

1 BIOLOGICAL SCIENCES

2 **Cost, risk and avoidance of inbreeding in a cooperatively breeding bird**

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13 **Abstract**

14 Inbreeding is often avoided in natural populations by passive processes such as sex-biased  
15 dispersal. But, in many social animals, opposite-sexed adult relatives are spatially clustered,  
16 generating a risk of incest and hence selection for active inbreeding avoidance. Here we show that  
17 in long-tailed tits (*Aegithalos caudatus*), a cooperative breeder that risks inbreeding by living  
18 alongside opposite-sex relatives, inbreeding carries fitness costs and is avoided by active kin  
19 discrimination during mate choice. First, we identified a positive association between  
20 heterozygosity and fitness, indicating that inbreeding is costly. We then compared relatedness  
21 within breeding pairs to that expected under multiple mate choice models, finding that pair  
22 relatedness is consistent with avoidance of first-order kin as partners. Finally, we show that the  
23 similarity of vocal cues offers a plausible mechanism for discrimination against first-order kin  
24 during mate choice. Long-tailed tits are known to discriminate between the calls of close kin and  
25 non-kin, and they favor first-order kin in cooperative contexts, so we conclude that long-tailed tits  
26 use the same kin discrimination rule to avoid inbreeding as they do to direct help towards kin.

27 **Keywords:** Inbreeding, kin discrimination, cooperative breeder, mate choice.

28 **Significance statement**

29 Inbreeding reduces fitness leading to selection for incest avoidance in many organisms. Passive  
30 processes, such as sex-biased dispersal, may reduce inbreeding risk, but when dispersal is limited,  
31 inbreeding may still be minimized by animals actively recognizing and discriminating kin from  
32 non-kin when choosing mates. We investigated inbreeding costs, risk and avoidance in a  
33 cooperative bird species in which opposite-sex adults disperse locally to breed and frequently  
34 associate. We identified a reduction in fitness in inbred individuals, and show that despite a

35 substantial inbreeding risk, breeders alleviate this by discriminating against close kin as partners.  
36 We show that the increased vocal similarity among relatives offers a probable recognition  
37 mechanism for this observed level of kin discrimination during mate choice.

## 38 **Introduction**

39 Inbreeding is generally maladaptive because it increases homozygosity and hence the unmasking  
40 of deleterious recessive alleles, which, when expressed, result in a reduction in fitness among  
41 inbred individuals termed inbreeding depression<sup>1,2</sup>. Inbreeding may be tolerated<sup>3,4</sup>, however, if  
42 avoidance is costly, or if the costs of inbreeding are outweighed by the inclusive fitness benefits  
43 accrued from breeding with or interacting socially with relatives<sup>5,6</sup>. Thus, the selection pressures  
44 on alternative inbreeding strategies depend on the fitness consequences of inbreeding, typically  
45 inferred by the strength of inbreeding depression, and the costs of inbreeding avoidance.  
46 Inbreeding depression is often difficult to quantify in natural populations<sup>7</sup>, but it has been shown  
47 to select for various avoidance mechanisms<sup>8,9,10</sup>. Passive processes that disrupt opposite-sex kin  
48 associations, such as sex-biased dispersal, are widespread<sup>11,12</sup>, but when dispersal is constrained<sup>13</sup>  
49 or when there is countervailing selection for kin association<sup>14</sup>, individuals may frequently  
50 encounter kin as potential mates. This is the case in most cooperative breeders, where delayed  
51 natal dispersal creates structured populations within which opposite-sex kin associate beyond  
52 reproductive maturity<sup>15</sup>. In such situations, inbreeding may be minimized by extra-group  
53 matings<sup>16-20</sup> or by abstention from breeding<sup>21-23</sup>. The latter often results in strong reproductive  
54 skew, with reproduction monopolized by a minority of dominant individuals within groups, aided  
55 by subordinate helpers<sup>24-26</sup>.

56 Most cooperative species live in discrete groups that occupy exclusive territories, but in some  
57 others, helping (providing care to others' offspring) follows local natal dispersal that results in  
58 continued association among relatives across extended social networks known as 'kin  
59 neighbourhoods'<sup>27</sup>. Kin neighbourhoods are characterized by a diffuse kin structure where mean  
60 relatedness among socially interacting individuals is low. This degree of social organisation also  
61 exists in colonial breeders, such as sociable weavers (*Philetairus socius*), in which males and  
62 females may recruit as breeders within their natal colony<sup>28</sup>. Such social structures select for strong  
63 kin discrimination in helping behaviour because of the risk of directing care towards non-kin<sup>29</sup>,  
64 and if adult associations include opposite-sex relatives, then strong inbreeding depression would  
65 also be expected to select for a mechanism for active incest avoidance.

66 However, the extent to which variation in relatedness across social systems influences inbreeding  
67 risk and the strength of kin discrimination exercised during mate choice remain relatively  
68 understudied. Fitness costs of inbreeding<sup>30</sup> or of being inbred have been identified in several  
69 cooperative breeders<sup>19,20,31</sup>, and active incest avoidance has been demonstrated in western  
70 bluebirds (*Sialia mexicana*)<sup>32</sup> and inferred in red-winged fairy-wrens (*Malurus elegans*)<sup>33</sup> and  
71 grey-crowned babbblers (*Pomatostomus temporalis*)<sup>34</sup>. But, the discrimination rules used to avoid  
72 inbreeding and the recognition mechanisms that effectively minimize its costs have not been  
73 determined.

74 Here, we present a comprehensive study of inbreeding depression, inbreeding risk and inbreeding  
75 avoidance in long-tailed tits (*Aegithalos caudatus*). Long-tailed tits breed in kin neighbourhoods  
76 and exhibit redirected helping, whereby failed breeders acquire indirect fitness by helping to  
77 provision non-descendant kin<sup>35</sup>. Although dispersal is female-biased, natal dispersal distances of  
78 both sexes are short<sup>36</sup>, creating fine-scale genetic structure within breeding populations<sup>37</sup>. This kin-

79 structure facilitates kin-selected helping, but also results in both kin and non-kin being available  
80 as partners when monogamous pairs form each spring<sup>38</sup>. Using a long-term genetic and life-history  
81 dataset<sup>39</sup>, we assess the evidence for inbreeding depression and a risk of incest, and test putative  
82 rules for inbreeding avoidance to determine the likely kin recognition mechanism<sup>40,41</sup>.

### 83 **Results**

84 Reduced heterozygosity in inbred individuals is a major source of inbreeding depression, and  
85 associations between heterozygosity at microsatellite markers and variation in fitness are widely  
86 used as an indirect measure of inbreeding depression when pedigree-derived inbreeding  
87 coefficients are unreliable<sup>42</sup>. We tested for an association between standardized heterozygosity at  
88 17 microsatellite markers (H) and fitness using four fitness-associated life-history traits: whether  
89 an individual recruited to the breeding population; the proportion of eggs that hatched in a female's  
90 first clutch; the probability that a breeder produced recruits; and the direct fitness of breeders that  
91 produced recruits. Here, direct fitness is a measure of individuals' lifetime reproductive success  
92 that corrects for the contribution of helpers (see Methods). This is important because the presence  
93 of helpers has a very substantial effect on fledgling recruitment<sup>35</sup>, and this social effect must be  
94 removed to reveal the fitness that most closely reflects an individual's intrinsic 'quality'.  
95 Heterozygosity was positively associated with the hatching success of females' clutches (Fig. 1b)  
96 and the direct fitness of breeders that produced recruits (Fig. 1d), but there was no association  
97 between H and an individual's probability of recruitment (Fig. 1a), nor on the probability that a  
98 breeder produced recruits (Fig. 1c). In our analyses, both hatching success and direct fitness are  
99 adult traits, and this reduction in fitness of inbred adults indicates that inbreeding has long-term,  
100 negative fitness consequences.

101 Long-tailed tits exhibit a significantly enhanced level of relatedness between adult males and  
102 females within 600m<sup>37</sup>, a range within which pairing typically occurs (Fig. 2). However, based on  
103 the pedigree, only one out of 609 pairs (0.2%) were first-order relatives, and a further two pairings  
104 (0.3%) were between second-order kin (Table S1). Genetic relatedness estimates ( $r_{QG}$ )<sup>43</sup> revealed  
105 a similar frequency of close inbreeding (2/609, 0.3%), but substantially more cases of moderate  
106 inbreeding (94/609, 15.4%; Table S2). These results suggest active avoidance of close kin when  
107 pairing, rather than retrospective extra-pair mating to avoid inbreeding with a related partner.  
108 Indeed, the relatively low levels of promiscuity in long-tailed tits<sup>44,45</sup>, make extra-pair mating an  
109 unlikely mechanism of inbreeding avoidance. Instead, we examined whether inbreeding was  
110 actively avoided when choosing a social mate.

111 The relatedness of observed pairs was compared with that expected under a series of mate choice  
112 models that assumed all first-year, widowed or divorced opposite-sex breeders present in the same  
113 year were available as potential partners, within ranges of 300m, 600m, and further 300m  
114 increments up to 2100m. Mean  $r_{QG}$  to a chosen partner was significantly lower than that expected  
115 for females selecting partners at random from within 300m (generalized linear mixed-effects  
116 model (GLMM),  $n = 2420$ ,  $t = 7.23$ ,  $P < 0.001$ ), 600m (GLMM,  $n = 2433$ ,  $t = 3.93$ ,  $P < 0.001$ ),  
117 900m (GLMM,  $n = 2433$ ,  $t = 3.03$ ,  $P < 0.01$ ), but not 1200m ( $n = 2433$ ,  $t = 1.9$ ,  $P = 0.06$ ; Fig. 3a).  
118 Mean  $r_{QG}$  to a chosen partner was lower than predicted for males selecting mates from within  
119 300m ( $n = 2416$ ,  $t = 7.84$ ,  $P < 0.001$ ), 600m ( $n = 2432$ ,  $t = 5.14$ ,  $P < 0.001$ ), 900m ( $n = 2432$ ,  $t =$   
120  $3.79$ ,  $P < 0.001$ ) and 1200m ( $n = 2432$ ,  $t = -0.84$ ,  $P = 0.01$ ; Fig. 3b). These results demonstrate  
121 strong discrimination against kin as partners within the range that mates are normally chosen,  
122 suggesting that inbreeding depression may be sufficiently strong to cause selection for inbreeding  
123 avoidance.

124 To identify a plausible discrimination rule for incest avoidance, we compared observed and  
125 expected pair  $r_{QG}$  assuming either avoidance of first-order kin ( $r_{QG} \geq 0.375$ ), or avoidance of first-  
126 and second-order kin ( $r_{QG} \geq 0.125$ ), by removal of these kin from the pool of potential partners at  
127 pairing ranges within 1200m. When first-order kin were removed, observed and expected pair  $r_{QG}$   
128 did not differ significantly if females selected mates within 300m (GLMM;  $n = 2420$ ,  $t = 0.36$ ,  $P$   
129  $= 0.72$ ), 600m ( $n = 2433$ ,  $t = -1.32$ ,  $P = 0.18$ ), 900m ( $n = 2433$ ,  $t = -1.15$ ,  $P = 0.25$ ) and 1200m  
130 ( $n = 2433$ ,  $t = -1.46$ ,  $P = 0.14$ ; Fig. 3a). The same was true for males when they were assumed to  
131 select mates from within these ranges (GLMM; 300m:  $n = 2416$ ,  $t = 1.47$ ,  $P = 0.14$ ; 600m:  $n =$   
132  $2432$ ,  $t = -0.05$ ,  $P = 0.96$ ; 900m:  $n = 2432$ ,  $t = -0.29$ ,  $P = 0.77$ ; 1200m:  $n = 2432$ ,  $t = -0.84$ ,  $P =$   
133  $0.39$ ; Fig. 3b). In contrast, when both first- and second-order kin were removed, observed pair  $r_{QG}$   
134 was higher than expected at all ranges for both females (GLMM; 300m:  $n = 2420$ ,  $t = -9.9$ ,  $P <$   
135  $0.001$ ; 600m:  $n = 2420$ ,  $t = -11.46$ ,  $P < 0.001$ ; 900m:  $n = 2420$ ,  $t = -11.52$ ,  $P < 0.001$  and 1200m:  
136  $n = 2433$ ,  $t = -11.8$ ,  $P < 0.001$ ; Fig. 3a) and males (GLMM; 300m:  $n = 2416$ ,  $t = -9.16$ ,  $P < 0.001$ ;  
137 600m:  $n = 2416$ ,  $t = -11.04$ ,  $P < 0.001$ ; 900m:  $n = 2416$ ,  $t = -11.19$ ,  $P < 0.001$  and 1200m:  $n =$   
138  $2432$ ,  $t = 11.54$ ,  $P < 0.001$ ; Fig. 3b). Thus, the observed relatedness of breeding pairs closely  
139 matches the pattern expected by avoidance of first-order kin as mates. This degree of  
140 discrimination can effectively reduce inbreeding because first-order relatives are the category of  
141 kin most likely to be encountered nearby in long-tailed tit populations<sup>36</sup>, although the substantially  
142 lower risk of pairing with second-order and more distant kin remains.

143 Long-tailed tits can discriminate kin from non-kin using learned vocal cues<sup>41</sup>, a mechanism that is  
144 consistent with helpers preferentially aiding close kin<sup>37,40</sup>. We investigated whether the same  
145 mechanism may enable inbreeding avoidance. Our analyses focused on the churr call, a short-  
146 range contact call that is highly repeatable within individuals through time<sup>46</sup>. The similarity of the

147 churr calls of opposite-sex breeders varied with relatedness: first-order kin ( $n = 20$  dyads) had  
148 more similar calls than second-order (GLMM;  $n = 249$  dyads,  $t = -3.02$ ,  $P = 0.002$ ) or non-kin  
149 (GLMM;  $n = 1078$  dyads,  $t = -3.62$ ,  $P < 0.001$ ). Crucially, the calls of males and females within  
150 breeding pairs were significantly less similar than those of opposite-sex first-order kin within  
151 pairing range (Fig. 4). In contrast, there was no significant difference in vocal similarity between  
152 observed pairs and second-order kin or non-kin (Fig. 4). These results suggest that vocal similarity  
153 provides a plausible mechanism for avoidance of first-order kin as partners, although we cannot  
154 exclude the possibility that other phenotypic cues are also involved.

## 155 **Discussion**

156 We have shown that inbreeding carries long-term fitness costs in long-tailed tits, but detected no  
157 short-term cost on recruitment. Inbreeding depression may be masked in the short-term because  
158 external factors such as nest predation have large impacts on offspring fitness in early life.  
159 Alternatively, inbreeding depression may affect embryo development or chick survival during the  
160 first few days after hatching<sup>47</sup>. We genotyped chicks at 11 days old, so inbred individuals would  
161 be a missing fraction in our data if inbreeding depression occurs prior to this age. Furthermore, the  
162 probability of both individual recruitment and recruit production are likely to be largely governed  
163 by stochastic events, such as predation, whereas hatching success and direct fitness may have a  
164 stronger genetic component. The presence of helpers may also mitigate some of the fitness  
165 consequences of inbreeding depression. Maternal care buffers inbreeding depression in the burying  
166 beetle (*Nicrophorus vespilloides*)<sup>48</sup>, and in long-tailed tits the probability that an individual recruits  
167 and its own production of recruits are both correlated with helper number<sup>49</sup>. Investigation into the  
168 heritability of life-history traits such as hatching success would further elucidate the mechanism  
169 by which inbreeding reduces fitness.



170 Long-tailed tits actively avoid close inbreeding, despite the substantial risk of incest, by avoidance  
171 of first-order kin as mates. By contrast, the observed frequency of pairings between second-order  
172 kin was relatively high (15.4% of pairs) when using genetic relatedness estimates, although not  
173 when using the pedigree (0.3% of pairs). The kin structure of long-tailed tit populations means that  
174 after excluding first-order kin, the proportion of birds (of either sex) that are second-order kin  
175 within 600m is 14.7% using genetic relatedness estimates and 2.7% of birds using pedigrees<sup>37</sup>.  
176 Thus, our observed frequencies of second-order kin pairings are close to what would be expected  
177 from random pairing among birds that are not first-order kin, further supporting our proposed rule  
178 for kin discrimination during mate choice (Fig. 3). Together, the significant inbreeding depression  
179 and pattern of inbreeding avoidance observed support the hypothesis that there is selection for  
180 inbreeding avoidance.

181 These findings are consistent with previous studies demonstrating recognition of first-order kin in  
182 a cooperative context<sup>37,40</sup>. They are also consistent with the idea that kin recognition in long-tailed  
183 tits requires a period of association during development, when vocalisations are learned<sup>41</sup>. It is  
184 very likely that first-order kin (siblings, parents and offspring) associate during rearing, whereas  
185 second-order kin are likely to be reared apart. Consequently, vocalisations are more similar among  
186 first-order relatives than among second-order or non-kin<sup>50</sup>. There are two instances in which this  
187 is not the case: extra-pair paternity and when pair-bonds last more than one year so that full siblings  
188 are produced in different nests. However, long-tailed tits are not very promiscuous<sup>44</sup>, and their low  
189 mate fidelity across seasons<sup>38</sup>, high annual mortality and low chance of successful reproduction<sup>51</sup>  
190 mean that the probability of either instance is low. Avoidance of first-order, but not second-order  
191 kin as mates, therefore supports familiarity as the mechanism of kin recognition. However, because

192 long-tailed tits do not live in stable kin groups throughout their life, recognition of familiar  
193 individuals still relies on phenotypic rather than spatial cues.

194 Our results suggest that a single kin discrimination rule may explain inbreeding avoidance and kin  
195 preference in helping in long-tailed tits, with observational evidence showing that vocal cues offer  
196 a plausible mechanism for kin recognition. However, there is an intriguing contrast between the  
197 observations that while distant and non-kin are frequently helped<sup>35</sup>, close inbreeding is extremely  
198 rare. A single recognition mechanism can produce variable outcomes depending on the position of  
199 the acceptance threshold, which may shift according to the relative fitness costs and benefits  
200 associated with acceptance and rejection errors<sup>52,53</sup>. These in turn will be determined by the  
201 probability of encountering a relative and the fitness consequences of the associated behaviour.  
202 Assuming that there is some overlap in the similarity of cues produced by close kin and by distant  
203 or non-kin<sup>50</sup> (Fig. 4), an acceptance threshold that includes most close kin, but also some distant  
204 or non-kin would explain the observed pattern of helping<sup>35</sup>. The same recognition threshold could  
205 also operate during mate choice but with the reverse effect that almost all close kin, and  
206 presumably some distant or non-kin, are rejected as partners, resulting in the infrequent close  
207 inbreeding that we observed. A recognition threshold that is generous in the context of helping and  
208 stringent in the context of mate choice makes intuitive sense in long-tailed tits. Redirected helping  
209 by failed breeders is likely to incur little cost but potentially substantial benefit when kin-  
210 directed<sup>35</sup>. In contrast, inbreeding depression (Fig. 2) suggests selection for strict avoidance of  
211 close kin as partners. Therefore, we conclude that a single kin discrimination mechanism has  
212 evolved to serve two functions, driving kin association in one context and kin avoidance in the  
213 other.

## 214 **Materials and Methods**

215 **Study population:** A population of 17-72 (mean *c.*50) pairs of long-tailed tits was studied during  
216 the breeding season (February-June) between 1994 and 2017 in the Rivelin Valley, Sheffield, UK  
217 (53°38'N 1°56'W). The site is approximately 2.5km<sup>2</sup> and comprises predominantly deciduous  
218 woodland and scrub. The population is open: approximately 40% of breeders hatched in the study  
219 site and are referred to as native, while the remaining immigrant adults are assumed to have  
220 dispersed into the study site during their first year, based on the observation that individuals have  
221 high site fidelity following their first breeding year<sup>49</sup>. Each year, almost all individuals (> 95%)  
222 were marked with a British Trust for Ornithology (BTO) ring and a unique combination of two  
223 color rings. Native birds were ringed as 11-day old nestlings and immigrant adults were captured  
224 in mist nests under BTO license before or during their first breeding season. When ringed, a sample  
225 of 5-30µl of blood was taken by brachial venipuncture under Home Office license. All breeding  
226 attempts were closely monitored and GPS coordinates were taken for each nest (*n* = 1461); a  
227 Cartesian coordinate system (UTM) was used to describe geographic distance between nests.

228 **Social pedigree:** We used the social pedigree to predict the correlation between heterozygosity  
229 and individual inbreeding coefficients, and to identify matings among known kin in our population.  
230 The pedigree was created using 23 years of field observations (1994-2017, *n* = 3068 birds). For  
231 further details on pedigree construction, see<sup>37</sup>. To calculate social relatedness (*r*) among dyads, an  
232 additive relationship matrix was generated from the pedigree in R (version 3.5.0, 2018) using the  
233 *nadiv* package<sup>54</sup>. Six breeding birds in our study population (0.2%) were from cross-fostered  
234 broods in 1996-1998, but given that birds raised together treat each other as kin<sup>41</sup>, we included  
235 them in the social pedigree. For the same reason, while there is a low rate of extra-pair paternity  
236 (11% chicks in 30% nests) in long-tailed tits<sup>44</sup>, it has not been corrected for in the social pedigree.

237 **Inbreeding coefficients:** Inbreeding coefficients were calculated from the social pedigree. It was  
238 possible to infer reliable  $f$  values from the pedigree for 129 birds (native individuals with all  
239 grandparents known).  $f$  values from an additional nine birds that were offspring of presumed  
240 immigrant siblings, based on genetic sibship reconstruction, were also included. As more distant  
241 shared ancestors than grandparents, if known, would cause individual inbreeding coefficients to  
242 increase,  $f$  values are likely to be under-estimated based on incomplete pedigree information.

243 **Molecular genetics:** Individuals were genotyped at 17 microsatellite loci<sup>55</sup>. Population allele  
244 frequencies were generated in CERVUS (version 3.0.7, 2007). All available genotypes were used  
245 (1994-2017,  $n = 3304$  birds) to maximize accuracy and ensure non-zero estimates for all alleles.  
246 The genetic relatedness of dyads was estimated using coefficient of relatedness estimates<sup>43</sup>,  $r_{QG}$ ,  
247 in SPAGeDi (version 1.1.5, 2002). This estimate is reliable when tested against our social  
248 pedigree<sup>56</sup>.

249 **Inbreeding:** Inbreeding cases were identified using the social pedigree and genetic relatedness  
250 estimates. Genetic ( $r_{QG}$ ) and social ( $r$ ) relatedness of all breeding pairs from 1994-2016 in which  
251 both adults were ringed and genotyped was calculated. Measurements were taken from distinct  
252 pairs. Occasionally, long-tailed tits swap partners within a breeding season, in which case, the first  
253 pairing of that year was used. Individuals often breed in multiple years, either with the same partner  
254 or a new partner. The dataset used in this study contained 609 pairs made up of 445 females and  
255 412 males in 1994-2016. Pairs were considered closely or moderately inbred if they comprised  
256 known first-order ( $r = 0.5$ ) or second-order ( $r = 0.25$ ) kin, respectively. As incomplete social  
257 pedigrees may underestimate incest rates in open populations, inbreeding was also quantified using  
258 genetic relatedness estimates ( $r_{QG}$ ). The  $r_{QG}$  estimate of known first-order kin ( $r = 0.5$ ) was  $0.454$   
259  $\pm 0.149$  (mean  $\pm$  SD,  $n = 1211$  dyads). For known second-order kin ( $r = 0.25$ ),  $r_{QG}$  was  $0.198 \pm$

260 0.154 (mean  $\pm$  SD,  $n = 515$  dyads). The  $r_{QG}$  estimate of all other dyads of known parentage ( $r <$   
261 0.25) was  $0.002 \pm 0.131$  (mean  $\pm$  SD,  $n = 54521$  dyads). The distribution of  $r_{QG}$  estimates among  
262 known first-order, second-order and non-kin are shown in Fig. S1. Based on these distributions, a  
263 lower  $r_{QG}$  threshold of 0.375 was set to approximate first-order kin (mean  $r_{QG} \pm$  SD =  $0.503 \pm$   
264  $0.094$ ,  $n = 1438$ ) and 0.125 to approximate second-order kin (mean  $r_{QG} \pm$  SD =  $0.197 \pm 0.059$ ,  $n$   
265 = 11979). The mean  $r_{QG}$  of observed pairs was  $0.002 \pm 0.123$  (mean  $\pm$  SD,  $n = 609$ ).

266 **Mate choice models:** For each focal breeder, their  $r_{QG}$  to their chosen partner was compared with  
267 their mean  $r_{QG}$  to all potential partners, each breeding year (1994-2016), under the pairing  
268 constraints of a series of mate choice models assuming all first-year, widowed or divorced  
269 opposite-sex breeders present in the same year were available as potential partners, within  
270 concentric ranges of radius 300m, 600m, 900m, and further 300m increments up to 2100m.

271 **Heterozygosity-fitness correlations:** Pedigree-derived inbreeding coefficients can be estimated  
272 only when parentage can be traced back at least two generations, but both sets of grandparents  
273 were known for only 5.3% of native birds ( $n = 138$ ). Therefore, standardized multi-locus  
274 heterozygosity (H) was estimated for all genotypes (1994-2016,  $n = 3182$ ). Heterozygosity is  
275 standardized by dividing the proportion of typed loci for which an individual was heterozygous by  
276 the mean heterozygosity of those loci at which the individual was typed<sup>57</sup>. Heterozygosity-fitness  
277 correlations can only be regarded as providing evidence for inbreeding depression if  
278 heterozygosity is a predictor of individual inbreeding coefficients. We used the analytical  
279 derivations outlined in<sup>39</sup> to predict the correlation between heterozygosity and  $f$  in our population  
280 as  $r(H, f) = -0.43$  ( $n = 138$ , mean  $f = 0.03$ , variance in  $f = 0.004$ , number of loci = 17, mean  
281 heterozygosity of loci = 0.759). This value is relatively large compared to other studies predicting  
282 the relationship between inbreeding coefficient and heterozygosity, including populations where

283 inbreeding depression has been demonstrated. For example, the correlation coefficient  $r(H, f)$  in  
284 red deer (*Cervus elaphus*)<sup>58</sup> and song sparrows (*Melospiza melodia*)<sup>59</sup> are -0.25 and -0.22,  
285 respectively<sup>39</sup>. Thus, genetic diversity at marker loci reflects genetic diversity throughout the  
286 genome, including at unknown loci that affect trait variation; i.e. marker and fitness loci are in  
287 identity disequilibrium<sup>42</sup>. This validates the use of heterozygosity as a proxy for inbreeding  
288 coefficient in our study. Measurements were taken from distinct samples.

289 **Direct fitness:** Direct fitness was calculated as lifetime reproductive success quantified in terms  
290 of genetic offspring equivalents and corrected for extra-pair paternity and the offspring gained by  
291 having helpers. The fraction of recruits in a brood that was attributable to helpers was estimated  
292 using a mixed effects model of the effect of helper number on recruitment<sup>49</sup>. This fraction was  
293 subtracted from the total number of recruits produced over an individual's lifetime. The remaining  
294 fraction was halved to reflect the relatedness between a single parent and its offspring. The  
295 assumption that parents and their offspring have a relatedness coefficient of 0.5 does not account  
296 for higher relatedness of inbred offspring to their parents<sup>60</sup>. However, the almost complete absence  
297 of close inbreeding and the low incidence of inbreeding among more distant relatives indicate that  
298 errors in our estimation of direct fitness introduced by this simplifying assumption will be small.

299 **Acoustic recordings:** A short-distance contact call, the churr, was recorded from adults using a  
300 Sennheiser ME67/K6 shotgun microphone fitted with a Rycote windjammer. Recordings were  
301 made onto a Roland R-05 version 1.03 WAV/MP3 recorder with a 6GB SanDisk memory card,  
302 set to a sample rate of 48kHz with WAV-16bit accuracy. The microphone input level was set to  
303 60db with a low-cut frequency of 400Hz. All recordings were made between 06:00 and 18:00 BST.  
304 Birds were recorded at a distance of approximately 3-15m, to minimize sound degradation and  
305 reverberation. Birds were recorded at the nest and identified by their unique color ring

306 combinations. If more than one bird was present, vocalizations were assigned to individuals by  
307 observing movements of the bill and throat feathers. At the start of each recording, date, time, nest  
308 number and recording number were dictated into the microphone. When caller ID could be  
309 identified with certainty, this was dictated into the microphone after each call. In total, 213  
310 recordings were made in 2015-2017, containing 1116 churr calls from 98 birds (mean  $\pm$  SD =  
311  $11.39 \pm 10.24$  per bird; range 1 – 42).

312 **Acoustic analysis:** The sampling frequency was converted to 22.05 KHz and recordings were  
313 visualized spectrographically to assess call quality, with a frequency resolution of 188Hz and a  
314 time resolution of 2.7ms in Avisoft SAS-Lab Pro version 4.52 (Avisoft Bioacoustics). Recordings  
315 with extreme background noise were excluded. All useable calls were isolated, stored and  
316 measured in Luscinia (version 2.16.10.29.01, <https://rflachlan.github.io/Luscinia/>). Vocal  
317 similarity was assessed by dynamic time-warping analysis (DTW) implemented in Luscinia. DTW  
318 analysis generates a score representing the amount of warping required to match one signal to  
319 another. The acoustic features used in the DTW analysis were weighted as: time = 1, fundamental  
320 frequency = 2, change in fundamental frequency = 2, compression factor = 0.1, minimum element  
321 length = 10, time SD weighting = 1, ArcTan transform weight for frequency slope = 0.02,  
322 maximum warp = 100%. These settings generated a DTW algorithm that correctly matched  
323 visually similar vocalizations, assessed using a dendrogram and multidimensional scaling plot.  
324 The low compression factor optimizes the capture of acoustic complexity. This increased  
325 weighting of frequency parameters to time is also in line with previous studies suggesting that  
326 frequency parameters show greater individuality than temporal parameters and are particularly  
327 important for kin recognition in this species<sup>46</sup>.

328 **Call similarity and pairing:** Among the breeding pairs for which we had recordings of both  
329 breeders ( $n = 51$ ), there were no cases of pairing among known first-order or second-order kin,  
330 based on the social pedigree. Based on genetic relatedness estimates, there were no cases of pairing  
331 among first-order kin ( $r_{QG} \geq 0.375$ ) and 13 (25.5%) cases of pairing among second-order kin ( $r_{QG}$   
332  $\geq 0.125$ ). Dyadic vocal similarity (DTW score) was compared among: breeding pairs; potential  
333 pairs of first-order kin ( $r_{QG} \geq 0.375$ ); potential pairs of second-order kin ( $0.375 > r_{QG} \geq 0.125$ ) and  
334 potential pairs of non-kin ( $r_{QG} < 0.125$ ) within 1350m, the range within which 95% pairs are  
335 formed. Genetic estimates of pedigree relationships were used for consistency with our analysis of  
336 putative discrimination rules. Potential pairings were dyads of opposite-sex first-year, widowed or  
337 divorced breeders present in the breeding population in the same year. The distance between adults  
338 was based on the location of an individual's first breeding attempt in a given year.

339 **Statistical analysis:** All statistical analyses were carried out in R (version 3.5.0, 2018).  
340 Associations between heterozygosity and fitness traits (HFCs) were tested using generalized linear  
341 mixed-effects models in the lme4 package. Recruitment was modelled as a binary response  
342 variable with a binomial error distribution and logit link. The fixed effects were: H, sex, to control  
343 for male-biased philopatry, fledge date (days since March 1st), because offspring fledging earlier  
344 in the year have a greater probability of recruitment<sup>61</sup>, and number of helpers at natal nest, as helper  
345 number has been shown to increase recruitment probability<sup>51</sup>. Hatching success was modelled as  
346 a proportional response variable with a binomial error distribution and logit link. The fixed effects  
347 were: H, lay date and female mass as a nestling. The probability of producing recruits was  
348 modelled as a binary response variable with a binomial error distribution and logit link. The fixed  
349 effects were: H and fledgling sex ratio (proportion of male fledglings produced, to control for male  
350 philopatry). Direct fitness was modelled as a continuous response variable with a Gamma error



351 distribution and inverse link, with H, sex and fledgling sex ratio fitted as fixed effects. In all HFC  
352 models, genetic brood was fitted as a random effect to avoid pseudoreplication of H estimates and  
353 control for seasonal differences. In hatching success models, breeding year was also fitted as a  
354 random effect.

355 Analyses of the mating options available to males and females were conducted in separate mate  
356 choice models. As the same allele frequencies are used to calculate  $r_{QG}$  across years, the  $r_{QG}$  of  
357 unique dyads across years is consistent. However, due to demographic factors such as divorce,  
358 migration, birth, death, and dispersal, the mean  $r_{QG}$  of focal breeders to their potential partners  
359 under each mate choice model will vary across years. To quantify inbreeding avoidance and  
360 identify a putative decision rule with regard to kinship, we fitted linear mixed-effects models with  
361 restricted maximum likelihood. For focal males and females, we compared  $r_{QG}$  to chosen partner  
362 with (i) mean  $r_{QG}$  to potential partners under random mate choice (with respect to kinship), (ii)  
363 mean  $r_{QG}$  to potential partners after the removal of close kin, and (iii) mean  $r_{QG}$  to potential partners  
364 after the removal of close and distant kin, within pairing ranges of 300m, 600m, 900m and 1200m.  
365 When pairs persisted across years, the first year a pair was observed was used in the analysis. Year  
366 nested within focal bird ID was fitted as a random effect, to generate comparisons within  
367 individuals in a given year.

368 Churr call dissimilarity was compared among four groups of individuals (breeding pairs, potential  
369 breeding pairs of first-order kin, potential breeding pairs of second-order kin and potential  
370 breeding pairs of non-kin) using generalized linear mixed-effects models. DTW score was  
371 modelled as a continuous response variable with a Gamma distribution and inverse link function.  
372 The fixed effect was group, with male ID and female ID both fitted as random effects. The  
373 relationship between churr call dissimilarity and kinship was tested using a separate model that

374 included all genotyped breeders, irrespective of pairing status. In this model, the independent  
375 variable was kinship. DTW score was modelled as a continuous response variable with a Gamma  
376 distribution and inverse link function. The fixed effect was kinship, with male ID and female ID  
377 both fitted as random effects.

378 **Data availability:** Source datasets and code for this manuscript will be made available in a Dryad  
379 Digital Repository.

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### 389 **Author contributions**

390 BJH conceived and managed the long-tailed tit study and supervised the project with EJHR. AEL,  
391 BJH, SPS and MS designed the study and collected data. AEL performed all analyses and wrote  
392 the manuscript. JS supported analysis of inbreeding costs. RFL supported bioacoustic analysis.  
393 JPG calculated direct fitness. All authors contributed to revisions. The authors declare no  
394 competing interests.

**References**

- 396 1. B. Charlesworth, D. Charlesworth, The genetic basis of inbreeding depression. *Genet. Res.*  
397 **74**, 329-340 (1999).
- 398 2. L. F. Keller, D. M. Waller, Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**,  
399 230-241 (2002).
- 400 3. I. G. Jamieson, S. S. Taylor, L. N. Tracy, H. Kokko, D. P. Armstrong, Why some species  
401 of birds do not avoid inbreeding: insights from New Zealand robins and saddlebacks.  
402 *Behav. Ecol.* **20**, 575–584 (2009).
- 403 4. C. Wang, X. I. N. Lu, Female ground tits prefer relatives as extra-pair partners: driven by  
404 kin-selection? *Mol. Ecol.* **20**, 2851-2863 (2011).
- 405 5. H. Kokko, I. Ots, When not to avoid inbreeding. *Evolution* **60**, 467-475 (2006).
- 406 6. G. A. Parker, Sexual conflict over mating and fertilization: an overview. *Philos. Trans. R.*  
407 *Soc. B* **361**, 235-259 (2006).
- 408 7. A. B. Duthie, J. M. Reid, Evolution of inbreeding avoidance and inbreeding preferences  
409 through mate choice among interacting relatives. *Am. Nat.* **188**, 651-667 (2016).
- 410 8. L. Lehmann, N. Perrin, Inbreeding Avoidance through Kin Recognition: Choosy Females  
411 Boost Male Dispersal. *Am. Nat.* **162**, 638–652 (2003).
- 412 9. A. E. Pusey, M. Wolf, Inbreeding avoidance in animals. *Trends Ecol. Evol.* **11**, 201–206  
413 (1996).
- 414 10. C. Riehl, C. A. Stern, How cooperatively breeding birds identify relatives and avoid incest:  
415 New insights into dispersal and kin recognition. *BioEssays* **37**, 1303–1308 (2015).
- 416 11. P. J. Greenwood, Mating systems, philopatry and dispersal in birds and mammals. *Anim.*  
417 *Behav.* **28**, 1140–62 (1980).

- 418 12. M. A. Du Plessis, Obligate cavity-roosting as a constraint on dispersal of green (red-billed)  
419 woodhoopoes: consequences for philopatry and the likelihood of inbreeding. *Oecologia*  
420 **90**, 205–11 (1992).
- 421 13. A. Cockburn, H. L. Osmond, R. A. Mulder, D. J. Green, M. C. Double, Divorce, dispersal  
422 and incest avoidance in the cooperatively breeding superb fairy-wren *Malurus cyaneus*. *J.*  
423 *Anim. Ecol.* **72**, 189–202 (2003).
- 424 14. P. Stacey, J. Ligon, Territory Quality and Dispersal Options in the Acorn Woodpecker, and  
425 a Challenge to the Habitat-Saturation Model of Cooperative Breeding. *Am. Nat.* **130**, 654-  
426 676 (1987).
- 427 15. B. J. Hatchwell, The evolution of cooperative breeding in birds: kinship, dispersal and life  
428 history. *Philos. Trans. R. Soc. B* **364**, 3217–3227 (2009).
- 429 16. M. G. Brooker, I. Rowley, M. Adams, P. R. Baverstock, Promiscuity: An inbreeding  
430 avoidance mechanism in a socially monogamous species? *Behav. Ecol. Sociobiol.* **26**, 191-  
431 199 (1990).
- 432 17. K. Tarvin, M. Webster, E. Tuttle, S. Pruett-Jones, Genetic similarity predicts the level of  
433 extra-pair paternity in splendid fairy-wrens. *Anim. Behav.* **70**, 945-955 (2005).
- 434 18. C. W. Varian-Ramos, M. S. Webster, Extrapair copulations reduce inbreeding for female  
435 red-backed fairy-wrens, *Malurus melanocephalus*. *Anim. Behav.* **83**, 857-864 (2012).
- 436 19. G. K. Hajduk *et al.*, Inbreeding, inbreeding depression, and infidelity in a cooperatively  
437 breeding bird. *Evolution* **72**, 1500–1514 (2018).
- 438 20. W. Lichtenauer, M. De Pol, A. Cockburn, L. Brouwer, Indirect fitness benefits through  
439 extra-pair mating are large for an inbred minority, but cannot explain widespread infidelity  
440 among red-winged fairy-wrens. *Evolution* **73**, 467–480 (2019).

- 441 21. H. Kokko, J. Ekman, Delayed Dispersal as a Route to Breeding: Territorial Inheritance,  
442 Safe Havens, and Ecological Constraints. *Am. Nat.* **160**, 468–484 (2002).
- 443 22. W. D. Koenig, J. Haydock, Incest and incest avoidance in *Ecology and Evolution of*  
444 *Cooperative Breeding in Birds*, W. D. Koenig, J. L. Dickinson Eds. (Cambridge University  
445 Press, 2004).
- 446 23. R. D. Magrath, R. G. Heinsohn, R. A. Johnstone, Reproductive skew in *Ecology and*  
447 *Evolution of Cooperative Breeding in Birds*, W. D. Koenig, J. L. Dickinson Eds.  
448 (Cambridge University Press, 2004).
- 449 24. W. D. Koenig, M. T. Stanback, J. Haydock, Demographic consequences of incest  
450 avoidance in the cooperatively breeding acorn woodpecker. *Anim. Behav.* **57**, 1287-1293  
451 (1999).
- 452 25. W. D. Koenig, J. Haydock, M. T. Stanback, Reproductive roles in the cooperatively  
453 breeding acorn woodpecker: incest avoidance versus reproductive competition. *Am. Nat.*  
454 **151**, 243-255 (1998).
- 455 26. C. Riehl, Kinship and Incest Avoidance Drive Patterns of Reproductive Skew in  
456 Cooperatively Breeding Birds. *Am. Nat.* **190**, 774–785 (2017).
- 457 27. J. L. Dickinson, B. J. Hatchwell, Fitness consequences of helping in *Ecology and Evolution*  
458 *of Cooperative Breeding in Birds*, W. D. Koenig, J. L. Dickinson Eds. (Cambridge  
459 University Press, 2004).
- 460 28. R. E. van Dijk, R. Covas, C. Doutrelant, C. N. Spottiswoode, B. J. Hatchwell, Fine-scale  
461 genetic structure reflects sex-specific dispersal strategies in a population of sociable  
462 weavers (*Philetairus socius*). *Mol. Ecol.* **24**, 4296–4311 (2015).

- 463 29. C. K. Cornwallis, S. A. West, A. S. Griffin, Routes to indirect fitness in cooperatively  
464 breeding vertebrates: kin discrimination and limited dispersal. *J. Evol Biol.* **22**, 2445-2457  
465 (2009).
- 466 30. S. B. McRae, Family values: costs and benefits of communal nesting in the moorhen. *Anim.*  
467 *Behav.* **52**, 225–245 (1996).
- 468 31. A. K. Townsend *et al.*, Disease-mediated inbreeding depression in a large, open population  
469 of cooperative crows. *Proc. R. Soc. B* **276**, 2057–2064 (2009).
- 470 32. J. L. Dickinson, C. Akçay, E. D. Ferree, C. A. Stern, A hierarchical analysis of incest  
471 avoidance in a cooperative breeder. *Behav. Ecol.* **27**, 1132-1140 (2016).
- 472 33. L. Brouwer, M. Van De Pol, E. Atema, A. Cockburn, Strategic promiscuity helps avoid  
473 inbreeding at multiple levels in a cooperative breeder where both sexes are philopatric.  
474 *Mol. Ecol.* **20**, 4796–4807 (2011).
- 475 34. C. J. Blackmore, R. Heinsohn, Variable mating strategies and incest avoidance in  
476 cooperatively breeding grey-crowned babblers. *Anim. Behav.* **75**, 63–70 (2008).
- 477 35. B. J. Hatchwell, P. R. Gullett, M. J. Adams, Helping in cooperatively breeding long-tailed  
478 tits: a test of Hamilton’s rule. *Philos. Trans. R. Soc. B* **369**, 20130565 (2014).
- 479 36. S. P. Sharp, M. B. Baker, J. D. Hadfield, M. Simeoni, B. J. Hatchwell, Natal dispersal and  
480 recruitment in a cooperatively breeding bird. *Oikos* **117**, 1371-1379 (2008).
- 481 37. A. E. Leedale, S. P. Sharp, M. Simeoni, E. J. H. Robinson, B. J. Hatchwell, Fine-scale  
482 genetic structure and helping decisions in a cooperatively breeding bird. *Mol. Ecol.* **27**,  
483 1714–1726 (2018).

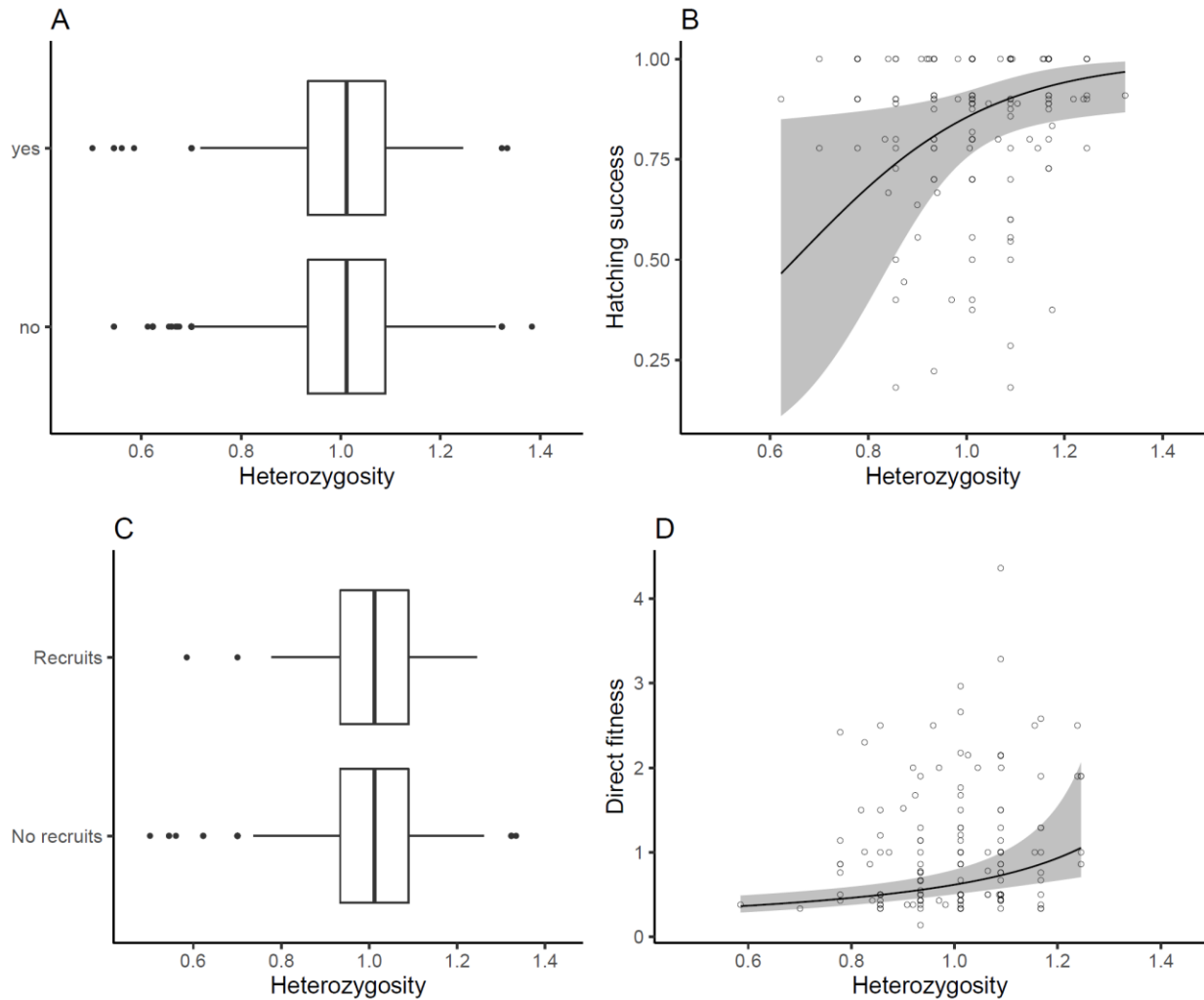
- 484 38. B. J. Hatchwell, A. F. Russell, D. J. Ross, M. K. Fowlie, Divorce in cooperatively breeding  
485 long-tailed tits: a consequence of inbreeding avoidance? *Proc. R. Soc. B* **267**, 813-819  
486 (2000).
- 487 39. J. Slate *et al.* Understanding the relationship between the inbreeding coefficient and  
488 multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* **93**, 255-  
489 265 (2004).
- 490 40. A. F. Russell, B. J. Hatchwell, Experimental evidence for kin-biased helping in a  
491 cooperatively breeding vertebrate. *Proc. R. Soc. B* **268**, 2169-2174 (2001).
- 492 41. S. P. Sharp, A. McGowan, M. J. Wood, B. J. Hatchwell, Learned kin recognition cues in a  
493 social bird. *Nature* **434**, 1127-1130 (2005).
- 494 42. M. Szulkin, N. Bierne, P. David, Heterozygosity-fitness correlations: a time for reappraisal.  
495 *Evolution* **64**, 1202-1217 (2010).
- 496 43. D. C. Queller, K. F. Goodnight, Estimating relatedness using genetic markers. *Evolution*  
497 **43**, 258-275 (1989).
- 498 44. J. P. Green, B. J. Hatchwell, Inclusive fitness consequences of dispersal decisions in a  
499 cooperatively breeding bird, the long-tailed tit (*Aegithalos caudatus*). *Proc. Natl. Acad.*  
500 *Sci. U.S.A.* **115**, 12011–12016 (2018).
- 501 45. B. J. Hatchwell, D. J. Ross, N. Chaline, M. K. Fowlie, T. Burke, Parentage in the  
502 cooperative breeding system of long-tailed tits, *Aegithalos caudatus*. *Anim. Behav.* **64**, 55-  
503 63 (2002).
- 504 46. S. P. Sharp, B. J. Hatchwell, Individuality in the contact calls of cooperatively breeding  
505 long-tailed tits (*Aegithalos caudatus*). *Behaviour* **142**, 1559-1575 (2005).

- 506 47. N. L. Hemmings, J. Slate, T. R. Birkhead, Inbreeding causes early death in a passerine bird.  
507 *Nat. Commun.* **3**, 863 (2012).
- 508 48. N. Pilakouta, S. Jamieson, J. A. Moorad, P. T. Smiseth, Parental care buffers against  
509 inbreeding depression in burying beetles. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8031–8035  
510 (2015).
- 511 49. A. McGowan, B. J. Hatchwell, R. J. W. Woodburn, The effect of helping behaviour on the  
512 survival of juvenile and adult long-tailed tits *Aegithalos caudatus*. *J. Anim. Ecol.* **72**, 491-  
513 499 (2003).
- 514 50. A. E. Leedale, R. F. Lachlan, E. J. H. Robinson, B. J. Hatchwell, Helping decisions and  
515 kin recognition in long-tailed tits: is call similarity used to direct help towards kin? *Philos.*  
516 *Trans. R. Soc. B* 20190565. doi:10.1098/rstb.2019.0565 (2020).
- 517 51. B. J. Hatchwell, S. P. Sharp, A. P. Beckerman, J. Meade, Ecological and demographic  
518 correlates of helping behaviour in a cooperatively breeding bird. *J. Anim. Ecol.* **82**, 486-  
519 494 (2013).
- 520 52. H. K. Reeve, The evolution of conspecific acceptance thresholds. *Am. Nat.* **133**, 407-435  
521 (1989).
- 522 53. S. G. Downs, F. L. W. Ratnieks, Adaptive shifts in honey bee (*Apis mellifera* L.) guarding  
523 behavior support predictions of the acceptance threshold model. *Behav. Ecol.* **11**, 326-333  
524 (2000).
- 525 54. M. E. Wolak, nadiv: an R package to create relatedness matrices for estimating non-  
526 additive genetic variances in animal models. *Methods Ecol. Evol.* **3**, 792-796 (2012).
- 527 55. M. Simeoni *et al.*, Characterization of 20 microsatellite loci in the long-tailed tit *Aegithalos*  
528 *caudatus* (*Aegithalidae*, *AVES*). *Mol. Ecol. Notes* **7**, 1319-1322 (2007).

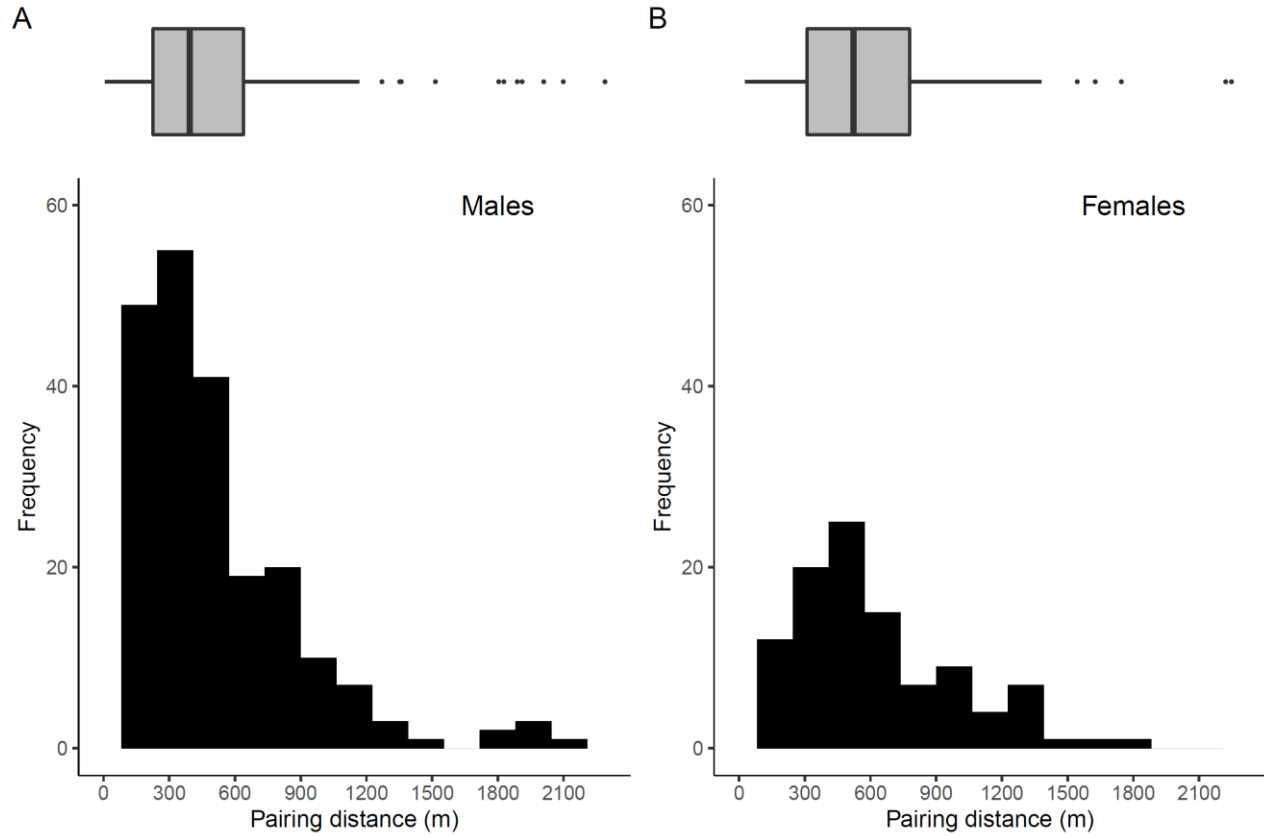


- 529 56. K. -B. Nam, M. Simeoni, S. P. Sharp, B. J. Hatchwell, Kinship affects investment by  
530 helpers in a cooperatively breeding bird. *Proc. R. Soc. B* **277**, 3299-3306 (2010).
- 531 57. D. W. Coltman, J. G. Pilkington, J. A. Smith, J. M. Pemberton, Parasite-mediated selection  
532 against inbred Soay sheep in a free-living island population. *Evolution* **53**, 1259-1267  
533 (1999).
- 534 58. T. C. Marshall *et al.*, Estimating the prevalence of inbreeding from incomplete pedigrees.  
535 *Proc R Soc B* **269**, 1533–1539 (2002).
- 536 59. L. F. Keller, Inbreeding and its fitness effects in an insular population of sparrows  
537 (*Melospiza melodia*). *Evolution* **52**, 240–250 (1998).
- 538 60. J. M. Reid *et al.*, Variation in parent-offspring kinship in socially monogamous systems  
539 with extra-pair reproduction and inbreeding. *Evolution*, **70**, 1512–1529 (2016).
- 540 61. B. J. Hatchwell *et al.*, Helpers increase long-term but not short-term productivity in  
541 cooperatively breeding long-tailed tits. *Behav. Ecol.* **15**, 1–10 (2004).

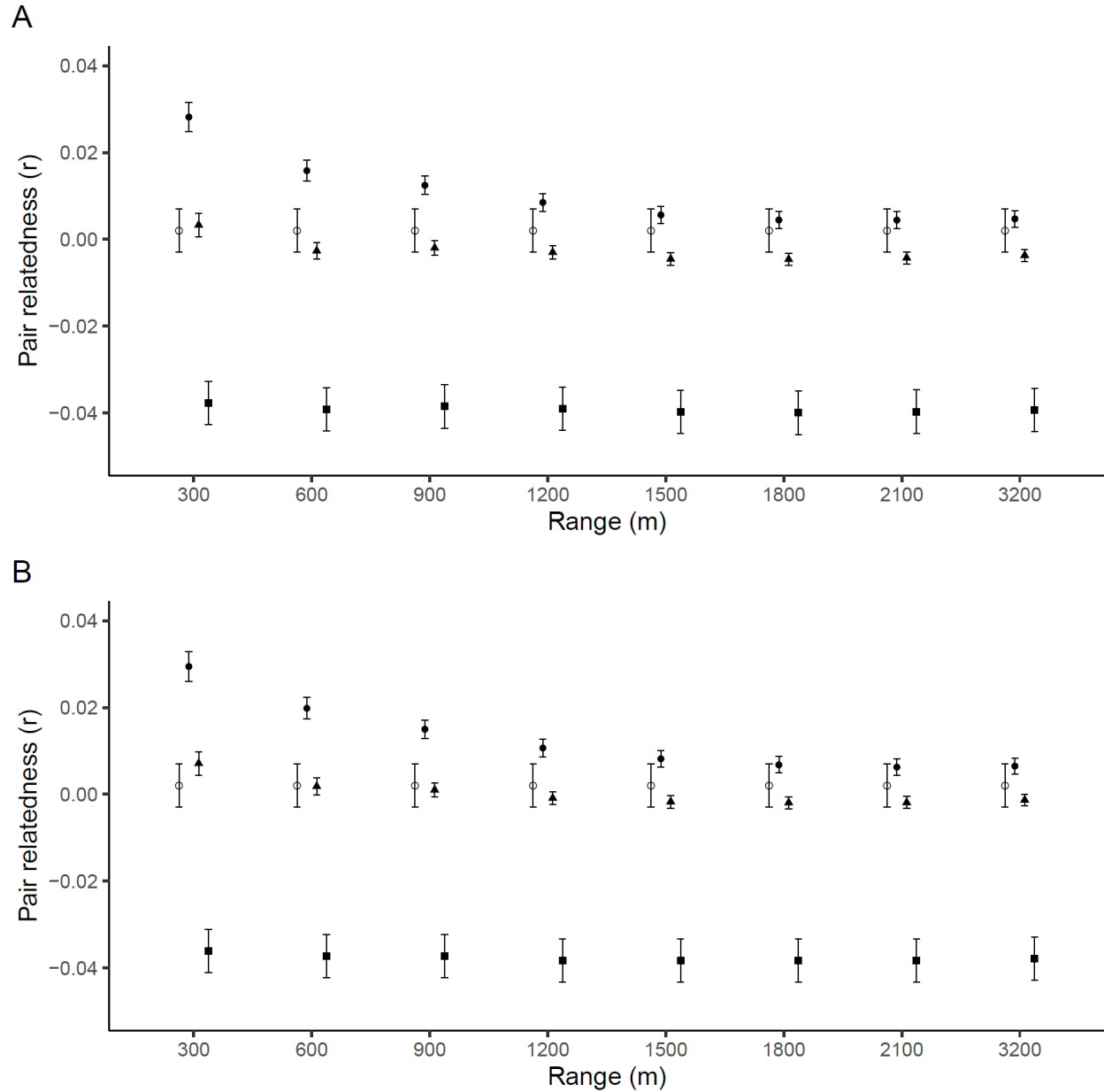
542



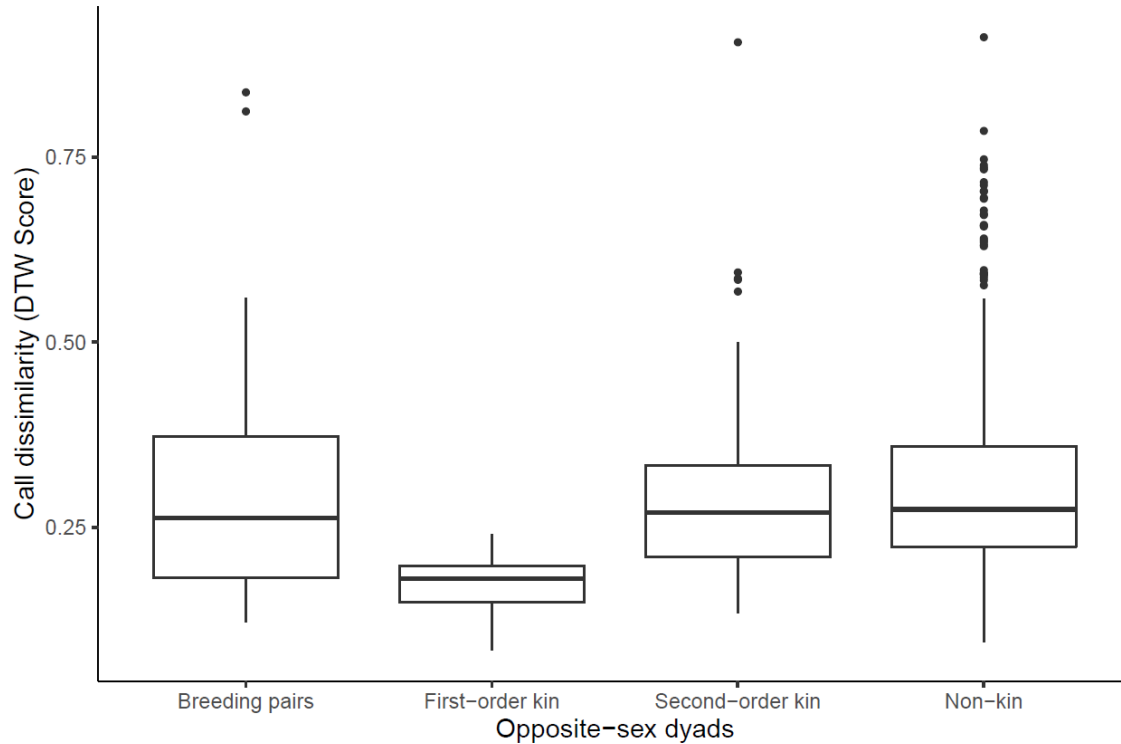
543 **Fig. 1.** The relationship between heterozygosity at microsatellite loci (H) and fitness components.  
 544 (A) probability of recruitment was not associated with H (GLMM,  $N = 1924$ ,  $z = 0.40$ ,  $P = 0.69$ );  
 545 (B) females' hatching success was positively associated with H (GLMM,  $N = 142$ ,  $z = 2.32$ ,  $P =$   
 546  $0.02$ ); (c) probability of producing recruits was not associated with H (GLMM,  $N = 744$ ,  $z = -1.77$ ,  
 547  $P = 0.07$ ); (d) the direct fitness of breeders that produced recruits was positively associated with  
 548 H (GLMM,  $N = 151$ ,  $t = -4.65$ ,  $P < 0.001$ ). Full model outputs are reported in Tables S3-6. Lines  
 549 represent model predictions  $\pm 95\%$  CI constructed using fixed effects, boxplots represent median  
 550  $\pm 1.5x$  IQR.



551 **Fig. 2.** Frequency distribution and median (+IQR) pairing ranges of (A) male (median = 393m,  $N$   
 552 = 230) and (B) female (median = 523m,  $N$  = 109) breeders, calculated as the distance between an  
 553 individual's natal nest and their first breeding attempt. Boxplots represent median  $\pm$  1.5x IQR.



554 **Fig. 3.** Mean genetic relatedness of breeding pairs formed within increasing ranges (open circles),  
 555 and the expected relatedness if (A) females ( $N = 445$ ) or (B) males ( $N = 412$ ) selected mates at  
 556 random with respect to kinship (closed circles), avoided kin with  $r_{QG} > 0.375$  (closed triangles),  
 557 and avoided kin with  $r_{QG} > 0.125$  (closed squares). Expected relatedness was the mean relatedness  
 558 of focal birds to all opposite-sex available breeders within each range under each mate choice  
 559 model. Error bars represent the standard error around the mean.



560 **Fig. 4.** Dissimilarity of churr calls among groups of opposite-sex dyads: breeding pairs ( $N = 51$ );  
 561 first-order kin ( $N = 11$ ); second-order kin ( $N = 155$ ); non-kin ( $N = 735$ ). Dyads that were not  
 562 breeding pairs comprised available breeders within pairing range ( $\leq 1350\text{m}$ , 95% pairs) present in  
 563 the same breeding year. Call dissimilarity was measured using dynamic time warping analysis  
 564 (DTW). Call dissimilarity within breeding pairs was higher than that within potential pairs of first-  
 565 order kin (GLMM;  $N = 952$ ,  $t = 2.87$ ,  $P = 0.004$ ) but not second-order ( $t = 0.06$ ,  $P = 0.94$ ), or non-  
 566 kin ( $t = -1.63$ ,  $P = 0.10$ ). Boxplots represent median  $\pm 1.5$  IQR.