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Supplemental Information for:

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

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SI1 Reconstruction of the long-tailed tit social pedigree

Parentage analysis

The long-tailed tit social pedigree was constructed using detailed field observations of marked birds in the Rivelin Valley population from 1994-2016 (n=3182). A small proportion (<2%) of recruits first ringed as adults were philopatric birds that were not ringed as chicks because they fledged from inaccessible nests. To assign parentage to these birds, we used the likelihood approach implemented in CERVUS to perform a parentage analysis on all genotyped putative immigrants that appeared in the population from 1997-2016; analysis was done on a year by year basis, with all adults present in the population in the recruit's birth-year considered as potential parents, based on the assumption that long-tailed tits disperse from their natal area during their first winter (McGowan *et al.* 2003; Sharp *et al.* 2008a). Due to incomplete sampling early in the project, 1994-1996 were excluded. For each year, simulations were run with 100,000 offspring, assuming: 80% of candidate parents were sampled (proportion of recruits genotyped), 98.3% of loci were typed (calculated from the allele frequency data) and a mistyping rate of 0.01, with 10 as the minimum number of typed loci. Previous analyses have shown that the true error rate is less than 1% (M. Simeoni, S.P. Sharp & B.J. Hatchwell, unpublished data). We then carried out parent pair analyses with reference to critical delta scores for 99% confidence derived from the simulations. Having identified parent-offspring relationships, we also compared this to the r_{QG} estimates calculated for each pair. Parent-offspring relationships were considered to be genuine only if the parents were known to have paired together and fledged unringed chicks in the recruit's birth-year.

Sibship reconstruction

As this is an open population, our social pedigree is incomplete. However, long-tailed tits are known to disperse in sibling coalitions during their first year (McGowan *et al.* 2003; Sharp *et al*. 2008b). We tested for the presence of full siblings among each yearly cohort of genotyped immigrants, using the 'Descending Ratio' sibship reconstruction algorithm implemented in KINGROUP v.2. (Konovalov *et al*. 2004). A primary hypothesis of full siblings was tested against a null hypothesis of unrelated pairs; separate analyses were carried out for each year from 1995 to 2016. Having identified sibships, we performed likelihood ratio tests based on pairwise r_{QG} estimates using the same hypotheses and carried out 10,000 simulations. The results from the sibship reconstruction were considered to match those from likelihood ratio tests if individuals in a dyad with a significant likelihood ratio ($p < 0.05$) were placed in the same sibling group (or if those in a dyad with a non-significant likelihood ratio were placed in different groups). Sibling relationships were added to the social pedigree only if all siblings in a given group matched and had high pairwise r_{QG} estimates. Birds were included in our reconstructed social pedigree only if their parentage was known, or if they had been assigned a sibling group ($n = 2815$).

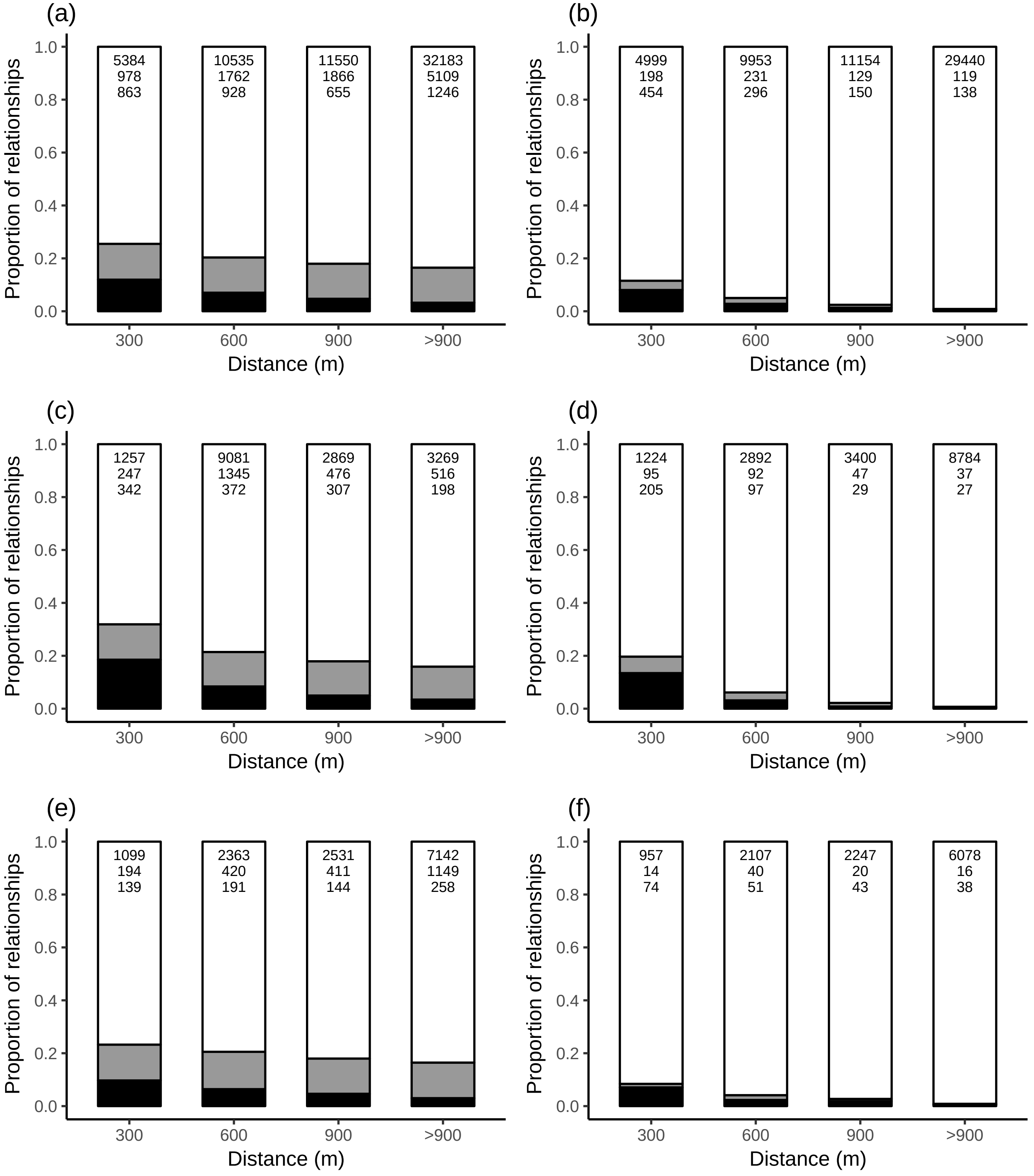
SI2 Distribution of kin and non-kin relationships among breeding long-tailed tits

Using both genetic data from microsatellites (n = 1022) and the social pedigree (n = 866), we calculated the frequency of first order kin ($r_A \geq 0.5$; $r_{QG} > 0.25$), second order kin ($0.5 > r_A \geq 0.25$; $0.25 \geq r_{QG} \geq 0.125$) and non-kin ($r_A < 0.25$; $r_{QG} < 0.125$) available to helpers in the breeding population. The frequency of these relationships was measured over four defined distance bands, based on the nest at which birds first appeared as an adult: 0-300m, 300-600m, 600-900m, >900m. The relationship between distance and the frequency of kin relationships was analysed using a Pearson's Chi-squared test. Within distance bands, the distribution of male and female kin was compared using a series of Pearson's Chi-squared tests.

Based on genotypes, on average the long-tailed tit breeding population was made up of 5.1% first order relationships (n = 3702), 13.3% second order relationships (n = 9715) and 81.6% non-kin relationships (n = 59652). Based on the social pedigree, the population contained just 1.8% first order (n = 1038) and 1.2% second order kin relationships (n = 677), with the remaining 97% relationships between non-kin (n = 55546). Among males, relationships measured using genetic data (6.1% first order, n = 1219; 12.7% second order, n = 2584; 81.2% non-kin, n = 16476) were again higher than those estimated from the social pedigree (2.2% first order, n = 358; 1.6% second order, n = 271; 96.2% non-kin, n = 16300). Among females, the same pattern emerged, with genetic data (4.6% first order, n = 732; 13.5% second order, n = 2174; 81.9% non-kin, n = 13135) resulting in higher estimates of kinship than the social pedigree (1.8% first order, n = 206; 0.8% second order, n = 90; 97.4% non-kin, n = 11389). These rather low observed frequencies of kin relationships are consistent with the low mean r_{QG} estimates observed across the population as a whole.

As the distance between dyads of birds increased, the proportion of kin decreased (genetic data: $\chi2 = 1101.3$, df = 6, $p < 0.001$, Fig. SI2a; social pedigree: $\chi2 = 2144.3$, df = 6, $p < 0.001$, Fig. SI2b). This strong distance effect was apparent among males (genetic data: $\chi2 = 696.7$, df = 6, $p < 0.001$, Fig. SI2c; social pedigree: $\chi2 = 1476.9$, df = 6, $p < 0.001$, Fig. SI2d) and among females (genetic data: $\chi2 = 160.91$, df = 6, $p < 0.001$, Fig. SI2e; social pedigree: $\chi2 = 279.92$, df = 6, $p < 0.001$, Fig. SI2f). The proportion of kinships was greater among males than among females within 300m (genetic data: $\chi2 = 51.17$, df = 2, $p < 0.001$; social pedigree: $\chi2 = 67.42$, df = 2, $p < 0.001$), at 300-600m (genetic data: $\chi2 = 10.19$, df = 2, $p < 0.01$; social pedigree: $\chi2 = 10.65$, df = 2, $p < 0.01$), at 600-900m for the social pedigree ($\chi2 = 14.64$, df = 2, $p < 0.001$) but not for genetic data ($\chi2 = 0.51$, df = 2, $p = 0.78$), and over 900m (genetic data: $\chi2 = 6.43$, df = 2, $p < 0.05$; social pedigree: $\chi2 = 10.80$, df = 2, $p < 0.01$).

Fig. SI2. Proportion of 1st order (black), 2nd order (grey) and non-kin (white) in the breeding population over four bands of distance between dyads. (a) genotype data for all birds, (b) social pedigree for all birds, (c) genotype data for males, (d) social pedigree for males, (e) genotype data for females, (f) social pedigree for females. The number of pairwise relationships in each distance band is displayed (top to bottom: non-, 2nd order and 1st order kin).


(a)
Proportion of relationships
1.0
0.8
0.6
0.4
0.2
0.0
5384
978
863
10535
1762
928
11550
1866
655
32183
5109
1246
300
600
900
>900
Distance (m)
(b)
Proportion of relationships
4999
198
454
9953
231
296
11154
129
150
29440
119
138
300
600
900
>900
Distance (m)
(c)
Proportion of relationships
1257
247
342
9081
1345
372
2869
476
307
3269
516
198
300
600
900
>900
Distance (m)
(d)
Proportion of relationships
1224
95
205
2892
92
97
3400
47
29
8784
37
27
300
600
900
>900
Distance (m)
(e)
Proportion of relationships
1099
194
139
2363
420
191
2531
411
144
7142
1149
258
300
600
900
>900
Distance (m)
(f)
Proportion of relationships
957
14
74
2107
40
51
2247
20
43
6078
16
38
300
600
900
>900
Distance (m)

SI3 Allele distributions across microsatellite markers

Table SI3. Distribution of alleles among 17 microsatellite loci used to estimate genetic relatedness in long-tailed tits.

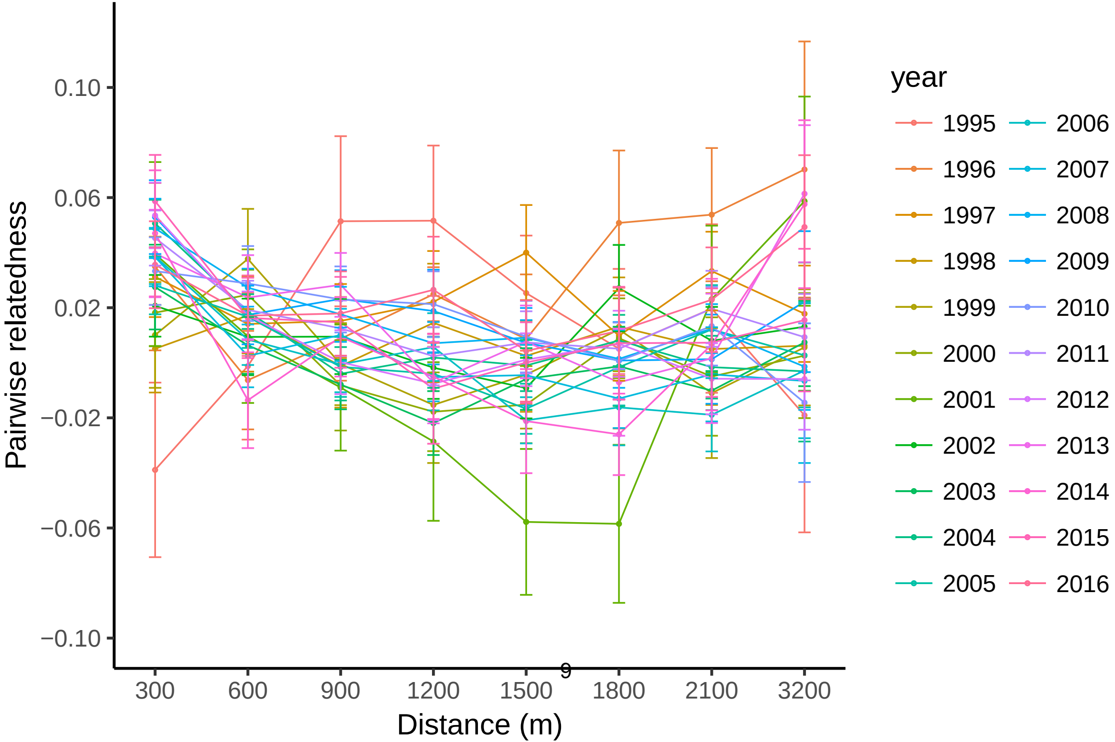
Locus	Number of alleles
CAM01	26
CAM23	5
Tgu_01.040	7
Tgu_04.012	5
Tgu_05.053	9
Tgu_13.017	5
Ase.37	18
CAM03	14
CAM15	8
Pca.4	13
Ase18	17
Ase64	6
Hru2	6
Hru6	53
Pca3	7
PmaD22	43
Ppi2	22
Total	264

SI4 Annual male-female genetic structure

Table SI4. Temporal variation in mean pairwise relatedness, *r_{QG},* between male and female long-tailed tits. Approximate SE were calculated by jackknifing over loci.

Year	r_{QG}	SE	n (birds)	n (comparisons)
1994	0.2459	0.0501	10	24
1995	0.0195	0.0213	28	196
1996	0.0214	0.0109	48	551
1997	0.0221	0.0115	57	806
1998	0.0079	0.0112	55	756
1999	0.0062	0.0156	68	1152
2000	0.0017	0.0128	93	2160
2001	0	0.019	39	374
2002	0.0079	0.0101	75	1386
2003	-0.0027	0.0071	87	1862
2004	0.0082	0.0064	126	3944
2005	0.0026	0.0087	91	2070
2006	-0.0001	0.0081	98	2385
2007	0.0033	0.0084	89	1968
2008	0.0168	0.0061	119	3498
2009	0.0189	0.0088	75	1404
2010	0.0185	0.011	106	2808
2011	0.0139	0.0081	104	2703
2012	0.007	0.0091	111	3068
2013	0.0153	0.0096	55	756
2014	0.0019	0.0118	56	783
2015	0.0146	0.013	58	841
2016	0.0207	0.0142	71	1254

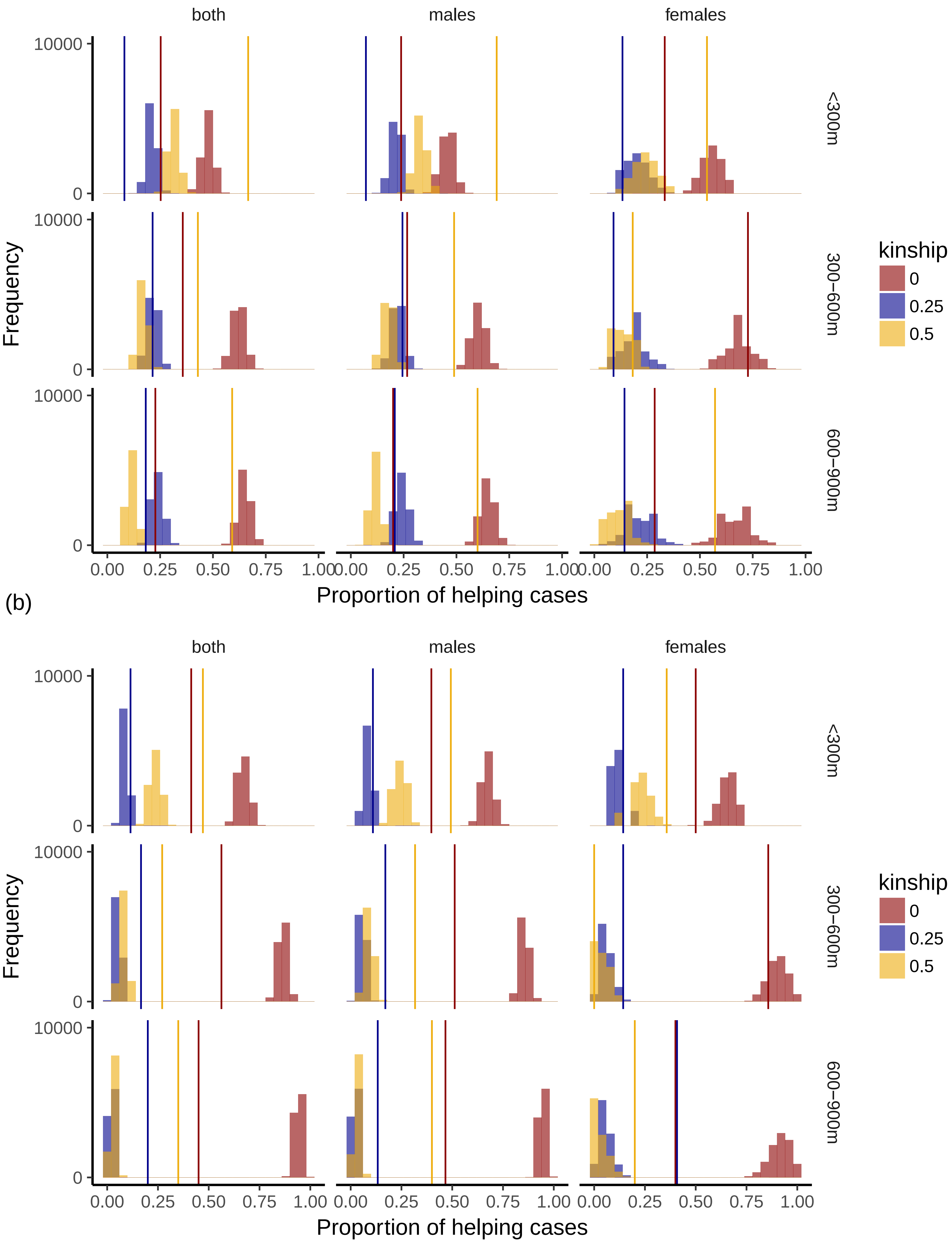
Fig. SI4. Mean pairwise relatedness (r_{QG}) in a long-tailed tit population over eight bands of distance between males and females from 1995-2016. Data from 1994 is excluded due to low sample size. Error bars approximate SE of relatedness estimates from jackknifing over loci.



Pairwise relatedness
0.10
0.06
0.02
−0.02
−0.06
−0.10
300
600
900
1200
1500
1800
2100
3200
Distance (m)
year
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016

SI5 Helping patterns and availability of kin

Fig. SI5. Proportion of help given to nests belonging to at least one 1st order kin (green), at least one 2nd order kin (blue), or two non-kin (red) over three distance bands between helpers and recipients. Within each band, the proportion of kin helped (vertical line) is compared with that expected if help was given randomly within that range, based on 10000 permutations of potential nests for focal helpers within years (histogram). Relatedness between helpers and recipients is estimated using (a) genetic data and (b) the social pedigree. Analyses were carried out on all helpers, male helpers and female helpers. The observed proportion was considered statistically significant it fell outside the 95% ($p<0.05$), 99% ($p<0.01$) or 99.9% ($p<0.001$) confidence interval of the random distribution.


both
males
females
<300m
300–600m
600–900m
10000
0
Frequency
kinship
0
0.25
0.5
0.00
0.25
0.50
0.75
1.00
Proportion of helping cases
(b)
both
males
females
<300m
300–600m
600–900m
10000
0
Frequency
kinship
0
0.25
0.5
0.00
0.25
0.50
0.75
1.00
Proportion of helping cases