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Napper, C.J. and Hatchwell, B.J. orcid.org/0000-0002-1039-4782 (2016) Social dynamics in nonbreeding flocks of a cooperatively breeding bird: causes and consequences of kin associations. Animal Behaviour, 122. pp. 23-35. ISSN 0003-3472

https://doi.org/10.1016/j.anbehav.2016.09.008

Social dynamics in non-breeding flocks of a cooperatively breeding bird: causes and consequences of kin associations

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Word count (main text only): 9111

Word count (text + references): 11070

Kin selection is regarded as a key process in the evolution of avian cooperative breeding, and kinship influences helper decisions in many species. However, the effect of kinship on non-breeding social organisation is still poorly understood despite its potential fitness implications. Here, we investigated the origins and consequences of kin associations in non-breeding flocks of long-tailed tits *Aegithalos caudatus*, an atypical cooperative breeder where helpers are failed breeders that redirect care towards relatives living in kin neighbourhoods. We found that kinship is an important factor in initial grouping decisions; all members of a nuclear family initially joined the same flock and failed breeders chose to flock with their relatives. Flocks that merged during the non-breeding season also contained relatives. In contrast to these findings of positive kin association, when long-tailed tits switched flocks they tended to disperse into flocks with fewer relatives, although such switches often occurred with kin. In a playback experiment, we found no evidence that aggression shown towards members of other flocks was affected by kinship, indicating that kin associations result from a preference to flock with relatives rather than a constraint on flocking with non-relatives. Finally, using social network analysis, we show that fine-scale non-breeding associations among individuals were positively related with kinship, and that these non-breeding associations were reflected in helping decisions in the subsequent breeding season, in addition to the previously reported effects of kinship and proximity. We conclude that long-tailed tits prefer to associate with kin when not breeding, and suggest that by doing so they gain either nepotistic benefits within flocks or future indirect benefits during breeding.

Keywords: Aegithalos caudatus; cooperative breeding; helper; kin neighbourhood; kin selection; long-tailed tit; relatedness; social network; winter flock.

Cooperatively breeding birds typically live in groups that include close relatives (Hatchwell, 2009; Riehl, 2013) and kin selection is generally regarded as a major driver of helping behaviour (Dickinson & Hatchwell, 2004; West, Griffin, & Gardner, 2007). Indeed, an effect of kinship on the alloparental investment of helpers has been extensively documented (e.g. Curry, 1989; Dickinson, 2004; Wright, McDonald, te Marvelde, Kazem, & Bishop, 2010), but much less is known about the effect of kinship on social interactions in contexts other than breeding, possibly because interactions outside the breeding season are often deemed to be less important in the evolution of sociality. Nevertheless, kin-based winter sociality has been suggested to distinguish species that breed cooperatively from those that do not (Ekman, 1989; Noske, 1991), and to act as an intermediate stage in the transition from asociality to cooperative breeding (Drobniak, Wagner, Mourocq, & Griesser, 2015). Furthermore, individuals that delay dispersal to associate with close kin during the winter have been shown to derive nepotistic benefits relative to immigrants in western bluebirds *Sialia mexicana* (Dickinson, Euaparadorn, Greenwald, Mitra, & Shizuka, 2009; Dickinson, Ferree, Stern, Swift & Zuckerberg 2014) and Siberian jays *Perisoreus infaustus* (Ekman, Bylin, & Tegelstrom, 2000). However, few other studies have examined the role that relatedness plays in social interactions both within and between groups outside of the breeding season, even though such interactions may have important consequences for the cooperative behaviour of individuals during subsequent breeding events.

Most cooperatively breeding bird species spend the non-breeding season in stable family groups that form when mature offspring delay dispersal and remain on their parents' territory (Covas & Griesser, 2007; Ekman, Hatchwell, Dickinson, & Griesser, 2004; Emlen, 1982). Helping can also occur within extended family networks or 'kin neighbourhoods' (Dickinson & Hatchwell, 2004). In such systems, non-breeding group membership is often less stable than in typical cooperative breeders, with individuals dispersing between groups and groups merging or disbanding. Dispersive behaviour is generally thought to disrupt the kin structure of a population (Emlen, 1997; Gardner & West, 2006; Perrin & Goudet, 2001), raising the question of how kin neighbourhoods develop in dispersive species. Several potential mechanisms exist, the most obvious of which is localised natal dispersal that results in relatives living in close proximity to each other (Dickinson et al., 2009; Preston, Briskie, Burke, & Hatchwell, 2013; Sharp, Simeoni, & Hatchwell, 2008). There is also growing evidence for the coordinated dispersal of kin in cooperative breeders (e.g. Koenig, Hooge, Stanback, & Haydock, 2000; Pollack & Rubenstein, 2015; Ridley, 2012; Williams & Rabenold, 2005), including among species that help within kin neighbourhoods (Sharp, Baker, Hadfield, Simeoni, & Hatchwell, 2008). Of course, there may also be costs of associating with relatives, especially if it entails kin competition over resources (Griffin & West, 2002) or a risk of inbreeding (Pusey, 1987). Therefore, for certain categories of individuals, such as juveniles of one particular sex, there may also be countervailing selection for dispersal away from relatives, resulting in sex-biased natal dispersal (Greenwood, 1980). Alternatively, inbreeding may be avoided via active discrimination against kin as social or sexual partners within kin neighbourhoods that comprise male and female relatives (Dickinson, Akçay, Ferree & Stern 2016; Riehl & Stern, 2015).

Besides the immediate benefits of living alongside relatives (Ekman et al., 2004), if helping decisions are influenced by familiarity as well as kinship, non-breeding social associations in kin neighbourhoods may also have important fitness consequences during subsequent breeding events. Within kin neighbourhoods, individuals may interact with both relatives and non-relatives, so that shared group membership may be insufficient for effective kin discrimination, even though this may be needed for individuals to maximise their inclusive fitness (Cornwallis, West & Griffin, 2009). The effects of kinship and familiarity on helping behaviour are hard to distinguish in species that live in discrete family groups because helpers are usually both related to and associated with any potential recipients of their help. However, distinguishing the effects of these factors may be more straightforward when helping occurs within kin neighbourhoods comprising both kin and non-kin (e.g. Kraaijeveld & Dickinson, 2001; McGowan, Fowlie, Ross, & Hatchwell, 2007).

We investigated the origins and consequences of kin associations in non-breeding flocks of long-tailed tits, *Aegithalos caudatus*. Long-tailed tits have a kin-selected cooperative breeding system where redirected helping occurs within kin neighbourhoods. Birds do not delay dispersal or breeding to help; instead, at the beginning of each breeding season all birds attempt to breed in pairs. Helpers are breeders whose own nests have failed and whose care is redirected to the brood of another pair. Helping is typically kin-directed and usually occurs between brothers, although a small minority of helpers are female and a small proportion care at the nests of non-relatives (Hatchwell, Gullett, &

Adams, 2014; Nam, Simeoni, Sharp, & Hatchwell, 2010; Russell & Hatchwell, 2001). Following breeding, long-tailed tits form mixed-sex flocks usually comprising 5-25 birds. Flock members forage together during the day and roost together in linear huddles at night, thereby gaining thermoregulatory benefits (Hatchwell, Sharp, Simeoni & McGowan, 2007). Flocks occupy large non-exclusive ranges that typically contain both adults and juveniles from multiple families as well as unrelated immigrants that disperse between flocks during their first winter. These immigrants include both sexes, although in our study population the majority are females due to female-biased natal dispersal (Sharp, Simeoni, McGowan, Nam & Hatchwell, 2011). Most birds flock with at least one close relative ($r \geq 0.25$) during the non-breeding season (Ezaki, Miyawaza & Sakikawa, 1991; Hatchwell, Anderson, Ross, Fowlie, & Blackwell, 2001; McGowan et al., 2007), but how these patterns of kinship arise is not well understood. Likewise, while it is known that the ranges of related flocks overlap more than those of unrelated flocks (Hatchwell, Anderson et al., 2001), and that siblings often disperse together (Sharp, Baker, et al., 2008; Sharp, Simeoni, et al., 2008), flock membership is not fixed with both adults and juveniles switching between flocks, and flocks coalescing or disbanding through the non-breeding season; the influence of relatedness on these flock mergers and switches has not been examined. Finally, although several factors determining a failed breeder's propensity to help have been described previously, including condition (Meade & Hatchwell, 2010), relatedness (Russell & Hatchwell 2001) and date (MacColl & Hatchwell 2002), the potential influence of prior association during the non-breeding season is unknown.

In this paper, we first examined whether flock membership, flock mergers and flock switches were influenced by the relatedness of flock members. We then used a playback experiment to test whether observed kin associations were caused by differential aggression towards unrelated intruders. Third, we used social network analysis to study the effect of kinship on fine-scale social interactions among individual birds. Finally, we investigated the effect of these associations on helping behaviour in the following breeding season.

METHODS

Study System

Field Observations

We have studied a population of long-tailed tits occupying a 3 km^2 site in the Rivelin Valley, Sheffield, UK (53°23'N, 1°34W) since 1994. At the start of each breeding season, pairs (mean = 49 *per annum*) were located and colour-ringed (>95% of all adults are ringed by the end of each breeding season). Long-tailed tits are single-brooded; their nests were found by following pairs and subsequently monitored closely until they failed or broods fledged. During the nestling period, nests were observed for approximately one hour every other day and the identities of all provisioning adults (parents and any helpers) recorded. Nestlings in accessible nests were colour-ringed when 11 days old. Flocks were observed during the non-breeding seasons of 1996-1997 (October-March, 35 observation days), 1997-1998 (May-February, 56 days), 1998-1999 (May-February, 57 days), 2011-2012 (May-March, 87 days) and 2012-2013 (May-March, 80 days). Flocks were followed for up to 4h, until contact was lost or until all birds in the group were identified. All flock members could rarely be identified in a single observation period, so we assumed that sighting of two or more known flock members was a reliable indicator of flock identity. Flock size was defined as the number of ringed birds in each flock. This is a minimum estimate because most flocks also contained a small number of unringed immigrants that dispersed into our study site in their first winter. Flock position was recorded every two minutes on to large-scale maps (scale 1 cm: 50 m) in 1996-1999, and every minute using a Garmin Geko 201 GPS in 2011-2013. Map registrations were converted to map coordinates for analysis at a resolution of 10 m.

Pedigree Construction

We used social pedigrees derived from 19 years of field observations to estimate dyadic relatedness among individuals in our population. Long-tailed tits can use social pedigree information provided by calls that they learn from carers to recognise kin (Sharp, McGowan, Wood, & Hatchwell, 2005), and these cues provide a reliable estimate of genetic relatedness because brood parasitism and extra-pair paternity are infrequent (Hatchwell, Ross, Chaline, Fowlie, & Burke, 2002). A cross-fostering experiment (*N* = 9 broods) produced seven cross-fostered recruits in 1996 – 1998 (Hatchwell, Ross, Fowlie & McGowan, 2001), these were considered as relatives of their foster family rather than their biological family. Dyads were classified as first-order relatives (*r* = 0.5), second-order relatives (*r* = 0.25), third order relatives (*r* = 0.125) or unrelated (*r* = 0). Social pedigrees were inevitably incomplete because our population is open, with immigrants dispersing into the study site during their first winter. Therefore, we performed sibship reconstructions for these immigrants as well as parentage analyses for all birds that were first ringed as adults, as detailed below.

Blood samples (approx. 10µl per bird) were taken from 84.8% of observed flock members (*N* = 495) by brachial venipuncture under UK Home Office Licence. Genomic DNA was extracted from blood, amplified and genotyped at twenty microsatellite loci: Ase18, Ase37, Ase64, Hru2, Hru6, LOX1, Pca3, Pma22, Ppi2, CAM01, CAM03, CAM15, CAM17, CAM23, P2DP8, Pca4, Tgu_01-040, Tgu_04-012, Tgu_05-053 and Tgu_013-017; mean number of alleles = 14.6, range = 2 – 53 (Adams, Robinson, Mannarelli, & Hatchwell, 2015; Simeoni et al., 2007). Birds were sexed using standard molecular genetic techniques (Griffiths, Double, Orr, & Dawson, 1998).

The great majority of birds ringed as adults were likely to be immigrants, but some may have fledged unringed from inaccessible nests in the study site. To ensure that we did not misclassify these birds as immigrants, we performed a parentage analysis using the likelihood approach in CERVUS v. 3.0 (Kalinowski, Taper, & Marshall 2007). Allele frequencies were estimated using all genotyped individuals in all years (1994-2012, *N* = 2755) to maximise accuracy in estimating the frequency of rare alleles and to ensure non-zero allele frequencies. For each year, CERVUS was run with the following simulation parameters: 100,000 offspring, the true number and sampled proportion of candidate parents, 96.3% loci typed (calculated from allele frequency data), 10 as the minimum number of loci typed and a mistyping rate of 0.01. We classified all birds that were first ringed as adults as ‘offspring’ and all breeders and helpers from the previous year as ‘candidate parents’; this is justified because natal dispersal in long-tailed tits occurs during an individual’s first year (McGowan, Hatchwell, & Woodburn, 2003; Sharp, Baker, et al., 2008) so any immigrants in a given year are likely to have hatched in the previous year. We then carried out parentage analyses with known sexes, but no prior information about how the sexes were paired, using critical LOD scores for 99% confidence derived from the simulations. To ensure the reliability of parentage assignments in our pedigree matrix, parent-offspring relationships were considered to be genuine only if the parents were known to have been paired and to have fledged unringed chicks in the year in question. This resulted in 12 (2.4%) of the birds we observed in flocks being assigned to a natal nest within the study site (one bird from 1997-1998 flocks, seven birds from three nests in 2011-2012 flocks and four birds from two nests in 2012-2013 flocks).

Long-tailed tits often disperse with relatives (Sharp, Simeoni, et al., 2008), so to determine whether sibling groups existed among immigrants we reconstructed sibships using KINGROUP v.2 for all years from 1994 to 2012. All immigrants in a given year were considered to be potential siblings and we tested for the presence of full siblings using the ‘descending ratio’ algorithm (Konovalov et al., 2004), and the allele frequencies used in the parentage analysis described above. The results from sibship reconstruction were considered to match those of likelihood ratio tests if the score for a dyad was $P < 0.05$. Sibling relationships were added to our social pedigrees only if all siblings in a given group were mutually significantly matched in the analysis. This resulted in two sibgroups in flocks in 1996-1997 (two groups of two birds), three sibgroups in 1997-1998 (three groups of two birds), four sibgroups in 1998-1999 (four groups of two birds), eight sibgroups in 2011-2012 (five groups of two birds, one group of three birds and two groups of four birds) and four sibgroups in 2012-2013 (three groups of two birds and one group of seven birds). These sibgroups were in addition to those sibgroups assigned to nests within the study site in the parentage analysis.

Flock Dynamics

Flock Membership

To investigate whether kinship influenced flock membership, we first recorded whether all individuals associated with a successful nest (parents, offspring and any helpers) initially joined the same flock. Secondly, we investigated whether failed breeders that did not become helpers joined flocks containing relatives. To do this, we determined how many nearby flocks were available for a failed breeder to join on the day they were first observed in a flock; flocks were considered nearby if they were within the maximum distance (1260 m) travelled by any failed breeder to join a flock from their last known breeding attempt. We fitted generalised linear mixed models (GLMMs) with a binomial error structure and logit link function using the ‘lmer’ function in the R package ‘lme4’ (Bates & Maechler, 2010) to determine the role of kinship in flock-joining decisions. Whether or not a flock was ‘joined’ (1 or 0) was the response term; ‘relatedness’, ‘distance’ and an interaction between these two variables were fitted as predictor variables, and the ‘ID’ of the failed breeder was fitted as a random term. ‘Relatedness’ was defined as the proportion of each flock that was related to the failed breeder at three levels ($r = 0.5$, $r \geq 0.25$ and $r \geq 0.125$) calculated using pedigree information supplemented by parentage and sibship analyses (see above). ‘Distance’ was the distance between a bird’s last breeding attempt and the location of the flock on the day that they were first observed in it. Failed breeders were included only if they did not help, had a choice of nearby flocks to join, and were first observed in a flock in May or June of 1997, 1998, 2011 or 2012 so that only initial flocking decisions were considered. Lastly, if a failed breeder appeared in the dataset in multiple years, we used the observation from their first year only.

From the three global models (one for each measure of relatedness), we generated candidate models containing all possible combinations of the predictors. These were then ranked using AICc and models within two AICc of the top model were deemed to be supported. If multiple models were supported, a final model was obtained by averaging parameter estimates according to their Akaike weights (Burnham & Anderson, 2002) using the R package ‘MuMIn’ (Barton, 2012). The relative importance of each explanatory variable in the final model was calculated as a sum of all the model weights in which that variable appears, so that if a variable appeared in all models it would have a relative importance of one. All statistical analyses were carried out in the R environment, v 2.12.0 (R Development Core Team, 2010).

Flock Mergers

Two flocks were considered to have merged if they joined to form a permanent new flock, with no members remaining in their original flocks. Flocks occasionally coalesced briefly as they foraged in a shared part of their ranges, before splitting again; these ‘temporary mergers’ were excluded from analysis. All flock mergers occurred soon after broods fledged, when unringed birds were rare in the study site, so flock size was defined here as the total number of birds in each flock on the merger date, including any unringed birds. To investigate the effect of kinship on flock mergers, we compared the relatedness (at three levels: $r = 0.5$, $r \geq 0.25$ and $r \geq 0.125$, as above) of neighbouring flocks that merged with those that did not. If an individual was related to multiple birds in another flock, we defined relatedness from its closest relative in that flock. Flocks were considered to be neighbours if they were within 480m (the greatest observed distance between flocks that merged) on the merger date and if no other flocks occupied the space between them. Too few flock mergers were observed for statistical analysis.

Flock Switches

Individuals observed in multiple flocks over the course of the non-breeding season were considered to have switched flocks. Direct observations of flock switches were rare, so switches were assumed to have occurred on the midpoint between the dates the bird was last observed in their original flock and first identified in a new flock. Birds that switched between the same two flocks within a month of each other were considered to have switched together, unless they were known to have switched at different times. Occasionally, birds switched back and forth between the same two flocks, remaining in the new flock only for a short period of time (median duration of temporary switches = 23.5 days, range = 3 - 149; note that this is the maximum duration of switches and most birds that switched temporarily were observed in their new flock only once). These ‘temporary switches’ were excluded from analysis. If a bird switched flocks more than once during the study, we used only their first switch; this excluded 9 birds that switched flocks in more than one season. To determine whether flock switches were influenced by kinship, we compared a bird’s relatedness to members of its original flock and to its new flock on its switch date using Wilcoxon signed-rank tests in R (R Development Core Team, 2010). Relatedness was defined as the proportion of each flock that was related to the individual at three levels ($r = 0.5$, $r \geq 0.25$ and $r \geq 0.125$), as above. Flock size was defined here as the total number of ringed birds in each flock on the switch date. Tests were performed using all switches, and also separately for each sex, age class (adult or juvenile) and status in the previous breeding season (successful breeder, failed breeder or helper).

Playback Experiment

A playback experiment was used to investigate behaviour involved in flocking decisions, specifically testing whether the response of flocks towards the calls of non-flock members was influenced by kinship. We used the short-range 'churr' call that is often produced when long-tailed tits meet an unfamiliar flock or individual. This call is highly individual-specific (Sharp & Hatchwell, 2005) and during breeding pairs react less aggressively towards the playback of churr calls of relatives than those of non-relatives (Sharp et al., 2005). Churr calls were recorded from individually marked breeding adults close to their nests in 2011 and 2012 at a distance of <15 m. Recordings were made onto a Tascam DR-100 with a sampling frequency of 44.1 kHz and 24-bit accuracy using a Sennheiser MKH60P48 microphone fitted with a standard basket windshield and windjammer. Spectograms were produced for all recordings using Avisoft SASLab Pro (version 5.1.20) and the 'cleanest' churr call was selected for each individual. All selected calls were of similar amplitude to each other and to those produced in the field. A highpass frequency filter of 1kHz was applied to minimise background noise without altering the minimum frequency of calls. A 1-minute sequence of 36 randomly spaced copies of the call was then created and this sequence was looped five times to create a 5-minute sequence for playback.

Playback experiments were conducted during the non-breeding season of 2011-2012 (3 trials) and 2012-2013 (5 trials). Focal flocks were located and each experienced two treatments: the churr calls of a relative ($r \geq 0.25$ to at least one flock member; mean number of flock members bird was related to = 3.375, range = 1 - 6), and the churr calls of a randomly selected non-relative ($r < 0.125$ to all flock members). Relatedness was determined using social pedigrees. All calls used for playbacks were from birds that were alive but living in a different flock to the one subject to playback. Trials consisted of a control period of five minutes of no playback followed by five minutes of playback. Calls were broadcast using an iPod touch and an X-mini II portable speaker, and all flocks were within 20 m of the speaker at the start of playback. To measure flock responses, we recorded the closest approach to the speaker, the time spent within 20 m of the speaker, and the rate of churr and triple calls (a non-aggressive contact call) during the whole trial and during playback. Treatment order was randomised and the observer, who stood >20 m from the speaker, was blind to which treatment was taking place. Trials of kin and non-kin playback to a focal flock took place a week apart.

Wilcoxon signed-rank tests were used to determine whether flocks responded differently to calls of relatives and non-relatives. The difference between treatments in closest approach to the speaker, time spent within 20 m of the speaker and net vocal response per flock member (the difference in call rate between the control and playback periods when controlled for flock size) was used to examine responses. There was no significant difference between years so data were pooled for analyses. All analyses were performed in R version 2.12.0 (R Core Development Team, 2010).

Analysis of Individual Associations

Social Network Analysis

Direct physical interactions between identifiable individuals are rarely observed, so two birds were considered associated if they were seen together in the same flock on the same day. This ‘gambit of the group’ (Franks, Ruxton & James, 2010) is appropriate for this species because all flock members forage and roost together so the assumption that each individual is associated with every other individual is met. Weighted association indices were calculated for each dyad using the half weight index (HWI) in the program SOCPROG 2.4 (Whitehead, 2009). This index was chosen because it is the most appropriate when individuals are located infrequently, creating a bias in favour of sighting only one individual from a dyad in a given sampling period (Cairns & Schwager, 1987). The HWI is defined as:

$$\mathrm{HWI} = y_{ab}/(y_{ab} + y_0 + 0.5(y_a + y_b)),$$

where y_{ab} = number of sampling periods in which both bird ‘a’ and bird ‘b’ were seen in the same group, y_a = number of sampling periods in which bird ‘a’ was observed but bird ‘b’ was not, y_b =

number of sampling periods in which bird ‘b’ was observed but bird ‘a’ was not and y_0 = number of sampling periods in which birds ‘a’ and ‘b’ were seen in different groups. Only birds present in the study site for the entire non-breeding season were included in the weighted network to ensure that mortality/dispersal did not affect analyses. Birds were also excluded from the weighted network if they were observed on fewer than three sampling days (14 birds in 1996-1997, 8 birds in 1997-1998, 2 birds in 1998-1999, 19 birds in 2011-2012 and 17 birds in 2012-2013) because rarely observed birds tended to have low maximum association strengths.

To determine whether dyads associated more or less frequently than expected by chance, we compared the observed weighted association matrix with randomly permuted association matrices using the SOCPROG program. Random observations of individuals in different groups were swapped so that the total number of groups each individual was seen in and the number of individuals in each group were kept constant (Bejder, Fletcher & Bräger, 1998; Manly, 1995). Association matrices were permuted sequentially beginning with the observed matrix and so were not independent. Therefore, we performed 1,000 permutations each containing 1,000 trial flips (after which *P*-values stabilised to within 0.01) to assess the significance of the differences in the standard deviation (SD) and coefficient of variance (CV) between the observed matrix and the permuted matrices. We also assessed the significance of the difference in the proportion of non-zero edges (i.e. associations) in the observed matrix and permuted matrices. Significantly higher SD and CV of real data compared to random data indicates preferred associations and a lower proportion of non-zero indices in the observed data compared to the permuted data indicates that individuals avoid each other. *P*-values were calculated based on the proportion of permuted CV or SD values that are lower than the observed values, for example, $P = 0.70$ indicates that the real value is larger than 70% of the random ones. If the real value fell within the top or bottom 2.5% of the random distribution ($P > 0.975$ or $P < 0.025$), we rejected the null hypothesis that the real value could have arisen by chance.

Social Association and Relatedness

To determine whether social association was influenced by kinship, we examined the significance of correlations between matrices of dyadic association and dyadic relatedness using

Mantel tests with 1,000 permutations in SOCPROG. This approach was used to investigate associations among all birds, between males, between females (although there were too few females in our networks to perform this test in 1996-1997) and between the sexes. We repeated these tests while controlling both measures for a third matrix of the distance between nests in the previous breeding season to control for an effect of philopatry causing related individuals to live in close spatial proximity and therefore to be more likely to be associated by chance.

Social Association and Helping

To study the effect of non-breeding associations on helping decisions, we first identified helpers that (a) were in the weighted network in the winter prior to helping, (b) helped at a nest at which at least one member of the breeding pair was also in the network, and (c) had a choice of active nests (i.e. contained nestlings on the day they started helping) where at least one member of each pair was in the network. If a helper helped at multiple nests in the year in question, we considered only their first helping decision; 5/22 helpers considered in this analysis helped at a second nest after their first choice nest had been depredated. We fitted generalised linear mixed models (GLMMs) with a binomial error structure and logit link function using the 'lmer' function in the R package 'lme4' (Bates & Maechler, 2010) to determine whether association in the previous winter influenced which pair the helper chose to assist. Whether a nest was 'helped' or not was used as the response term in this analysis; 'association strength', 'kinship' and 'distance' were fitted as predictor variables and 'helper ID' was fitted as a random effect. Association strength was defined here as the association index between the potential helper and the most closely associated member of a breeding pair; kinship was defined as the relatedness between the potential helper and the brood; and distance was measured between the helper's last known breeding attempt and the nest in question. From this global model we generated a set of candidate models containing all possible combinations of the predictor variables which were then ranked using AICc. There were no models within two AICc of the top model so we calculated parameter estimates from this model (Burnham & Anderson, 2002). GLMM analysis was carried out in R v2.12.0 (R Development Core Team, 2010). Finally, we used Mantel tests with 1000 permutations to assess the correlation between the distance between two individuals' nests and their association strength in the previous non-breeding season's social network.

RESULTS

Flock Dynamics

The number of flocks in our study area varied within and between years. In 1996-1997, there were just four flocks (containing 63 ringed birds) that retained their separate status from October to March. In 1997-1998, two of the six initial flocks merged shortly after fledging, leaving five flocks ($N = 93$ birds) that remained distinct for the rest of the season. Likewise, in 1998-1999, two of 11 flocks observed at the start of the non-breeding season merged soon after fledging, leaving 10 flocks ($N = 117$ birds) that persisted until March. The study population was generally larger during the second period of non-breeding observations: in 2011-2012, there were initially 20 flocks, but mergers in early June resulted in 13 flocks ($N = 177$ birds) that retained separate status for the rest of the year. In 2012-2013, following mergers and one disbandment, 13 initial flocks ($N = 156$ birds) became 10 flocks that remained distinct until the following season. Average flock size across all years was 16.45 ± 10.6 SD ($N = 42$; range = 2 - 42) ringed birds (this is a minimum flock size due to the presence of some unringed birds). The non-breeding ranges of flocks that remained after flocks had merged or disbanded (Fig. 1) illustrate the extensive overlap in flock home ranges previously described by Hatchwell, Anderson et al. (2001).

Flock Membership

At the end of each breeding season, all birds associated with a successful nest (parents, offspring and any helpers) flocked together (with one exception in 2011 where the female and helpers joined a different flock to the male and fledglings following disruption caused by depredation of some offspring during fledging). Typical fledged brood size is 7.8, so this means that juveniles usually had several first-order relatives in their initial flock. Failed breeders either joined a family group or formed a new flock if there were no families nearby. The average distance between the last breeding attempt of a failed breeder and the flock it joined was 373 m (range = 40 – 1260 m, $N = 90$; five failed breeders were observed joining flocks in two years, but appear only once in the GLMM analyses). Most failed breeders (78.8%, $N = 85$) had a choice of flocks within a range of 1260 m, and they generally joined closer flocks (Table 1, Fig. 2a). Failed breeders were also most likely to join a flock when they were more closely related to its members (Table 1, Fig. 2b). This was true for all three levels of relatedness, but kinship had the strongest effect on the flock membership decisions of failed breeders when only first-order relatives ($r = 0.5$) were considered (Table 1). There was also an interaction between distance and kinship; birds were most likely to join flocks with their relatives if they joined closer flocks, probably due to the effect of philopatry (Table 1). This apparent preference for flocking with relatives resulted in 77.8% ($N = 36$) of failed breeders with a relative present in a nearby flock joining a flock containing at least one relative ($r \geq 0.125$). The remaining 49 failed breeders had no nearby relatives and hence joined flocks with non-kin ($r < 0.125$).

Flock Mergers

We observed nine mergers between eight pairs and one trio of flocks. Five mergers occurred between flocks with members related at the level of $r = 0.5$, eight occurred between flocks related at $r \geq 0.125$ and just one was between unrelated flocks. Of the 19 flocks involved in mergers, only seven had a choice of neighbouring flocks within the maximum distance of 480 m. The mean ± SD relatedness of focal flocks to the flock they merged with (as measured by the proportion of the flock that were relatives at the $r \geq 0.125$ level) was 0.638 ± 0.377 ($N = 7$) and their relatedness to the flocks they did not merge with was 0.429 ± 0.437 ($N = 7$). The small sample size precludes further analysis.

Flock Switches

Most birds remained in the same flock for the whole of the non-breeding season; 85.1% of all birds ($N = 604$) were observed in one flock only (although some of these will have died or dispersed from the study site at some stage). We observed 147 switches, but after exclusion of temporary switches and repeated switches by the same bird, we analysed 69 switches between non-breeding flocks. Across all years, there was no significant difference in the probability of switching according to sex (females 15.3%, $N = 274$; males 10.7%, $N = 327$; chi-square test: $\chi^2_1 = 2.55$, $P = 0.12$), or age (adults 13.7%, $N = 342$; juveniles 11.9%, $N = 236$; $\chi^2_1 = 0.286$, $P = 0.59$). In addition, the probability of switching was not significantly associated with the status of adults at the end of the previous breeding season (successful breeders 15.6%, $N = 96$; failed breeders 13.4%, $N = 164$; helpers 14.0%, $N = 43$; chi-square test: $\chi^2_2 = 0.24$, $P = 0.89$). Flock switches took place throughout the non-breeding season, although they were most common soon after fledging (Fig. 3a). This pattern of flock switches is similar to the timing of disappearances of ringed birds from our study population during the non-breeding season (Fig. 3b), although the latter must include mortality as well as dispersal events.

In general, birds switched to flocks containing fewer relatives (Table 2). The proportion of first-order relatives in a flock had a greater influence on an individual's switching decision than the proportion of relatives with $r \geq 0.25$ or 0.125 in a flock. Closer scrutiny of flock switches showed that juveniles tended to switch flocks to one containing fewer opposite-sex relatives, but only when considering first-order kin (Table A1). Finally, any sex differences in switching behaviour in relation to kinship were of marginal significance, but males ($r = 0.5$) moved to flocks where they were less closely related to other birds (Table 2, Table A1). The sex ratio of ringed birds in original and destination flocks did not differ significantly (Table A2), suggesting that flock switches were not driven by a lack of potential mates in the current flock.

Individuals moved between flocks together in 57.1% ($N = 147$) of all observed switches, with a mean ± SD group size of 2.8 ± 1.69 birds ($N = 30$ groups; range = 2 - 9). Males (65.6%, $N = 32$) and females (56.8%, $N = 37$) were equally likely to switch flock in a group (chi-square test: $\chi^2_1 = 0.26$, $P = 0.613$), but juveniles (76.9%, $N = 26$) tended to switch flocks with others more often than adults (51.2%, $N = 43$; chi-square test: $\chi^2_1 = 3.50$, $P = 0.061$). Of the birds that switched flock in a group, 61.9% ($N = 42$) did so with at least one first-order relative and 71.4% ($N = 42$) switched flock with at least one relative ($r \geq 0.125$). In these group switches, birds did not preferentially switch with relatives from their flock of origin; indeed, for those birds that switched permanently, relatedness to birds they switched flock with (mean $r = 0.146 \pm 0.136$ SD, $N = 42$) was significantly lower than their relatedness to all members of their flock of origin (mean $r = 0.228 \pm 0.206$ SD, $N = 42$; Wilcoxon Test $V = 85$, $N = 42$, $P < 0.001$). Switching groups typically included both sexes (18/30; 60%), the remainder being all male (26.7%) or all female (13.3%) groups. When siblings dispersed together ($N = 14$ sibling groups) there was no indication of sex-bias either; seven sibling groups were mixed sex and seven were single sex (five male, two female).

Playback Experiments

In all trials, flocks responded immediately to the start of playback by approaching the speaker (mean ± SD closest approach = 8.31 ± 4.69 m, $N = 16$ trials, 8 flocks), but often lost interest and resumed foraging while the playback was still being broadcast (mean ± SD duration <20 m from the speaker = 194 ± 114 s, $N = 16$ trials). Flocks also responded aggressively, with more churr calls (Wilcoxon signed-rank test: $V = 1$, $N = 16$ trials, $P < 0.001$) and fewer triple calls ($V = 105$, $N = 16$, $P = 0.011$; Fig. 4) relative to the control period. Thus, there was a rapid, aggressive, but short-lived response to the simulated presence of a non-flock member. However, there was no differential response towards calls of relatives versus non-relatives; the flock's vocal response to playback, the closest approach to the speaker and the time spent within 20m of the speaker did not differ significantly between the two treatments (Table 3).

Individual associations

We observed a total of 606 birds in flocks across all years, but only those present throughout a non-breeding season and observed on at least three occasions were included in analyses, giving a sample of 164 birds in weighted networks (15 in 1996-1997, 34 in 1997-1998, 44 in 1998-1999, 53 in 2011-2012, and 18 in 2012-2013). Unsurprisingly, given their flocking behaviour, long-tailed tits exhibit significant pairwise associations and avoidances. In all years, the CV and SD of observed association indices were higher than those of at least 99.9% of random permutations, and the number of non-zero edges in observed networks was significantly lower than in random networks (Table 4). On average, individuals had 8.22 associates (*N* = 164 birds, range = 0 - 18) and the average association strength between associated birds was 0.394 (*N* = 673 links, range = 0.059 - 1.000).

Social Association and Relatedness

Across all years, 69.5% (*N* = 164) of birds had a relative ($r \geq 0.125$) in the population. Relatedness and social association were closely linked, with the pedigree relatedness matrix and half weight association matrix being significantly correlated in all years (Fig. 5, Table 5). Overall, the mean ± SD association strength of relatives ($r \geq 0.125$) was 0.406 ± 0.289, while for non-relatives ($r < 0.125$) it was 0.061 ± 0.160. Mean ± SD association strength between first-order relatives (0.513 ± 0.243) was much higher than for second (0.118 ± 0.213) or third-order relatives (0.124 ± 0.143), suggesting that the link between social networks and kinship is driven mainly by the association of close kin. When analysing the sexes separately, the strength of links between males, between females and between the sexes increased with kinship, with the exception of males in 1996-1997 and females in 2011-2012 (Table 5). These patterns persisted when both matrices were controlled against a third matrix of distances between last known nests in the previous breeding season, although correlations among females tended to be either non-significant or weaker than those for males (Table 5).

Social Association and Helping

In the breeding seasons following our observations of non-breeding flocks, 26 birds from our weighted networks helped at a nest where at least one member of the breeding pair was also in the network; 84.6% (*N* = 26) of these helpers assisted a bird they had previously been associated with. However, 75.3% (*N* = 73) of failed breeders that we had observed in networks did not help even though they had an associate in the population with an active nest after their own nest had failed. Furthermore, 31.5% of these non-helpers were related to that associate, indicating that the presence of an associated relative with an active nest does not necessarily lead to helping. We also observed eight failed breeders helping an unrelated pair, six (75%) of which were known to be associated with at least one member of that pair in the previous non-breeding season. Finally, 22 helpers in our weighted networks had a choice of previous associates to help. GLMM analysis showed that association strength was a significant predictor of which associate they chose to help (Fig. 6), as was relatedness to the brood and the distance from the helper's last breeding attempt; birds were most likely to help associated relatives whose nests were closest to their own (Table 6).

DISCUSSION

The non-breeding flock dynamics of long-tailed tits were strongly influenced by kinship. This was evident in initial grouping decisions, where all individuals associated with a successful nest flocked together and failed breeders joined flocks containing relatives, when available. Likewise, the few mergers observed were mostly of related flocks. On the other hand, flock-switches tended to result in dispersal to flocks with fewer relatives, although it was common for birds to switch flocks in groups with their relatives, especially for juveniles. When we investigated non-breeding social interactions at the individual level using social network analysis, associations were again influenced by kinship, especially for males. A playback experiment indicated that these flocking decisions were not a function of nepotistic behaviour by members of the destination flock, because we observed no difference in flock responses to the calls of kin and non-kin. Finally, social links in non-breeding flocks were positively associated with helping decisions in the subsequent breeding season, in addition to the previously reported effects of kinship and distance.

Our finding that all individuals associated with a successful nest (parents, offspring and helpers) initially flocked together is unsurprising because adult long-tailed tits provision fledglings until they are independent, about three weeks after fledging. More interesting is that failed breeders chose to flock with relatives even if they were not associated with them in the preceding breeding season and had travelled further from their last breeding attempt to do so, indicating that kin association is not simply a function of extended parental care and philopatry. This kin association that we observed outside the breeding season replicates the kin preference of failed breeders in helping decisions (Russell & Hatchwell, 2001; Nam et al., 2010; Hatchwell et al., 2014). In both cases, kin associations are not simply a function of birds having only relatives to interact with, but rather appears to result from an active choice of relatives as associates in both breeding and non-breeding contexts. This study also confirms previous results showing a substantial proportion of unrelated birds within non-breeding flocks of long-tailed tits. Importantly, our finding that unrelated failed breeders could join a flock even though they had not helped any flock members supports McGowan et al.'s (2007) conclusion that helping is not payment for group membership in long-tailed tits.

Flock mergers mostly involved related flocks, reinforcing the kin structure that already exists due to their initial family-based formation. Long-tailed tit flocks do not occupy stable, exclusive territories during the non-breeding season and the ranges of related flocks are more likely to overlap than those of unrelated flocks (Hatchwell, Anderson et al., 2001), perhaps predisposing them to merge. Such mergers have been recorded in many species, usually in the context of fission-fusion social dynamics (Aureli et al., 2008), but the degree of group cohesion and the frequency and permanence of fission-fusion events is extremely variable across species, as is the role of kinship. In some cases, relatedness is strongly associated with such events (e.g. Archie, Moss & Alberts, 2006; Holekamp, Smith, Strelioff, Van Horn & Watts, 2012), while in others the effect of relatedness is less pronounced (e.g. Lee, Lee & Hatchwell, 2010; Wolf & Trillmich, 2008), or even absent (e.g. Arnberg, Shizuka, Chaine & Lyon, 2015; Liker et al., 2009). In the only previous study of mergers of non-breeding groups in cooperatively breeding birds, small breeding groups of apostlebirds *Struthidea cinerea* merge to form larger non-breeding flocks, but the effect of relatedness on mergers is unknown (Griesser et al., 2009).

Permanent flock switches were frequent and, in contrast to initial grouping decisions, resulted in movement to flocks containing fewer relatives. Sharp, Baker et al. (2008) reported that many birds in our population, especially females, disperse beyond the study site boundary, but mean natal dispersal distance within the study site was <500m, so many dispersers would be expected to move to adjacent flocks. The timing of switches that we observed also coincided with disappearances from the study site (Fig. 3) so switches are presumably such short-distance dispersal events. If inbreeding avoidance is a major driver of dispersal, juveniles are predicted to be more dispersive than adults (Greenwood, 1980; Pusey, 1987). We could not directly compare the frequencies of flock switches by adults and juveniles, because many juveniles disperse beyond the boundaries of our study site while adults do not, and because the mortality rate of juveniles is much higher than that of adults (Sharp, Baker, et al., 2008). However, the fact that destination flocks for juveniles that switched contained fewer relatives than flocks of origin is consistent with the idea that switches reduce the risk of inbreeding.

On the other hand, dispersal is usually assumed to dilute population kin structure, but we found two ways in which kin structure is maintained by dispersing long-tailed tits. First, they often dispersed with relatives; and second, even after dispersing and attempting to breed independently, failed breeders joined non-breeding flocks containing relatives. Several other cooperative breeders disperse in coalitions (e.g. Koenig et al., 2000; Ridley, 2012; Williams & Rabenold, 2005) and our finding that it is common for long-tailed tits to switch flock with relatives within our study site complements previous work by Sharp, Simeoni, et al. (2008) who found that related immigrants often enter our study population together. In contrast to other cooperative species where dispersing relatives are usually of the same sex, it is notable that long-tailed tits frequently switched flocks with opposite-sex relatives. Therefore, although flock switching by long-tailed tits may well be a strategy to reduce the risk of inbreeding, it does not remove that risk entirely because dispersers often moved with opposite-sex kin and destination flocks usually contained kin. Dickinson et al. (2016) have recently shown in western bluebirds, another kin neighbourhood cooperative breeder, that males pairing within or nearby their winter group risked breeding with kin but actively avoided related females as partners, implying effective inbreeding avoidance through kin recognition. A similar mechanism for inbreeding avoidance would be expected in long-tailed tits, although it has not yet been demonstrated.

We have assumed that individuals decide which flocks to join, but these decisions may also depend on interactions between existing flock members and newcomers. This idea that aggressive interactions between residents and dispersers play a major role in dispersal and settlement decisions is supported in several cooperative breeders (e.g. Kleiber, Kyle, Rockwell & Dickinson, 2007; Mares, Young, Levesque, Harrison & Clutton-Brock, 2011; Radford, 2003; Mueller & Manser, 2007), and in

Siberian jays, aggression by residents influences the probability of settlement by potential dispersers (Griesser, Nystrand, Eggers & Ekman, 2008). In our playback experiment, despite rapid and aggressive responses to the churr calls of non-group members, the flock's hostility to playback did not depend on the kinship of a subset of flock members to the simulated newcomer. This result suggests that aggression towards non-relatives does not cause kin-oriented flocking decisions in long-tailed tits, although the small number of playback experiments we were able to perform limits our confidence in concluding that such decisions are made entirely by the disperser. In western bluebirds, Kleiber et al. (2007) found that aggression towards intruders was driven by sexual competition rather than by an aggressor's relatedness to other group members. However, in our study, the large flock size and rapid movements of birds in focal flocks precluded assessment of individual responses, so we could not test whether responses were a function of individuals' relatedness to either other flock members or to the bird whose call was being played.

The apparent absence of kin discrimination in response to playback by flocks during the non-breeding season is consistent with Napper, Sharp, McGowan, Simeoni & Hatchwell (2013), who found little evidence for a kinship effect on social interactions during roost formation in captive wintering flocks of long-tailed tits. It also contrasts with the outcome of two previous playback experiments conducted during the nestling period, each showing kin discrimination with the same sample size (Hatchwell, Ross et al., 2001; Sharp et al., 2005). Why should responses be so different in breeding and non-breeding contexts? Two explanations are likely. First, cooperative interactions during breeding occur among a small number of related individuals (Nam et al., 2010), while in flocks, groups are much larger and comprise both kin and non-kin. Second, the fluid nature of flock composition, the presence of multiple families and low average relatedness, may reduce the opportunity for any particular kin group to assert dominance over immigrants. This is in sharp contrast to the situation in Siberian jays, where dispersers attempt to join small, stable family kin-groups as subordinates (Griesser et al., 2008).

The dispersal decisions that we have described lead to kin-structured non-breeding populations of long-tailed tits. Demographic traits also contribute to this kin structure, specifically the skewed reproductive success of pairs that results in a small effective population size (Beckerman, Sharp & Hatchwell, 2011). The availability of kin subsequently plays a critical role in the expression of cooperative behaviour in long-tailed tits by generating the opportunity for kin selection to operate (Hatchwell et al., 2014). However, the kin neighbourhoods within which cooperative breeding occurs are very different to the nuclear family groups that are the key social unit of typical avian cooperative breeders because most individuals with which a focal long-tailed tit associates outside breeding, and most close neighbours during the breeding season are unrelated to them. Therefore, for kin-selected helping to evolve it is important that failed breeders are able to recognise kin and discriminate in their favour when making helping decisions. The final question that we addressed, therefore, was whether interactions among individuals during the non-breeding season influence behaviour during breeding.

Each sex was more closely associated with birds of the same or opposite sex if they were related; this was particularly true of males, even after controlling for the effect of philopatry. This makes sense because dispersal in long-tailed tits is female-biased (Sharp, Baker, et al., 2008) so social bonds among related females are weaker than among males. Indeed, the finding that females are more closely associated with kin than non-kin is perhaps surprising because females are less likely to help than males, and Sharp, Simeoni, McGowan, Nam & Hatchwell (2011) suggested that this was because they are not closely associated with relatives during the non-breeding season. This is clearly not the case and our results support Sharp et al.'s (2011) alternative suggestion that females are simply less likely to help than males, perhaps because they are in poorer condition than males after breeding.

The strength of prior association was a significant factor in helper decision-making; most helpers fed at the nests of associates, and association strength was a significant predictor of who was helped even after controlling for the effects of relatedness and spatial factors. Thus, long-tailed tits behave in a similar way to more typical cooperative breeders in which helping usually occurs between familiar relatives on the same territory, but they must be more discriminating than most other species to achieve this (Cornwallis et al., 2009). However, a third of non-helpers had associated relatives in the population but did not assist them when the opportunity arose, so the presence of an associated relative nearby does not guarantee helping. Meade and Hatchwell (2010) attributed this failure to help when the opportunity arose to poor condition of these 'non-helpers'. We also found that the few helpers caring for non-kin usually helped prior associates. Long-tailed tits gain no direct benefits from helping (Meade & Hatchwell, 2010), and it is possible that help for non-kin results from recognition errors. Kin recognition in this species is based on calls learned during development (Sharp et al., 2005). If learning extends into the non-breeding season, association with non-kin could lead to categorisation of non-kin as kin. Our finding that instances of apparently misdirected care occurred among birds that were associated during the non-breeding season is consistent with this idea.

In conclusion, long-tailed tits tend to flock with their relatives, even after dispersal, suggesting either that there are benefits of flocking with kin, or costs of flocking with non-kin. Differential interactions between kin and non-kin could occur in several contexts, including anti-predator behaviours (e.g. Griesser & Ekman, 2004; Maklakov, 2002), food-sharing (e.g. Dickinson et al., 2009), and access to communal roosts (e.g. McGowan, Sharp, Simeoni & Hatchwell, 2006), although Napper et al. (2013) found little effect of kinship on dominance status that might influence these social interactions. We think it is more likely that the inclusive fitness benefits of kin-directed helping behaviour in long-tailed tits (Hatchwell et al., 2014) selects for prolonged association with kin outside the breeding season to maximise individuals' ability to recognise and discriminate in favour of relatives during breeding. Our finding that non-breeding social interactions influence cooperative breeding behaviour indicates that prior association is an important factor in helpers' investment decisions. Further studies are needed to determine whether this is common among species where helping occurs within kin neighbourhoods, or indeed in any other cooperative species where helpers have a choice of broods that they may care for.

Acknowledgments

We are grateful to all those who have contributed to the long-term data that has contributed to this study, especially Douglas Ross and Martin Fowlie who collected data on winter flocks in 1996-1999, and Ben Ellison-Smith, Angela Chira and Thomas Mondain-Monval who assisted with the playback experiment. Thanks also to landowners in the Rivelin Valley who gave us access to their land. Andy Radford and Terry Burke provided helpful feedback on the study. This work was funded by a Natural Environment Research Council PhD studentship (CJN) and grant NE/1027118/1 (BJH).

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Table 1 Results of GLMM analyses investigating the effects of kinship (proportion of the flock that were relatives at $r = 0.5$, $r \geq 0.25$ and $r \geq 0.125$) and distance (measured from a failed breeder's last breeding attempt to the centre of a flock's range) on failed breeders' flocking decisions

	Model parameter	Relative importance	Estimate	Unconditional SE	Lower CI	Upper CI
$r = 0.5$	(Intercept)		-1.932	0.236	-2.394	-1.470
	Proportion of relatives	1.00	2.508	0.596	1.339	3.677
	Distance	1.00	-3.061	0.478	-3.999	-2.123
	Proportion of relatives*Distance	1.00	2.525	0.895	0.771	4.279
$r \geq 0.25$	(Intercept)		-1.912	0.219	-2.342	-1.482
	Proportion of relatives	1.00	0.809	0.485	-0.144	1.761
	Distance	0.71	-3.089	0.452	-3.978	-2.200
	Proportion of relatives*Distance	0.49	1.304	0.625	0.073	2.534
$r \geq 0.125$	(Intercept)		-1.986	0.235	-2.446	-1.527
	Proportion of relatives	1.00	1.267	0.360	0.562	1.973
	Distance	1.00	-3.295	0.484	-4.244	-2.346
	Proportion of relatives*Distance	1.00	1.884	0.601	0.705	3.062

Standardised parameter estimates, unconditional standard errors and relative importance of explanatory variables were obtained by averaging across three models within two AICc of the top model when relatedness was defined as $r \geq 0.25$. There were no models within two AICc of the top model when relatedness was $r = 0.5$ and $r \geq 0.125$ so estimates were calculated from the top model alone.

Table 2 The relatedness of long-tailed tits to other members of the flock of origin and the destination flock from birds that switched permanently between flocks

Dataset	Relatedness	Status in previous season	Proportion of flock of origin that were relatives (mean ± SD)	Proportion of destination flock that were relatives (mean ± SD)	*N*	*V*	*P*
All	0.5	All	0.083 ± 0.174	0.052 ± 0.185	69	273.5	0.043
Males		All	0.138 ± 0.227	0.076 ± 0.245	32	131.0	0.049
All	≥ 0.25	Chick	0.216 ± 0.286	0.100 ± 0.267	26	169.5	0.065

P-values were determined using Wilcoxon signed-rank tests and *V* is the sum of ranks assigned to differences with a positive sign. Analyses were conducted at three levels of relatedness ($r = 0.5$, $r \geq 0.25$, $r \geq 0.125$), for all birds combined and separately for each sex, age class and for adults of each breeding status at the end of the last breeding season. Only results where $P < 0.10$ are presented.

Table 3 The effect of kinship on a flock's vocal response to playback, closest approach to the speaker or time spent within 20m of the speaker

Response	Kin (mean ± SD)	Non-kin (mean ± SD)	*V*	*P*
Net churr rate	1.16 ± 1.71	1.23 ± 1.45	15	0.74
Net triple rate	-0.62 ± 1.17	-0.74 ± 0.55	20	0.84
Closest approach (m)	8.25 ± 4.89	8.38 ± 4.81	18	1.00
Time within 20 m (s)	198 ± 116	190 ± 121	11	1.00

P-values were determined using Wilcoxon signed-rank tests and *V* is the sum of ranks assigned to differences with a positive sign.

Table 4 Results of permutation tests for preferred and avoided associations

Year	Real			Random			*P*		
	SD	CV	Non-zero edges	SD	CV	Non-zero edges	SD	CV	Non-zero edges
1996-1997	0.247	1.937	0.248	0.149	1.104	0.523	0.999	0.999	0.001
1997-1998	0.235	1.818	0.310	0.120	0.891	0.676	1.000	1.000	0.001
1998-1999	0.216	2.167	0.220	0.102	1.080	0.553	0.999	1.000	0.000
2011-2012	0.135	2.574	0.171	0.094	1.742	0.280	0.999	0.999	0.001
2012-2013	0.213	2.318	0.196	0.113	1.264	0.444	1.000	0.999	0.000

P-values are based on the proportion of permuted CV or SD values that are lower than the observed values. If the real value fell within the top or bottom 2.5% of the random distribution ($P > 0.975$ or $P < 0.025$, two-tailed test), we rejected the null hypothesis that the real value could have arisen by chance.

Table 5 Results of Mantel tests on the correlation between pedigree relatedness and half weight association matrices both alone and when controlled for nest location in the previous breeding season

Year	Number of individuals	Alone		Controlled for nest location	
		Matrix correlation	*P*	Matrix correlation	*P*
1996-1997	15	0.353	0.003	0.267	0.018
males	9	0.198	0.154	0.145	0.233
females	6	-	-	-	-
males-females		0.490	0.003	0.360	0.013
1997-1998	34	0.407	<0.001	0.305	<0.001
males	18	0.415	<0.001	0.316	0.001
females	16	0.435	<0.001	0.314	0.002
males-females		0.396	<0.001	0.298	0.001
1998-1999	44	0.578	<0.001	0.474	<0.001
males	31	0.711	<0.001	0.648	<0.001
females	13	0.493	0.006	0.269	0.033
males-females		0.452	<0.001	0.304	0.002
2011-2012	53	0.494	<0.001	0.427	<0.001
males	32	0.661	<0.001	0.612	<0.001
females	21	0.079	0.121	0.030	0.285
males-females		0.464	<0.001	0.408	<0.001
2012-2013	18	0.691	<0.001	0.609	<0.001
males	11	0.670	0.001	0.634	<0.001
females	7	0.610	0.046	0.381	0.144
males-females		0.732	<0.001	0.640	<0.001

Table 6 Results of GLMM analyses investigating the effects of association strength, kinship and distance from the helper's last breeding attempt on helping decisions

Model Parameter	Estimate	Standard Error	Lower Confidence Interval	Upper Confidence Interval
Intercept	-3.710	0.574	-4.835	-2.585
Association strength	1.557	0.533	0.512	2.602
Kinship	-1.819	0.823	-3.432	-0.206
Distance	1.746	0.449	0.866	2.626

There were no models within two AICc of the top model so standardised parameter estimates and standard errors were calculated from the top model alone.

Table A1 Number of opposite sex relatives in flocks of origin and destination flocks for birds that switched permanently between flocks

Dataset	No. of opposite sex relatives in flock of origin (mean ± SD)	No. of opposite sex relatives in destination flock (mean ± SD)	V	P
$r = 0.5$				
All	0.449 ± 1.165	0.261 ± 1.093	121.5	0.115
Males	0.656 ± 1.153	0.188 ± 0.738	74.5	0.041
Females	0.270 ± 0.962	0.324 ± 1.334	7.0	1.000
Adults	0.209 ± 0.833	0.279 ± 1.241	13.5	1.000
Chicks	0.846 ± 1.287	0.231 ± 0.815	56.0	0.041
Male chicks	1.125 ± 1.408	0.313 ± 1.014	46.0	0.060
Female chicks	0.400 ± 0.966	0.100 ± 0.316	1.0	1.000
$r \geq 0.25$				
All	0.609 ± 1.140	0.522 ± 1.313	259.5	0.574
Males	0.688 ± 1.230	0.656 ± 1.285	95.8	0.900
Females	0.541 ± 1.538	0.405 ± 1.343	41.5	0.469
Adults	0.419 ± 1.348	0.605 ± 1.530	41.5	0.501
Chicks	0.923 ± 1.383	0.385 ± 0.852	97.5	0.119
Male chicks	1.188 ± 1.515	0.563 ± 1.031	70.5	0.251
Female chicks	0.500 ± 1.080	0.100 ± 0.316	3.0	0.371
$r \geq 0.125$				
All	0.768 ± 1.673	0.956 ± 1.859	292.0	0.519
Males	0.688 ± 1.230	1.031 ± 1.823	79.0	0.525
Females	0.838 ± 1.993	0.946 ± 1.914	71.5	0.830
Adults	0.581 ± 1.776	0.721 ± 1.623	45.5	0.418
Chicks	1.077 ± 1.468	1.423 ± 2.157	101.5	0.635
Male chicks	1.188 ± 1.515	1.313 ± 2.089	52.5	1.000
Female chicks	0.900 ± 1.449	1.600 ± 2.366	10.0	0.550

Analysis using Wilcoxon signed rank tests.

Table A2 The sex ratio (proportion male among ringed birds) of flocks of origin and destination flocks for birds that switched permanently between flocks

Dataset	Sex ratio in flock of origin (mean ± SD)	Sex ratio in destination flock (mean ± SD)	*V*	*P*
All	0.594 ± 0.178	0.522 ± 0.130	904.5	0.261
Males	0.577 ± 0.201	0.541 ± 0.119	167.0	0.637
Females	0.590 ± 0.157	0.508 ± 0.139	303.5	0.281
Adults	0.573 ± 0.185	0.504 ± 0.126	375.5	0.185
Chicks	0.602 ± 0.169	0.554 ± 0.135	115.0	1.000
Male chicks	0.653 ± 0.148	0.573 ± 0.148	43.0	0.397
Female chicks	0.520 ± 0.175	0.532 ± 0.120	19.0	0.410

Analysis using Wilcoxon signed rank tests. Note that actual sex ratio is close to parity, and the apparent male bias is because unringed birds are more likely to be female.

Figure Legends

Figure 1. The non-breeding ranges of four flocks in 1996-1997 (a), five flocks in 1997-1998 (b), ten flocks in 1998-1999 (c), thirteen flocks in 2011-2012 (d), and ten flocks in 2012-2013 (e). Colours are not used consistently between years. Ranges are presented as minimum convex polygons.

Figure 2. The distance from failed breeders' last breeding attempts (a), and first-order relatedness (b) of failed breeders to the flocks that they chose to join and those that they did not join. Boxes represent the upper and lower quartiles, bold lines represent the median and whiskers extend to the most extreme data point that is within 1.5 interquartile ranges of the box. Circles represent any data points that fell outside 1.5 interquartile ranges of the box.

Figure 3. Time in the non-breeding season of flock switches within the study site (a), and disappearances of ringed individuals from the study site due to death or dispersal (b).

Figure 4. The number of churr calls (a) and triple calls (b) produced by each flock member in the five minutes before (control) and during playback (playback). Boxes represent the upper and lower quartiles, bold lines represent the median and whiskers extend to the most extreme data point that is within 1.5 interquartile ranges of the box. Circles represent any data points that fell outside 1.5 interquartile ranges of the box.

Figure 5. Weighted network of individual long-tailed tits in 1997-1998, illustrating typical pattern of associations. Dots represent individuals, black lines represent ties between non-relatives, green lines represent ties between associated relatives and red lines represent individuals that are linked by kinship but were not associated in the network. Line thickness represents the strength of association between two individuals. See Table 5 for statistical analysis of all years.

Figure 6. The association strength between helpers and the most closely associated member of the breeding pair at nests they chose to help and those they did not in all years. Boxes represent the upper and lower quartiles, bold lines represent the median and whiskers extend to the most extreme data point that is within 1.5 interquartile ranges of the box. Circles represent any data points that fell outside 1.5 interquartile ranges of the box.

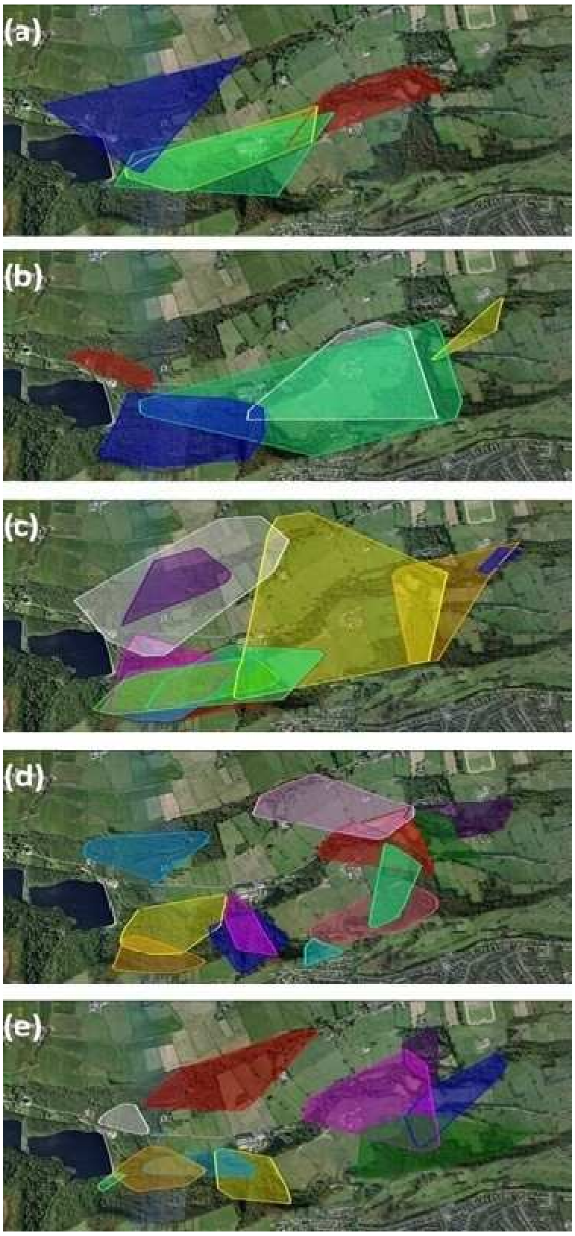

Figure 1

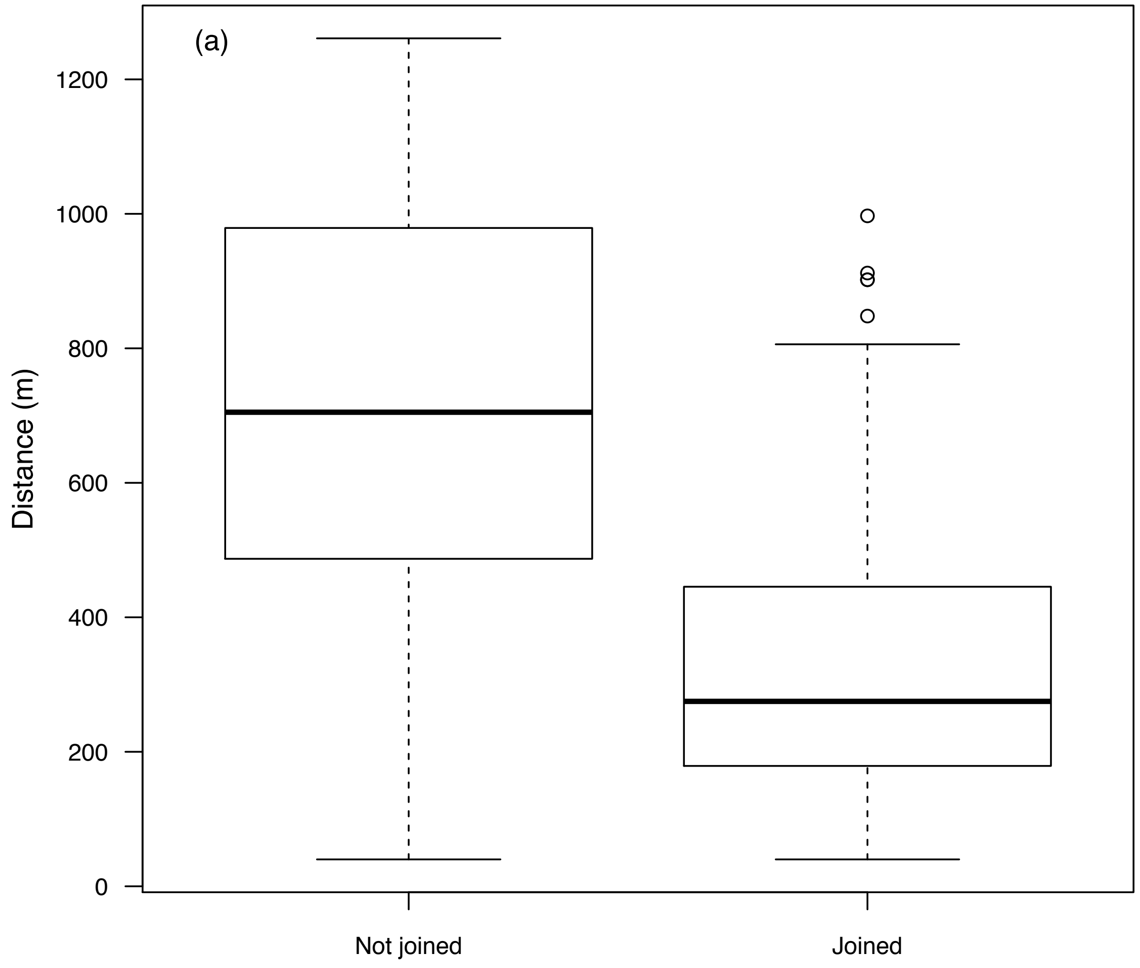

Figure 2

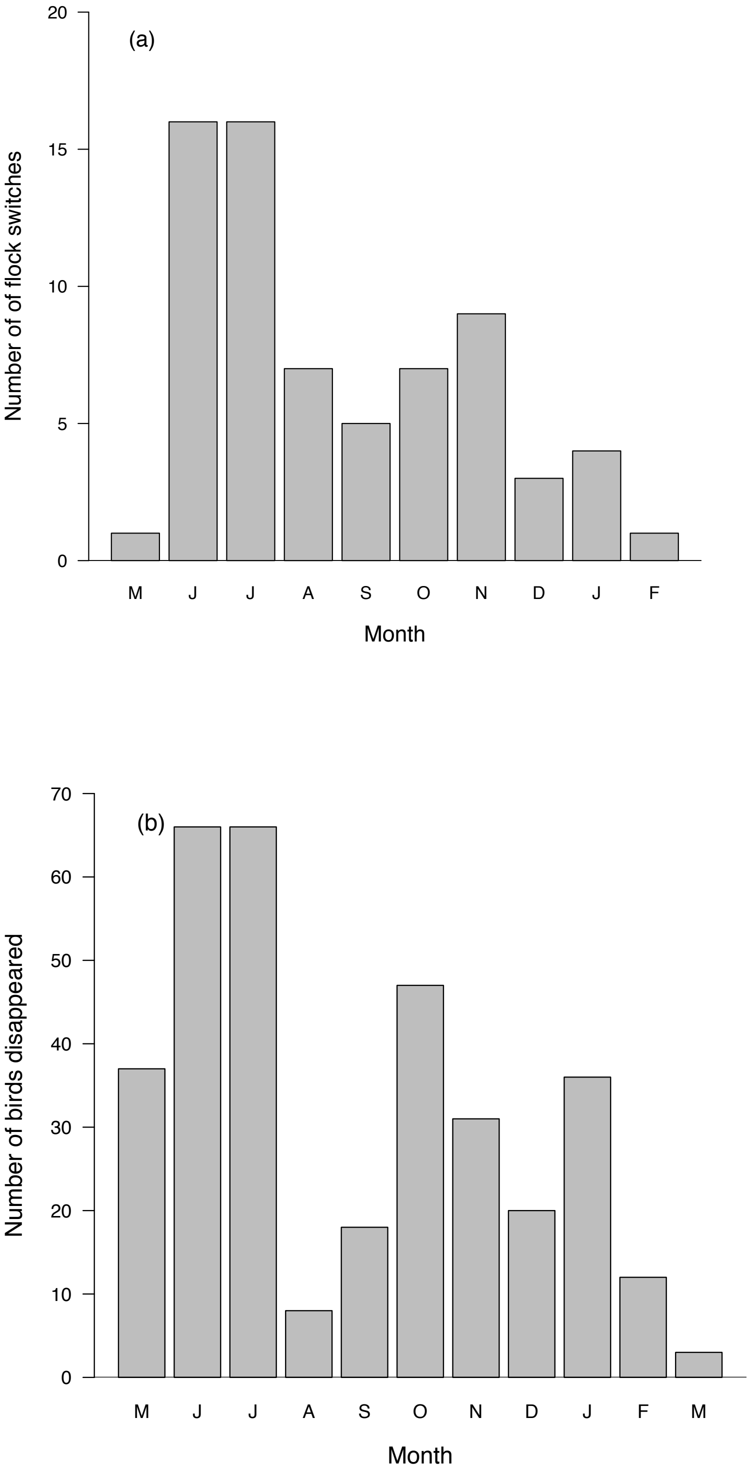
(a)
Number of of flock switches
0
5
10
15
20
M
J
J
A
S
O
N
D
J
F
Month
(b)
Number of birds disappeared
0
10
20
30
40
50
60
70
M
J
J
A
S
O
N
D
J
F
M
Month

Figure 3

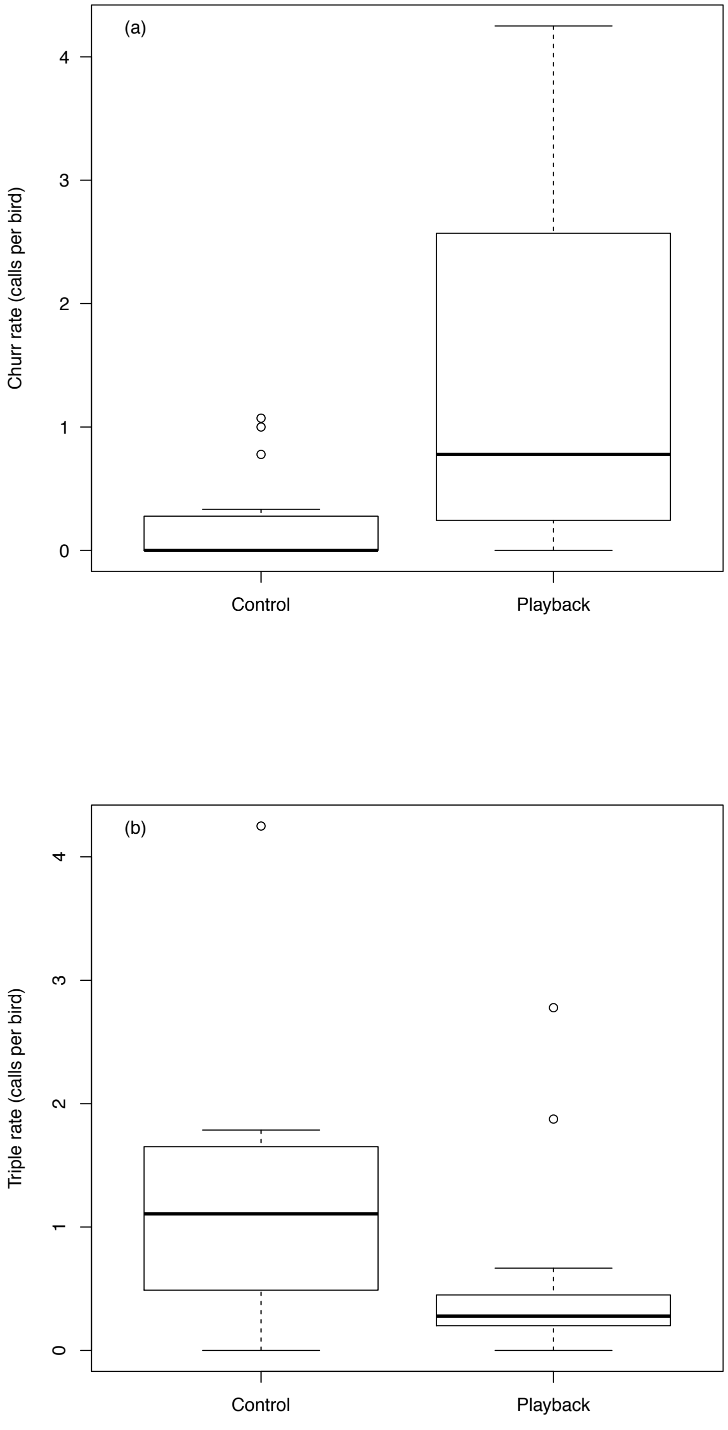
(a)
Churr rate (calls per bird)
0
1
2
3
4
Control
Playback
(b)
Triple rate (calls per bird)
0
1
2
3
4
Control
Playback

Figure 4

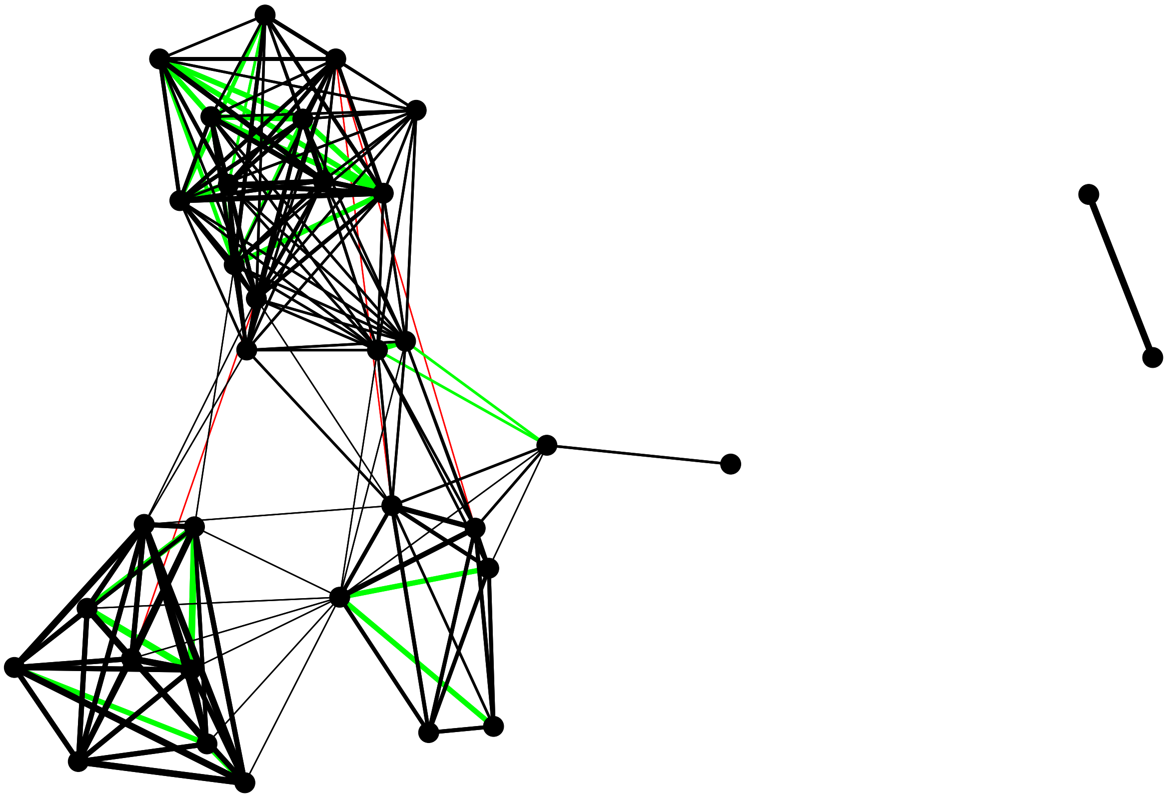

Figure 5

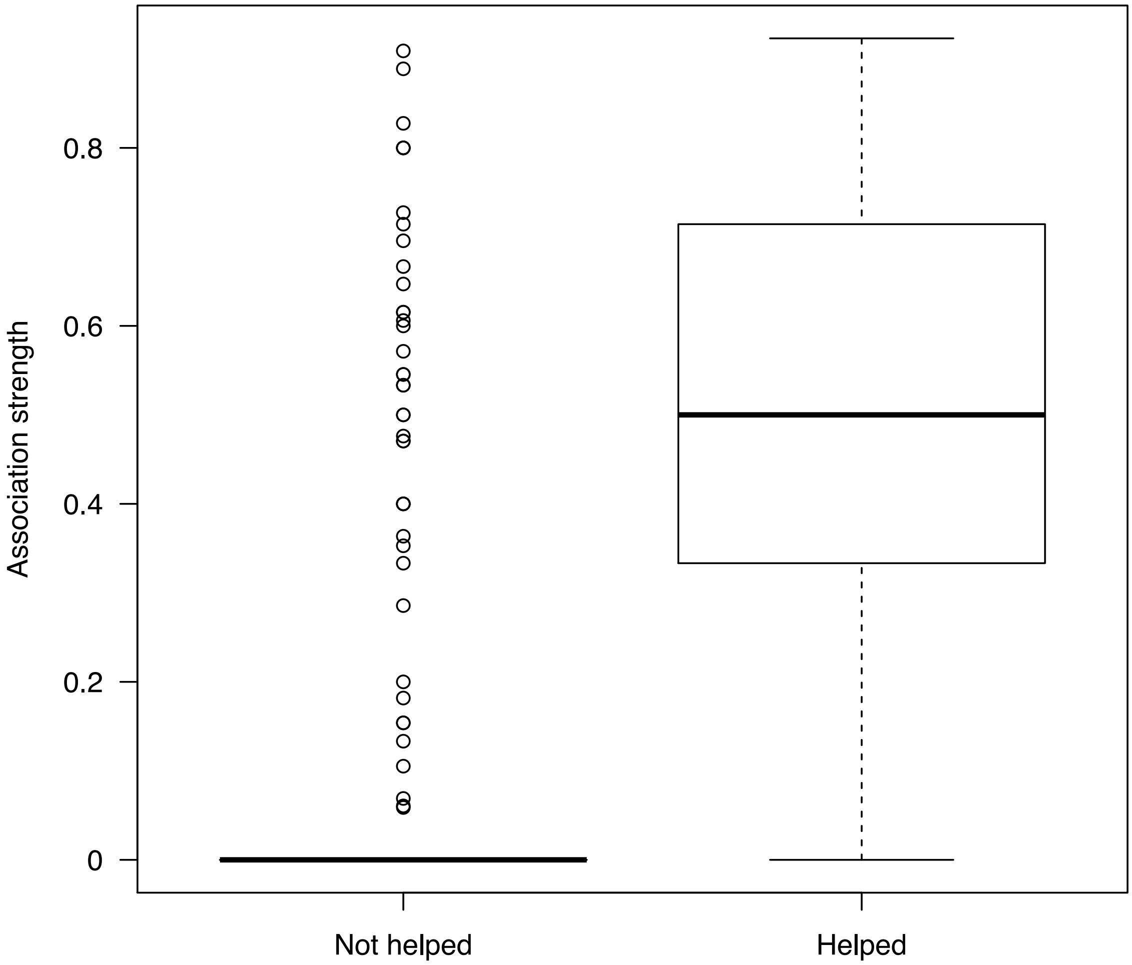

Figure 6