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

2 Running title: Fine-scale genetic structure in social birds

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9 **Abstract**

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

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

26

27 **Introduction**

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

30 Genetic structuring occurs when gene flow is limited, resulting in an increase in genetic
31 differentiation with geographical distance, or 'isolation-by-distance' (Wright 1943). Gene flow can
32 be restricted by physical barriers (Frantz et al. 2010) or ecological traits (Edelaar et al. 2012), such
33 as species' dispersal capacity (Watts et al. 2004) and migration patterns (Rolshausen et al. 2013).
34 At large spatial scales, genetic structure can lead to local adaptation and speciation (Winker et al.
35 2013), while at finer scales, behavioural traits such as territoriality (Lee et al. 2010) or natal
36 philopatry (Woxvold et al. 2006) can lead to the spatial clustering of relatives, or kin structure,
37 within populations. This can have important implications for inbreeding (Keller & Waller 2002)
38 and the evolution of sociality (Hamilton 1964; Bourke 2011). The nature of genetic structuring in
39 wild populations can reveal much about a species' ecology and the strength of selection for
40 phenotypic traits. For instance, since flight increases dispersal capacity, gene flow in avian
41 populations is generally assumed to be high, and genetic structure is expected to be observed at
42 large spatial scales (van Treuran et al. 1999; Crochet 2000). However, in cooperatively breeding
43 birds, dispersal patterns characteristic of social living can lead to fine-scale genetic structure,
44 observed at the level of social groups or across territories (Emlen 1997; van Dijk et al. 2015).
45 Studies combining molecular and field data can reveal how behavioural and demographic traits
46 shape genetic structure, and predict the evolutionary consequences of such spatial-genetic
47 distributions.

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

52 This genetic structure facilitates kin selection (Hamilton 1964) because to gain indirect fitness
53 benefits from cooperation, relatedness between the donor and recipient of aid must be higher, on
54 average, than between randomly selected individuals within the population (Queller 1994).
55 Essentially, kin-selected helping can evolve only when individuals have the opportunity to interact
56 with kin, so population viscosity is regarded as a necessary precursor to the evolution of kin-
57 selected cooperative breeding (Dickinson & Hatchwell 2004; West et al. 2007). As well as
58 alloparental care, there are other contexts in which long-term kin associations may be beneficial,
59 such as communal investment in public goods (van Dijk et al. 2014) or predator defence (Griesser
60 & Ekman 2005). On the other hand, increased levels of relatedness among neighbouring
61 individuals can also incur fitness costs through kin competition for mates or resources (Taylor
62 1992; West et al. 2002), and an increased likelihood of incestuous matings (Koenig & Haydock
63 1994). The risk of inbreeding and subsequent inbreeding depression (Keller & Waller 2002) may
64 be alleviated through sex-biased dispersal (Pusey & Wolf 1996), and in many cooperative species,
65 philopatry by breeders is biased strongly toward one sex (Greenwood 1980; Walters et al. 2004;
66 Double et al. 2005). However, in the absence of effective spatial separation, recognition
67 mechanisms that permit accurate kin discrimination may be necessary to mitigate the costs
68 associated with long-term interactions with relatives, as well as to maximise inclusive fitness
69 benefits via cooperation (Komdeur & Hatchwell 1999). The extent of kin discrimination expected
70 in cooperative societies depends on the benefits of accurate kin recognition, and the costs of
71 recognition errors, which both depend heavily on the genetic structure of the breeding population
72 (Griffin & West 2003; Cornwallis et al. 2009). Note that we follow Sherman et al. (1997) in using
73 ‘kin discrimination’ to refer to the differential treatment of conspecifics differing in genetic
74 relatedness, and ‘kin recognition’ to describe any mechanism by which this is effected.

75 In most avian cooperative breeders, individuals within a population are organised into discrete
76 groups, in which relatedness among members is generally high and predictable (Cornwallis et al.
77 2009). Studies of the fine-scale genetic structure of cooperative species has therefore tended to
78 focus on gene flow between such groups, rather than on dyadic genetic differentiation at broader
79 spatial scales or across the population as a whole. However, for a small number of cooperative
80 breeders, such as western bluebirds *Sialia mexicana* (Dickinson et al. 1996) and riflemen
81 *Acanthisitta chloris* (Preston et al. 2013), cooperative social interactions extend beyond discrete
82 nuclear or extended family units to less defined, connected networks of relatives, known as ‘kin
83 neighbourhoods’ (Dickinson & Hatchwell 2004). In such cases, the relatedness between socially
84 interacting individuals is much more variable and relatively low overall (Cornwallis et al. 2009).
85 The underlying genetic structure resulting from this complex social pattern remains largely
86 unexplored, having been measured only in the bell miner *Manorina melanophrys* (Painter et al.
87 2000) and sociable weaver *Philetairus socius* (Covas et al. 2006).

88 Here, we describe the previously undefined population genetic structure underlying the social
89 organisation of long-tailed tits *Aegithalos caudatus* and investigate how this relates to the
90 opportunities and patterns of helping with respect to kinship. By quantifying the distribution of
91 genetically similar individuals within populations, we can gain a deeper understanding of the
92 opportunity for kin selection to operate in this species, and provide insights into the level of kin
93 discrimination required for helpers to maximise inclusive fitness. Long-tailed tits are facultative
94 cooperative breeders, but unlike most cooperative species, adult offspring do not delay natal
95 dispersal or breeding to help their parents to raise young (Hatchwell 2016). Instead, all adults
96 disperse during their first winter and attempt to breed independently the following spring, and may

97 decide to help at a relative's nest only if their own reproductive attempts fail (Russell & Hatchwell
98 2001). Local recruitment is male-biased, yet natal dispersal distances of both sexes are short (Sharp
99 et al. 2008a) and siblings often disperse together (Sharp et al. 2008b). Long-tailed tits also exhibit
100 strong kin associations during winter, which are reflected in their helping decisions the following
101 spring (Napper & Hatchwell 2016). Another aspect of the long-tailed tit's life history that
102 strengthens genetic structure is the pattern of offspring mortality that results in a small effective
103 population size (Lehmann & Balloux 2007). High nest predation rates cause frequent removal of
104 whole broods at the nestling stage, and in the following year result in high recruitment rates of
105 close kin from the small proportion of successful nests (Beckerman et al. 2011). Together, these
106 demographic and life-history patterns permit the characteristic flexibility in reproductive strategy
107 from independent breeding to helping within an individual's lifetime. Around 40% of nests are
108 helped, usually by one or two helpers, and helpers gain indirect fitness benefits by increasing the
109 productivity of helped broods (Hatchwell et al. 2004; MacColl & Hatchwell 2004). In contrast, no
110 significant direct fitness benefits of helping have been identified (McGowan et al. 2003; Meade &
111 Hatchwell 2010). Helpers are overwhelmingly male (Sharp et al. 2011) and move away from their
112 last failed breeding attempt to redirect care (Hatchwell 2016), often skipping the nearest available
113 nest in search of one belonging to a relative (Russell & Hatchwell 2001). Furthermore, helpers
114 adjust their effort according to kinship, provisioning more closely related broods at higher rates
115 (Nam et al. 2010). These patterns suggest that kin-biased helping is not solely a result of kin
116 structure, but involves active discrimination among individuals. In the absence of reliable spatial
117 cues to kinship, helpers use vocal cues learned during early development to recognize and
118 preferentially aid close relatives (Hatchwell et al. 2001; Sharp et al. 2005). However, the precise
119 relationship between dispersal patterns, genetic structure and helping decisions remains unclear.

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

134

135 **Materials and Methods**

136 Study area and field methods

137 A population of 17-72 (mean c.50) pairs of long-tailed tits was studied during the breeding season
138 (February-June) between 1994 and 2016 in the Rivelin Valley, Sheffield, UK (53°38'N 1°56'W).
139 The site is approximately 2.5km² and comprises predominantly deciduous woodland and scrub.
140 The site also encompasses areas of farmland, gardens and a golf course, and is surrounded by low-

141 quality habitat. This is an open population, with approximately 40% of breeders hatched in the
142 study site (A.E. Leedale, unpublished data). The remaining adults are assumed to be first year
143 breeders that emigrated from outside the study site, based on the observation that individuals have
144 high site fidelity following their first breeding year (McGowan et al. 2003). Almost all individuals
145 (>95%) were marked with a metal BTO ring and a unique combination of two plastic colour rings
146 for field identification. Native birds were ringed as 11-day old nestlings and immigrant adults were
147 captured in mist nests under BTO licence before or during breeding. When ringed, a sample of 5-
148 30µl of blood was taken by brachial venepuncture under Home Office licence. All breeding
149 attempts were closely monitored and GPS coordinates were taken for each nest (n = 1461); a
150 Cartesian coordinate system (UTM) was used to describe geographic distance between nests.

151 Molecular analyses

152 Molecular markers were used to estimate genetic relatedness between individuals and define
153 population genetic structure. Genomic DNA was extracted from blood samples and amplified. All
154 sampled individuals were sexed using the P2-P8 sex-typing primers (Griffiths et al. 1998).
155 Individuals ringed between 1994 and 2006 were genotyped at 8 microsatellite loci (Ase18; Ase37;
156 Ase64; Hru2; Hru6; Pca3, PmaD22, Ppi2). Thereafter, individuals were genotyped at an additional
157 9 loci (CAM01, CAM03, CAM15, CAM23, Tgu_01.040, Tgu_04.012, Tgu_05.053, Tgu_13.017,
158 Pca4). For further details on genotyping procedures, see Simeoni et al. (2007) and Adams et al.
159 (2015). The population allele frequencies used in all analyses were generated using all genotyped
160 individuals (1994-2016, n = 3182) in CERVUS v3.0.7 (Kalinowski et al. 2007), to maximise
161 accuracy in estimating the frequency of rare alleles and to ensure non-zero frequencies for all alleles
162 in the dataset. The genetic relatedness between pairs of individuals was estimated using Queller

163 and Goodnight's (1989) r_{QG} coefficient of relatedness in SPAGeDi v1.1.5 (Hardy & Vekemans
164 2002). This relatedness estimate has been found to be reliable when tested against our social
165 pedigree (Nam et al. 2010).

166 Social pedigree

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

175 Spatial analysis of genetic relatedness

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

182 To assess fine-scale genetic structure within our population, we performed spatial autocorrelation
183 analyses of relatedness (r_{QG}) as a function of geographic distance: (i) among all individuals, (ii)

184 among males, (iii) among females, and (iv) between males and females. The distance between
185 breeding birds was based on the locations of their first nests in a given year. We compared the
186 observed r_{QG} values within defined distance bands with the corresponding frequency distributions
187 of r_{QG} when random permutations of the data were performed. The median natal dispersal distance
188 within our study site was 393m for males and 522m for females, and the median distance travelled
189 by established breeders between years was 312m (A.E. Leedale, unpublished data). We therefore
190 set distance intervals of 300m as a scale on which to examine population structure, from pairwise
191 comparisons of individuals at the same nest (0m), to those 2100m away; with a final distance band
192 (>2100m) containing pairwise comparisons of individuals from the most distant nests (mean \pm SD
193 distance between birds = 1033m \pm 596; maximum distance = 3195m). These bands generated
194 enough variation in dyadic genetic relatedness, while maintaining a large enough sample size at
195 each distance interval to ensure meaningful analyses. Long-tailed tits are relatively short-lived, so
196 the genetic structure of the population may fluctuate over time due to demographic processes such
197 as dispersal, mortality and recruitment (Balloux & Lugon-Moulin 2002; Hatchwell et al. 2013).
198 Therefore, although all genetic and spatial analyses were performed on the long-term dataset, these
199 were restricted to within-year comparisons among individuals.

200 Spatial autocorrelation among all individuals, among males and among females were analysed
201 separately using SPAGeDi. Individual locations were permuted 20,000 times for tests on each
202 distance band and all tests were two-tailed. SPAGeDi cannot be used to perform spatial
203 autocorrelation analyses both within-years and between opposite-sex pairs of individuals only, so
204 to analyse male-female genetic structure, an equivalent randomisation procedure was conducted
205 using R version 3.3.2 (R Core Team 2015). Mean observed r_{QG} estimates were calculated for each

206 defined distance band. The observed r_{QG} values within each band were replaced with an equal-
207 sized sample of r_{QG} values selected at random across all distance bands. From these values, the
208 mean null r_{QG} was calculated, and permuted 20,000 times to simulate the random frequency
209 distribution. In all analyses, the observed r_{QG} in each distance band was considered statistically
210 significant if the mean fell outside the 95% confidence interval of the random distribution.

211 The genetic structure of males and females was compared using mixed effects models (GLMM).
212 The typical natal dispersal distance was <400m for philopatric male recruits, and <600m for female
213 recruits (Sharp et al. 2008a; see above), and neither sex exhibited significant kin structure beyond
214 600m (see Results). Therefore, we compared relatedness among males with that observed among
215 females within two distance bands (0-300m and 300-600m) only. For each distance band, r_{QG}
216 values were modelled with sex fitted as a fixed effect and the IDs of both birds fitted as random
217 effects.

218 Helping decisions

219 The range within which failed breeders search for helping opportunities is likely to interact with
220 population genetic structure to affect the probability of helping kin. Using both genetic data ($n =$
221 1022) and the social pedigree ($n = 866$), we calculated the frequency of first order kin (pedigree r_A
222 ≥ 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
223 non-kin ($r_A < 0.25$; $r_{QG} < 0.125$) in the breeding population (see SI2, Supplemental Information).
224 Only relationships between breeders that were present in the same year were considered. Helping
225 distance was calculated as the distance between helpers' last failed breeding attempt in a given year
226 and the nest at which they first appeared as a helper the same year. Distance between nests was

227 measured in UTM coordinates and was calculated in the R package, raster version 2.5-8 (Hijmans
228 2016).

229 To assess the impact of kin structure on helping behaviour, we quantified the proportion of cases
230 in which help was given to broods belonging to at least one first order kin, at least one second order
231 kin, or two unrelated breeders over the three distance bands in which helping is likely to occur: 0-
232 300m, 300-600m and 600-900m. We used Pearson's Chi-squared tests to determine whether the
233 proportion of helpers assisting kin was affected by distance, and whether the proportion of helpers
234 assisting kin differed between males and females. We also calculated the probability that helpers
235 would provide care to broods belonging to kin if they helped a random brood within 0-300m, 300-
236 600m and 600-900m. For each helping event, a nest was selected at random from the pool of nests
237 the focal helper could have chosen. This pool contained all nests present in the year the helping
238 event occurred within the same distance band as the chosen nest. The proportion of cases in which
239 help was given to nests belonging to at least one first order kin, at least one second order kin or
240 two unrelated breeders was calculated based on this random sample. The procedure was repeated
241 10,000 times to generate a distribution of expected proportions for each distance band if nests were
242 selected randomly with respect to kinship. To determine the effect of distance and helper sex on
243 the probability of helping kin, we carried out Pearson's Chi-squared tests using the mean of the
244 randomly generated proportions. Finally, we compared the expected probability of helping at a nest
245 belonging to a relative to the observed proportion of birds helping kin. The observed proportion of
246 helped nests within each distance band in which the helper was a first order, second order or non-
247 relative was considered statistically significant if it fell outside the 95% confidence interval of the
248 randomly generated distribution. This allowed us to determine whether kin structuring alone could

249 explain observed patterns of helping behaviour, and if not, the degree of discrimination required to
250 direct care towards kin. All analyses were carried out on all helpers and separately by sex, using
251 both genetic data and the social pedigree.

252

253 **Results**

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

263 Spatial analysis of genetic relatedness

264 Mean \pm SE population-level relatedness was 0.011 ± 0.003 among all birds (1719 observations of
265 1022 birds, 73069 within-year comparisons), 0.012 ± 0.004 among males (909 observation of 529
266 males, 20279 comparisons), 0.012 ± 0.005 among females (810 observation of 493 females, 16041
267 comparisons) and 0.009 between males and females (1719 observations of 1022 birds, 36749
268 comparisons). The standard error of relatedness estimates was not quantifiable for male-female
269 genetic structure across years (see Methods), but for within-year estimates of mean relatedness

270 between males and females with standard errors from jack-knifing over loci see SI4, Supplemental
271 Information.

272 Spatial analyses revealed fine-scale genetic structure within our breeding population, with nearby
273 individuals being the most genetically similar (Fig. 1). Pairwise relatedness among all individuals
274 was higher than expected (based on permuted pairwise relatedness) within a radius of 300m and
275 between 300m and 600m (both $p < 0.001$, Fig. 1a). Within each sex, relatedness was higher than
276 expected within 300m and 300-600m for both males (both $p < 0.001$, Fig. 1b) and females (both p
277 < 0.001 , Fig. 1c). Although slightly lower than the within-sex comparisons, dyadic relatedness
278 between males and females was also significantly higher than expected by chance within a radius
279 of 300m and 300-600m (both $p < 0.001$, Fig. 1d). Within-year comparisons between males and
280 females exhibit a similar spatial pattern (SI4, Supplemental Information). The distance at which
281 kin structure breaks down in all comparisons is beyond 600m (Fig. 1). Within this distance,
282 relatedness among males was significantly higher than relatedness among females at 0-300m
283 (GLMM: $F = 20.63$, $df = 1,780$, $p < 0.001$), but not at 300-600m (GLMM: $F = 2.29$, $df = 1,888$, p
284 $= 0.13$).

285 Helping decisions

286 The median distance travelled by failed breeders to help another breeding pair was 263m for males
287 ($n = 164$) and 346m for females ($n = 37$), with most helpers travelling within the 0-300m and 300-
288 600m distance bands (Fig. 2). Using genetic estimates of relatedness, r_{QG} , mean \pm SD relatedness
289 of all helpers to recipient breeding pairs was 0.14 ± 0.16 (95% CI = -0.10-0.36). In the majority of
290 cases ($n = 181$), helpers assisted at least one first or second order relative ($r = 0.5$, 56.9%; $r = 0.25$,
291 13.3%), but a substantial minority of helpers were unrelated to the recipients ($r = 0$, 29.8%).

292 Estimated helper-recipient relatedness was lower using the social pedigree, although a majority of
293 helped nests were again helped by at least one first or second order relative ($r = 0.5$, 39.4%; $r =$
294 0.25 , 14.2%; $r = 0$, 46.5%; $n = 150$ cases).

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

305 Comparing the relatedness of male and female helpers to the recipients of their care, overall males
306 tended to help kin more often than females (Table 1). This was significant using the genetic data
307 (males: $r = 0.5$, 61.8%; $r = 0.25$ 13.9%; $r = 0$, 24.3%; $n = 144$ cases; females: $r = 0.5$, 42.4%; $r =$
308 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
309 (males: $r = 0.5$, 42.6%; $r = 0.25$, 13.2%; $r = 0$, 44.2%; $n = 129$ cases; females: $r = 0.5$, 23.1%; $r =$
310 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
311 probability of helping kin was driven by a relatively small number of unrelated female helpers in
312 the 300-600m distance band (Table 1).

313 The randomisation tests that we conducted to determine the random probability of helping a
314 relative showed, not surprisingly given the kin structure of our population, that the random
315 probability of helping first-order kin decreased with distance for all helpers (genetic data: $\chi^2 = 13.2$,
316 $df = 2$, $p < 0.01$; social pedigree: $\chi^2 = 28.5$, $df = 2$, $p < 0.001$; Fig 3, Table 1), for male helpers
317 (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
318 female helpers (genetic data: $\chi^2 = 7.4$, $df = 2$, $p < 0.001$; social pedigree: $\chi^2 = 32.5$, $df = 2$, $p <$
319 0.001 ; Table 1). There was no significant difference in the opportunity to help a relative between
320 male and female helpers travelling within 0-300m (genetic data: $\chi^2 = 2.41$, $df = 2$, $p = 0.29$; social
321 pedigree: $\chi^2 = 0.88$, $df = 2$, $p = 0.69$; Table 1), 300-600m (genetic data: $\chi^2 = 1.7$, $df = 2$, $p = 0.45$;
322 social pedigree: $\chi^2 = 2.01$, $df = 2$, $p = 0.42$; Table 1) or 600-900m (genetic data: $\chi^2 = 0.31$, $df = 2$,
323 $p = 0.89$; social pedigree: $\chi^2 = 1.33$, $df = 2$, $p = 0.56$; Table 1) to provide help. Crucially, first-order
324 kin were helped significantly more often than expected at random irrespective of distance travelled
325 to provide help (Fig. 3; Table 1). This kin-bias in helping was also apparent among male helpers
326 within all distance bands, and also among female helpers for all but one comparison (Table 1).

327

328 **Discussion**

329 Long-tailed tits breed cooperatively in diffuse family structures, or kin neighbourhoods, such that
330 interactions among non-kin and kin of varying relatedness are frequent. We used a combination of
331 long-term field observations and population genetic analyses to investigate genetic structure and
332 patterns of helping in this atypical social system. Our results showed significant, fine-scale genetic
333 structure in long-tailed tit populations, with positive spatial autocorrelation of dyadic relatedness
334 estimates among breeding birds. Crucially, this pattern exists after natal dispersal, and was strong

335 among males, among females and between the sexes. These findings contrast with most studies of
336 cooperatively breeding birds that have measured spatial-genetic autocorrelation in breeders of both
337 sexes, and show genetic structure in adult males only due to complete female-biased dispersal
338 (Painter et al. 2000; Double et al. 2005; Temple et al. 2006; Woxvold et al. 2006), although fine-
339 scale genetic structure among both sexes has also been demonstrated in sociable weaver colonies
340 (van Dijk et al. 2015). We found that genetic structure was stronger in males than females, but both
341 males and females remained spatially associated with same-sex relatives once they started
342 breeding. This reflects previously reported patterns of dispersal in this species: although females
343 disperse further than males (Sharp et al. 2008a), some adults of each sex disperse only short
344 distances from their natal area to become independent breeders. This underlying genetic structure
345 is also consistent with known patterns of social association during the non-breeding season (Napper
346 and Hatchwell 2016).

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

357 between dyads increased, the proportion of kinships decreased so that only 3% of dyads over 900m
358 were close kin (Fig. SI2, Supplemental Information). Interestingly, using genetic relatedness
359 estimates the proportion of second order kin did not decrease with distance.

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

375 Overall, where they differ from the social pedigree, genetic relatedness estimates may be more
376 reliable. However, pedigree data is essential for understanding how accurately individuals are able
377 to recognise kin, particularly when the mechanism depends on socially learned cues (Sharp et al.
378 2005). Kin recognition via associative learning is likely to be effective where there is a reliable

379 correlation between genetic relatedness and association during a sensitive phase of development, a
380 requirement probably satisfied in most species demonstrating parental care (Komdeur and
381 Hatchwell, 1999). Associative learning is therefore considered the most widespread mechanism of
382 kin recognition in cooperatively breeding birds, where extended brood care at the nest provides a
383 period of association among relatives, and has been identified in many species, including
384 Galápagos mockingbirds *Nesomimus parvulus* (Curry 1998; Curry & Grant 1990), Seychelles
385 warblers *Acrocephalus sechellensis* (Richardson et al. 2003; Komdeur et al. 2004) and splendid
386 fairy wrens *Malurus splendens* (Payne et al. 1988). On the other hand, in some species, such as
387 stripe-backed wrens *Campylorhynchus nuchalis* (Price 1998; 1999) and green woodhoopoes
388 *Phoeniculus purpureus* (Radford, 2005), kin recognition has been suggested to have a genetic
389 component, and studies on bell miners (Wright et al. 2010) and long-tailed tits (Nam et al. 2010)
390 found significant effects of genetic relatedness on helper effort, indicating a mechanism more
391 sophisticated than associative learning alone. Further studies of phenotypic cues in relation to
392 genetic similarity and social information are necessary to determine the precise mechanisms of kin
393 recognition in such species.

394 The social organisation of long-tailed tits gives helpers a choice of whom to aid in a situation where
395 simple decision rules based on spatial cues are insufficient, even at close proximity, for effective
396 kin discrimination. Here, we have shown that helpers assist far more first order kin than expected
397 if help were given randomly with respect to kinship. This is consistent with the finding of Russell
398 and Hatchwell (2001) that helpers exhibit a kin preference in their choice of brood to help.
399 Although most helpers choose to help kin within the range of kin clustering, improving their
400 chances of encountering a relative, 67% helped at a nest belonging to at least one first order kin,

401 compared with a 31% probability of encountering a first order relative within 300m (Table 1).
402 Furthermore, the proportion of helpers that assisted kin did not, in general, decrease with distance;
403 the marginally lower probability of helping first order kin at 300-600m was driven by a small
404 number of female helpers within this range that helped non-kin. Therefore, even when fewer kin
405 were available, helpers still identified a similar proportion of first order kin to help. This degree of
406 kin discrimination is unusual in cooperative breeders; helping more often occurs indiscriminately
407 within closely related family groups (Cornwallis et al. 2009). This is because cooperation typically
408 precedes dispersal, with offspring staying within natal territories to help their parents raise young
409 (Ligon & Stacey 1991). Such viscous populations exhibit strong kin structure and the probability
410 of helping kin is predictably high within the nuclear family unit. In a minority of cooperative
411 breeders helping behaviour occurs post-dispersal and extends beyond the confines of a stable group
412 (Ligon & Burt 2004; Hatchwell 2009) within extended social networks such as neighbourhoods of
413 western bluebirds (Dickinson et al. 1996) and long-tailed tits (Hatchwell 2016), clans of white-
414 fronted bee-eaters *Merops bullockoides* (Emlen & Wrege 1992) or coterries of bell miners (Clarke
415 & Fitz-Gerald 1994). A crucial characteristic of such social structures is that the proportion of kin
416 available to helpers is relatively low, and relatedness among individuals is too variable to favour
417 indiscriminate cooperation (Cornwallis et al. 2009). In such populations, helpers must exercise a
418 degree of discrimination to reliably direct help towards kin, so it is no coincidence that
419 observational and experimental studies of these species have revealed some of the strongest
420 evidence for active kin discrimination in social birds (Emlen & Wrege 1988; Hatchwell et al. 2001;
421 Russell & Hatchwell 2001; Sharp et al. 2005; McDonald & Wright 2011; Akcay et al. 2013;
422 Dickinson et al. 2016).

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

441 Within the range that most helpers travelled to help, there was no difference between the sexes in
442 the opportunity to help kin. Thus, the fact that females comprise only 15% of helpers in this
443 population (Hatchwell et al. 2004) is not because they have few opportunities to help. This result
444 is consistent with a previous study of another population by Sharp et al. (2011), showing that when

445 males and females exhibited similar levels of philopatry, there was still a strong male bias in
446 helping. Therefore, the male bias in helping behaviour is not due to male philopatry, and hence
447 helping opportunities, per se. Instead, the decision to help by failed breeders is probably related to
448 individual condition; failed breeders that choose to become helpers tend to be in better condition
449 than those that do not (Meade & Hatchwell 2010). Since egg-laying, incubating and brooding are
450 performed exclusively by females (Hatchwell 2016), the fitness costs associated with parental care
451 may be more pronounced in females than in males, reducing their likelihood of becoming helpers
452 when breeding attempts fail. We also found an intriguing trend for female helpers to assist fewer
453 close kin and more non-kin than male helpers did. However, we treat this result with some caution
454 because the sample of female helpers available for comparison is still relatively small.

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

462 In conclusion, we have revealed fine-scale genetic structure in our long-tailed tit population that is
463 consistent with the kin clustering expected from known demographic and dispersal patterns. This
464 provides an opportunity for kin selection to operate, and, in the absence of any other known benefits
465 of interacting socially with kin (Napper et al. 2013; Napper & Hatchwell 2016), is likely to have
466 emerged as a result of selection for kin-directed helping behaviour. However, despite this genetic

467 structure, the random probability of helping at the nest of a relative is still relatively low,
468 demonstrating that in the kin-selected cooperative breeding system of long-tailed tits, active
469 discrimination of kin from non-kin is required when choosing at which nest to help in order to
470 maximise inclusive fitness. The learned vocal cues used by long-tailed tits to recognise kin provides
471 one such mechanism (Sharp et al. 2005), although the degree to which they can discriminate among
472 kin of variable relatedness requires further study. Overall, this study demonstrates how population
473 genetic structure, generated by dispersal and life-history traits, determines the opportunity for
474 interactions among relatives, and the selective pressure these exert on kin discrimination rules in
475 social animals.

476

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485

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710 substantial care. *Proceedings of the Royal Society B-Biological Sciences*, **227**, 437-445.

711

712 **Data Accessibility**

713 Sampling locations, microsatellite genotypes and social pedigree to be archived in Dryad.

714

715 **Author Contributions**

716 BJH conceived and managed the long-tailed tit study, and supervised the project with EJHR.

717 AEL, BJH, SPS and MS designed the study and collected long-term data. AEL conducted

718 analyses and wrote the manuscript, and all authors contributed to revisions.

719 **Table 1.** Proportion of help given to nests belonging to at least one 1st order kin, at least one 2nd
720 order kin, or two non-kin over three distance bands between helpers and recipients. Within each
721 band, the proportion of kin helped is compared with that expected if help was given randomly
722 within that range, based on 10000 permutations of potential nests for focal helpers within years
723 (1994-2016). Relatedness between helpers and recipients is estimated using both genetic data and
724 the social pedigree. Analyses were carried out on all helpers, male helpers and female helpers. The
725 observed proportion was considered statistically significant if fell outside the 95% ($p < 0.05$), 99%
726 ($p < 0.01$) or 99.9% ($p < 0.001$) confidence interval of the random distribution.

data	sex	distance (m)	kinship	helped nests	observed proportion	potential nests	expected proportion (mean \pm SD)	p	
genetic	both	0-300	0.5	66	0.67	184	0.31 \pm 0.03	<0.001	
			0.25	8	0.08	127	0.21 \pm 0.02	<0.001	
			0	25	0.25	296	0.47 \pm 0.03	<0.001	
		300-600	0.5	24	0.43	182	0.17 \pm 0.02	<0.001	
			0.25	12	0.21	281	0.21 \pm 0.03	NS	
			0	20	0.36	831	0.62 \pm 0.03	<0.001	
		600-900	0.5	13	0.59	138	0.12 \pm 0.02	<0.001	
			0.25	4	0.18	325	0.24 \pm 0.03	NS	
			0	5	0.23	921	0.65 \pm 0.03	<0.001	
		males	0-300	0.5	58	0.69	162	0.33 \pm 0.03	<0.001
				0.25	6	0.07	105	0.21 \pm 0.03	<0.001
				0	20	0.24	244	0.45 \pm 0.03	<0.001
	300-600		0.5	22	0.49	161	0.18 \pm 0.03	<0.001	
			0.25	11	0.24	245	0.22 \pm 0.03	NS	
			0	12	0.27	700	0.60 \pm 0.03	<0.001	
	600-900		0.5	9	0.60	109	0.11 \pm 0.02	<0.001	
			0.25	3	0.20	262	0.24 \pm 0.03	NS	
			0	3	0.20	767	0.64 \pm 0.03	<0.001	
	females		0-300	0.5	8	0.53	22	0.24 \pm 0.05	<0.001
				0.25	2	0.13	22	0.21 \pm 0.05	NS
				0	5	0.33	52	0.55 \pm 0.04	<0.001
		300-600	0.5	2	0.18	21	0.13 \pm 0.04	NS	
			0.25	1	0.09	36	0.18 \pm 0.06	NS	
			0	8	0.73	131	0.68 \pm 0.06	NS	
600-900		0.5	4	0.57	29	0.12 \pm 0.05	<0.001		
		0.25	1	0.14	63	0.21 \pm 0.06	NS		
		0	2	0.29	154	0.67 \pm 0.07	<0.001		
pedigree		both	0-300	0.5	41	0.47	111	0.24 \pm 0.03	<0.001
				0.25	10	0.11	35	0.09 \pm 0.01	NS
				0	36	0.41	316	0.67 \pm 0.03	<0.001
	300-600		0.5	13	0.27	65	0.08 \pm 0.02	<0.001	
			0.25	8	0.17	47	0.05 \pm 0.01	<0.001	
			0	27	0.56	827	0.86 \pm 0.02	<0.001	
	600-900	0.5	7	0.35	23	0.03 \pm 0.01	<0.001		
		0.25	4	0.20	27	0.03 \pm 0.03	<0.001		
		0	9	0.45	960	0.94 \pm 0.02	<0.001		
	males	0-300	0.5	36	0.49	101	0.24 \pm 0.03	<0.001	
			0.25	8	0.11	31	0.08 \pm 0.02	<0.05	
			0	29	0.39	261	0.67 \pm 0.03	<0.001	

	300-600	0.5	13	0.32	60	0.09 ± 0.02	<0.001
		0.25	7	0.17	40	0.06 ± 0.02	<0.001
		0	21	0.51	717	0.86 ± 0.02	<0.001
	600-900	0.5	6	0.40	20	0.03 ± 0.01	<0.001
		0.25	2	0.13	16	0.02 ± 0.01	<0.001
		0	7	0.47	803	0.95 ± 0.02	<0.001
females	0-300	0.5	5	0.36	10	0.22 ± 0.05	<0.01
		0.25	2	0.14	4	0.12 ± 0.03	NS
		0	7	0.50	55	0.66 ± 0.05	<0.01
	300-600	0.5	0	0.00	5	0.04 ± 0.03	NS
		0.25	1	0.14	7	0.06 ± 0.03	<0.05
		0	6	0.85	110	0.90 ± 0.05	NS
	600-900	0.5	1	0.20	7	0.03 ± 0.03	<0.001
		0.25	2	0.40	7	0.05 ± 0.03	<0.001
		0	2	0.40	157	0.92 ± 0.05	<0.001

727

728 **Figure Legends**

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

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

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





