

1 **Helping decisions and kin recognition in long-tailed tits: is call similarity used to**
2 **direct help towards kin?**

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8 **Abstract**

9 Most cooperative breeders live in discrete family groups, but in a minority, breeding
10 populations comprise extended social networks of conspecifics that vary in relatedness.
11 Selection for effective kin recognition may be expected for more related individuals in
12 such kin neighbourhoods to maximise indirect fitness. Using a long-term social pedigree,
13 molecular genetics, field observations and acoustic analyses, we examine how vocal
14 similarity affects helping decisions in the long-tailed tit *Aegithalos caudatus*. Long-tailed
15 tits are cooperative breeders in which help is typically redirected by males that have failed
16 in their own breeding attempts towards the offspring of male relatives living within kin
17 neighbourhoods. We identify a positive correlation between call similarity and kinship
18 suggesting that vocal cues offer a plausible mechanism for kin discrimination.
19 Furthermore, we show that failed breeders choose to help males with calls more similar
20 to their own. However, although helpers fine-tune their provisioning rates according to
21 how closely related they are to recipients, their effort was not correlated with their vocal
22 similarity to helped breeders. We conclude that although vocalisations are an important

23 part of the recognition system of long-tailed tits, discrimination is likely to be based on
24 prior association and may involve a combination of vocal and non-vocal cues.

25 **Keywords:** Cooperative breeding, kin recognition, kin discrimination, call similarity,
26 acoustics.

27 **Introduction**

28 Kin selection is often invoked to explain the evolution of cooperation in kin-structured
29 communities, and is expected to result in selection for some mechanism to discriminate
30 kin from non-kin (1). A recognition mechanism that permits the differential treatment of
31 conspecifics according to their genetic similarity (2) enables individuals to avoid
32 inbreeding (3, 4) and maximise inclusive fitness (1, 5) in populations where kin and non-
33 kin associate beyond reproductive maturity. However, although the adaptive functions of
34 kin recognition are well known, the proximate mechanisms, including sensory cues and
35 cognitive thresholds, are often difficult to determine. Our current understanding of kin
36 recognition in social animals is that discriminating individuals acquire cues to kinship
37 from a referent (oneself, a subset of kin, or the local environment), which are used to form
38 internal templates (6) at a sensitive phase during development (7). Although, it may also
39 be possible that cues and templates are genetically determined (8). Templates are later
40 compared with the phenotypes of encountered conspecifics, and discriminatory behaviour
41 is performed based on the perceived similarity between templates and encountered
42 phenotypes (9). Thus, any cue that reliably co-varies with relatedness may be used to
43 discriminate kin from non-kin.

44 Most animal societies exhibit a substantial degree of kin structure, whereby individuals
45 are organised in to more or less discrete family units of parents and their retained offspring

46 (10-12). If the probability of encountering a relative is high, individuals can maximise
47 indirect fitness by indiscriminately cooperating within their group (13, 14) and avoid
48 inbreeding by selecting partners from outside the group (15). In less viscous societies,
49 such spatial cues to kinship may be unreliable. For example, in a small number of
50 cooperatively breeding birds, cooperation occurs after natal dispersal, across extended
51 networks of relatives known as *kin neighbourhoods* (16). Here, the relatedness among
52 spatially clustered individuals is less predictable, so kin recognition based on the
53 phenotypic cues of potential social partners may be necessary (17). In such situations,
54 selection should favour effective discrimination, but any recognition system is prone to
55 error because phenotypic cues overlap between non-kin and kin of varying relatedness
56 due to individual variation (6). Thus, kin recognition is likely to involve a certain rate of
57 *acceptance errors*, where non-kin are perceived as kin, and *rejection errors*, where kin
58 are perceived as non-kin (2, 6). The accuracy of kin recognition, and hence the frequency
59 of such errors, depends on their relative costs, which, in turn, is determined by the
60 probability of encountering a relative and the fitness consequences of the associated
61 behaviours (18). This theoretical framework is supported empirically by intraspecific
62 studies showing shifts in acceptance thresholds as the costs of error change (19), and by
63 comparative analyses that demonstrate stronger kin discrimination in cooperatively
64 breeding vertebrates where the benefits of helping are greater (20), and when the average
65 relatedness within a group is lower and more variable (13).

66 Kin recognition often requires prior association; individuals learn the phenotypes of kin
67 encountered during a sensitive phase and distinguish these familiar individuals from
68 unfamiliar ones later in life (7). Alternatively, recognition may involve phenotype-
69 matching, whereby individuals form a generalised template against which the phenotypes

70 of unknown individuals are compared (21). Phenotype matching does not require a period
71 of previous association between matching individuals (22), but relies on a positive
72 correlation between template-phenotype similarity and degree of genetic relatedness (23).
73 Whether kin are recognised through prior association or phenotype matching can be
74 difficult to determine; both mechanisms involve matching phenotypes to learned cues,
75 yet they differ in template specificity (6), such that mechanisms involving phenotype
76 matching permit individuals to recognise unfamiliar kin and distinguish between kin of
77 varying relatedness.

78 Kin recognition based on familiarity may often be sufficient for individuals to maximise
79 inclusive fitness by directing help towards relatives, and prior association is indeed
80 thought to be the most common mechanism of kin recognition in cooperatively breeding
81 birds (24-26). However, studies on long-tailed tits *Aegithalos caudatus* (27) and bell
82 miners *Manorina melanophrys* (28), species in which helping occurs within kin
83 neighbourhoods, found that helpers modify provisioning effort according to their degree
84 of relatedness to recipient broods. In such situations, the risk of caring for non-kin is high,
85 so kin recognition mechanisms with low error rates are likely to be selected for (13).
86 Moreover, finely tuned adjustment of provisioning behaviour in relation to kinship could
87 indicate a relatively sophisticated mechanism of kin recognition that involves phenotype
88 matching. Vocalisations are used as kin recognition cues in both species (29, 30) and
89 more widely in birds (31), although olfactory kin recognition has also been described in
90 a few species (32-34). In bell miners, a relationship between genetic relatedness and vocal
91 similarity has been reported (30), but whether this relationship exists in other species
92 remains to be tested.

93 This study aimed to identify the mechanism permitting kin-directed cooperation and
94 flexible helper investment in long-tailed tits, a kin-neighbourhood cooperative breeder
95 that exhibits effective kin recognition in the absence of spatial cues (35). Helpers are
96 failed breeders that redirect their care following unsuccessful attempts at independent
97 breeding. Around 50% of successful nests receive help (36), typically from one or two
98 helpers, but not all failed breeders choose to become helpers (37). Although our study
99 population is kin-structured during breeding, most neighbours are non-kin and help is
100 directed towards close kin more often than expected by indiscriminate helping (38).
101 Furthermore, helpers provision more closely related broods at higher rates (27). Helpers
102 are overwhelmingly male, and gain indirect fitness benefits by increasing the productivity
103 of related broods (39, 40). In contrast, no direct fitness benefits of helping have been
104 identified (41, 42). Vocalisations play a major role in in the coordination of cooperative
105 behaviour (29). Previous studies have demonstrated individuality in the churr call: a
106 short-range contact call often used at the nest (43). Playback and cross-fostering
107 experiments have shown that individuals can recognize siblings using the churr call, and
108 that these calls are learned during development (29). However, whether churr call
109 similarity is used to assess relatedness when making helping decisions remains untested.

110 Here, we quantified variation in churr call structure within and between adult long-tailed
111 tits and determined which sound parameters explained this variation. We also tested for
112 an association between call similarity and relatedness, and examined whether degree of
113 vocal similarity influenced helping decisions by analysing the churr call similarity of
114 helpers to the breeders they helped and to nearby breeders they did not help. Finally, we
115 investigated whether long-tailed tits helpers adjust their provisioning effort according to
116 how similar their churr calls are to the helped breeders.

117 **Methods**

118 *Study site and field methods*

119 Fieldwork was carried out on a population of 31-46 breeding pairs of long-tailed tits in
120 the Rivelin Valley, Sheffield, UK (53°38'N 1°56'W) from 2015 to 2017. The site is
121 approximately 2.5km² and comprises predominantly deciduous woodland, scrub and
122 farmland. This population of *c.*50 pairs (range: 18 – 72) has been studied extensively
123 since 1994. The population is open: approximately 40% of breeders hatched in the study
124 site, and are referred to as 'native' (A. E. Leedale & B. J. Hatchwell, unpublished data,
125 2017), while the remaining 'immigrant' adults are assumed to have dispersed in to the
126 study area during their first winter, because individuals show high breeding site fidelity
127 following their first breeding year (40). Almost all individuals (*c.*95%) were uniquely
128 colour-ringed for field identification. Native birds were ringed as 11-day old nestlings
129 and immigrants were captured in mist nests and ringed under British Trust for
130 Ornithology licence during breeding. A sample of 5-30µl of blood was taken by brachial
131 venepuncture under Home Office licence. All nesting attempts were closely monitored to
132 record breeding events and life-history traits such as timing of breeding, clutch size,
133 incubation period and brood size, and the identity of parents and helpers. Nest locations
134 were recorded using GPS receivers to an accuracy of 8m. For most nests, provisioning
135 behaviour was observed every two days from day two of the nestling period (day 0 = day
136 of hatching; long-tailed tit broods hatch synchronously) to fledging (typically day 16 or
137 17) or until nest failure. Most observation periods lasted 1h, during which the identities
138 and visit rate of all carers were recorded. For further details of provisioning observations,
139 see (27, 39).

140 The churr call is disyllabic, consisting of an initial syllable of one or two unique elements,
141 followed by a second syllable comprising a single element repeated several times (44).
142 The churr calls of adult carers were recorded at the nest using a Sennheiser ME67/K6
143 shotgun microphone onto a Roland R-05 version 1.03 WAV/MP3 recorder, with a sample
144 rate of 48kHz, WAV-16bit accuracy, an input level of 60db and a low-cut frequency of
145 400Hz. All recordings were made in dry conditions between 06:00 and 18:00 BST. Birds
146 were recorded at a distance of approximately 3-15m, to minimize the effects of sound
147 degradation and reverberation. Birds were identified by their colour ring combinations.
148 During recording, bird ID was dictated into the microphone after each call. In total, 213
149 recordings were made, containing 1116 churr calls from 98 individuals (mean = $11.39 \pm$
150 10.24 SD per bird; range 1 – 42).

151 *Bioacoustic analysis*

152 Recordings were digitized with 16-bit accuracy at a sampling rate of 48kHz.
153 Spectrograms were produced in Avisoft SAS-Lab Pro version 4.52 (Avisoft Bioacoustics,
154 Raimund Specht, Berlin, Germany) using a 256-point FFT length with a Hamming
155 window, 100% frame size and 50% window overlap, generating a frequency resolution
156 of 188Hz and a time resolution of 2.7ms. All recordings were visualized
157 spectrographically to assess quality. Some background noise was removed using a high-
158 pass filter of 1.5 kHz, though recordings with extreme background noise were excluded.
159 The sampling frequency was converted to 22.05 kHz for further analysis. As long-tailed
160 tit calls range from 2-9kHz, this re-sampling does not affect the acoustic signal. All
161 useable calls were stored and measured in Luscinia version 2.16.10.29.01
162 (<https://rflachlan.github.io/Luscinia/>).

163 A subset of data was tested for individual repeatability. To determine the minimum
164 number of calls required to capture individual variation, the cumulative repertoire size
165 (number of distinct calls, based on number of syllables) was plotted against the number
166 of calls considered to that point, for 100 churr calls, 10 from each of 10 birds recorded on
167 at least two days in 2015. The resulting plots generally levelled off before the number of
168 calls reached six (mean calls needed to reach asymptote = 5.5 ± 2.89 SD, range 2-10).
169 Therefore, repeatability tests were carried out on all calls from individuals with recordings
170 of at least six calls from at least two days between 2015 and 2017: 907 churr calls from
171 54 individuals (mean = 17.46 ± 10.02 SD per bird; range 6 – 42). Within-individual
172 repeatability was tested using two approaches. The first approach compared within and
173 between-individual variation in overall call structure using dynamic time-warping
174 (DTW), implemented in Luscinia. The second tested the individual repeatability of
175 specific vocal characteristics (defined in Supplementary Table S1).

176 Dynamic time warping (DTW) is a distance-based programming technique used to search
177 for an optimal alignment of two signals, which has been implemented for use in
178 bioacoustics. The algorithm calculates a distance score between signals based on certain
179 acoustic features, with greater distance meaning lower similarity. The acoustic features
180 used in the DTW analysis were weighted as: time = 1, fundamental frequency = 2, change
181 in fundamental frequency = 2, compression factor = 0.1, minimum element length = 10,
182 time SD weighting = 1, ArcTan transform weight for frequency slope = 0.02, maximum
183 warp = 100%. These settings generated a DTW algorithm that correctly matched visually
184 similar vocalizations, assessed using a dendrogram and multidimensional scaling plot.
185 This is also in line with previous studies suggesting that frequency parameters show
186 greater individuality than temporal parameters and are particularly important for kin

187 recognition in this species (43). Pairwise comparisons of individual calls generated a
188 matrix of DTW scores for each pair of calls. To compare call similarity within and
189 between individuals, pairwise comparisons were assigned a value according to whether
190 the comparison was made between calls from the same individual (0) or from two
191 individuals (1). The DTW scores were aggregated and mean call similarity within and
192 between individuals was compared. Since this analysis contained calls from across years,
193 the measures of call similarity were also compared within and between years.

194 *Relatedness*

195 Individuals were genotyped at 17 microsatellite loci. Genetic relatedness was estimated
196 using coefficient of relatedness (r_{QG}) (45) in SPAGeDi version 1.1.5 (46). This
197 relatedness estimate is reliable when tested against our social pedigree (27). For further
198 details on genotyping, see (47, 48). The population allele frequencies used in analyses
199 were generated using all genotyped individuals (1994-2017, $n = 3304$) in CERVUS
200 version 3.0.7 (49) to ensure non-zero frequencies for all alleles. To calculate social
201 relatedness among dyads, an additive relationship matrix was generated from the social
202 pedigree (1994-2017, $n = 3068$) in R version 3.5.0 (50), using the nadiv package (51). For
203 further details on social and genetic relatedness estimates, see (38). Hereafter, genetic
204 relatedness refers to the r_{QG} coefficients calculated from the microsatellite markers,
205 whereas kinship refers to social relationships derived from the pedigree.

206 *Call similarity, relatedness and helping*

207 Vocal similarity between individuals (1116 calls from 98 individuals) was quantified by:
208 (i) DTW analysis, and (ii) the difference in repeatable ($R > 0.2$) acoustic parameters
209 (Table 2), measured as Euclidean distances using the R package, spaa (52). To investigate

210 how vocal similarity varied with relatedness, we tested for a relationship between churr
211 call similarity and both genetic relatedness and kinship. For the latter, three degrees of
212 kinship were considered: first-order ($r = 0.5$), second-order ($r = 0.25$), or non-kin ($r <$
213 0.25); non-kin relationships included only those birds for which the parentage of both
214 birds in the dyad was known.

215 Helpers observed in 2015 – 2017 were related to male but not female parents of the broods
216 they provisioned (see Results), so our analyses focused on helpers' vocal similarity to
217 breeding males. If individuals use vocal similarity as a cue to relatedness, in order to
218 direct helping effort towards close kin, helpers were expected to be more vocally similar
219 to the breeders they helped than the breeders they did not help. For each helper, vocal
220 similarity to male breeders at their first chosen nest in a given year ($n = 19$) was compared
221 with their mean vocal similarity to a sample of potential males (excluding those helped)
222 nesting within 750m that year ($n = 272$), the range in which the majority of failed breeders
223 travel to provide help (mean = 337.4m \pm 253.4 SD, 95% CI = 744.1m, $n = 220$). Helping
224 distance was calculated as the distance between a helper's last failed breeding attempt
225 and the nest at which they first appeared as a helper in the same year.

226 To investigate whether helpers use vocal similarity to modify their provisioning effort,
227 we tested for a relationship between the provisioning rates of helpers and their vocal
228 similarity to the helped males. Because vocal similarity is a putative cue to relatedness
229 (53), we also tested for a relationship between provisioning rate and relatedness, using
230 genetic relatedness estimates and kinship from the social pedigree. Although the fitness
231 consequences of helping depend on genetic relatedness, pedigree data is essential for
232 understanding how accurately individuals are able to recognize kin, particularly when the

233 mechanism depends on socially learned cues (29). Provisioning rate was therefore
234 expected to correlate most strongly with kinship.

235 *Statistical analysis*

236 Statistical analysis was carried out using R version 3.5.0 (50). Overall similarity in call
237 structure within and between individuals was compared using a generalized linear mixed-
238 effects model (GLMM) fitted by restricted maximum likelihood (REML) in the lme4
239 package (54). The square root of DTW distance score was fitted as the dependent variable,
240 comparison type (within or between individuals) as a fixed effect and bird ID 1 and bird
241 ID 2 as nested random effects. To test for differences across years, within or between
242 years was also fitted as a fixed effect and year 1 and year 2 fitted as nested random effects.
243 Individual repeatability based on call parameters was carried out using multiple GLMMs
244 in the rptR package (55, 56). Year and ID were set as grouping variables, allowing for
245 effects of year and ID to be tested. Gaussian models were used to test the repeatability of
246 continuous variables and Poisson models were used to test the repeatability of count
247 variables. To test for sex differences in call characteristics, GLMMs were built with each
248 sound parameter as the dependent variable, sex as a fixed effect and bird ID and year as
249 random effects. The significance of fixed effects was reported using Satterthwaite's
250 degrees of freedom in the lmerTest package (57).

251 The relationships between vocal similarity and relatedness among adult breeders were
252 analysed using Mantel tests based on Spearman correlations of ranked distances with
253 10000 permutations using the R package, ecodist (58). The relatedness of helpers to
254 breeders that were helped and those that were not helped was compared using a Pearson's
255 chi-squared test for kinship data and a general linear model fitted in lme4 for genetic

256 relatedness estimates. Vocal similarity within observed helper-breeder dyads was
257 compared to mean vocal similarity within potential dyads for each focal helper using
258 Wilcoxon signed-rank tests. Significance values were based on two-tailed tests and
259 sample sizes are reported with the results. The vocal similarity of helpers to: (i) helped
260 kin, (ii) helped non-kin, and (iii) non-kin that were not helped was compared using a
261 GLMM fitted with DTW score measured as a continuous variable with a Gamma
262 distribution and log link, and helper ID as a random effect.

263 To investigate whether helper provisioning rates varied with respect to their vocal
264 similarity to male breeders, we used linear mixed-effects models fitted by REML. Genetic
265 relatedness, kinship and vocal similarity were expected to be closely correlated, so their
266 effect on provisioning rate was analysed in three separate models. In each model, the
267 provisioning rate of helpers (number of visits/hour) was the response variable. In the first
268 model, the explanatory variables were: nestling age, brood size, group size and genetic
269 relatedness, all of which influence the provisioning rates of helpers (36). In the second
270 model, the explanatory variables were: nestling age, brood size, group size and kinship.
271 In the third model, the explanatory variables were: nestling age, brood size, group size
272 and vocal similarity. Genetic relatedness was the r_{QG} estimate between helpers and male
273 breeders, measured as a continuous variable. Kinship was the relationship between
274 helpers and male breeders according to the social pedigree (three factor levels: $r = 0$, $r =$
275 0.25 and $r = 0.5$). Vocal similarity was the DTW score of churr calls between helpers and
276 male breeders. Nestling age was measured in days from hatching (day 0). Brood size was
277 the number of chicks in the nest on day 11, a good indicator of brood size from hatching
278 because nestling starvation is rare (37). Group size was the number of adults that
279 provisioned a nest (parents and helpers). Bird identity and nest identity were included as

280 random effects, to control for non-independence of repeated observations of feeding rates
281 by the same birds, and repeated observations of feeding rates at the same nest. All
282 explanatory covariates were initially included in full models and then dropped
283 sequentially unless doing so significantly reduced the amount of variance explained,
284 generating three minimum adequate models containing either genetic relatedness, kinship
285 or vocal similarity as explanatory variables.

286 **Results**

287 *Individual repeatability*

288 Visual inspection of spectrograms suggested that churr calls from the same individual
289 were more similar in acoustic structure than those of different individuals. This was
290 confirmed quantitatively, because the distance score from DTW for within-individual
291 comparisons was significantly lower than that for between-individual comparisons
292 (GLMM, estimate \pm SE = -0.08 ± 0.008 , $df = 1561$, $t = -9.9$, $p < 0.001$; Fig. 1). Whether
293 comparisons were made between calls recorded in the same or different years did not
294 affect DTW distance score (GLMM, estimate = -0.003 ± 0.005 , $df = 4917$, $t = -0.65$, $p =$
295 0.55). Churr calls were repeatable within individuals based on all of the parameters tested,
296 with maximum fundamental frequency across the churr call showing the greatest
297 individual repeatability (Table 1). There was no effect of recording year or sex on any of
298 the parameters tested (Supplementary Table S2-S3).

299 *Call similarity, relatedness and helping*

300 Although there was substantial variation in vocal similarity among breeders in all
301 pedigree categories (Supplementary Fig. S1), churr call similarity correlated with kinship

302 in several acoustic parameters: DTW score (Mantel test, $R = -0.06$, $p < 0.01$), difference
303 in mean frequency ($R = -0.04$, $p = 0.03$) and difference in frequency bandwidth ($R = -$
304 0.04 , $p = 0.03$; Fig. 2a, Table 2). In contrast, churr call similarity did not correlate with
305 genetic relatedness (Fig. 2b, Table 2).

306 Based on the social pedigree 32% (6/19) of helpers in 2015 – 2017 were known first-
307 order relatives of the male, 16% (3/19) were second-order relatives of the male, and 55%
308 (10/19) were apparently unrelated to the male. Thus, the mean relatedness of helpers to
309 male breeders from the social pedigree was $r = 0.19 \pm 0.2$ SD ($n = 19$). The mean genetic
310 relatedness of helpers to male breeders was $r = 0.17 \pm 0.2$ SD ($n = 15$), showing that
311 estimates of kinship from our pedigree closely match genetically estimated relatedness.
312 In contrast, there were no cases of help given to known female kin, and the mean genetic
313 relatedness of helpers to females was $r = -0.04 \pm 0.12$ SD ($n = 13$). As expected, the
314 kinship between helpers and males that they did not help within 750m was significantly
315 lower: 10.6% ($n = 226$) of relationships in which kinship was known were first order
316 kinships, 4.4% were second order kinships, and 84.9% dyads were unrelated (Pearson's
317 Chi-squared test, $\chi^2 = 17.3$, $p < 0.001$). Likewise, the mean genetic relatedness of helpers
318 to breeding males within 750m that were not helped was $r = 0.07 \pm 0.18$ SD ($n = 272$),
319 significantly lower than that observed for males that were helped (GLM, $t = 2.55$, $p =$
320 0.01).

321 The decision of which male to help was positively associated with call similarity, as
322 predicted. Failed breeders helped males that had more similar churr calls than those they
323 did not help, based on DTW score (Wilcoxon signed-rank test: $v = 20$, $n = 19$, $p < 0.01$;
324 Table 3). Furthermore, there was no significant difference in the call similarity of helpers

325 to helped kin and helped non-kin (GLMM: $t = -0.29$, $n = 19$, $p = 0.77$). In contrast, call
326 similarity of helpers to non-kin that were helped was significantly greater than to non-kin
327 that were not helped (GLMM: $t = -2.52$, $n = 19$, $p = 0.01$; Fig. 3). For full model outputs,
328 see Supplementary Table S4.

329 Finally, we examined whether helper effort was modified according to relatedness and/or
330 call similarity, analysing the provisioning data that were available for 14 of the 19 cases
331 of helping. These included 41 observation periods of 14 helpers at 11 nests over 3 years
332 (mean duration of observation = $180.1 \text{ min} \pm 145.3 \text{ SD}$ per nest, range = 1 – 8h, mean
333 feeding rate (visits per hour) = $5.05 \pm 2.56 \text{ SD}$ per helper, range = 1 – 10.4). The
334 provisioning rate of helpers increased with kinship between helper and male breeder
335 (GLMM: $\chi^2 = 5.68$, $p = 0.02$), an effect that increased with group size (GLMM: $\chi^2 = 11.61$,
336 $p < 0.001$). The provisioning rate of helpers also increased with genetic relatedness
337 between helper and male breeder when group size was large (GLMM: $\chi^2 = 5.94$, $p = 0.01$).
338 In contrast, the vocal similarity between helper and male breeder had no effect on helper
339 provisioning rate (GLMM: $\chi^2 = 0.01$, $p = 0.9$). For full model comparisons, see
340 Supplementary Table S5.

341 **Discussion**

342 Vocalisations are clearly a critical component of the kin-selected cooperative breeding
343 system of long-tailed tits. Our results show, along with previous studies, that these calls
344 are individual-specific (43) and that churr call similarity is positively associated with
345 kinship (53), although this was the case for the social pedigree but not for genetic
346 relatedness estimated from microsatellite data. This finding is consistent with previous
347 experimental studies indicating that churr calls are learned from provisioning adults in

348 early development (29). The sample of helpers included in this study showed a strong
349 preference for kin relative to their availability, a finding that is again consistent with
350 previous observational and experimental studies (35, 38). Importantly, we found that
351 churr calls offer a potential mechanism to facilitate this kin preference because the calls
352 of helpers were more similar to males they helped than to those they did not. Moreover,
353 some helpers assisted unrelated recipients, and we found that call similarity between
354 helpers and these non-kin recipients was greater than that between helpers and non-kin
355 they could have helped. By contrast, there was no difference in the call similarity of
356 helpers to kin and non-kin recipients. Finally, contrary to expectations, we found that
357 although the provisioning effort of helpers was correlated with kinship, again supporting
358 the findings of a previous study (27), helper effort was not predicted by call similarity to
359 the helped male breeder.

360 Previous studies have revealed a strong preference for kin by helpers in long-tailed tits
361 (35, 38), as reported in many other cooperatively breeding vertebrates (e.g. 13, 20, 24,
362 30, 59). Studies of other species have also shown that kin recognition is achieved using
363 vocal cues (e.g. 60-62), and this study provides further direct evidence that vocal
364 similarity is the mechanism of kin recognition that permits kin-directed helping in long-
365 tailed tits. However, there remains the persistent puzzle that a significant proportion of
366 helpers in this species help broods to which they are unrelated (27, 38, 41), even though
367 they appear to gain no benefit from doing so (42). As vocalisations are learned in the nest,
368 it is possible that helpers (whether related or unrelated) could gain future direct benefits
369 through increasing the chances of being identified as kin by the grown offspring of the
370 broods they helped. However, reciprocal helping is rare; in just 3.7% cases did helpers
371 choose to help breeders that had helped at their natal nest. Indeed, the high annual

372 mortality rate provides little opportunity for reciprocity from helped broods (42), and
373 most helping occurs among siblings (27, 35). It therefore seems unlikely that the
374 opportunity to be identified as kin by helped broods could drive helper decisions. Instead,
375 our results support an earlier suggestion that this counter-intuitive behaviour arises from
376 recognition errors (41).

377 The theoretical framework of the acceptance threshold model argues that an actor
378 categorises conspecifics depending on an acceptance threshold: a degree of template-
379 phenotype dissimilarity below which it will accept and above which it will reject
380 conspecifics as kin (6). Our results suggest that long-tailed tits use degree of vocal
381 similarity to recognise close kin, but also that their threshold for kin discrimination does
382 lead to acceptance errors, with non-kin sometimes helped despite there being no known
383 benefit of doing so (38, 41, 42). There are two reasons why long-tailed tit helpers may be
384 'generous' with their help and inclined to make acceptance errors. First, although failed
385 breeders may prefer to help close kin, given that relatives are clustered within the range
386 that most helping occurs (38), there might still be a reasonable chance of gaining some
387 indirect fitness by helping an unfamiliar individual because they could be more distantly
388 related. This suggestion is supported by the finding of Leedale *et al.* (38) that the
389 frequency of helping second order relatives was as expected from random choice among
390 nearby males. Secondly, Hatchwell *et al.* (41) argued that the costs of helping are low in
391 long-tailed tits because there is no cost of lost breeding opportunity (all helpers are
392 breeders that have failed to reproduce successfully) and help is provided for only a short
393 period during the nestling and post-fledging stage. In contrast, the potential benefit of
394 helping, via the increased recruitment of relatives is high. Therefore, a permissive
395 threshold for acceptance of another individual as kin should be selected for (6). The

396 critical finding from that previous study (41) is that even with low mean relatedness
397 between helpers and the brood they care for ($r = 0.17$), Hamilton's rule for the evolution
398 of altruistic helping was satisfied.

399 Our finding that social pedigree was a better predictor of vocal similarity than genetic
400 relatedness estimates was expected given that churr calls are learned (29). Several other
401 species of cooperatively breeding birds have family- or group-specific vocalisations that
402 are also consistent with this mechanism (60, 63-66). Learned kin recognition cues in any
403 sensory modality are expected to be reliable if they are acquired at a time when
404 associating individuals are close kin. In long-tailed tits, churr calls develop in the nest,
405 learned from tutors that are likely to be first-order relatives, increasing vocal similarity
406 among first-order kin relative to the general population (29). Nevertheless, a continuous
407 positive correlation between call similarity and relatedness, rather than a threshold effect,
408 could arise if calls are learned from parents; for example, half-siblings that share one
409 parent could be less vocally similar than full siblings that share two parents, or uncles,
410 aunts and even cousins could conceivably retain some family-specific vocal traits.
411 Although genetic relatedness estimates are reliable when tested against our social
412 pedigree (27), the variation and overlap in genetic relatedness estimates for first-order,
413 second-order and non-kin (Supplementary Fig. S1) may explain the weak correlation
414 between vocal similarity and genetic relatedness estimates compared with pedigree
415 kinship (53).

416 Our results do not exclude a genetic influence on vocal variation; indeed, high individual
417 repeatability suggests some innate individual differences in long-tailed tits. Yet, any
418 recognition system that relies entirely on genetically acquired cues may be susceptible to
419 rejection errors because mutation and recombination would cause even close kin to be

420 genetically dissimilar at some loci (67, 68). Genetic recognition cues have been reported
421 in several non-avian taxa (69-70), but in social birds, kin recognition typically requires a
422 critical period of learning during which recognition templates are formed (7). However,
423 very little is known about how socially learned recognition cues develop; for example,
424 which adults act as tutors, or whether offspring can distinguish between helpers and
425 parents during learning is unknown in any cooperative breeder. Further investigation into
426 the learning and development of vocal cues in situations where there are multiple potential
427 tutors is a worthwhile avenue for further study.

428 Familiarity is the most widely supported mechanism of kin recognition in cooperatively
429 breeding birds (71, 72), with kin association during extended brood care providing the
430 sensitive period during which reliable recognition templates can form (7). In long-tailed
431 tits, it is very likely that first-order kin are associated during this crucial period, but there
432 are instances in which this is not the case. First, extra-pair paternity occurs at low rates
433 and results in half-siblings being raised together (73). Second, offspring presumably
434 acquire recognition templates from any second-order kin or non-kin that helped them as
435 a nestling. Third, pair-bonds that last more than one year may produce full-siblings that
436 have not been raised together, although the high annual mortality rate (42), high divorce
437 rate (74) and low probability of successful reproduction (39) together make this a rare
438 event. But, despite these complicating factors, the pattern that long-tailed tits usually help
439 at the nest of individuals with whom they have had close prior association (29, 75)
440 supports the idea that familiarity is the principal driver of helping decisions. Familiarity
441 is also a stronger predictor of cooperative behaviour than genetic relatedness in Galápagos
442 mockingbirds *Nesomimus parvulus* (24) and Seychelles warblers *Acrocephalus*
443 *sechellensis* (26). In the latter species, helpers provision the offspring of breeding females

444 that provisioned them as a nestling, suggesting the context of prior association influences
445 helper decisions (76).

446 Although kinship to male breeders explained a considerable amount of the variation in
447 the provisioning rates of individual helpers, helpers did not adjust their provisioning rates
448 according to vocal similarity to those breeders, suggesting that churr call similarity alone
449 is not responsible for the fine-tuning of helping effort in relation to kinship. Therefore,
450 although vocalisations may convey kinship cues, assessment of relatedness based solely
451 on degree of call similarity is unlikely. This contrasts with studies of the bell miner, which
452 identified ‘mew’ call similarity as the cue to relatedness that allows helpers to make fine-
453 scale adjustments in their provisioning effort (28, 30). However, whether the reported
454 relationship between provisioning effort and call similarity in bell miners is continuous
455 or threshold-based is unclear. Bell miners live in complex societies in which membership
456 of a coterie does not guarantee kinship and there is no evidence of a period of call learning,
457 suggesting that ‘mew’ calls are innate (30). Such genetically acquired cues would permit
458 bell miners to recognise relatives in a population where familiarity does not signal
459 kinship. By contrast, the social structure of long-tailed tits is relatively simple. The
460 proximity of non-kin and kin of variable relatedness within breeding populations also
461 necessitates active kin recognition, but the period of more or less exclusive association
462 between close kin in early life provides an opportunity to learn the identity of kin that is
463 missing in bell miners.

464 **Conclusions**

465 Our results indicate that vocal similarity is part of a combination of cues that allows
466 individual long-tailed tits to recognise familiar individuals. The positive relationship

467 between provisioning effort and relatedness to the brood may reflect a decision to help
468 more familiar kin at a higher rate than less familiar individuals that are likely to be more
469 distant kin. Discrimination based on prior association or familiarity would permit this
470 adjustment. Regarding the precise cues used for discrimination, whether they are learned
471 or genetic, a recognition mechanism that depends on variation in a single trait may be
472 unstable; individuals bearing common cues are more likely to be accepted as kin than
473 those with rare cues, leading to phenotypic convergence or fixation, and subsequent
474 breakdown of the recognition system (77). A recognition system based on multi-
475 component kin 'signatures' would be less vulnerable to such processes.

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681 **Table 1.** Repeatability of long-tailed tit churr call parameters based on caller identity (*n*
682 = 907 calls from 54 birds).

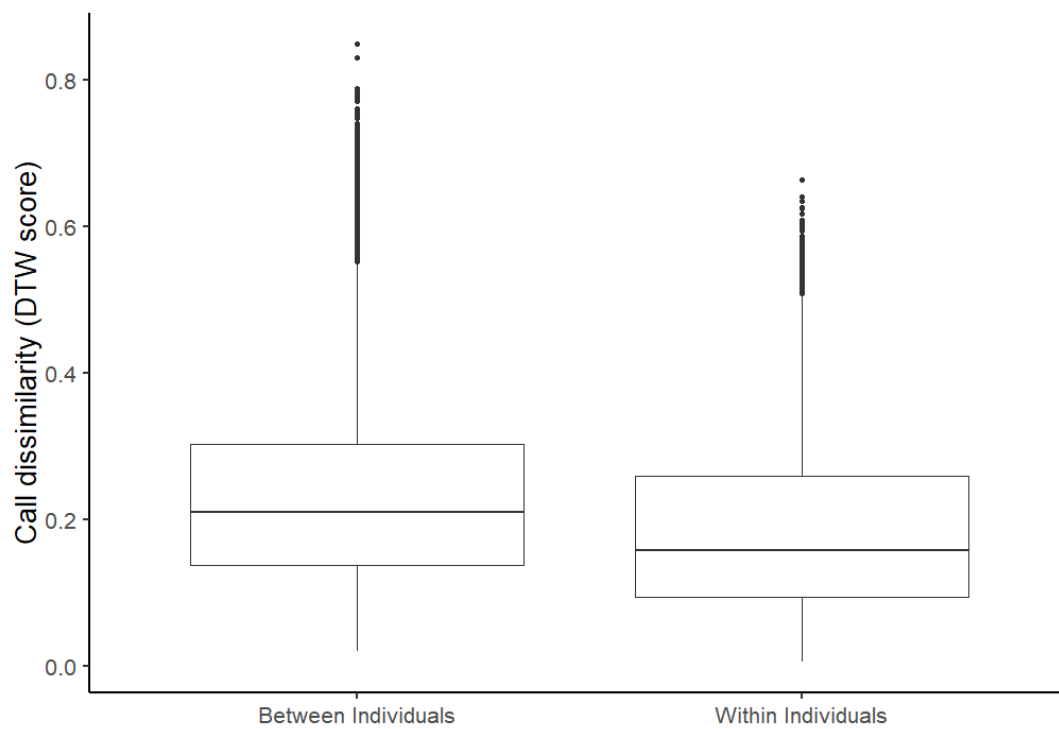
Call parameter	<i>R</i> ± SE	CI	<i>p</i>
Duration (ms)	0.33 ± 0.05	0.22, 0.42	< 0.001
Number of repeats	0.07 ± 0.02	0.02, 0.09	< 0.001
Fundamental frequency (Hz)	0.29 ± 0.05	0.19, 0.38	< 0.001
Maximum fundamental frequency (Hz)	0.5 ± 0.07	0.35, 0.61	< 0.001
Bandwidth (Hz)	0.21 ± 0.05	0.13, 0.3	< 0.001
Weiner Entropy	0.19 ± 0.04	0.11, 0.27	< 0.001

683 **Table 2.** The correlation between churr call dissimilarity and relatedness in long-tailed
684 tits based on dynamic time-warping analysis (DTW) and the difference (Δ) in a range of
685 acoustic parameters. The results and significance values from Mantel tests are reported
686 for dyadic comparisons among breeders based on degree of kinship calculated from the
687 social pedigree ($n = 80$), and genetic relatedness estimates ($n = 88$).

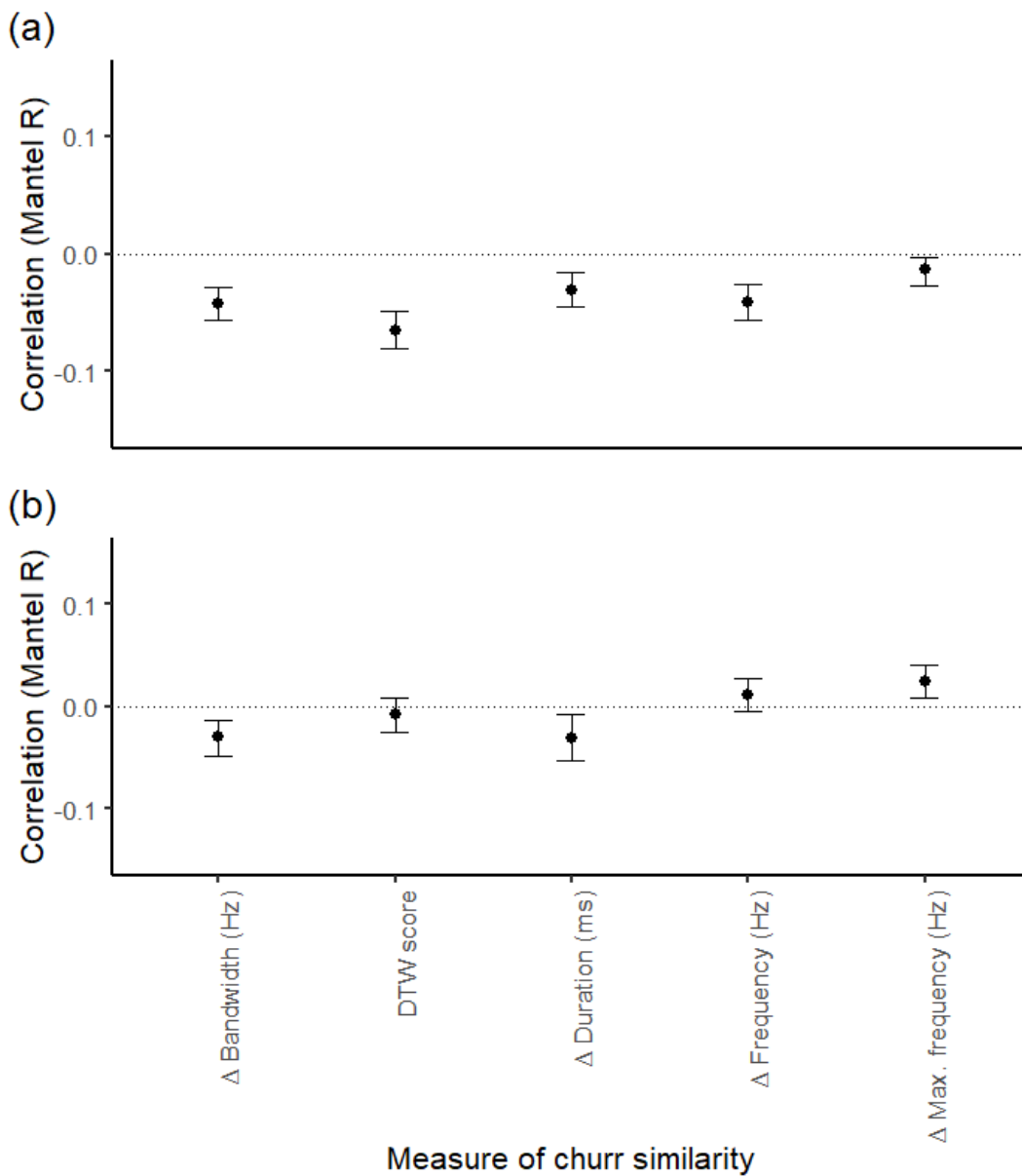
Measure of call dissimilarity	Relatedness variable	Mantel R	Lower 5% CI	Upper 95% CI	p
DTW	Kinship	-0.06	-0.08	-0.05	0.001
Δ Bandwidth (Hz)	Kinship	-0.04	-0.05	-0.03	0.028
Δ Mean Frequency (Hz)	Kinship	-0.04	-0.05	-0.02	0.029
Δ Max. Frequency (Hz)	Kinship	-0.01	-0.02	0.01	0.500
Δ Duration (ms)	Kinship	-0.03	-0.04	-0.01	0.116
DTW	Genetic	-0.01	-0.02	0.01	0.819
Δ Bandwidth (Hz)	Genetic	-0.03	-0.04	-0.01	0.281
Δ Mean Frequency (Hz)	Genetic	0.01	-0.01	0.02	0.661
Δ Max. Frequency (Hz)	Genetic	0.02	0.01	0.04	0.278
Δ Duration (ms)	Genetic	-0.03	-0.05	-0.01	0.227

688 **Table 3.** Dissimilarity of churr calls between helpers and helped male breeders ($n = 19$)
689 compared with the mean call dissimilarity of those helpers to the potential males they did
690 not help ($n = 272$). Potential males were those breeding within 750m of the helpers last
691 failed nest in the same year. Call dissimilarity was measured using dynamic time warping
692 analysis (DTW), and the difference (Δ) in a range of acoustic parameters.

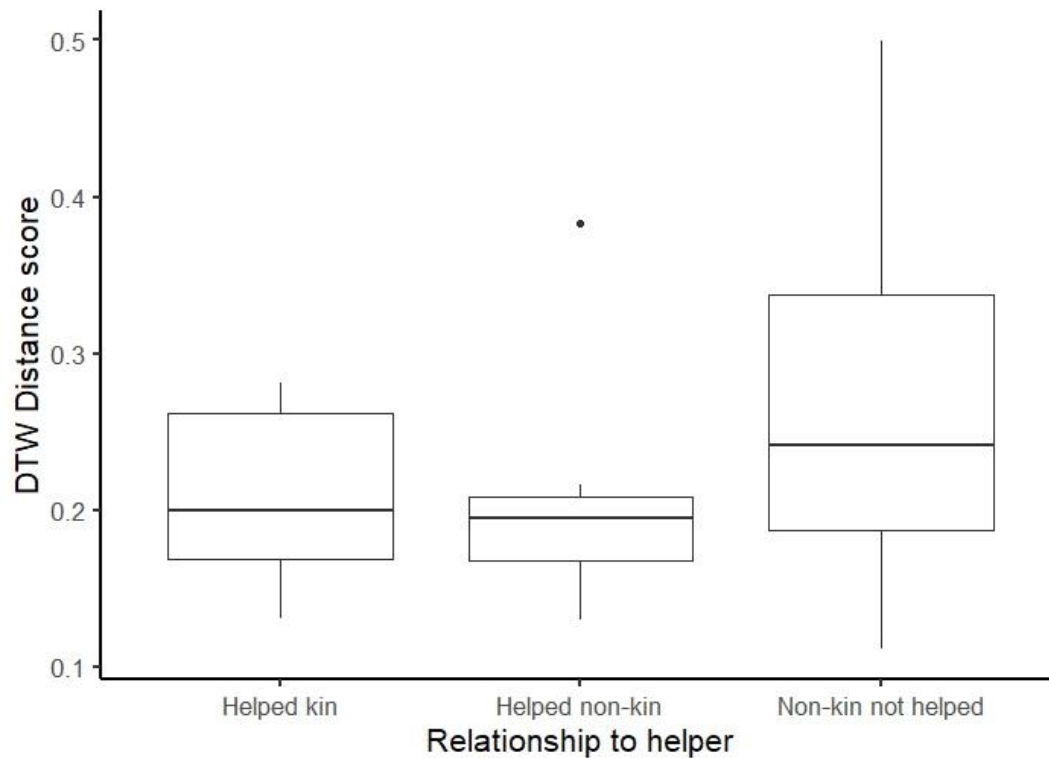
Measure of call dissimilarity	Helped males ($n = 19$ dyads)	Potential males ($n = 252$ dyads)	Wilcoxon's signed rank	
	mean \pm SD	mean \pm SD	ν	p
DTW score	0.21 \pm 0.06	0.26 \pm 0.06	20	0.002
Δ Bandwidth (Hz)	149.54 \pm 138.48	193.93 \pm 103.52	61	0.18
Δ Mean frequency (Hz)	274.07 \pm 207.49	318.93 \pm 117.59	63	0.21
Δ Maximum frequency (Hz)	243.61 \pm 179.51	326.04 \pm 126.57	47	0.05
Δ Duration (ms)	25.68 \pm 16.79	30.96 \pm 11.21	60	0.17



693 **Fig. 1.** Dissimilarity of the long-tailed tit churr call ($n = 907$ calls from 54 birds) within
694 and between individuals, measured using distance scores generated by dynamic-time
695 warping analysis in *Luscinia*.



696 **Fig. 2.** The relationship between churr call dissimilarity and relatedness in long-tailed tits
 697 based on dynamic time-warping analysis (DTW score) and the difference (Δ) in a range
 698 of acoustic parameters. Mantel R correlations are shown for dyadic comparisons among
 699 breeders based on (a) degree of kinship calculated from the social pedigree ($n = 80$), and
 700 (b) genetic relatedness estimates ($n = 88$).



701 **Fig. 3.** Dissimilarity of churr calls among helper-breeder dyads: (i) helpers and related (r
702 ≥ 0.25) breeding males they helped ($n = 9$); (ii) helpers and unrelated breeding males they
703 helped ($n = 8$) and (iii) helpers and unrelated breeding males within helping range (\leq
704 750m) that they did not help ($n = 218$). Call dissimilarity was measured using dynamic
705 time warping analysis (DTW). Boxplots represent median \pm IQR. A full model output is
706 reported in Supplementary Table S4.