social structure in more fine-grained analyses, including analyses of each sex separately (in the event that sex-biased migration might limit kin selection to one sex), and separate analyses for each social community. The complex winter societies of golden-crowned sparrows appear to be based on cooperative benefits unrelated to kin selection.**

*Keywords*: genetic network, golden-crowned sparrow, kinship, migration, social network, winter social structure, *Zonotrichia*

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## Introduction

Group living is common in a wide diversity of organisms. Groups can vary from temporary, fleeting associations to complex societies with long-term social associations. Understanding the factors that favour such stable social groups remains an important focus of social evolution research. Kinship is often an important correlate of stable and complex societies (Hamilton 1964; Alexander 1974; Wilson 1975). For example, the stable cooperative societies of many birds, mammals, invertebrates and microbes often involve family groups or extended families (Bourke & Franks 1995; Solomon & French 1996; Koenig & Dickinson 2004; Clutton-Brock 2006; Hatchwell 2010; Kuzdzal-Fick *et al.* 2011; Strassmann *et al.* 2011; Kamel & Grosberg 2013). However, social groups can also be favoured in the absence of kinship, such as where the benefits of group living are strong and are shared among all group members (Trivers 1971; Connor 1995). Moreover, simple ecological factors such as the distribution of resources in space

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and time can also lead to aggregations of individuals even in the absence of benefits of direct social affiliations (Ramos-Fernández *et al.* 2006). Thus, disentangling the importance of kinship relative to other influences on social structure is important for understanding the evolutionary origins of complex societies.

Social organization and kinship have both been particularly well studied in birds, but the vast majority of this research has focused either on reproduction or in societies that remain together year round – typically cooperative breeding species (Emlen 1982; Koenig & Dickinson 2004; Ekman 2006). Many birds, however, are migratory, and for these species, we have a very rudimentary understanding of social behaviour in migration or on the wintering grounds. For example, the most basic pattern of whether individuals are solitary or live in groups (flocks) is well understood, but we know little about the composition or stability of these groups. The conceptual framework of fission–fusion dynamics (Aureli *et al.* 2008) provides a useful perspective for thinking about the range of possible social patterns that flocking migratory birds could have – from completely dynamic, unstable groupings to social groups with considerable stability (Shizuka *et al.* 2014; Silk *et al.* 2014). Generally, studies of fission–fusion societies in nonavian taxa are beginning to reveal that complex and stable societies may be more widespread than previously realized. The societies that are particularly stable and close knit are also the ones where kinship may be most likely to be present (e.g. Kraaijeveld & Dickinson 2001; Krutzen *et al.* 2003; Archie *et al.* 2006; Ostrowski *et al.* 2008; Portelli *et al.* 2009; Rollins *et al.* 2012; see also Silk *et al.* 2014).

Here, we explore the relationship between social complexity and kinship in wintering communities of a migratory sparrow. Our recent study of winter social organization in the golden-crowned sparrow (*Zonotrichia atricapilla*) revealed unsuspected social complexity and associations between individuals that persist across several years (Shizuka *et al.* 2014). Golden-crowned sparrows forage in small flocks (typically 2–10 individuals) on their wintering grounds, and social network analysis revealed that although individual membership in short-term foraging flocks is dynamic, flock members come from larger, more stable social communities. These communities are also very stable across years in both membership and home range location: returning sparrows prefer to associate with the same individuals with whom they had associated in the previous years, and analyses show that this pattern is explained more by social preference than shared use of space (Shizuka *et al.* 2014). This suggests that some individual sparrows form close associations with others that could last their entire lifetimes, at least during the winter portions of their lives. Although group living can provide general benefits to individuals irrespective of the identity of group membership, for example by decreasing the risk of predation (Hamilton 1971; Bertram 1978), these general benefits cannot easily explain why individuals prefer to associate with certain members of the population but not others, and why these social associations are so stable across time. The question we address here is whether kinship plays any role in the tight social affiliations we detected in particular, and community structure more generally.

Determining patterns of kinship in winter social groups of migratory species is somewhat more challenging than for breeding groups because we lack information on pedigrees and must use indirect approaches to estimate pairwise relatedness patterns. We used multilocus genotype data (microsatellite DNA) to estimate patterns of relatedness in our study population. We genotyped the same individuals used in our social network study (Shizuka *et al.* 2014), which allows us to determine whether degree of relatedness correlates with social network metrics. The genetic analysis revealed that many individuals had a high coefficient of relatedness with at least one other individual in the population (equivalent to a second- or first-order relative) with which they could have potentially associated. We investigated the relationship between kinship and social structure in two different ways. First, to determine whether relatives tend to cluster in the same social communities, we compared social networks based on social affiliations with genetic networks based on relatedness patterns. Second, we investigated social affiliations on a finer scale and asked whether pairwise relatedness estimates correlate with the strength of the social affiliation between pairs of individuals. We did these analyses for the entire population, for individual communities and for each sex separately. For one of the seasons, we also analysed whether patterns of kinship and social associations differed across age classes, as inferred by their capture dates. Finally, we investigated whether the social communities we detected were genetically distinct from each other as might be expected if communities form around kin groups.

## Materials & methods

### *Study site and subjects*

We studied golden-crowned sparrow flocks at the University of California, Santa Cruz Arboretum. The approximate seven-hectare study area includes a mixture of shrubs, trees and open grassy areas where the birds forage in flocks. Golden-crowned sparrows typically arrive at this wintering site in October and depart for the breeding grounds in April. We conducted flock observations in two seasons: January to March 2010 (hereafter 'Season 1') and October 2010 to March 2011 (hereafter 'Season 2'). To identify individual birds in flocks, we caught birds at regular feeding stations using Potter-style traps baited with millet. We gave each captured bird a unique combination of coloured vinyl leg bands (Hill 1992), and we also collected a blood sample for genetic determination of gender and microsatellite analysis.

### *Behavioural data to determine social structure*

We used flock censuses to determine the social structure of the population (further details presented in Shizuka *et al.* 2014; data available at Dryad Digital Repository, doi: 10.5061/dryad.d3 m85). We defined a flock as a group of birds within an approximately 5-metre radius. In our study, these flocks are short-lived associations of individuals and flock membership can change dramatically over short periods of time. For each flock, we recorded as many colour-banded individuals as could be accurately identified. We only included individuals that were banded prior to the beginning of flock observations for the year. For example, the Season 1 analysis includes birds banded early in the season (October–December 2009), as our flock censuses began in January, but it does not include any birds banded later than December. In contrast, the Season 2 analysis only includes birds banded in previous seasons because flock censuses began in October (Shizuka *et al.* 2014). This means that some yearling birds could have been included in the data set for Season 1, but not in Season 2. To ensure that our flocks represent independent observations, we excluded flocks that were seen within 20 min of each other and contained two or more of the same individuals. Most flock observations were made on days when we did not provide food at feeders, but 10% of the flock observations were made on days when food was provided and we excluded any observations made at feeding sites. The social network results are unchanged if we omit flock observations carried out on days where food was provided. In Season 1, we observed 77 flocks with 3.3 ± 1.9 (mean ± SD) banded individuals, and in Season 2, we observed 340 flocks with 2.2 ± 1.7 banded individuals (Shizuka *et al.* 2014). A large proportion of flock members were banded: 74.9% were banded in 2009 (based on all 77 flocks) and 74.8% in 2010 (based on 193 flocks where flock size was confidently estimated).

We used the flock censuses to build social networks of flock comembership. In each network, nodes represent individual birds and edges represent the simple ratio association index (Cairns & Schwager 1987). We then applied a network-based community detection process to identify clusters of individuals that are more strongly associated with each other than with others (Newman & Girvan 2004). Specifically, we used the algorithm proposed by Clauset *et al.* (2004) to find groupings that maximize the proportion of association indices that occur within clusters. The result is an assignment of individuals into social communities within a network as well as a measure of discreteness of the social clusters (termed *modularity*). Our previous work showed that this population contained three main social communities in both seasons (see Fig. 2 below), and these assignments were robust to sampling error (Shizuka *et al.* 2014).

### *Genetic data*

We collected a small blood sample (~20 μL) from the brachial vein of each individual and stored it in Queen's lysis buffer (Dawson *et al.* 1998). Some samples were then frozen (-20C), and some were stored in the refrigerator (2C). DNA extraction was performed with either standard phenol–chloroform procedure or Qiagen DNeasy Blood & Tissue Kit (QIAGEN). The phenol–chloroform procedure produced higher DNA yields.

*Sexing protocol.* We used the primers P2 and P8 for sexing individuals (Griffiths *et al.* 1998). PCR amplification was carried out in a total volume of 10 μL. The final reaction conditions were as follows: 0.5 μL of 25 mM MgCl2, 1.5 μL of 2 mM dNTPs, 2 μL of 2.5 μM of each primer, 200 ng template DNA, 1 μL of 10× buffer (Qiagen), 1 μL of 1 mg/mL BSA, 0.12 μL of Taq DNA polymerase (Qiagen) and 1.38 μL ddH20. PCR was performed on an Applied Biosystems GeneAmp 9700 thermal cycler. An initial denaturing step at 92 °C for 2 min was followed by 26 cycles of 92 °C for 30 s, 54 °C for 45 s and 72 °C for 45 s. Final extension was 72 °C for 5 min. Bands were scored by hand on a 3% NuSieve agarose gel: two bands were present for a female and one band present for a male (Chaine *et al.* 2011).

*Microsatellite protocol.* As there were no published microsatellite primers available for golden-crowned sparrows, we screened potential primers from other closely related species (Primmer *et al.* 1996; Stenzler *et al.* 2004; Poesel *et al.* 2009). Thirty primers were initially screened of which 24 were optimized for PCRs. The final 14 primers selected for genetic analysis (see below for explanation) were initially optimized for white-crowned sparrows *Zonotrichia leucophrys* (13 primers from Poesel *et al.* 2009) and golden-winged warblers *Vermivora chrysoptera* (1 primer from Stenzler *et al.* 2004.)

PCRs were carried out for all individuals. We set up 25 μL PCRs containing 10 ng template DNA, 10× *Ex Taq* buffer ($Mg^{2+}$ free), 0.2 mM each dNTP, between 1.0 and 3.5 mM $MgCl_2$, 0.05 U *TaKaRa Ex Taq* polymerase (Takara Bio Inc.), 0.015 mM forward primer tailed with M13 (Boutin-Ganache *et al.* 2001), 0.2 mM reverse primer and 0.2 mM M13 labelled with fluorescent dye (Appendix 1). We used the Dye Set DS-33 (Applied Biosystems Inc.) to fluorescently label primers with 6-FAM, VIC, NED and PET.

PCRs were run under the following conditions: initial denaturation at 94 °C for 3 min, then 33 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 40 s (65 °C for 40 s for GCSPR19 only), extension at 72 °C for 40 s, concluding with a final extension at 72 °C for 5 min. We verified PCR amplification by visualizing PCR products on 3% NuSieve agarose gels stained after casting with GelRed Nucleic Acid Gel Stain, 3× in water (Biotium).

To identify alleles, all amplified PCR products were suspended in formamide and analysed on an ABI Prism 3100 Genetic Analyzer (Applied Biosytstems Inc.) at the University of California, Berkeley, DNA Sequencing Facility using a LIZ-labelled 600 internal standard (Applied Biosystems Inc.). The data were analysed visually using PEAK SCANNER Software v1.0 (Applied Biosystems Inc.). All samples were retyped, and 98% were confirmed correct. Scores that differed by 1 bp or less were considered to be equal for all tetranucleotide repeats.

As our study solely relies on microsatellite data to quantify kinship, we were diligent in our selection of appropriate loci by utilizing a microsatellite screening protocol (Selkoe & Toonen 2006). We included loci that met three conditions: (i) they were accurately scored genotypes (we rescored a subset of genotypes and calculated an error rate of <1%) that (ii) were void of null alleles and (iii) did not exhibit linkage disequilibrium. Although we sought to maximize the number of loci used in our study, we were aware that loci that did not pass this screening process would decrease the accuracy of our genetic estimates. We compared observed and expected heterozygosity and deviations from Hardy–Weinberg (HW) expectations using ML-RELATE (Kalinowski *et al.* 2006) using Bonferroni correction of alpha levels for multiple comparisons (Table 1). We tested for linkage disequilibrium between the microsatellite loci using GENEPOP version 4.0.10 (Raymond & Rousset 1995; Rousset 2008). We excluded loci with null alleles, which were detected using Microchecker (Van Oosterhout *et al.* 2004). We used 14 microsatellite loci that passed all three criteria to calculate relatedness coefficients (see Appendix 1).

*Relatedness estimation*. We generated a pairwise coefficient of relatedness between all pairs of individuals using ML-RELATE software (Kalinowski *et al.* 2006) to calculate maximum-likelihood estimates. This method is considered to be more accurate than other estimators (Milligan 2003). Pairwise coefficients of relatedness between individuals were calculated separately for each season. Estimation of pairwise genetic relatedness was carried out by entering the genotypes of all individuals in the programme as if the individuals comprised a single population, as no prior reference data were available about this population.

### Statistical analysis

*Correlating social associations with genetic relatedness.* Our goal was to determine whether relatedness and degree of social association were correlated. Our sample included all 31 individuals in the network for Season 1

**Table 1** Fourteen microsatellite loci used to calculate genetic relatedness. The *P* value for GCSPR17, *P* = 0.0036, exceeds the adjusted sequential Bonferroni alpha level of 0.0023 (0.05/21; 21 loci were screened)

| Locus | Size Range | No. of Alleles | Observed heterozygosity (HO) | Expected heterozygosity (HE) | P value for Hardy-Weinberg test |
|---|---|---|---|---|---|
| GCSPR1* | 300–524 | 34 | 0.978 | 0.96 | 0.51 |
| GCSPR2* | 167–207 | 11 | 0.966 | 0.87 | 0.90 |
| GCSPR3* | 252–288 | 9 | 0.903 | 0.83 | 0.59 |
| GCSPR5* | 226–274 | 12 | 0.915 | 0.85 | 0.15 |
| GCSPR9* | 194–243 | 9 | 0.919 | 0.83 | 0.76 |
| GCSPR10* | 186–280 | 18 | 0.9 | 0.9 | 0.25 |
| GCSPR11* | 171–215 | 12 | 0.881 | 0.89 | 0.28 |
| GCSPR12* | 221–261 | 18 | 0.951 | 0.87 | 0.93 |
| GCSPR14* | 188–244 | 13 | 0.919 | 0.87 | 0.73 |
| GCSPR15* | 186–230 | 10 | 0.857 | 0.83 | 0.69 |
| GCSPR16* | 230–278 | 12 | 0.932 | 0.86 | 0.87 |
| GCSPR17* | 200–288 | 16 | 0.722 | 0.89 | 0.00 |
| GCSPR19* | 172–216 | 12 | 0.622 | 0.58 | 0.16 |
| GCSPR30† | 312–352 | 13 | 0.812 | 0.8 | 0.16 |

*Poesel *et al.* 2009; †Stenzler *et al.* 2004.

and 40 of 43 individuals in the network for Season 2 (three individuals were excluded due to lack of a DNA sample). We compared the network of social associations with the network of pairwise relatedness for each year (Fig. 2) in multiple ways.

We first assessed whether dyads with a high coefficient of relatedness ($r \geq 0.25$) occurred within the same social communities more than expected by chance. We used a Fisher's exact test to compare the number of dyads in the same or different communities that had relatedness coefficients above or below 0.25. We then conducted three separate analyses to ask whether genetic relatedness correlated with different levels of social associations. First, we tested for correlations between pairwise relatedness coefficients and association indices for all dyads. The second set of analyses used data for sex-specific dyads (females only and then males only) to test for the possibility that relatedness might affect associations in same sex dyads only. Third, we tested whether kinship influenced associations only within social communities, by comparing pairwise relatedness and pairwise-association indices separately for each of the six communities as determined by social network analysis (three communities in Season 1; three communities in Season 2). All correlations of pairwise matrices were tested by the Mantel randomization tests with 10,000 permutations using the 'ecodist' package (Goslee & Urban 2007) using R statistical computing software (version 3.1.2).

*Winter social structure in relation to breeding population structure.* One alternative mechanism that could influence the genetic structure of winter populations and lead to the appearance of kinship effects is breeding season structure coupled with nonrandom winter associations based on breeding site. Specifically, individuals within communities might share alleles not due to relatedness but due to a shared breeding population with allele frequencies distinctive from other breeding populations (and winter communities). This type of connectivity between breeding populations and social communities within wintering grounds could lead to the appearance of kinship. The theoretical false signature of kinship we suggest here is related to the Wahlund effect, whereby population substructure can lead to a reduction in heterozygosity relative to levels expected for a panmictic population (Sinnock 1975). If birds from distinct breeding populations form social communities within our winter population, then this could bias our estimates of kinship. We currently do not have data regarding the breeding provenance of our wintering population. However, a study of golden-crowned sparrows in a nearby population (~150 km) found that four birds from one winter population all bred in separate breeding populations along the Pacific coast of Alaska (Seavy *et al.* 2012), suggesting that winter populations could contain individuals from a mix of breeding populations. To rule out a Wahlund-type effect, we assessed population structure using $F_{st}$ values and checked for deficiency of heterozygotes against Hardy–Weinberg expectations. Pairwise $F_{st}$ values within and between social communities and across all loci were calculated separately for each season using the online version of GENEPOP v. 4.2. (Raymond & Rousset 1995). A null distribution of $F_{st}$ values was generated through 1000 permutations of the haplotypes between populations, and the $P$ value represents the proportion of permutations leading to an $F_{st}$ value larger than or equal to the observed value.

## Results

### *Patterns of genetic relatedness*

Most dyads show low relatedness based on our estimates of pairwise relatedness values (Fig. 1). Nonetheless, a few dyads showed relatedness estimates consistent with first- or second-order kinship (Fig. 1), suggesting the potential for some kin associations. In Season 1, 2.4% of dyads from the same communities had relatedness values $r \geq 0.25$, whereas 1.9% of all pairwise relatedness values were greater or equal to 0.25. In Season 2, 4.9% of dyads from the same communities had relatedness values $r \geq 0.25$, whereas the 3.6% of all pairwise relatedness values were greater or equal to 0.25.

Although the fraction of all dyads estimated to involve at least second-order relatives is low, what may matter more to kin cooperation is whether individuals have any close relatives at all in the population with which they could associate. We therefore determined the fraction of individuals in the population that had at least one-second-order relative ($r \geq 0.25$) and at least one-first-order relative ($r \geq 0.5$) present in the population with which they could have potentially associated. In Season 1, 48% of the individuals in the population ($N = 31$) had at least one-second-order relative present in the population and 16% had at least one-first-order relative present. In Season 2, 80% of the individuals we studied ($N = 40$) had at least one-second-order relative present in the population, while 20% had at least one-first-order relative present. The sexes did not differ in the likelihood of having a close relative in the population (Fisher's exact test: $P = 0.11$ and $P = 0.17$ for seasons 1 and 2). In summary, a substantial fraction of the population each year did have at least one relative with which they could have associated and we conducted several analyses (below) to determine whether kin-based associations actually occurred.

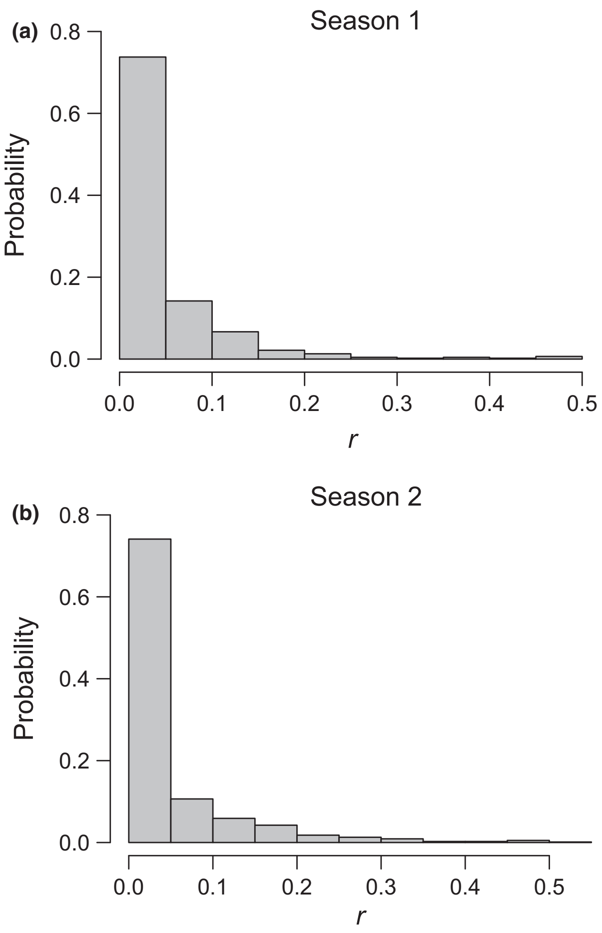

**Fig. 1** Distributions of pairwise relatedness values ($r$) for Season 1 ($n$ = 465) and Season 2 ($n$ = 781).

### *Social association and genetic relatedness*

At the broad level of community affiliation, relatives did not occur in the same communities more often than expected by chance (Fig. 2; Table 2: Fisher's exact test for Season 1, $P = 0.32$; for Season 2, $P = 0.84$). A comparison of social networks based on social affiliations and genetic networks based on relatedness values reveals that the two networks are not correlated in either year of the study (Fig. 2; Table 3). Moreover, we further analysed pairwise correlations at several different levels. There was no significant correlation between the strength of individual social association and genetic relatedness at the population level or within individual social communities (Table 3). Because the strong and consistent skew in sex ratio of our population suggested differential migration patterns (74% and 75% of the population were female in seasons 1 and 2, respectively), we tested to see whether correlations between social association and relatedness might exist within one sex but not the other. However, we did not detect any significant correlations within either sex (Table 3).

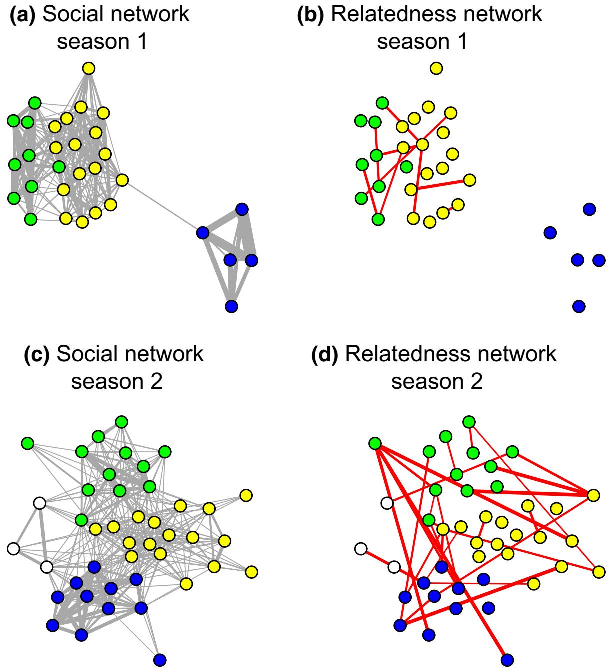

**Fig. 2** Social and relatedness networks are not correlated. (a), (c) The social networks were generated based on flock association patterns in each season. Each node (circles) represents an individual bird, and edge widths (grey lines) represent the association index between each pair of birds. Node colours represent social community membership. The placement of nodes was determined by a force-directed algorithm that displays clusters of nodes that are tightly connected. (b), (d) Relatedness networks are displayed using the same node colour and placement as the corresponding social network, but the edge widths (red lines) now representing $r$-values. For clarity, we only display edges corresponding to $r \geq 0.25$. Note that the relatedness patterns do not follow social community structure.

**Table 2** Two-way contingency tables of first- and second-order relatives with high coefficient of relatedness ($r \geq 0.25$) occurring within or across communities

| Season | | $r \geq 0.25$ | $r < 0.25$ |
|---|---|---|---|
| 1 | Same Community | 5 | 177 |
| | Different Community | 4 | 279 |
| 2 | Same Community | 9 | 228 |
| | Different Community | 19 | 524 |

### *Social association and population structure*

One final way to assess the link between social affiliation and relatedness is to determine whether communities correlate with the genetic structure of the population. Across the 14 microsatellite loci, there was no indication that social communities reflected the genetic structure of the population ($F_{st}$ range 0–0.06 in Season 1, 0–0.01

**Table 3** Correlation between pairwise genetic relatedness and social association (simple ratio) among golden-crowned sparrows. Correlation coefficients ($r$) were calculated by matrix correlation, associated $P$ values were calculated by Mantel randomization tests with 10 000 permutations; $n$ = number of individuals with number of dyads used in the tests given in parentheses. $P$ values are one-tailed, as provided by the software (ECODIST)

| | Season 1 | | | Season 2 | | |
|---|---|---|---|---|---|---|
| Sample | $n$ (dyads) | Mantel $r$ | $P$ | $n$ (dyads) | Mantel $r$ | $P$ |
| All dyads | 31 (930) | −0.003 | 0.51 | 40 (1560) | −0.045 | 0.90 |
| Male–male dyads | 8 (56) | −0.188 | 0.83 | 10 (90) | −0.307 | 0.99 |
| Female–female dyads | 23 (506) | −0.019 | 0.59 | 30 (870) | 0.019 | 0.33 |
| Social Community 1 | 9 (72) | −0.112 | 0.73 | — | — | — |
| Social Community 2 | 17 (272) | −0.058 | 0.75 | — | — | — |
| Social Community 3 | 5 (20) | 0.426 | 0.20 | — | — | — |
| Social Community 4 | — | — | — | 11 (110) | −0.098 | 0.79 |
| Social Community 5 | — | — | — | 17 (272) | 0.036 | 0.30 |
| Social Community 6 | — | — | — | 11 (110) | −0.096 | 0.71 |

in Season 2; Table 4). For both seasons, the $F_{st}$ values between each pair of social groups within seasons were all 0.

### *Robustness of relatedness estimates*

A possible explanation for our failure to link kinship with social behaviour in our study could be that we failed to obtain reasonable estimates of relatedness. We can test this hypothesis because we estimated relatedness separately for each of the 2 years. Despite the considerable population turnover between years, 19 birds occurred in both seasons and we can determine repeatability of the relatedness estimates for 171 dyads from these 19 individuals (Becker 1985). The relatedness estimates were highly repeatable ($r = 0.96 \pm 0.01$, $F_{2,171} = 51.65$, $P < 0.0001$); our kinship estimates are clearly very robust.

**Table 4** $F_{st}$ values for each locus, with social community as a grouping category, calculated with GENEPOP

| Loci | Season 1 $F_{st}$ | Season 2 $F_{st}$ |
|---|---|---|
| GCSPR 1 | 0.005 | 0 |
| GCSPR 2 | 0 | 0 |
| GCSPR 3 | 0 | 0.009 |
| GCSPR 5 | 0 | 0.001 |
| GCSPR 9 | 0.016 | 0.018 |
| GCSPR 10 | 0 | 0 |
| GCSPR 11 | 0 | 0 |
| GCSPR 12 | 0 | 0 |
| GCSPR 14 | 0 | 0 |
| GCSPR 15 | 0 | 0 |
| GCSPR 16 | 0 | 0 |
| GCSPR 17 | 0 | 0 |
| GCSPR 19 | 0.061 | 0 |
| GCSPR 30 | 0 | 0.007 |

## Discussion

Our previous work demonstrated that our study population of wintering golden-crowned sparrows was subdivided into relatively discrete and highly stable social communities within which most flock associations occurred (Shizuka *et al.* 2014). Here, we tested the hypothesis that social interaction strength was correlated with pairwise kinship. Genetic networks based on relatedness showed no correspondence with social networks, indicating that relatives are not more likely to belong to the same social community. Exploring this pattern at the individual level rather than community level, we showed that the strength of pairwise affiliations was not correlated with pairwise relatedness. Finally, we also investigated whether there were subtle patterns of kin associations that would not have been picked up using a global comparison of kinship and flock associations. For example, sexes show different migration patterns in some birds (Altwegg *et al.* 2000; Liu *et al.* 2013) and migrate different distances (Ketterson & Nolan 1983). Indeed, our winter population is consistently female biased (~75% females in both years), suggesting such differential migration in this species. This migration pattern would preclude kinship associations across sexes, but kin of the same sex may still associate closely with each other. However, there were no detectable relationships between genetic relatedness and social associations for dyads involving the same sex. We also isolated social associations and genetic relatedness within social communities to test whether we could detect more subtle patterns found only within individual communities, but no patterns were detected. Overall, these findings provide convincing support that kinship plays no role in the striking multi-year social affiliations we previously documented in golden-crowned sparrows (Shizuka *et al.* 2014).

Assessing relatedness on the wintering grounds for migrant birds introduces a potential complication for studies that do detect kin associations. Because pedigree information is not feasible, relatedness in winter populations must be inferred from the patterns of shared alleles. The inference takes into account the frequency of given alleles in the population, but with migratory species, the question becomes what is the relevant population from which to assess gene frequencies? For example, if winter social communities contain individuals from the same breeding population, and different communities are populated from different breeding areas (that also differ in allele frequencies), genetic differences between winter social communities could occur even in the absence of kin-based social cohesion. Treating the entire winter population as a panmictic population when estimating relatedness patterns could potentially lead to a false signature of kin structure. In our study, this is clearly not an issue because we failed to find any evidence for kinship effects on sociality, but this factor should be considered by any future studies that do find evidence for kin-structured wintering groups in migratory species.

Overall, the average relatedness between pairs of birds was low in our winter population of sparrows, and social interactions occurred mostly between unrelated birds. Only 2–5% of all possible dyads were considered close kin (pairwise assignments of $r \geq 0.25$ by maximum-likelihood method). These results align with other recent studies that have also detected a low percentage of close kin in winter bird aggregations (Liker *et al.* 2009; Fleskes *et al.* 2010; Lee *et al.* 2010; Liu *et al.* 2013). However, although our study revealed no relation between kinship and social structure, it was somewhat surprising that many individuals did appear to have at least one close relative ($r \geq 0.25$) in the population. The question remains as to how these putative relatives end up in the same winter population even though they do not associate more than expected by chance. We suggest two explanations. First, relatives could migrate together to the same wintering grounds even though they do not then associate with each other. Dispersal of kin groups has recently been reported for a cooperative breeding species (Sharp *et al.* 2008). Second, unrelated birds from the same breeding populations could migrate together and different breeding populations differ sufficiently in genetic structure, which could create the appearance of relatedness as we described above. Both hypotheses make the prediction that the apparent close relatives arrive together in their first winter at the wintering site.

More broadly, the emerging literature on genetic structure of animal social networks suggest that social groups within populations can vary dramatically with respect to kinship, including those with highly cohesive kin-based groups (e.g. African elephants (*Loxodonta africana*), Archie *et al.* 2006; spotted hyenas (*Crocuta crocuta*), Holekamp *et al.* 2012), groups with moderate levels of genetic relatedness due to limited dispersal (e.g. Galápagos sea lion (*Zalophus wollebaeki*), Wolf & Trillmich 2008; eastern grey kangaroos (*Macropus giganteus*), Best *et al.* 2014; vinous-throated parrotbills (*Paradoxornis webbianus*), Lee *et al.* 2010), or groups with little to no genetic relatedness (e.g. this study; tufted duck (*Aythya fuligula*) Liu *et al.* 2013). These patterns of variation provide opportunities for exploring how ecological factors interact with kinship to produce variations in the structures of animal societies.

As kinship does not explain the stability of social affiliations in wintering golden-crowned sparrows, the question remains as to how and why these winter social groupings are so highly stable, even across several winters. Our previous study showed that stable social structure is shaped by across-year fidelity to flock-mates, and individuals that return to the population across years flock with the same birds as the previous year more than expected based on shared use of space (Shizuka *et al.* 2014). What then is the ultimate driver of such long-term stability? Theory predicts that long-term social associations can help stabilize reciprocal cooperation and counter the destructive consequences of cheating (Trivers 1971; Axelrod & Hamilton 1981; Connor 1995). For example, stable partners help maintain cooperative egg trading in chalk bass (*Serranus tortugarum*) (Fischer 1984; Petersen 1995) or bloodmeal sharing in vampire bats (*Desmodus rotundus*) (Carter & Wilkinson 2013). We do not yet have evidence that golden-crowned sparrows exhibit such forms of overt cooperation but many forms of cooperation should be enhanced with group stability. Possibilities for wintering birds include mitigating the costs of unnecessary aggression when competition can be mediated by individual recognition (Godard 1991; Tibbetts & Dale 2007), shared predator vigilance (Pulliam 1973), food sharing (Ekman & Rossander 1992) and shared defence of group territories (Kraaijeveld & Dickinson 2001). While these benefits do not necessarily require long-term associations across years, such multi-year associations may further enhance benefits over individual lifetimes. Note also that these costs and benefits will manifest themselves during many types of social interactions. Foraging groups that we have studied (Shizuka *et al.* 2014) provide one social context for the benefits of sociality to apply, and roosting behaviour could provide an additional such social context (Ward & Zahavi 1973; Marzluff *et al.* 1996). Regardless of the exact cause of group stability, the lack of kinship as an important factor generating long-term social structure suggests that there are important benefits to stable social associations that we have yet to uncover.

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N.N.A. conducted the molecular work and genetic analyses and wrote the first draft of the manuscript. B.E.L. and A.S.C. collected the field observations, and D.S. ran the social network analyses. B.E.L., A.S.C. and D.S. edited the manuscript.

## Data accessibility

Microsatellite loci and alleles used to calculate relatedness; pairwise relatedness and social association indices: Dryad doi:10.5061/dryad.ss250.

Raw data for the social network analyses, including date, time and bird identity: Dryad doi:10.5061/dryad.d3 m85.

## Appendix 1

Primer sequences listed by locus. PCRs were optimized given the following salt concentrations (MgCl2) and annealing temperatures (TA). The repeat motif for each microsatellite is given in base pairs (bp). NA provides the number of unique alleles for that locus across all samples. The size range of each locus is listed in base pairs.

| Locus | Primer Sequence (5′-3′) | MgCl2 (μL) | TA | Repeat Motif (bp) | NA | Size Range (bp) |
|---|---|---|---|---|---|---|
| GCSPR1 | F: TATCGAGCATTGCCCTCCC<br>R: GCAGAGTATGAGGTTTTCCTTCC | 2.5 | 59C | 4 | 34 | 300–524 |
| GCSPR2 | F: GCAGCCATTTTGCTGTCATTC<br>R: CCATCTGTCTGTCTTTCTGTCTG | 3.5 | 59C | 4 | 11 | 167–207 |
| GCSPR3 | F: ACCCAAAGTGCAAATCCCATC<br>R: ACAAAGTCCCGTTTTCCTTGC | 3.5 | 59C | 4 | 9 | 252–288 |
| GCSPR5 | F: GCCAAACTCAGTGACCTGC<br>R: AGTTCCTGCACGGTTCTTC | 2.5 | 59C | 4 | 12 | 226–274 |
| GCSPR9 | F: CCAGCCTGATTTCCCATGC<br>R: TGTTGAGCATCTCTGGAGG | 2.5 | 59C | 4 | 9 | 194–243 |
| GCSPR10 | F: TGCCAGCAACTCTGCCTC<br>R: TGAGCTTCCAGCCCTTCAG | 2.5 | 59C | 4 | 18 | 186–279 |
| GCSPR11 | F: TCCATGCTTCTGAACTGCC<br>R: ACACCTGCTTTTCCTGACTG | 3.5 | 59C | 4 | 12 | 171–215 |
| GCSPR12 | F: TAGGCAGGGACAGCAAGAC<br>R: ACTACCAGAACCAACTAGGGG | 3.5 | 59C | 2 | 18 | 221–261 |
| GCSPR14 | F: AGAATGCTCTGGAACCGGC<br>R: AGGACCTGTGTGCCAATTAAG | 3.5 | 59C | 4 | 13 | 188–244 |
| GCSPR15 | F: CAGCCTGTTCCATGCATCC<br>R: GCTCGGTTCTTGCTCACAG | 2.5 | 59C | 4 | 10 | 186–230 |
| GCSPR16 | F: AACCAAGCCACCACAATGC<br>R: GACAGGCACTAGGATGGGAG | 3.5 | 59C | 4 | 12 | 230–278 |
| GCSPR17 | F: GCCACGTTACACATCCTGC20<br>R: CTGGCATTCCAAAGCTGGG | 2.5 | 59C | 4 | 16 | 200–288 |
| GCSPR19 | F: ACTGTTCTTTTCTCCACCCAC<br>R: GGTTGAATCCCAGGTGGAAAC | 2.5 | 65C | 4 | 12 | 172–216 |
| GCSPR30 | F: TGTCCTCCCCCTGTTTGTTTTA<br>R: ATTGTCCCCACTGCATCCTTCA | 1.5 | 59C | 3 | 13 | 313–352 |