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Fine-scale genetic structure and helping decisions in a cooperatively breeding bird

Running title: Fine-scale genetic structure in social birds

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Abstract

In animal societies, characteristic demographic and dispersal patterns may lead to genetic structuring of populations, generating the potential for kin selection to operate. However, even in genetically structured populations, social interactions may still require kin discrimination for cooperative behaviour to be directed towards relatives. Here, we use molecular genetics and long-term field data to investigate genetic structure in an adult population of long-tailed tits Aegithalos caudatus, a cooperative breeder in which helping occurs within extended kin networks, and relate this to patterns of helping with respect to kinship. Spatial autocorrelation analyses reveal fine-scale genetic structure within our population, such that related adults of either sex are spatially clustered following natal dispersal, with relatedness among nearby males higher than that among nearby females, as predicted by observations of male-biased philopatry. This kin structure creates opportunities for failed breeders to gain indirect fitness benefits via redirected helping, but crucially, most close neighbours of failed breeders are unrelated and help is directed towards relatives more often than expected by indiscriminate helping. These findings are consistent with the effective kin discrimination mechanism known to exist in long-tailed tits, and support models identifying kin selection as the driver of cooperation.

Keywords: cooperation, kin selection, population genetic structure, kin discrimination

Introduction

The genetic structure of populations, that is the frequency and distribution of genotypes in space, is a key biological feature that influences diverse behavioural and evolutionary phenomena.

Genetic structuring occurs when gene flow is limited, resulting in an increase in genetic differentiation with geographical distance, or ‘isolation-by-distance’ (Wright 1943). Gene flow can be restricted by physical barriers (Frantz et al. 2010) or ecological traits (Edelaar et al. 2012), such as species’ dispersal capacity (Watts et al. 2004) and migration patterns (Rolshausen et al. 2013). At large spatial scales, genetic structure can lead to local adaptation and speciation (Winker et al. 2013), while at finer scales, behavioural traits such as territoriality (Lee et al. 2010) or natal philopatry (Woxvold et al. 2006) can lead to the spatial clustering of relatives, or kin structure, within populations. This can have important implications for inbreeding (Keller & Waller 2002) and the evolution of sociality (Hamilton 1964; Bourke 2011). The nature of genetic structuring in wild populations can reveal much about a species’ ecology and the strength of selection for phenotypic traits. For instance, since flight increases dispersal capacity, gene flow in avian populations is generally assumed to be high, and genetic structure is expected to be observed at large spatial scales (van Treuran et al. 1999; Crochet 2000). However, in cooperatively breeding birds, dispersal patterns characteristic of social living can lead to fine-scale genetic structure, observed at the level of social groups or across territories (Emlen 1997; van Dijk et al. 2015). Studies combining molecular and field data can reveal how behavioural and demographic traits shape genetic structure, and predict the evolutionary consequences of such spatial-genetic distributions.

Animal societies characteristically exhibit a degree of genetic structure that can provide substantial fitness benefits to individual group members (Cornwallis et al. 2009; Hatchwell 2010). In cooperatively breeding animals, limited natal dispersal leads to the spatial clustering of relatives (Ekman et al. 2004; Heg et al. 2004; Clutton-Brock & Lukas 2012; Rubenstein & Abbott 2017).

This genetic structure facilitates kin selection (Hamilton 1964) because to gain indirect fitness benefits from cooperation, relatedness between the donor and recipient of aid must be higher, on average, than between randomly selected individuals within the population (Queller 1994). Essentially, kin-selected helping can evolve only when individuals have the opportunity to interact with kin, so population viscosity is regarded as a necessary precursor to the evolution of kin-selected cooperative breeding (Dickinson & Hatchwell 2004; West et al. 2007). As well as alloparental care, there are other contexts in which long-term kin associations may be beneficial, such as communal investment in public goods (van Dijk et al. 2014) or predator defence (Griesser & Ekman 2005). On the other hand, increased levels of relatedness among neighbouring individuals can also incur fitness costs through kin competition for mates or resources (Taylor 1992; West et al. 2002), and an increased likelihood of incestuous matings (Koenig & Haydock 1994). The risk of inbreeding and subsequent inbreeding depression (Keller & Waller 2002) may be alleviated through sex-biased dispersal (Pusey & Wolf 1996), and in many cooperative species, philopatry by breeders is biased strongly toward one sex (Greenwood 1980; Walters et al. 2004; Double et al. 2005). However, in the absence of effective spatial separation, recognition mechanisms that permit accurate kin discrimination may be necessary to mitigate the costs associated with long-term interactions with relatives, as well as to maximise inclusive fitness benefits via cooperation (Komdeur & Hatchwell 1999). The extent of kin discrimination expected in cooperative societies depends on the benefits of accurate kin recognition, and the costs of recognition errors, which both depend heavily on the genetic structure of the breeding population (Griffin & West 2003; Cornwallis et al. 2009). Note that we follow Sherman et al. (1997) in using ‘kin discrimination’ to refer to the differential treatment of conspecifics differing in genetic relatedness, and ‘kin recognition’ to describe any mechanism by which this is effected.

In most avian cooperative breeders, individuals within a population are organised into discrete groups, in which relatedness among members is generally high and predictable (Cornwallis et al. 2009). Studies of the fine-scale genetic structure of cooperative species has therefore tended to focus on gene flow between such groups, rather than on dyadic genetic differentiation at broader spatial scales or across the population as a whole. However, for a small number of cooperative breeders, such as western bluebirds Sialia mexicana (Dickinson et al. 1996) and riflemen Acanthisitta chloris (Preston et al. 2013), cooperative social interactions extend beyond discrete nuclear or extended family units to less defined, connected networks of relatives, known as 'kin neighbourhoods' (Dickinson & Hatchwell 2004). In such cases, the relatedness between socially interacting individuals is much more variable and relatively low overall (Cornwallis et al. 2009). The underlying genetic structure resulting from this complex social pattern remains largely unexplored, having been measured only in the bell miner Manorina melanophrys (Painter et al. 2000) and sociable weaver Philetairus socius (Covas et al. 2006).

Here, we describe the previously undefined population genetic structure underlying the social organisation of long-tailed tits Aegithalos caudatus and investigate how this relates to the opportunities and patterns of helping with respect to kinship. By quantifying the distribution of genetically similar individuals within populations, we can gain a deeper understanding of the opportunity for kin selection to operate in this species, and provide insights into the level of kin discrimination required for helpers to maximise inclusive fitness. Long-tailed tits are facultative cooperative breeders, but unlike most cooperative species, adult offspring do not delay natal dispersal or breeding to help their parents to raise young (Hatchwell 2016). Instead, all adults disperse during their first winter and attempt to breed independently the following spring, and may decide to help at a relative’s nest only if their own reproductive attempts fail (Russell & Hatchwell 2001). Local recruitment is male-biased, yet natal dispersal distances of both sexes are short (Sharp et al. 2008a) and siblings often disperse together (Sharp et al. 2008b). Long-tailed tits also exhibit strong kin associations during winter, which are reflected in their helping decisions the following spring (Napper & Hatchwell 2016). Another aspect of the long-tailed tit’s life history that strengthens genetic structure is the pattern of offspring mortality that results in a small effective population size (Lehmann & Balloux 2007). High nest predation rates cause frequent removal of whole broods at the nestling stage, and in the following year result in high recruitment rates of close kin from the small proportion of successful nests (Beckerman et al. 2011). Together, these demographic and life-history patterns permit the characteristic flexibility in reproductive strategy from independent breeding to helping within an individual’s lifetime. Around 40% of nests are helped, usually by one or two helpers, and helpers gain indirect fitness benefits by increasing the productivity of helped broods (Hatchwell et al. 2004; MacColl & Hatchwell 2004). In contrast, no significant direct fitness benefits of helping have been identified (McGowan et al. 2003; Meade & Hatchwell 2010). Helpers are overwhelmingly male (Sharp et al. 2011) and move away from their last failed breeding attempt to redirect care (Hatchwell 2016), often skipping the nearest available nest in search of one belonging to a relative (Russell & Hatchwell 2001). Furthermore, helpers adjust their effort according to kinship, provisioning more closely related broods at higher rates (Nam et al. 2010). These patterns suggest that kin-biased helping is not solely a result of kin structure, but involves active discrimination among individuals. In the absence of reliable spatial cues to kinship, helpers use vocal cues learned during early development to recognize and preferentially aid close relatives (Hatchwell et al. 2001; Sharp et al. 2005). However, the precise relationship between dispersal patterns, genetic structure and helping decisions remains unclear.

Using detailed observational and pedigree data, combined with molecular genetics, we first define the genetic structure among long-tailed tit breeders and relate this to the known patterns of dispersal for this species. We examine spatial genetic clustering both within and between sexes to determine whether this reflects female-biased dispersal and male-biased local recruitment. To assess how population structure impacts social behaviour, we also examine the distribution of close, distant and non-kin through the population to quantify the helping opportunities available to failed breeders in relation to distance. Finally, we compare the expected probability of helping kin based on population genetic structure with the observed patterns of helping to test whether kin-biased helping in long-tailed tits can be explained by this newly described genetic structure, or whether it is a result of active kin discrimination. We calculate kinship using both genetic data and a social pedigree. The measures differ because the population is open, so the social pedigree is inevitably incomplete. However, both measures are informative because although the fitness consequences of helping depend on genetic relatedness, social relatedness is the only information available to birds when making decisions, by way of socially learned recognition cues (Sharp et al. 2005).

Materials and Methods

Study area and field methods

A population of 17-72 (mean c.50) pairs of long-tailed tits was studied during the breeding season (February-June) between 1994 and 2016 in the Rivelin Valley, Sheffield, UK (53º38’N 1º56W). The site is approximately 2.5km^2 and comprises predominantly deciduous woodland and scrub. The site also encompasses areas of farmland, gardens and a golf course, and is surrounded by lowquality habitat. This is an open population, with approximately 40% of breeders hatched in the study site (A.E. Leedale, unpublished data). The remaining adults are assumed to be first year breeders that emigrated from outside the study site, based on the observation that individuals have high site fidelity following their first breeding year (McGowan et al. 2003). Almost all individuals (>95%) were marked with a metal BTO ring and a unique combination of two plastic colour rings for field identification. Native birds were ringed as 11-day old nestlings and immigrant adults were captured in mist nests under BTO licence before or during breeding. When ringed, a sample of 5-30µl of blood was taken by brachial venepuncture under Home Office licence. All breeding attempts were closely monitored and GPS coordinates were taken for each nest (n = 1461); a Cartesian coordinate system (UTM) was used to describe geographic distance between nests.

Molecular analyses

Molecular markers were used to estimate genetic relatedness between individuals and define population genetic structure. Genomic DNA was extracted from blood samples and amplified. All sampled individuals were sexed using the P2-P8 sex-typing primers (Griffiths et al. 1998). Individuals ringed between 1994 and 2006 were genotyped at 8 microsatellite loci (Ase18; Ase37; Ase64; Hru2; Hru6; Pca3, PmaD22, Ppi2). Thereafter, individuals were genotyped at an additional 9 loci (CAM01, CAM03, CAM15, CAM23, Tgu_01.040, Tgu_04.012, Tgu_05.053, Tgu_13.017, Pca4). For further details on genotyping procedures, see Simeoni et al. (2007) and Adams et al. (2015). The population allele frequencies used in all analyses were generated using all genotyped individuals (1994-2016, n = 3182) in CERVUS v3.0.7 (Kalinowski et al. 2007), to maximise accuracy in estimating the frequency of rare alleles and to ensure non-zero frequencies for all alleles in the dataset. The genetic relatedness between pairs of individuals was estimated using Queller and Goodnight's (1989) r_{QG} coefficient of relatedness in SPAGeDi v1.1.5 (Hardy & Vekemans 2002). This relatedness estimate has been found to be reliable when tested against our social pedigree (Nam et al. 2010).

Social pedigree

The social pedigree was created using 22 years of field observations as described above (n = 2815 birds). For further details on pedigree construction see SI1, Supplemental Information. To calculate pairwise social relationships (r_A), an additive relationship matrix was generated from the pedigree using the R package nadiv (Wolak 2012). Six breeding birds in our study population (0.2%) were from cross-fostered broods in 1996-1998, but given that birds raised together treat each other as kin (Hatchwell et al. 2001; Sharp et al. 2005), we include them in the social pedigree. Similarly, while there is a low rate of extra-pair paternity in long-tailed tits (Hatchwell et al. 2002), we have not corrected for it in the pedigree.

Spatial analysis of genetic relatedness

To describe the overall genetic structure of our breeding population (n = 1022), we use Weir and Cockerham's (1984) inbreeding coefficient, F_{IS}, and the microsatellite allele size-based genetic differentiation estimate, R_{IS} (Slatkin 1995), as calculated in SPAGeDi, following the recommendation by Balloux & Lugon-Moulin (2002). To calculate the approximate standard error of genetic relatedness and differentiation estimates, multilocus estimates were jack-knifed over loci and alleles permuted among individuals 20,000 times.

To assess fine-scale genetic structure within our population, we performed spatial autocorrelation analyses of relatedness (r_{QG}) as a function of geographic distance: (i) among all individuals, (ii) among males, (iii) among females, and (iv) between males and females. The distance between breeding birds was based on the locations of their first nests in a given year. We compared the observed r_{QG} values within defined distance bands with the corresponding frequency distributions of r_{QG} when random permutations of the data were performed. The median natal dispersal distance within our study site was 393m for males and 522m for females, and the median distance travelled by established breeders between years was 312m (A.E. Leedale, unpublished data). We therefore set distance intervals of 300m as a scale on which to examine population structure, from pairwise comparisons of individuals at the same nest (0m), to those 2100m away; with a final distance band (>2100m) containing pairwise comparisons of individuals from the most distant nests (mean ± SD distance between birds = 1033m ± 596; maximum distance = 3195m). These bands generated enough variation in dyadic genetic relatedness, while maintaining a large enough sample size at each distance interval to ensure meaningful analyses. Long-tailed tits are relatively short-lived, so the genetic structure of the population may fluctuate over time due to demographic processes such as dispersal, mortality and recruitment (Balloux & Lugon-Moulin 2002; Hatchwell et al. 2013). Therefore, although all genetic and spatial analyses were performed on the long-term dataset, these were restricted to within-year comparisons among individuals.

Spatial autocorrelation among all individuals, among males and among females were analysed separately using SPAGeDi. Individual locations were permuted 20,000 times for tests on each distance band and all tests were two-tailed. SPAGeDi cannot be used to perform spatial autocorrelation analyses both within-years and between opposite-sex pairs of individuals only, so to analyse male-female genetic structure, an equivalent randomisation procedure was conducted using R version 3.3.2 (R Core Team 2015). Mean observed r_{QG} estimates were calculated for each defined distance band. The observed r_{QG} values within each band were replaced with an equal-sized sample of r_{QG} values selected at random across all distance bands. From these values, the mean null r_{QG} was calculated, and permuted 20,000 times to simulate the random frequency distribution. In all analyses, the observed r_{QG} in each distance band was considered statistically significant if the mean fell outside the 95% confidence interval of the random distribution.

The genetic structure of males and females was compared using mixed effects models (GLMM). The typical natal dispersal distance was <400m for philopatric male recruits, and <600m for female recruits (Sharp et al. 2008a; see above), and neither sex exhibited significant kin structure beyond 600m (see Results). Therefore, we compared relatedness among males with that observed among females within two distance bands (0-300m and 300-600m) only. For each distance band, r_{QG} values were modelled with sex fitted as a fixed effect and the IDs of both birds fitted as random effects.

Helping decisions

The range within which failed breeders search for helping opportunities is likely to interact with population genetic structure to affect the probability of helping kin. Using both genetic data (n = 1022) and the social pedigree (n = 866), we calculated the frequency of first order kin (pedigree $r_A \geq 0.5$; relatedness coefficient $r_{QG} > 0.25$), second order kin ($0.5 > r_A \geq 0.25$; $0.25 \geq r_{QG} \geq 0.125$) and non-kin ($r_A < 0.25$; $r_{QG} < 0.125$) in the breeding population (see SI2, Supplemental Information). Only relationships between breeders that were present in the same year were considered. Helping distance was calculated as the distance between helpers' last failed breeding attempt in a given year and the nest at which they first appeared as a helper the same year. Distance between nests was measured in UTM coordinates and was calculated in the R package, raster version 2.5-8 (Hijmans 2016).

To assess the impact of kin structure on helping behaviour, we quantified the proportion of cases in which help was given to broods belonging to at least one first order kin, at least one second order kin, or two unrelated breeders over the three distance bands in which helping is likely to occur: 0-300m, 300-600m and 600-900m. We used Pearson's Chi-squared tests to determine whether the proportion of helpers assisting kin was affected by distance, and whether the proportion of helpers assisting kin differed between males and females. We also calculated the probability that helpers would provide care to broods belonging to kin if they helped a random brood within 0-300m, 300-600m and 600-900m. For each helping event, a nest was selected at random from the pool of nests the focal helper could have chosen. This pool contained all nests present in the year the helping event occurred within the same distance band as the chosen nest. The proportion of cases in which help was given to nests belonging to at least one first order kin, at least one second order kin or two unrelated breeders was calculated based on this random sample. The procedure was repeated 10,000 times to generate a distribution of expected proportions for each distance band if nests were selected randomly with respect to kinship. To determine the effect of distance and helper sex on the probability of helping kin, we carried out Pearson's Chi-squared tests using the mean of the randomly generated proportions. Finally, we compared the expected probability of helping at a nest belonging to a relative to the observed proportion of birds helping kin. The observed proportion of helped nests within each distance band in which the helper was a first order, second order or non-relative was considered statistically significant if it fell outside the 95% confidence interval of the randomly generated distribution. This allowed us to determine whether kin structuring alone could explain observed patterns of helping behaviour, and if not, the degree of discrimination required to direct care towards kin. All analyses were carried out on all helpers and separately by sex, using both genetic data and the social pedigree.

Results

Breeding adults were genotyped at 8 (n = 525) or 17 (n = 497) polymorphic microsatellite loci (multilocus averages used, mean number of alleles per locus = 15, effective alleles = 7.09 (Nielsen et al. 2003), allelic richness = 6.88, gene diversity corrected for sample size = 0.761 and individual inbreeding coefficient $F_i = 0.007$). In total, 264 alleles were detected (for the distribution of alleles among loci, see Table SI3, Supplemental Information). The average genetic variation among breeders was not significantly different from random, based on the population inbreeding coefficient ($F_{IS} = -0.002 \pm 0.004$, $p = 0.67$) and microsatellite-specific genetic differentiation estimate ($R_{IS} = -0.014 \pm 0.027$, $p = 0.36$), indicating no significant inbreeding or outbreeding in our study population.

Spatial analysis of genetic relatedness

Mean ± SE population-level relatedness was 0.011 ± 0.003 among all birds (1719 observations of 1022 birds, 73069 within-year comparisons), 0.012 ± 0.004 among males (909 observation of 529 males, 20279 comparisons), 0.012 ± 0.005 among females (810 observation of 493 females, 16041 comparisons) and 0.009 between males and females (1719 observations of 1022 birds, 36749 comparisons). The standard error of relatedness estimates was not quantifiable for male-female genetic structure across years (see Methods), but for within-year estimates of mean relatedness between males and females with standard errors from jack-knifing over loci see SI4, Supplemental Information.

Spatial analyses revealed fine-scale genetic structure within our breeding population, with nearby individuals being the most genetically similar (Fig. 1). Pairwise relatedness among all individuals was higher than expected (based on permuted pairwise relatedness) within a radius of 300m and between 300m and 600m (both $p < 0.001$, Fig. 1a). Within each sex, relatedness was higher than expected within 300m and 300-600m for both males (both $p < 0.001$, Fig. 1b) and females (both $p < 0.001$, Fig. 1c). Although slightly lower than the within-sex comparisons, dyadic relatedness between males and females was also significantly higher than expected by chance within a radius of 300m and 300-600m (both $p < 0.001$, Fig. 1d). Within-year comparisons between males and females exhibit a similar spatial pattern (SI4, Supplemental Information). The distance at which kin structure breaks down in all comparisons is beyond 600m (Fig. 1). Within this distance, relatedness among males was significantly higher than relatedness among females at 0-300m (GLMM: $F = 20.63$, df = 1,780, $p < 0.001$), but not at 300-600m (GLMM: $F = 2.29$, df = 1,888, $p = 0.13$).

Helping decisions

The median distance travelled by failed breeders to help another breeding pair was 263m for males ($n = 164$) and 346m for females ($n = 37$), with most helpers travelling within the 0-300m and 300-600m distance bands (Fig. 2). Using genetic estimates of relatedness, r_{QG}, mean ± SD relatedness of all helpers to recipient breeding pairs was 0.14 ± 0.16 (95% CI = -0.10-0.36). In the majority of cases ($n = 181$), helpers assisted at least one first or second order relative ($r = 0.5$, 56.9%; $r = 0.25$, 13.3%), but a substantial minority of helpers were unrelated to the recipients ($r = 0$, 29.8%).

Estimated helper-recipient relatedness was lower using the social pedigree, although a majority of helped nests were again helped by at least one first or second order relative ($r = 0.5$, 39.4%; $r = 0.25$, 14.2%; $r = 0$, 46.5%; $n = 150$ cases).

Overall, we found little effect of the distance travelled by helpers on their probability of helping kin. Using genetic estimates of relatedness, helpers were marginally less likely to help relatives when travelling between 300-600m ($\chi^2 = 10.24$, df = 4, $p < 0.05$, $n = 177$) compared to the shorter and longer distance bands (Fig. 3a, Table 1). However, this effect was not significant when considering only male helpers, ($\chi^2 = 8.92$, df = 4, $p = 0.06$, $n = 144$); there were too few female helpers falling into each category to conduct an equivalent analysis (Table 1). Based on the social pedigree, there was no effect of distance on the proportion of first order, second order or non-kin helped when analysing all helpers ($\chi^2 = 5.88$, df = 4, $p = 0.22$, $n = 155$ cases; Fig. 3b, Table 1), or just male helpers ($\chi^2 = 3.49$, df = 4, $p = 0.48$, $n = 129$; Table 1). Note that again there were too few data to analyse female helpers separately (Table 1).

Comparing the relatedness of male and female helpers to the recipients of their care, overall males tended to help kin more often than females (Table 1). This was significant using the genetic data (males: $r = 0.5$, 61.8%; $r = 0.25$ 13.9%; $r = 0$, 24.3%; $n = 144$ cases; females: $r = 0.5$, 42.4%; $r = 0.25$ 12.1%; $r = 0$, 45.5%; $n = 33$ cases; $\chi^2 = 6.05$, df = 2, $p < 0.05$), but not with the social pedigree (males: $r = 0.5$, 42.6%; $r = 0.25$, 13.2%; $r = 0$, 44.2%; $n = 129$ cases; females: $r = 0.5$, 23.1%; $r = 0.25$ 19.2%; $r = 0$, 57.7%; $n = 26$ cases; $\chi^2 = 3.51$, df = 2, $p = 0.17$). This sex difference in the probability of helping kin was driven by a relatively small number of unrelated female helpers in the 300-600m distance band (Table 1).

The randomisation tests that we conducted to determine the random probability of helping a relative showed, not surprisingly given the kin structure of our population, that the random probability of helping first-order kin decreased with distance for all helpers (genetic data: $\chi^2 = 13.2$, df = 2, $p < 0.01$; social pedigree: $\chi^2 = 28.5$, df = 2, $p < 0.001$; Fig 3, Table 1), for male helpers (genetic data: $\chi^2 = 16.1$, df = 2, $p < 0.01$; social pedigree: $\chi^2 = 28.1$, df = 2 $p < 0.001$; Table 1), and female helpers (genetic data: $\chi^2 = 7.4$, df = 2, $p < 0.001$; social pedigree: $\chi^2 = 32.5$, df = 2, $p < 0.001$; Table 1). There was no significant difference in the opportunity to help a relative between male and female helpers travelling within 0-300m (genetic data: $\chi^2 = 2.41$, df = 2, $p = 0.29$; social pedigree: $\chi^2 = 0.88$, df = 2, $p = 0.69$; Table 1), 300-600m (genetic data: $\chi^2 = 1.7$, df = 2, $p = 0.45$; social pedigree: $\chi^2 = 2.01$, df = 2, $p = 0.42$; Table 1) or 600-900m (genetic data: $\chi^2 = 0.31$, df = 2, $p = 0.89$; social pedigree: $\chi^2 = 1.33$, df = 2, $p = 0.56$; Table 1) to provide help. Crucially, first-order kin were helped significantly more often than expected at random irrespective of distance travelled to provide help (Fig. 3; Table 1). This kin-bias in helping was also apparent among male helpers within all distance bands, and also among female helpers for all but one comparison (Table 1).

Discussion

Long-tailed tits breed cooperatively in diffuse family structures, or kin neighbourhoods, such that interactions among non-kin and kin of varying relatedness are frequent. We used a combination of long-term field observations and population genetic analyses to investigate genetic structure and patterns of helping in this atypical social system. Our results showed significant, fine-scale genetic structure in long-tailed tit populations, with positive spatial autocorrelation of dyadic relatedness estimates among breeding birds. Crucially, this pattern exists after natal dispersal, and was strong among males, among females and between the sexes. These findings contrast with most studies of cooperatively breeding birds that have measured spatial-genetic autocorrelation in breeders of both sexes, and show genetic structure in adult males only due to complete female-biased dispersal (Painter et al. 2000; Double et al. 2005; Temple et al. 2006; Woxvold et al. 2006), although fine-scale genetic structure among both sexes has also been demonstrated in sociable weaver colonies (van Dijk et al. 2015). We found that genetic structure was stronger in males than females, but both males and females remained spatially associated with same-sex relatives once they started breeding. This reflects previously reported patterns of dispersal in this species: although females disperse further than males (Sharp et al. 2008a), some adults of each sex disperse only short distances from their natal area to become independent breeders. This underlying genetic structure is also consistent with known patterns of social association during the non-breeding season (Napper and Hatchwell 2016).

This degree of kin structure post-dispersal creates opportunities for failed breeders to gain indirect fitness benefits via redirected helping (Hatchwell et al. 2014). Although long-tailed tit societies are not organised into discrete family units of close kin, neither are related individuals distributed randomly in space, but organised into kin neighbourhoods, allowing kin selection to operate. Pairwise relatedness is highest within 300m, and males typically seek helping opportunities within this range. In the rarer instance that females become helpers, they tend to travel slightly further, but still within the range of kin clustering. The higher than expected relatedness among individuals living in close proximity is driven by the tendency of close kin to cluster together, but is low overall because non-relatives or distant kin also breed in the vicinity. In fact, genetic estimates showed that only 12% of dyadic relationships within 300m were between first order kin and as the distance between dyads increased, the proportion of kinships decreased so that only 3% of dyads over 900m were close kin (Fig. SI2, Supplemental Information). Interestingly, using genetic relatedness estimates the proportion of second order kin did not decrease with distance.

According to the social pedigree, however, the proportions of first and second order kinships both decreased over distance and were substantially lower overall than the estimated kinships using genetic data (Fig. SI2, Supplemental Information). Our genetic data may detect more kin relationships than our social pedigree for several reasons. First, our population is open, with over half the breeding adults dispersing into the study site to breed, and their parentage is unknown. Therefore, although we can use genetic data to inform the pedigree for a proportion of immigrants (see SI1 Supplemental Information), some kin relationships are likely to go undetected. Second, long-tailed tits are relatively short-lived and high nest predation rates generate a low effective population size (Beckerman et al. 2011). Therefore, even for birds born in the study site, it is rarely possible to trace their social pedigree further than one generation without using genetic data to fill in the gaps. Third, a small number of individuals may breed just outside the study site in their first year, before moving into the site in subsequent years, and so kin relationships may exist among some immigrants across years. Kinship estimates from the social pedigree may therefore under-estimate the proportion of kin. Finally, the number of individuals in each relatedness category obviously depends on how those categories are defined using either pedigree or genetic data.

Overall, where they differ from the social pedigree, genetic relatedness estimates may be more reliable. However, pedigree data is essential for understanding how accurately individuals are able to recognise kin, particularly when the mechanism depends on socially learned cues (Sharp et al. 2005). Kin recognition via associative learning is likely to be effective where there is a reliable correlation between genetic relatedness and association during a sensitive phase of development, a requirement probably satisfied in most species demonstrating parental care (Komdeur and Hatchwell, 1999). Associative learning is therefore considered the most widespread mechanism of kin recognition in cooperatively breeding birds, where extended brood care at the nest provides a period of association among relatives, and has been identified in many species, including Galápagos mockingbirds Nesomimus parvulus (Curry 1998; Curry & Grant 1990), Seychelles warblers Acrocephalus sechellensis (Richardson et al. 2003; Komdeur et al. 2004) and splendid fairy wrens Malurus splendens (Payne et al. 1988). On the other hand, in some species, such as stripe-backed wrens Campylorhynchus nuchalis (Price 1998; 1999) and green woodhoopoes Phoeniculus purpureus (Radford, 2005), kin recognition has been suggested to have a genetic component, and studies on bell miners (Wright et al. 2010) and long-tailed tits (Nam et al. 2010) found significant effects of genetic relatedness on helper effort, indicating a mechanism more sophisticated than associative learning alone. Further studies of phenotypic cues in relation to genetic similarity and social information are necessary to determine the precise mechanisms of kin recognition in such species.

The social organisation of long-tailed tits gives helpers a choice of whom to aid in a situation where simple decision rules based on spatial cues are insufficient, even at close proximity, for effective kin discrimination. Here, we have shown that helpers assist far more first order kin than expected if help were given randomly with respect to kinship. This is consistent with the finding of Russell and Hatchwell (2001) that helpers exhibit a kin preference in their choice of brood to help. Although most helpers choose to help kin within the range of kin clustering, improving their chances of encountering a relative, 67% helped at a nest belonging to at least one first order kin, compared with a 31% probability of encountering a first order relative within 300m (Table 1). Furthermore, the proportion of helpers that assisted kin did not, in general, decrease with distance; the marginally lower probability of helping first order kin at 300-600m was driven by a small number of female helpers within this range that helped non-kin. Therefore, even when fewer kin were available, helpers still identified a similar proportion of first order kin to help. This degree of kin discrimination is unusual in cooperative breeders; helping more often occurs indiscriminately within closely related family groups (Cornwallis et al. 2009). This is because cooperation typically precedes dispersal, with offspring staying within natal territories to help their parents raise young (Ligon & Stacey 1991). Such viscous populations exhibit strong kin structure and the probability of helping kin is predictably high within the nuclear family unit. In a minority of cooperative breeders helping behaviour occurs post-dispersal and extends beyond the confines of a stable group (Ligon & Burt 2004; Hatchwell 2009) within extended social networks such as neighbourhoods of western bluebirds (Dickinson et al. 1996) and long-tailed tits (Hatchwell 2016), clans of white-fronted bee-eaters Merops bullockoides (Emlen & Wrege 1992) or coteries of bell miners (Clarke & Fitz-Gerald 1994). A crucial characteristic of such social structures is that the proportion of kin available to helpers is relatively low, and relatedness among individuals is too variable to favour indiscriminate cooperation (Cornwallis et al. 2009). In such populations, helpers must exercise a degree of discrimination to reliably direct help towards kin, so it is no coincidence that observational and experimental studies of these species have revealed some of the strongest evidence for active kin discrimination in social birds (Emlen & Wrege 1988; Hatchwell et al. 2001; Russell & Hatchwell 2001; Sharp et al. 2005; McDonald & Wright 2011; Akcay et al. 2013; Dickinson et al. 2016).

We found that second order kin were helped much less frequently than first order kin, as reported by Nam et al. (2010). It is interesting that the probability of helping second order kin did not differ significantly from that expected by random helping, except within 300m, where it was lower than expected. We suggest two possible reasons for this. First, a kin recognition mechanism that depends on association during early development (Sharp et al. 2005) may result in reliable discrimination of first order kin (i.e. siblings and parents), but would offer less opportunity to learn more distant kin, apart from any helpers. Alternatively, birds may be able to recognise second order kin, but prefer to help first order kin to maximise fitness. Previous studies have shown that long-tailed tits modify provisioning effort contingent on kinship (Nam et al. 2010), indicative of a capacity to discriminate between kin of varying relatedness. However, a minority of helpers also assist non-kin, and the probability of doing so did not change with distance and did not reflect kin availability. We have detected no direct benefits of helping in log-tailed tits (Meade & Hatchwell 2010), so such help for non-kin may be due to recognition 'errors' caused by a permissive threshold for acceptance of kin (Downs & Ratnieks 2000; Hatchwell et al. 2014). In cases where the benefits of helping a relative greatly exceed the costs of helping a non-relative, selection should favour a recognition mechanism that reflects these relative costs despite the potential for recognition errors (Reeve 1989). More studies are required that focus on the precise mechanism of kin recognition to investigate this possibility further.

Within the range that most helpers travelled to help, there was no difference between the sexes in the opportunity to help kin. Thus, the fact that females comprise only 15% of helpers in this population (Hatchwell et al. 2004) is not because they have few opportunities to help. This result is consistent with a previous study of another population by Sharp et al. (2011), showing that when males and females exhibited similar levels of philopatry, there was still a strong male bias in helping. Therefore, the male bias in helping behaviour is not due to male philopatry, and hence helping opportunities, per se. Instead, the decision to help by failed breeders is probably related to individual condition; failed breeders that choose to become helpers tend to be in better condition than those that do not (Meade & Hatchwell 2010). Since egg-laying, incubating and brooding are performed exclusively by females (Hatchwell 2016), the fitness costs associated with parental care may be more pronounced in females than in males, reducing their likelihood of becoming helpers when breeding attempts fail. We also found an intriguing trend for female helpers to assist fewer close kin and more non-kin than male helpers did. However, we treat this result with some caution because the sample of female helpers available for comparison is still relatively small.

The spatial clustering of relatives also has important consequences in terms of inbreeding risk. The population genetic structure revealed by our study shows that the average relatedness between males and females is higher than expected at random within approximately 700m. Therefore, in addition to the cooperative benefits, prolonged kin-associations between males and females at the reproductive stage may carry fitness costs associated with incest and inbreeding depression (Keller & Waller 2002). Whether these kin associations persist over the range that mate choice occurs and whether kin are actively avoided during mate choice, remains to be investigated.

In conclusion, we have revealed fine-scale genetic structure in our long-tailed tit population that is consistent with the kin clustering expected from known demographic and dispersal patterns. This provides an opportunity for kin selection to operate, and, in the absence of any other known benefits of interacting socially with kin (Napper et al. 2013; Napper & Hatchwell 2016), is likely to have emerged as a result of selection for kin-directed helping behaviour. However, despite this genetic structure, the random probability of helping at the nest of a relative is still relatively low, demonstrating that in the kin-selected cooperative breeding system of long-tailed tits, active discrimination of kin from non-kin is required when choosing at which nest to help in order to maximise inclusive fitness. The learned vocal cues used by long-tailed tits to recognise kin provides one such mechanism (Sharp et al. 2005), although the degree to which they can discriminate among kin of variable relatedness requires further study. Overall, this study demonstrates how population genetic structure, generated by dispersal and life-history traits, determines the opportunity for interactions among relatives, and the selective pressure these exert on kin discrimination rules in social animals.

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References

Akcay C., Swift R. J., Reed V. A. and Dickinson J. L. (2013) Vocal kin recognition in kin neighborhoods of western bluebirds. Behavioral Ecology, **24**, 898-905.

Adams M. J., Robinson M. R., Mannarelli M-E., Hatchwell B. J. (2015) Social genetic and social environment effects on parental and helper care in a cooperatively breeding bird. Proceedings of the Royal Society B-Biological Sciences, **282**, 20150689.

Balloux F. and Lugon-Moulin N. (2002) The estimation of population differentiation with microsatellite markers. Molecular Ecology, **11**, 155-165.

Beckerman A. P., Sharp S. P. and Hatchwell B. J. (2011) Predation and kin-structured populations: an empirical perspective on the evolution of cooperation. Behavioral Ecology, **22**, 1294-1303.

Bourke A. F. G. Principles of Social Evolution (Oxford University Press, 2011).

Clarke M. F., Fitz-Gerald G. F. (1994) Spatial organisation of the cooperatively breeding bell miner Manorina melanophrys. Emu, **94**, 96–105.

Clutton-Brock, T. H. and Lukas, D. (2012) The evolution of social philopatry and dispersal in female mammals. Molecular Ecology, **21**, 472–492.

Cornwallis C. K., West S. A. and Griffin A. S. (2009) Routes to indirect fitness in cooperatively breeding vertebrates: kin discrimination and limited dispersal. Journal of Evolutionary Biology, **22**, 2445-2457.

Covas R., Dalecky A., Caizergues A., Doutrelant C. (2006) Kin associations and direct vs indirect fitness benefits in colonial cooperatively breeding sociable weavers Philetairus socius. Behavioral Ecology and Sociobiology, **60**, 323–331.

Crochet P. A. (2000) Genetic structure of avian populations - allozymes revisited. Molecular Ecology, **9**, 1463–1469.

Curry R. L. (1988) Influence of kinship on helping behaviour of Galápagos Mockingbirds. Behavioral Ecology and Sociobiology **22**: 141-152.

Curry R. L. and Grant P. R. (1990) Galápagos Mockingbirds. In: Cooperative breeding in birds: Long-term studies of ecology and behavior (eds P.B Stacey and W.D. Koenig), pp. 291-331. Cambridge, UK: Cambridge University Press.

Dickinson J. L., Akcay C., Ferree E. D. and C. A. Stern. (2016) A hierarchical analysis of incest avoidance in a cooperative breeder. Behavioral Ecology, **27**, 1132-1140.

Dickinson J. L. and Hatchwell B. J. (2004) The fitness consequences of helping. In: Ecology and Evolution of Cooperative Breeding in Birds (eds Koenig W. D., Dickinson J. L.), pp. 48-66. Cambridge, UK: Cambridge University Press.

Dickinson J. L., Koenig W.D. and Pitelka F. A. (1996) Fitness consequences of helping behavior in the western bluebird. Behavioral Ecology, **7**, 168-177.

van Dijk R. E., Covas R., Doutrelant C., Spottiswoode C. and Hatchwell B. J. (2015) Fine-scale genetic structure reflects sex-specific dispersal strategies in a population of sociable weavers (Philetairus socius). Molecular Ecology, **24**, 4296-4311.

van Dijk R. E., Kaden J. C., Argüelles-Ticó A, Dawson, D. A., Burke, T. and Hatchwell, B. J. (2014) Cooperative investment in public goods is kin directed in communal nests of social birds. Ecology Letters, **17**, 1141-1148.

Double M. C., Peakall R., Beck, N. R. and Cockburn, A. (2005) Dispersal, philopatry and infidelity: dissecting local genetic structure in superb fairy-wrens Malurus cyaneus. Evolution, **59**, 625-635.

Downs S. G. and Ratnieks F. L. W. (2000) Adaptive shifts in honey bee (Apis mellifera L.) guarding behavior support predictions of the acceptance threshold model. Behavioral Ecology, **11**, 326-333.

Edelaar P., Alonso D., Lagerveld S., Senar J. C., Bjorklund M. (2012) Population differentiation and restricted gene flow in Spanish crossbills: not isolation-by-distance but isolation-by-ecology. Journal of Evolutionary Biology, **25**, 417-430.

Ekman J., Dickinson J. L., Hatchwell B. J., Griesser M. (2004) Delayed dispersal. In Ecology and Evolution of Cooperative breeding in birds (eds Koenig W. D., Dickinson J. L.), pp. 35–47. Cambridge, UK: Cambridge University Press

Emlen S. T. (1997) Predicting family dynamics in social vertebrates. In: Behavioural Ecology: An Evolutionary Approach (eds Krebs J. R., Davies N. B.), pp. 228-253. Oxford, UK: Blackwell Science Ltd.

Emlen S. T, Wrege P. H. (1988) The role of kinship in helping decisions among white-fronted bee-eaters. Behavioral Ecology and Sociobiology, **23**, 305-315.

Emlen S. T., Wrege P. H. (1992) Parent–offspring conflict and the recruitment of helpers among bee-eaters. Nature, **356**, 331–333.

Frantz A. C., Pope L. C., Etherington T. R., Wilson G. J. and Burke T. (2010) Using isolation-by-distance-based approaches to assess the barrier effect of linear landscape elements on badger (Meles meles) dispersal. Molecular Ecology, **19**, 1663-1674.

Greenwood P. J. (1980) Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour, **28**, 1140-1162.

Griesser M. and Ekman J. (2005) Nepotistic mobbing behaviour in the Siberian jay, Perisoreus infaustus. Animal Behaviour, **69**, 345-352.

Griffin A. S. and West S. A. (2003) Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. Science, **302**, 634-636

Griffiths R., Double M. C., Orr K. and Dawson R. J. G (1998) A DNA test to sex most birds. Molecular Ecology, **7**, 1071-1075.

Hamilton W. D. (1964) The genetical evolution of social behaviour. Journal of Theoretical Biology, **7**, 1-52.

Hardy O. J. and Vekemans X. (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes, **2**, 618–620.

Hatchwell B. J. (2009) The evolution of cooperative breeding in birds: kinship, dispersal and life history. Philosophical Transactions of the Royal Society B-Biological Sciences, **364**, 3217–3227.

Hatchwell B. J. (2010) Cryptic kin selection: Kin structure in vertebrate populations and opportunities for kin-directed cooperation. Ethology, **116**, 203–216.

Hatchwell B. J. (2016) Long-tailed tits: ecological causes and fitness consequences of redirected helping. In: Ecology and Evolution of Cooperative Breeding in Birds (eds W. D. Koenig and J. L. Dickinson), pp. 39-57 Cambridge, UK: Cambridge University Press.

Hatchwell B. J., Gullett P. R. and Adams M. J. (2014) Helping in cooperatively breeding long-tailed tits: a test of Hamilton's rule. Philosophical Transactions of the Royal Society B-Biological Sciences, **369**, 20130565.

Hatchwell B. J., Ross D. J., Chaline N., Fowlie M. K., Burke T. (2002) Parentage in the cooperative breeding system of long-tailed tits, Aegithalos caudatus. Animal Behaviour, **64**, 55-63.

Hatchwell B. J., Ross D. J., Fowlie M. K. and McGowan A. (2001) Kin discrimination in cooperatively breeding long-tailed tits. Proceedings of the Royal Society B-Biological Sciences, **268**, 885-890.

Hatchwell B. J., Russell A. F., MacColl A. D. C., Ross D. J., Fowlie M. K. and McGowan A. (2004) Helpers increase long-term but not short-term productivity in cooperatively breeding long-tailed tits. Behavioural Ecology, **15**, 1–10.

Hatchwell B. J., Sharp S. P., Beckerman A. P. and Meade J. (2013) Ecological and demographic correlates of helping behaviour in a cooperatively breeding bird. Journal of Animal Ecology, **82**, 486-494.

Heg D., Bachar Z., Brouwer L. and Taborsky M. (2004) Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. Proceedings of the Royal Society B-Biological Sciences, **271**, 2367–2374.

Hijmans R. J. (2016) raster: Geographic Data Analysis and Modeling. R package version 2.5-8. https://CRAN.R-project.org/package=raster.

Kalinowski S. T., Taper M. L. and Marshall T. C. (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology, **16**, 1099-1106.

Keller L. F., Waller D. M. (2002) Inbreeding effects in wild populations. Trends in Ecology and Evolution, **17**, 23-241.

Koenig W. D., Haydock J. (2004) Incest and incest avoidance. In: Ecology and Evolution of Cooperative Breeding in Birds (eds Koenig W. D., Dickinson J. L.), pp. 142–156. Cambridge, UK: Cambridge University Press.

Komdeur J. and Hatchwell B. J. (1999) Kin recognition: function and mechanism in avian societies. Trends in Ecology and Evolution, **14**, 237-241.

Komdeur J., Richardson D. S. and Burke T. (2004) Experimental evidence that kin discrimination in the Seychelles warbler is based on association and not on genetic relatedness. Proceedings of the Royal Society B-Biological Sciences, **271**, 963-969.

Konovalov D. A., Manning C. and Henshaw M. T. (2004) KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. Molecular Ecology, **4**, 779-782.

Lee J-W., Simeoni M., Burke T., Hatchwell B. J. (2010) The consequences of winter flock demography for genetic structure and inbreeding risk in vinous-throated parrotbills, Paradoxornis webbianus. Heredity, **104**, 472-481.

Lehmann L. and Balloux F. (2007) Natural selection on fecundity variance in subdivided populations: Kin selection meets bet hedging. Genetics, **176**, 361-377.

Ligon J. D., Stacey P. B. (1991) The origin and maintenance of helping in birds. American Naturalist, **138**, 254–258.

Ligon J. D., Burt D. B. (2004) Evolutionary origins. In: Cooperative breeding in birds (eds Koenig W. D., Dickinson J. L.), pp. 5–34. Cambridge, UK: Cambridge University Press.

MacColl A. D. C. and Hatchwell B. J. (2004) Determinants of lifetime fitness in a cooperative breeder, the long-tailed tit Aegithalos caudatus. Journal of Animal Ecology, **73**, 1137–1148.

McDonald P. G. & Wright J. (2011) Bell miner provisioning calls are more similar among relatives and are used by helpers at the nest to bias their effort towards kin. Proceedings of the Royal Society B-Biological Sciences, **278**, 3403-3411.

McGowan A., Hatchwell B. J. and Woodburn R. J. W. (2003) The effect of helping behaviour on the survival of juvenile and adult long-tailed tits Aegithalos caudatus. Journal of Animal Ecology, **72**, 491-499.

Meade J. and Hatchwell B. J. (2010) No direct fitness benefits of helping in a cooperative breeder despite higher survival of helpers. Behavioral Ecology, **21**, 1186-1194.

Nam K.-B., Simeoni M., Sharp S. P. and Hatchwell B. J. (2010) Kinship affects investment by helpers in a cooperatively breeding bird. Proceedings of the Royal Society B-Biological Sciences, **277**, 3299-3306.

Napper C. J. and Hatchwell B. J. (2016) Social dynamics in nonbreeding flocks of a cooperatively breeding bird: causes and consequences of kin associations. Animal Behaviour, **122**, 23-35.

Napper C. J., Sharp S. P., McGowan A., Simeoni S. and Hatchwell B. J. (2013) Dominance, not kinship, determines individual position within the communal roosts of a cooperatively breeding bird. Behavioral Ecology and Sociobiology, **67**, 2029-2039.

Nielsen R., Tarpy D. R. and Reeve H. K. (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population. Molecular Ecology, **12**, 3157-3164.

Painter J. N., Crozier R. H., Poiani A., Robertson R. J. and Clarke M. F. (2000) Complex social organization reflects genetic structure and relatedness in the cooperatively breeding bell miner, Manorina melanophrys. Molecular Ecology, **9**, 1339-1347.

Payne R. B., Payne L.L. and Rowley I. (1988) Kin and social relationships in splendid fairy-wrens - recognition by song in a cooperative bird. Animal Behaviour, **36**, 1341-1351.

Price J. J. (1998) Family- and sex-specific vocal traditions in a cooperatively breeding songbird. Proceedings of the Royal Society B-Biological Sciences, **265**, 497-502.

Price J. J. (1999) Recognition of family-specific calls in stripe-backed wrens. Animal Behaviour, **57**, 483-492.

Preston S. A. J., Briskie J. V. and Hatchwell B. J. (2013) Adult helpers increase the recruitment of closely related offspring in the cooperatively breeding rifleman. Behavioral Ecology, **27**, 1617–1626.

Pusey A. E. and Wolf M. (1996) Inbreeding avoidance in animals. Trends in Ecology and Evolution, **11**, 201-206.

Queller D. C. and Goodnight K. F. (1989) Estimating relatedness using genetic markers. Evolution, **43**, 258-275.

Queller D. C. (1994) Genetic relatedness in viscous populations. Evolutionary Ecology, **8**, 70-73.

R Core Team. (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Radford A. N. (2005) Group-specific vocal signatures and neighbour-stranger discrimination in the cooperatively breeding green woodhoopoe. Animal Behaviour, **70**, 1227-1234.

Reeve H. K. (1989) The evolution of conspecific acceptance thresholds. American Naturalist, **133**, 407-435.

Richardson D. S., Burke T. and Komdeur J. (2003) Sex-specific associative learning cues and inclusive fitness benefits in the Seychelles warbler. Journal of Evolutionary Biology, **16**, 854-861.

Rolshausen G., Segelbacher G., Hermes C., Hobson K. A. and Schaefer H. M. (2013) Individual differences in migratory behavior shape population genetic structure and microhabitat choice in sympatric blackcaps (Sylvia atricapilla). Ecology and Evolution, **3**, 4278-4289.

Russell A. F. and Hatchwell B. J. (2001) Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate. Proceedings of the Royal Society B-Biological Sciences, **268**, 2169-2174.

Rubenstein D. R. and Abbot P. (Eds) (2017) Comparative Social Evolution. Cambridge University Press.

Sharp S. P., Baker M. B., Hadfield J. D., Simeoni M. and Hatchwell B. J. (2008a) Natal dispersal and recruitment in a cooperatively breeding bird. Oikos, **117**, 1371-1379.

Sharp S. P., Simeoni M., McGowan A., Nam K. and Hatchwell B. J. (2011) Patterns of recruitment, relatedness and cooperative breeding in two populations of long-tailed tits. Animal Behaviour, **81**, 843-849.

Sharp S. P., McGowan A., Wood M. J. and Hatchwell B. J. (2005) Learned kin recognition cues in a social bird. Nature, **434**, 1127–1130.

Sharp S. P., Simeoni M. and Hatchwell B. J. (2008b) Dispersal of sibling coalitions promotes helping among immigrants in a cooperatively breeding bird. Proceedings of the Royal Society B-Biological Sciences, **275**, 2125-2130.

Sherman P. W., Reeve, H. K. & Pfennig, D. W. (1997) Recognition Systems. In: Behavioral Ecology: An Evolutionary Approach (eds Krebs J. R. & Davies N. B.), pp. 69-96. Cambridge, UK: Blackwell Science Ltd. Simeoni M. (2011) PhD thesis, University of Sheffield.

Simeoni M., Dawson D. A., Ross D. J., Châline N., Burke T. and Hatchwell B. J. (2007) Characterization of 20 microsatellite loci in the long-tailed tit Aegithalos caudatus (Aegithalidae, AVES). Molecular Ecology Notes, **7**, 1319-1322.

Slatkin M. (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics, **139**, 457-462.

Taylor P. D. (1992) Altruism in viscous populations: an inclusive fitness model. Evolutionary Ecology, **6**, 352-356.

Temple H. J., Hoffman J. I. and Amos, W. (2006) Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the white-breasted thrasher Ramphocinclus rachyurus. Molecular Ecology, **15**, 3449-3458.

van Treuren R., Bijlsma R., Tinbergen J. M., Heg D., van de Zande L. (1999) Genetic analysis of the population structure of socially organized oystercatchers (Haematopus ostralegus) using microsatellites. Molecular Ecology, **8**, 181–187.

Walters J. R., Cooper C. B., Daniels S. J., Pasinelli G., Schiegg K. (2004) Conservation biology. In: Ecology and Evolution of Cooperative Breeding in Birds (eds Koenig W. D., Dickinson J. L.), pp. 197–209. Cambridge, UK: Cambridge University Press.

Watts P. C., Rouquette J. R., Saccheri I. J., Kemp S. J., Thompson D. J. (2004) Molecular and ecological evidence for small-scale isolation by distance in an endangered damselfly, Coenagrion mercuriale. Molecular Ecology, **13**, 2931-2945.

Weir B. S. and Cockerham C. C. (1984) Estimating F-statistics for the analysis of population structure. Evolution, **38**, 1358-1370.

West S. A., Griffin A. S. and Gardner A. (2007) Evolutionary explanations for cooperation. Current Biology, **17**, R661-R672.

West S. A., Pen I., Griffin A. S. (2002) Cooperation and competition between relatives. Science, **296**, 72-75.

Winker K., McCracken K. G., Gibson D. D., Peters J. L. (2013) Heteropatric speciation in a duck, Anas crecca. Molecular Ecology, **22**, 5922-5935.

Wolak, M. E. (2012) nadiv: an R package to create relatedness matrices for estimating non-additive genetic variances in animal models. Methods in Ecology and Evolution, **3**, 792-796.

Woxvold I. A., Adcock G. J., Mulder R. A. (2006) Fine-scale genetic structure and dispersal in cooperatively breeding apostlebirds. Molecular Ecology, **15**, 3139-3146.

Wright S. (1943) Isolation by distance. Genetics, **28**, 114-138.

Wright J., McDonald P. G., te Marvelde L., Kazem A. J. N. and Bishop C. M. (2010) Helping effort increases with relatedness in bell miners, but ‘unrelated’ helpers of both sexes still provide substantial care. Proceedings of the Royal Society B-Biological Sciences, **227**, 437-445.

Data Accessibility

Microsatellite genotypes, breeding locations and the social pedigree (1994-2016) are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.0dm8mv1.

Author Contributions

BJH conceived and managed the long-tailed tit study, and supervised the project with EJHR. AEL, BJH, SPS and MS designed the study and collected long-term data. AEL conducted analyses and wrote the manuscript, and all authors contributed to revisions.

Figure Legends

Figure 1. Mean pairwise relatedness (r) in a long-tailed tit population over eight bands of distance between dyads: (a) among all individuals, (b) among males, (c) among females, (d) between males and females. Dashed lines indicate the simulated null mean r and 95% CI in an unstructured population. Error bars approximate SE estimates from jackknifing over loci. Numbers above the x axis represent the number of pairwise comparisons. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$.

Figure 2. Frequency distribution and median (+IQR) helping ranges of (a) male ($n = 164$) and (b) female ($n = 37$) long-tailed tit helpers, calculated as the distance between an individual's first helped nest and their last reproductive attempt that year.

Figure 3. Proportion of help given to nests belonging to at least one 1st order kin (black), at least one 2nd order kin (grey), or two non-kin (white) over three bands of distance between helpers and recipients (obs). The respective proportions expected if help was given randomly within that range is also shown (exp). Relatedness between helpers and recipients is estimated using both (a) genetic data and (b) the social pedigree.