# Relatedness and age reduce aggressive male interactions over mating in domestic fowl

Charlotte Rosher[1, 2], Anna Favati[3], Rebecca Dean[4, 5], Hanne Løvlie[1]*

[1] IFM Biology, Linköping University, 58183 Linköping, Sweden.

[2] Faculty of Life Sciences, University of Manchester, M13 9LP Manchester, UK.

[3] Department of Zoology, Stockholm University, 10691 Stockholm, Sweden.

[4] School of Biological Sciences, Clayton Campus, Monash University, Victoria 3800, Australia.

[5] Department of Genetics, Evolution and Environment, University College London, WC1E 6BT, UK.

*corresponding author: hanne.lovlie@liu.se

Running title: Reduced male-male aggression towards kin

17 Abstract

18 Altruistic behaviour represents a fundamental challenge in evolutionary biology. It is often best

19 understood through kin selection, where favourable behaviour is directed towards relatives.

20 Kin selection can take place when males cooperate to enhance the reproductive success of

21 relatives. Here we focus on reduced male-male competition over mating as a case of

22 cooperation, by examining male tolerance of matings by related and unrelated competitors. A

23 suitable model for exploring whether relatedness affects male-male interactions over mating is

24 the domestic fowl, *Gallus gallus domesticus.* In this species, males form social hierarchies and

25 dominant males commonly interrupt subdominant males' copulation attempts. We investigated

26 whether dominant male fowl differentially direct aggressive interactions towards unrelated and

27 related subordinate males during mating attempts. Dominant male fowl were found to interrupt

28 mating attempts of male relatives less often than those of unrelated males. We further tested

29 whether male age mediates the magnitude of kin tolerance behaviour. However, we found no

30 support for this as both young and old dominant males were less likely to interrupt related,

31 compared to unrelated, subdominant males' copulations during male-male interactions. Our

32 results, consistent with kin selection, provide a rare experimental demonstration of relatedness

33 relaxing male-male competition over mating.

34

35 Keywords: Aggression, Chicken, *Gallus gallus,* Intra-sexual selection, Kin recognition,

36 Relatedness

37

<u>Introduction</u>

Kin selection directs aid-giving behaviour towards kin over non-kin in order to support and promote the success of shared genes (Hamilton 1964). In this way, individuals can increase their inclusive fitness both directly by producing offspring and indirectly by promoting the reproductive success of their relatives (Hamilton 1964). Mechanisms which allow individuals to differentially respond towards others based on their likely degree of relatedness include spatial distribution (such as sex-biased dispersal), social familiarity, phenotype matching, or recognition alleles (reviewed in Komdeur and Hatchwell 1999). Kin selection has been widely demonstrated in contexts such as predator evasion (Sherman 1977), colony defence and propagation in eusocial insects (Queller and Strassman 1998), parental care (Shields 1984), and selective cannibalism (Walls and Roudebush 1991).

In addition to promoting the survival of related individuals, kin selection can also promote the reproductive success of relatives. Studies of kin selection have more often focused on cooperation in terms of directing aid-giving behaviour towards relatives, rather than aggression towards or inhibition of the success of non-relatives. For instance, kin selection has been explored in the context of male-male cooperation for attracting mates, with mixed outcomes. In some species, cooperative male groups are more likely to be comprised of brothers than unrelated individuals (Tasmanian hens, *Tribonyx mortierii,* Maynard Smith and Ridpath 1972; Tasmanian hens, *Gallinula mortierii*, Goldizen et al. 2000; peacocks, *Pavo cristatus*, Petrie et al. 1999; wild turkeys, *Meleagris gallopavo,* Krakauer 2005), while in others they are equally likely to comprise related and unrelated males (lions*, Panthera leo,* Packer and Pusey 1982; long-tailed manakins, *Chiroxiphia linearis*, McDonald and Potts 1994). More often, however, males are not cooperating, but are in direct competition with each other over mating opportunities (Andersson 1994). Relatedness has the potential to affect the aggressiveness of these competitive interactions (Hamilton 1964; Pizzari and Gardner 2012; Díaz-Muñoz et al. 2014; Pizzari et al. 2015), as well as during copulation where unrelated rival males should allocate larger ejaculates during sperm competition than related rivals due to kin selected benefits (Parker 2000). However, empirical studies fail to detect such differential responses by males (Australian field cricket, *Teleogryllus oceanicus*, Thomas and Simmons 2008; bank voles, *Myodes glareolus,* Klemme and Ala-Honkola 2014; house mouse, *Mus musculus domesticus,* Ramm and Stockley 2009). Further, kin selection can moderate aggression when there are inclusive fitness benefits (Hamilton 1964; Waldman 1988; Pizzari et al. 2015). The capacity for relatedness to affect male-male competitive interactions has been demonstrated in

nematodes, where higher relatedness mitigates mortality in lethal male fighting (Kapranas et al. 2015). In *Drosophila melanogaster*, male-male aggression in terms of fighting was reduced among brothers (Carazo et al. 2014; Carazo et al. 2015; Martin and Long 2015; but see Chippendale et al. 2005). In a recent study on the red junglefowl (*Gallus gallus*), direct competition among males was reduced when males were related, although competition after copulation increased, alluding to potentially complex dynamics of relatedness and intra-sexual selection (Tan et al., in press). These results highlight how male aggression can be mediated according to relatedness with competitors.

Aggressive interactions among competitors may potentially also be mediated by male age. This is because as individuals senesce they undergo a decline in residual reproductive value (Fisher 1930) which reduces their reproductive success (Bouwhuis et al. 2009; Reed et al. 2008). Older males, with reduced ejaculate competitive ability (Jones & Elgar 2004; Dean et al. 2010), may therefore increase their overall aggressive interactions towards competitors to prevent sperm competition and protect their paternity. Alternatively, one way in which reproductive senescence may manifest in older males is through an overall decline in aggressive interactions towards competitors. Male age may therefore either increase or decrease the overall intensity of aggressive interactions directed towards both related and unrelated competitors.

A more nuanced way in which male age may affect aggressive interactions is through increased differential aggression towards kin and non-kin. While it is well established that age can affect direct fitness (Reed et al. 2008; Bouwhuis et al. 2009), researchers have also suggested implications for inclusive fitness (Libertini 1988; Lee 2003; Bourke 2007; Ronce et al. 2010). For example, aged individuals can increase their inclusive fitness through the transfer of resources or care when they involve closely related kin (Lee 2003; Bourke 2007). Studies of the interaction between kin selection and senescence have often focussed on females, most notably in relation to child care in humans where aging women can have increased inclusive fitness by caring for grandchildren rather than producing offspring themselves (Lahdenperä et al. 2004). This has also been framed in terms of preventing inter-generational reproductive competition among females within a family (Cant and Johnstone 2008). In contrast, the general role of male reproductive senescence in relation to kin selection remains relatively unexplored. Indeed, males may be particularly prone to reproductive decline with age because their high rates of gametogenesis over time cause greater risk of deleterious mutations accumulating in their germ line, negatively affecting their offspring (Reinhardt 2007; Pizzari et al. 2008). Old

106 males, with lower reproductive potential, may therefore have different costs and benefits of
107 competing with related or unrelated males, compared to younger males. Under this scenario,
108 we may expect older males to preferentially prevent unrelated males from mating compared to
109 related males. Despite the scope for age to influence kin selection through male-male
110 aggression, this interaction has not yet been investigated.

111

112 We investigated the role of relatedness in male-male competition among first-order relatives
113 that were of two age classes (either old or young, see below) in the sexually promiscuous
114 domestic fowl (*Gallus gallus domesticus*). The fowl social structure shows clear hierarchies in
115 which dominant males have privileged access to females and show aggression towards
116 subordinates (Collias and Collias 1996). Further, males face sperm competition (i.e. where the
117 ejaculates of two or more males compete over fertilisation of a female's ova, Parker 1970), and
118 dominant males employ a sperm competition defence strategy (Parker 1984) by interrupting
119 the copulation attempts of subordinate males (Pizzari 2001). When groups contain multiple
120 females or multiple subordinate males, dominant males may be unable to effectively interrupt
121 copulation attempts, especially when subordinates copulate simultaneously, creating a
122 constraint on copulation interruption. In addition, interrupting copulation may carry costs
123 resulting from aggressive behaviour. Under natural conditions, fowl have overlapping
124 generations, limited dispersal and no sex-biased dispersal, thus related individuals of different
125 age groups encounter each other, including sibling and parent-offspring relationships (Collias
126 and Collias 1996). Moreover, studies suggest that fowl recognise kin from non-kin (Pizzari et
127 al. 2004; Løvlie et al. 2013; Tan et al., in press). We tested dominant male aggression towards
128 related and unrelated subordinates by measuring the likelihood of the dominant male
129 interrupting subordinates' copulation attempts. We first tested whether dominant males were
130 less likely to interrupt copulation attempts of related subordinate competitors compared to
131 unrelated subordinate competitors. Secondly, we tested for an effect of male age on the overall
132 propensity to interrupt copulations. Finally, we tested for increased tolerance towards matings
133 of younger related competitors in aged dominant males, who have lower reproductive potential
134 (Dean et al. 2010; Cornwallis et al. 2014). To do this we manipulated groups, enabling us to
135 investigate kin tolerance in old and young age classes of dominant male fowl towards equally
136 related competitors, who are sons or full-sibling brothers respectively (degree of relatedness of
137 0.5). We demonstrate that both old and young dominant males interrupt a lower proportion of
138 related subordinate male copulation attempts than those of unrelated subordinates, suggesting
139 that male fowl show kin tolerance during male-male competition over mating. Older males

140 show an overall reduced level of copulation interruptions than younger males. However,

141 contrary to our predictions, interruptions of copulation attempts made by unrelated males were

142 no more pronounced when the dominant male was old.

143

144 Methods

145 Study population

146 We used individuals ($n_{males}$ = 39, $n_{females}$ = 54) from a population of an old Swedish game breed

147 of domestic fowl ('Gammal svensk dvärghöna' in Swedish, see references in e.g. Zidar et al.

148 2012; Favati et al. 2014a; Løvlie et al. 2014), kept under semi-natural conditions at Tovetorp

149 Research station, Stockholm University. Experiments took place in July - September 2014 and

150 2015 during the birds' breeding season (Løvlie and Pizzari 2007). This population (population

151 sizes: $n_{males}$ = 63, 57, $n_{females}$ = 60, 55, for 2014 and 2015, respectively) is bred under

152 uncontrolled, relaxed artificial selective pressures and are kept in >6 mixed sex, mixed age

153 groups (1 – 13 years old). Birds used were pedigree-bred for one generation, sexually mature

154 (>1 year old), had uniquely numbered metal leg rings for identification, and were housed in

155 outdoor aviaries (4.6m x 10m), with ad libitum access to perches, dust baths, shelter, food and

156 water. Visual, but not vocal, contact with neighbouring birds was prevented.

157

158 Age treatments

159 In order to investigate the role of male age on kin tolerance during mating attempts, groups

160 were generated which contained 'old' dominant males. Across groups, the dominant males in

161 old and young groups thus differed significantly in age (Mann Whitney U-test, $mean_{old}$ ± SE =

162 6.23 ± 0.43 years, n = 9, $mean_{young}$ ± SE = 2.56 ± 0.78 years, n = 9, w = 70.5, p = 0.008,

163 Supplementary Figure S1). In this population, several lines of evidence (Dean et al. 2010)

164 suggest that males 6-8 years old suffer from reduced fertilising capacity. First, linear declines

165 in sperm production were recovered across the population. Second, in an artificial insemination

166 experiment which controlled for sperm number between competing ejaculates, sperm from 2-

167 3 year olds had a fertilising advantage over sperm from 6-8 year olds, fertilising 77 ± 10% (±

168 SE) of the eggs. Finally groups of females with dominant males of 6-8 years had overall lower

169 fertility (54 ± 10%) than groups with dominant males that were 3 years old (73 ± 7%). Together,

170 these findings suggest that 6 year old males in this population show reduced fertilising capacity

171 at multiple stages of reproductive investment. Aggression scores (scored from 0 - 6, 6 being

172 most aggressive, see Favati et al. 2014a) obtained prior to the experiment, available for

173 dominant males in 17 out of the 18 groups studied, showed that old and young dominant males

6

174 did not differ significantly in aggression (Mann-Whitney U-test, $\text{mean}_{old} \pm SE = 4.0 \pm 0.63$, n

175 = 8, $\text{mean}_{young} \pm SE = 4.22 \pm 0.28$, n = 9, w = 32.5, p = 0.76). Observations of groups with old

176 or young dominant males were randomised throughout the breeding seasons.

177

178 Establishing male dominance

179 Each group was formed with three males and four females. The males consisted of a dominant

180 focal male, his relative and his non-relative. Subordinate males within each group ($n_{groups} = 18$)

181 were matched in age (Wilcoxon matched pair-test, $\text{mean}_{related} \pm SE = 2.89 \pm 0.47$ years,

182 $\text{mean}_{unrelated} = 2.59 \pm 0.51$ years, w = 182.5, p = 0.51, Supplementary Figure S1), body mass

183 (paired t-test, $\text{mean}_{related} \pm SE = 1210 \pm 19g$, $\text{mean}_{unrelated} \pm SE = 1191 \pm 28g$, t = 0.78, df = 13,

184 p = 0.45), comb sizes (paired t-test, $\text{mean}_{related} \pm SE = 72.3 \pm 2.2$ mm, $\text{mean}_{unrelated} \pm SE = 77.8$

185 $\pm 2.2$ mm, t = 1.46, df = 13, p = 0.17), and were unrelated according to the pedigree information.

186 This means that individuals used as 'unrelated' were always less related than first order

187 relatives, and often less related than second order, based on the 1-2 generation pedigree

188 information available. Young treatments used a young dominant male, his full-sibling brother

189 and a non-relative, while old treatments used an old dominant male, his son and a non-related

190 male. All groups consisted of a unique combination of males. Due to limitations in the number

191 of related males in the specific age classes, 3 dominant males and 1 unrelated subordinate had

192 to be reused, and 7 males were reused in alternative positions (dominant, related subordinate

193 or unrelated subordinate). Males from a total of 10 families were used.

194

195 Before a trial, the dominant male was left in the aviary overnight in order to facilitate his

196 dominance over the males who were later introduced, based on the prior residence effect

197 (Maynard Smith and Parker 1976). Two females were left with the dominant male as company

198 during this period and were removed the next morning. The other two males of a group were

199 then introduced. The order of the introduction of the related or unrelated male to the resident

200 male was alternated between groups. Dominance is established by pairwise agonistic

201 interactions and a male was assigned a subordinate rank if a minimum of three successive

202 avoidances of another individual were observed (*sensu* Favati et al. 2014a; Favati et al. 2014b).

203 Clear submission and dominance was observed in all groups within the first 2 hours of

204 observations, and positions within the dominance hierarchy did not change during the

205 experiment.

206

207 Mating trials

In the afternoon, after the males had established their hierarchy, four females unrelated to the males, were released simultaneously into the enclosure at the start of the trial. Hence, three males competed over access to four females, which is a natural sex ratio and group size observed in the wild (see references in Løvlie and Pizzari 2007). Birds used to constitute a group were temporarily socially unfamiliar, and had not been housed together for the last 14 days. This was done because previous mating history reduces mating propensity in both sexes (Løvlie and Pizzari 2007).

Males may be more likely to initiate copulations when females have high fecundity (e.g. when they are young, or if they are currently laying eggs, Løvlie et al. 2005; Løvlie and Pizzari 2007), but there was no significant difference in female age between groups with old and young dominant males (unpaired t-test, $mean_{old} \pm SE = 2.97 \pm 0.45$ years, $mean_{young} \pm SE = 3.86 \pm 0.33$ years, t = -1.61, df = 15, p = 0.13), or number of eggs laid in groups (measured as eggs laid between the first and second observation evening, unpaired t-test, $mean_{old} \pm SE = 1.78 \pm 0.32$ eggs, $mean_{young} \pm SE = 1.4 \pm 0.28$ eggs, t = 0.76, df = 15, p = 0.46).

Eighteen groups were observed for 2 evenings each ($mean_{total\ observation\ time} \pm SE = 445 \pm 18$ minutes). In feral populations of *Gallus g. domesticus*, optimal copulation time is during the evening, because this is when the probability is highest that an insemination will result in fertilisation and male mating activity levels peak (see Løvlie and Pizzari 2007; Løvlie et al. 2005). One group had few copulatory attempts observed over two evenings, so the recording period was extended by one additional evening. Observations started around 16:30 local time after females had been released with the males, and terminated when the last bird had been roosting for 10 minutes (*sensu* Løvlie and Pizzari 2007). In each observation, all copulations and copulation attempts were recorded, with the identity of the copulating male and female. Other males which interrupted the copulation or copulation attempt (interruption defined as if a male moved, most often by running, towards the copulating couple and caused the copulating male to stop copulating with the female), were recorded with their identity. Female behavioural resistance towards a male's copulation attempt was scored according Løvlie et al 2014. Observations were carried out by CR and HL.

The study was conducted according to the ethical requirements in Sweden (Linköping Ethical committee, ethical permit no. 114-12).

Statistical analyses

243 Since groups showed substantial differences in the total number of copulation attempts made

244 by each subordinate male (range = 2 - 46 copulations, Table 3, Supplementary Figure S2), we

245 analysed copulation interruptions by the dominant male as a proportion of the total number of

246 interruptions of each subordinate male's copulation attempts. This measure, 'proportion of

247 interruptions', was created as a two-vector response variable, comprising 'number of

248 copulation attempts interrupted by the dominant male' (binomial numerator) and 'total number

249 of subordinate copulation attempts' (binomial denominator) for each subordinate male (*sensu*

250 Zuur et al. 2013). Variation in proportion of interruptions was analysed in a Generalized Linear

251 Mixed Model (GLMM, R package lme4) with age of dominant male (old/young) and

252 relatedness of the subordinate to dominant male (related/unrelated) as fixed effects, including

253 their interaction. Group identity (1 - 18), subordinate male identity and dominant male identity

254 were given as random factors. The model was fitted with a Binomial distribution and was

255 confirmed to not have over-dispersal. Because the interaction between age of dominant male

256 and relatedness of subordinate was non-significant (see Results), the model was re-run without

257 the interaction and statistics in such cases for main effects presented from the latter model.

258

259 To explore whether other aspects of male or female behaviour influenced proportion of

260 interrupted copulations observed, we ran several additional models. To determine whether

261 dominant male age affected number of copulation attempts, we ran a GLMM with number of

262 copulation attempts carried out by dominant males as the response variable. Time observed

263 (hours) was given as a continuous effect and age of dominant male (old/young) as a fixed effect.

264 Group identity (1 - 18) and dominant male identity were given as random factors. The model

265 was fitted with a Poisson distribution and was confirmed to not have over-dispersal.

266

267 To determine whether mating behaviour of the subordinate males in a group was affected by

268 either the age of the dominant male or the relatedness of the subordinate male to the dominant,

269 we ran a GLMM with number of subordinate copulation attempts as the response variable.

270 Time observed (hours) was added as a continuous effect, age of dominant male (old/young)

271 and relatedness of the subordinate to the dominant male (related/unrelated) were added as fixed

272 effects, including their interaction. Group identity (1 - 18), subordinate male identity and

273 dominant male identity were given as random factors. The model was fitted with a Poisson

274 distribution and confirmed to not have over-dispersal.

275

276 To explore variation in female mating behaviour towards subordinate male copulation
277 attempts, a GLMM investigated the proportion of subordinate male copulation attempts
278 resisted by females. A two-vector response variable was created, comprising 'number of
279 subordinate copulation attempts resisted by the female' (binomial numerator) and 'total number
280 of subordinate copulation attempts' (binomial denominator) for each subordinate male (*sensu*
281 Zuur et al. 2013). Relatedness of the subordinate to the dominant male (related/unrelated) was
282 added as a fixed effect. Group identity (1 - 18) and subordinate male identity were given as
283 random factors. The model was fitted with a Binomial distribution and was not over-dispersed.
284
285 Similarly, a GLMM investigated the proportion of dominant male copulation attempts resisted
286 by females. A two-vector response variable was created, comprising 'number of dominant male
287 copulation attempts resisted by the female' (binomial numerator) and 'total number of
288 dominant male copulation attempts' (binomial denominator) for each dominant male (*sensu*
289 Zuur et al. 2013). Age of dominant male (old/young) was added as a fixed effect. Group identity
290 (1 – 18) and dominant male identity were given as random factors. The model was fitted with
291 a Binomial distribution and was not over-dispersed.
292
293 Statistics were performed using RStudio v.0.98.1074.
294
295 Results
296 We observed 786 individual copulation attempts across the groups of which 143 were
297 interrupted and 111 of these were interruptions were made by the dominant male (78%).
298
299 Old dominant males interrupted a lower proportion of subordinate copulation attempts than
300 young dominant males (mean$_{old}$ ± SE = 0.16 ± 0.02, mean$_{young}$ ± SE = 0.29 ± 0.07, Table 1
301 Figure 1). Dominant males interrupted a lower proportion of related subordinate copulation
302 attempts than unrelated subordinate copulation attempts (mean$_{related}$ ± SE = 0.15 ± 0.05,
303 mean$_{unrelated}$ ± SE = 0.35 ± 0.07, Table 1, Figure 1). However, these interruptions were not
304 explained by an interaction between relatedness of subordinate and age class of the dominant
305 male (Table 1, Figure 1). Therefore, old dominant males did not interrupt a higher proportion
306 of unrelated subordinate copulation attempts than young dominant males did.
307
308 Dominant males did not attempt more copulation attempts per hour when the dominant male
309 was old (mean$_{old}$ ± SE = 1.55 ± 0.37, n = 9, mean$_{young}$ ± SE = 2.55 ± 0.37, n = 9, Table 2).

310

The rate of subordinate male copulation attempts in a group was not affected by dominant male age (mean$_{old}$ ± SE = 3.78 ± 0.95, mean$_{young}$ ± SE = 3.95 ± 0.75, n = 9, Table 3) or relatedness (mean$_{related}$ ± SE = 2.10 ± 0.38, mean$_{unrelated}$ ± SE = 1.75 ± 0.34, n = 9, Table 3), confirming that our results were not influenced by differences in subordinate male behaviour in groups with young and old dominant males.

Differences in female mating behaviour towards males' copulation attempts could in principle affect the propensity of dominant males to interrupt copulations. However, the proportion of subordinate copulation attempts that were resisted by the female was not significantly different when the subordinate was related or unrelated to the dominant male (mean$_{related}$ ± SE = 0.77 ± 0.06, n = 18, mean$_{unrelated}$ ± SE = 0.81 ± 0.05, n = 18, Table 4A), or dependent on the age of the dominant male (mean$_{old}$ ± SE = 0.67 ± 0.13, n = 9, mean$_{young}$ ± SE = 0.90 ± 0.03, n = 9, Table 4B).

Discussion

In a setup providing male-male competition over mating opportunities, we aimed to test whether (i) relatedness of competitor affects dominant male competitive behaviour, (ii) male age affects male competitive behaviour, and whether (iii) the age of the dominant male mediated the level of tolerance towards related competitor matings. We provide evidence that dominant male fowl are less likely to interrupt copulation attempts of related subordinates than unrelated subordinates, but we found that although older males were overall less likely to interrupt copulations, this effect was not more pronounced when the dominant male was old.

Differential aggression towards kin and non-kin has been considered by other studies of kin selection (e.g. cannibalism, Walls and Roudebush 1991; lethal male fighting, Kapranas et al. 2015), including recent work on contexts related to mating (Carazo et al. 2014; Martin & Long 2015; Tan et al., in press; reviewed in Díaz-Muñoz et al. 2014; Pizzari et al. 2015). We present one of few empirical examples where males show increased tolerance towards kin during pre-copulatory male-male competition. This aligns with other avian studies which suggest males prefer cooperating with kin to attract mates (Maynard Smith and Ridpath 1972; Petrie et al. 1999; Goldizen et al. 2000; Krakauer 2005), but differs in that our study investigates competitive interactions rather than cooperative interactions between males.

344 We report that dominant males interrupt a higher proportion of unrelated males' copulation
345 attempts than related males. This finding could in principle have been explained if females
346 favour males that are unrelated to the dominant male, for example if females seek high
347 offspring genetic diversity (Jennions and Petrie 2000). However, we found no evidence of
348 differential female behaviour towards subordinates that were unrelated to the dominant male.
349 Females may differentially favour ejaculates from males unrelated to the dominant male
350 through cryptic female choice (e.g. ejaculate ejection, Pizzari and Birkhead 2000; Dean et al.
351 2011; Løvlie et al. 2013). No ejaculate ejections were observed during the current study, and
352 we did not investigate other mechanisms of cryptic female choice, such biases in female sperm
353 utilisation which may in principle also affect the dominant male's propensity to interrupt
354 copulations. Further studies are needed to investigate the potential for this, and the complex
355 interaction of both pre- and post-copulatory, male and female dynamics when related
356 individuals interact.

357

358 Although we show that males are less aggressive towards related competitors, the underlying
359 kin recognition mechanism is currently unknown in the fowl. Previous research in the fowl has
360 shown that individuals respond differentially to potential sexual partners dependent on their
361 genetic relatedness (Pizzari et al. 2004; Gillingham et al. 2009; Løvlie et al. 2013; Tan et al.,
362 in press), and this effect seems to not be explained by social familiarity being used as a proxy
363 for kin recognition (Pizzari et al. 2004; Løvlie et al. 2013). In our population, all individuals
364 are likely to be socially familiar due to the fact that same-aged birds are hatched in the same
365 artificial incubators or by the same females, the birds are housed in large groups over winter
366 and rotated across experiments during the breeding seasons, so any effect of social familiarity
367 on male aggression is likely to be balanced across treatments. In other species, for example
368 *Drosophila melanogaster*, an olfactory mechanism can affect responses to kin (Tan et al. 2013).
369 Previous work has demonstrated that birds from our study population respond differentially to
370 olfactory cues (Zidar and Løvlie 2012) and there may be an as yet unexplored basis for an
371 olfactory kin recognition mechanism in the fowl. Independent of the mechanism through which
372 kin recognition occurs, our experiment entered dominant males into a competitive mating
373 situation with their relatives, and by interrupting a lower proportion of their relative's
374 copulation attempts than those of unrelated subordinates, our focal males favoured kin over
375 non-kin.

376

We also investigated kin tolerance behaviour in two age classes of dominant males. Male ageing may have the potential to increase kin tolerance towards younger relatives. When old males are unable to fertilise all available eggs due to declining male fertility (Jones et al. 2007; Møller et al. 2009; Dean et al. 2010), the relative cost of allowing a related male to mate (particularly in situations with no sperm competition, e.g. with a novel female) may be lower for old males compared to young males. Hypothetically, an old male that can only fertilise 50% of the eggs available to him will gain equal inclusive fitness benefit if he permits a younger male with relatedness 0.5 to fertilise 100% of the eggs available. This scenario may be particularly relevant when females risk sperm limitation (Wedell et al. 2002), for example under female-biased sex ratios, or when polyspermy is required for fertilisation to occur (like in birds, Hemmings and Birkhead 2015). We observe that older dominant males interrupt a lower proportion of copulation attempts compared with young dominant males. This is likely a result of reduced activity of older males, complementing our result that old dominant males show a non-significant tendency to have a lower copulation rate, and previous work on our study population showing that age affects mating behaviour negatively in male fowl (Dean et al. 2010). However, we do not find an interaction between age of dominant male and relatedness of subordinate, thus tolerance of kin matings did not increase with dominant male age.

Research on kin selection and ageing has predominantly focussed on females (reviewed in Bourke 2007), but our study presents a scenario where male age can be investigated. Future studies which take into account age-dependent declines in also ejaculate fertilising efficiency and offspring quality, could shed light on the different ways senescence may affect kin selection during male-male competition over mating opportunities. Focus within kin selection has centred on conditional helping behaviours rather than conditional harming behaviours, but incorporating finite group sizes with small spatial areas highlights that individuals are also likely to compete for resources, including mating opportunities (Lehmann et al. 2009; Ronce et al. 2010). Our study measures aggressive interactions over competitive matings which have the potential to affect inclusive fitness benefits when relatives compete. We demonstrate that in a competitive mating situation, male fowl favour kin by interrupting the copulation attempts of unrelated subordinates more frequently. However, male fowl do not show more pronounced kin-biased behaviour with age.

419

420 Data accessibility

421 Analyses reported in this article can be reproduced using the data provided by Rosher et al
422 (2017).

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616

**Figures and tables.**

619 **Figure 1. Interruptions of copulation attempts by old and young dominant male domestic**

620 **fowl towards related and unrelated subordinate males.** 'Proportion of interruptions' refers

621 to the proportion of subordinate male copulations interrupted by the dominant male. Males

622 showed lower aggression towards related male competitors over unrelated male competitors

623 and this effect was not different between older (black columns) or younger (grey columns)

624 dominant males. Error bars represent standard error of the mean.

625



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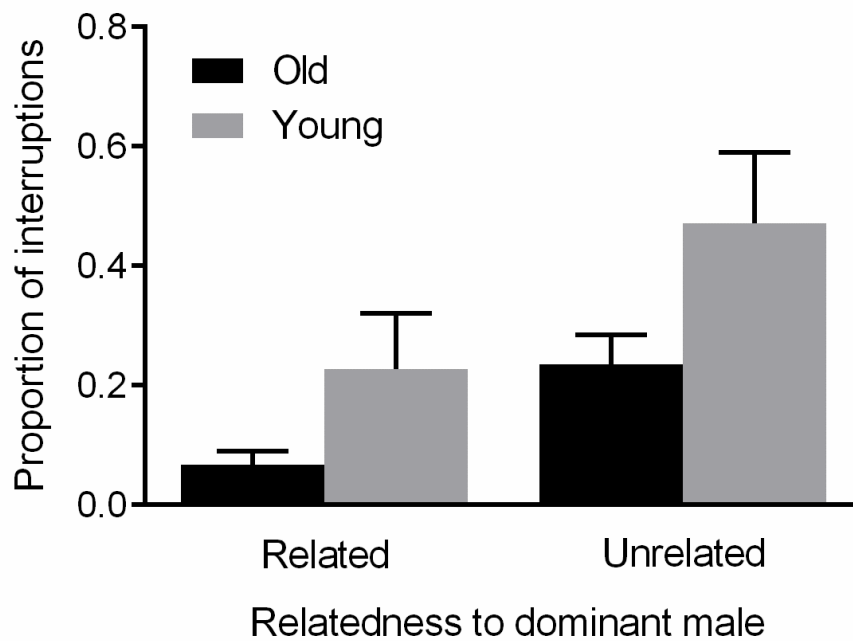
628    **Table 1. Variation in interrupted copulation attempts among male domestic fowl.**
629    Proportion of interrupted copulation attempts (i.e. number of subordinate copulation attempts
630    interrupted by the dominant divided by the total number of subordinate male copulation
631    attempts) in groups of domestic fowl was affected by: age of dominant male (young/old), and
632    relatedness (whether the subordinate male was related/unrelated to the dominant male). Output
633    is from Generalized linear mixed models with Binomial distributions. '*' denotes significance
634    at the $p < 0.05$ level, '**' denotes significance at the $p < 0.01$ level. Non-significant higher
635    order terms ($p > 0.1$) were removed from the final model.

| Factor | Parameter estimate | SE | $\chi^2$ | DF | P |
|---|---|---|---|---|---|
| **Proportion of interrupted copulation attempts** | | | | | |
| Age (categorical) | 0.68 | 0.21 | 7.09 | 1 | 0.0077** |
| Relatedness | 0.68 | 0.25 | 6.94 | 1 | 0.0084** |
| Age (categorical) x Relatedness | | | 1.93 | 1 | 0.17 |
| **Random** | | | | | **SD** |
| Group | | | | | 0.23 |
| Dominant male ID | | | | | 0.41 |
| Subordinate male ID | | | | | 0.62 |

636

637 **Table 2. Variation in mating propensity of dominant male domestic fowl.** Number of
638 copulation attempts by dominant males in groups of domestic fowl was not affected by time
639 (observation time for each group in hours), and age of dominant male (young/old) only had a
640 non-significant tendency. Output is from Generalized linear mixed model with Poisson
641 distribution.

| Factor | $\chi^2$ | DF | P |
|---|---|---|---|
| Time | 0.03 | 1 | 0.85 |
| Age | 2.82 | 1 | 0.09 |
| **Random** | | | SD |
| Group | | | 0.57 |
| Dominant male ID | | | 0.002 |

642

643

644

645 **Table 3. Variation in mating propensity of subordinate male domestic fowl.** Number of
646 copulation attempts by subordinate males in groups of domestic fowl was not affected by: time
647 (observation time for each group in hours), age of dominant male (young/old), and relatedness
648 (whether the subordinate male was related/unrelated to the dominant male). Output is from
649 Generalized linear mixed model with Poisson distribution. Non-significant higher order terms
650 (p > 0.1) were removed from the final model.

| Factor | $\chi^2$ | DF | P |
|---|---|---|---|
| Time | 2.35 | 1 | 0.13 |
| Age | 0.84 | 1 | 0.36 |
| Relatedness | 0.37 | 1 | 0.54 |
| Age x Relatedness | 2.01 | 1 | 0.16 |
| **Random** | | | **SD** |
| Group | | | 0.23 |
| Dominant male ID | | | 0.41 |
| Subordinate male ID | | | 0.62 |

651

**Table 4. Variation in mating resistance of female domestic fowl**. Females did not differ in their proportion of resistance (i.e. number of male copulation attempts resisted by the female divided by the total number of male copulation attempts) towards copulation attempts of (A) subordinate males, or (B) dominant males. For model A: relatedness (whether the subordinate male was related/unrelated to the dominant male). For model B: age of dominant male (young/old). Output is from Generalized linear mixed models with Binomial distributions.

| Factor | $\chi^2$ | DF | P |
|---|---|---|---|
| **(A) Female resistance of subordinate male copulation attempts** | | | |
| Relatedness | 0.094 | 1 | 0.76 |
| **Random** | | | **SD** |
| Group | | | <0.001 |
| Subordinate male ID | | | <0.001 |
| **(B) Female resistance of dominant male copulation attempts** | | | |
| Age | 0.011 | 1 | 0.92 |
| **Random** | | | **SD** |
| Group | | | <0.001 |
| Dominant male ID | | | <0.001 |

660 **Supplementary information for Rosher et al. 'Relatedness and age reduce**
661 **aggressive male interactions over mating in male domestic fowl'.**
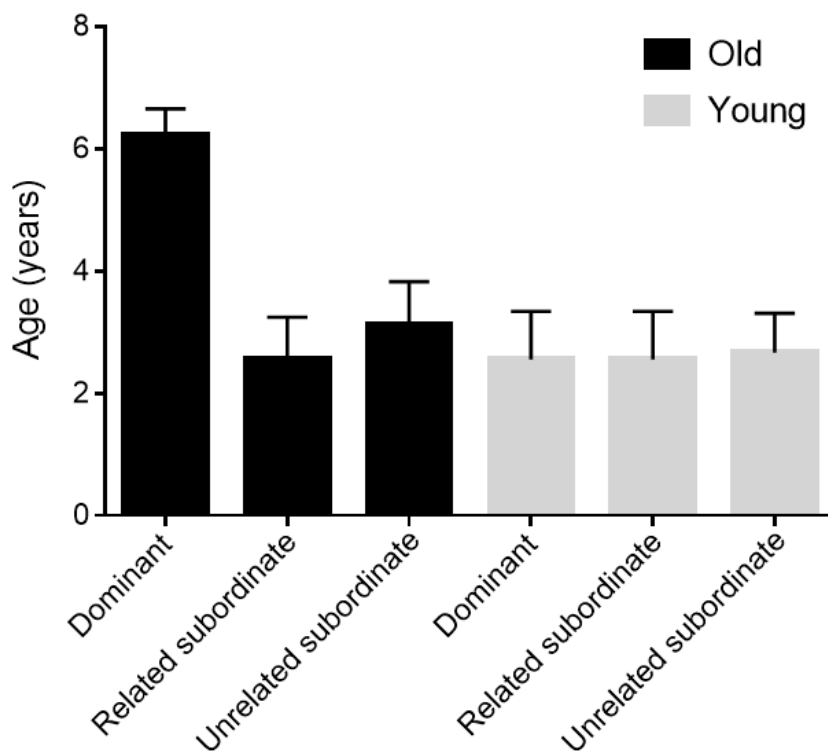
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663 This supplementary information includes:

664 Figure S1. Distribution of ages of male fowl used in the current study.

665 Figure S2. Distribution of subordinate male copulation attempts across groups of male fowl
666 used in the current study.

667

668



669

670 **Figure S1**. Mean ages for each male category for groups with old dominant males (black
671 bars) and groups with young dominant males (grey bars). Old dominant males were older
672 than all other categories of males, while no other categories differed in age (see main
673 manuscript for further details). Error bars represent standard error of the mean.

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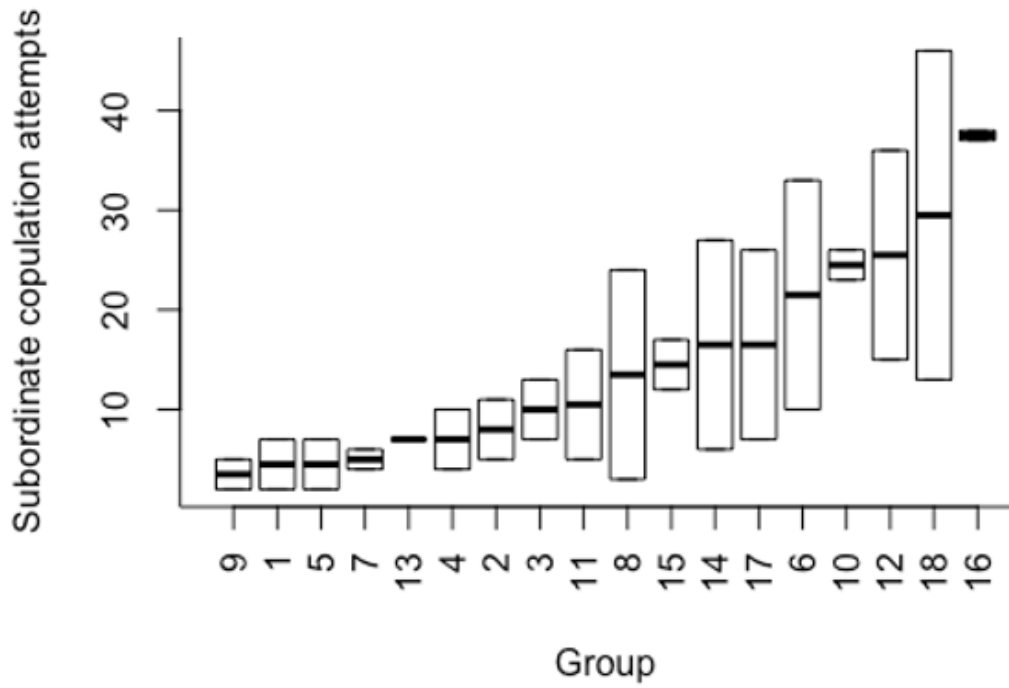
675

676 **Figure S2**. Median number of copulation attempts of subordinate males in individual groups
677 differed substantially among groups (see main manuscript for further details). Boxes
678 represent maximum and minimum number of copulation attempts.