

1 **Citation:**

2 **Wilson DR, Bayly KL, Nelson XJ, Gillings M, Evans CS (2008) Alarm calling best predicts mating and**
3 **reproductive success in ornamented male fowl, *Gallus gallus*. *Animal Behaviour*, 76: 543–554. doi:**
4 **10.1016/j.anbehav.2008.03.026**

5
6
7 Alarm calling best predicts mating and reproductive success in ornamented male fowl, *Gallus gallus*

8
9

10 **WILSON ET AL.: BEHAVIOUR PREDICTS PATERNITY IN FOWL**

11
12

13 DAVID R. WILSON^{*}, KAREN L. BAYLY^{*}, XIMENA J. NELSON^{*}, MICHAEL GILLINGS[†], AND CHRISTOPHER S.
14 EVANS^{*}

15
16

^{*}Centre for the Integrative Study of Animal Behaviour, Macquarie University

17
18

[†]Genes to Geoscience Research Centre, Department of Biological Sciences, Macquarie University

19
20

Correspondence: D. R. Wilson, Centre for the Integrative Study of Animal Behaviour, Macquarie

21 *University, Sydney, NSW 2109, Australia (email: david@galliform.bhs.mq.edu.au). K. L. Bayly, X. J.*

22 *Nelson, & C. S. Evans are at the Centre for the Integrative Study of Animal Behaviour, Macquarie*

23 *University, Sydney, NSW 2109, Australia. M. Gillings is at the Genes to Geoscience Research Centre,*

24 *Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia.*

25
26

Word Count: 5429 words, excluding title page, abstract, references

27

28 Studies of female mate choice in fowl typically invoke ornament size as the best predictor of male
29 reproductive success. The strongest evidence comes from experiments in which a hen is presented with
30 two unfamiliar and physically separated males that she can evaluate and mate with for up to 120
31 minutes. This design controls for prior experience and male-male competition, but deprives females of
32 information available only from longer sampling periods and a more natural context. In the wild, fowl
33 spend their lives in stable social groups. We observed birds under naturalistic conditions to evaluate the
34 biological significance of ornament size and to explore other potential predictors of male mating and
35 reproductive success. For each male, we measured morphology and several behaviours related to food,
36 predators, dominance, and courtship. Using principal components analysis and multiple regression, we
37 show that behaviour is the best predictor of male mating and reproductive success under natural
38 conditions, and that the most salient behaviours are dominance and the rate of antipredator signalling.
39 Dominance probably affects an individual's reproductive success by determining access to receptive
40 females, but the mechanism responsible for the role of alarm calling is less clear. Costly alarm signals
41 may advertise male quality, or they may reflect judicious risk-taking by males that have achieved
42 mating success.

43
44 *Keywords:* alarm call, fowl, *Gallus gallus*, ornament, paternity, referential signal, sexual selection

45 The hallmark of sexual selection is that phenotypic traits predict assortative mating and differential
46 reproductive success (Andersson 1994). Identifying such traits is thus an essential first step in
47 determining whether sexual selection is operating in a given species. Subsequent experiments can then
48 be designed to test for a causal effect on reproductive success.

49

50 Individuals assessing a prospective mate or opponent often consider multiple cues that reflect an
51 underlying quality (e.g., genetic quality, resource provisioning ability, fighting ability; Hagelin 2002;
52 Candolin 2003). If preferred cues are unavailable, or if there is insufficient time to assess them
53 accurately, animals may be forced to use less-reliable secondary cues (Zuk et al. 1992; Sullivan 1994). It
54 is therefore important that experiments designed to identify cues relevant to sexual selection consider
55 the life history of the species in question, and provide assessors with the gamut of cues and the
56 integration time available to them in a more natural context (Sullivan 1990). Species that
57 characteristically encounter rivals or prospective mates only briefly may have to rely on static
58 morphological cues or transient displays that can readily be assessed. In contrast, when encounters
59 with conspecifics are repeated or prolonged, as in species that form stable social groups, individuals can
60 also consider facultative traits that require greater assessment time. A particularly striking example of
61 such a process is provided by the superb fairy wren, *Malurus cyaneus*, in which female choice is
62 dependent upon the date on which males moult into their nuptial plumage months earlier (Mulder &
63 Magrath 1994).

64

65 Characters of particular interest to females have often been identified by presenting them with a
66 simultaneous choice between a pair of unfamiliar and physically separated males (Zuk et al. 1990a).
67 Similarly, observing the outcomes of aggressive interactions between dyads of unfamiliar males is a
68 useful approach for identifying consistent differences between winners and losers of male-male
69 competition (Hagelin 2002). However, female choice and male-male competition can act
70 contemporaneously. Under these conditions, individual reproductive success will likely reflect an

71 interaction between the two mechanisms of sexual selection, as opposed to the independent effect of
72 either one (Qvarnström & Forsgren 1998). The possibility of such interactions requires verification of
73 the importance of traits identified in tests of either female choice or male-male competition in a more
74 natural context, in which both mechanisms can play a role (Moore & Moore 1999).

75

76 Fowl are a classic and ideal system for studies of both female choice and male-male competition
77 (Darwin 1871). Males are covered with long, brilliant, plumage, and their resistance to parasites is
78 reflected in the size and condition of fleshy red ornaments (Zuk et al. 1990b; Parker & Ligon 2003). They
79 engage in a courtship display known as 'waltzing', provision females with food, and protect them from
80 predators (Kruijt 1964; Pizzari 2003). Females mate with multiple males (Ligon & Zwartjes 1995a) and
81 exert cryptic female choice following insemination (Pizzari & Birkhead 2000). During fights for territory
82 and rank, males use sharp spurs as weapons (Andersson 1994), and then assert their dominance by
83 crowing and by a visual display known as 'wingflapping' (Kruijt 1964). Other males often interfere with
84 copulation and intense sperm competition follows insemination (Kratzer & Craig 1980; Froman et al.
85 2002).

86

87 The vocal behaviour of fowl has received less attention from a functional perspective. Males have a
88 large vocal repertoire (Collias 1987), which includes crowing and at least three types of referential
89 signal (Evans 1997). Crowing is energetically inexpensive (Horn et al. 1995) and advertises a male's
90 social status (Leonard & Horn 1995). Dominant males approach the crows of other dominant
91 individuals, while females and subordinate males do not respond to crowing by males of any status
92 (Leonard & Horn 1995). The three referential signals specifically predict the presence of food (Evans &
93 Evans 1999), aerial predators and terrestrial predators (Evans et al. 1993). Companions respond in
94 functionally appropriate ways: searching for food (Evans & Evans 1999), crouching while looking
95 upward as though to detect a hawk, or standing erect while scanning the horizon as though to detect a
96 fox (Evans et al. 1993). Food and aerial alarm calls are not produced reflexively, but rather depend upon

97 the presence of a suitable audience. The necessary characteristics vary: any conspecific is sufficient to
98 induce aerial alarm calling (Karakashian et al. 1988), while only hens provide an adequate audience for
99 food calling (Evans & Evans 1999). Ground alarm calls are produced by both sexes and do not require an
100 audience (Evans 1997).

101
102 We surveyed the literature for direct correlations between the phenotype and mating success of
103 male fowl. In several mate choice experiments devoid of male-male competition (Zuk et al. 1990a, b, c;
104 Zuk et al. 1992; Ligon & Zwartjes 1995a, b; Zuk et al. 1995a; Chappell et al. 1997; Ligon et al. 1998),
105 females were presented with two unfamiliar and separated males for up to two hours. Females
106 consistently preferred the male with the larger ornament (reviewed in Parker & Ligon 2003), suggesting
107 that non-random mating is a function of female preference for parasite-resistant males (Zuk et al.
108 1990b). Several other studies, which did not measure ornamentation, found that a male's dominance is
109 positively related to his mating success, suggesting that male-male competition is also important (Guhl
110 et al. 1945; Guhl & Warren 1946; Kratzer & Craig 1980; Cheng & Burns 1988; Johnsen et al. 2001; Pizzari
111 2001, 2003).

112
113 In the present study, we measured possible correlates of male mating success in fowl living under
114 naturalistic conditions. In addition to dominance and ornamentation, we evaluated the role of
115 courtship behaviour and of referential signals evoked by food and predators. Courtship and food
116 provisioning have been inconsistently associated with male mating success in previous studies (e.g. Zuk
117 et al. 1995b; Pizzari 2003), but these traits are facultative and females may require more time to assess
118 them than is available in conventional choice tests (Sullivan 1990). Antipredator behaviour is positively
119 associated with male dominance (Pizzari 2003), but its relation with male mating success has not
120 hitherto been assessed. Finally, we tested whether the traits identified as predictors of male mating
121 success also predicted male reproductive success, to establish whether these are subject to selection.

122

METHODS

123

124

125 **General Methods**

126

127 Subjects were 64 male and 66 female sexually mature (1-6 year-old) fowl (*Gallus gallus*) derived
128 from flocks of golden Sebrights that had been breeding freely for several generations. This strain has
129 not been artificially selected for rapid growth or egg production. Although morphologically distinct from
130 junglefowl, they possess very similar behavioural and vocal repertoires (Collias 1987; Zuk et al. 1990c).
131 All individuals were assigned at random to one of 22 social groups. These were each composed of three
132 males and three females - a size and age structure consistent with that described for free-ranging fowl
133 (Collias et al. 1966).

134

135 Birds were observed under naturalistic conditions in large outdoor aviaries during the austral
136 breeding seasons (August-March) of 1999/2000 (season 1: three groups, $N_{\text{males}} = 9$), 2000/2001 (season
137 2: four groups, $N_{\text{males}} = 12$), 2005/2006 (season 3: 11 groups, $N_{\text{males}} = 31$; two males used in season 3 had
138 been in groups tested previously and so did not contribute data to the analysis) and 2006/2007 (season
139 4: four groups, $N_{\text{males}} = 12$). A maximum of two groups were tested sequentially in any given aviary in
140 any given season. Birds not involved in testing were housed in an indoor colony (see Evans & Evans
141 1999 for details). All birds were fitted with numbered and coloured leg bands to facilitate individual
142 identification.

143

144 Groups were formed by simultaneously releasing all six birds into one of several large (10 m x 20 m),
145 outdoor aviaries. These each contained a coop fitted with a perch for roosting, *ad libitum* food and
146 water, grass with patches of bare ground for dustbathing, and a gazebo structure affording shelter from
147 the sun. Aviaries were constructed of 1-cm² nylon mesh (A&A Contract Services, Qld, Australia), which
148 provided birds with an unobstructed view of their surroundings. Following their initial release, we

149 monitored all birds for signs of stress (e.g. panting). Overt aggression usually lasted less than one
150 minute, always less than three minutes, and usually terminated when one bird signalled subordinate
151 status by turning away. Following group formation, birds were given at least one week to establish
152 stable social structure, acclimate to the new surroundings and habituate to humans, prior to data
153 collection.

154

155 **Behavioural Observations**

156

157 We used continuous recording of a focal animal (Altmann 1974). In seasons 1 and 2, each male was
158 observed for one 20-minute session per day for 12-25 days (range: 240-500 minutes/male; $X \pm SE = 411$
159 ± 17 minutes, $N_{\text{males}} = 21$). Data collection for individual birds alternated daily between the morning (2-3
160 hours after sunrise) and afternoon (2-3 hours before sunset), and the order of observation of the three
161 males in a group was randomized. The observer (KLB) either sat or stood in the middle of the aviary and
162 scored behaviour using a notebook and a stopwatch. Observation of a group ended for the season
163 when two of the three hens became broody (and hence sexually unreceptive). The operational sex ratio
164 within groups therefore became male-biased during the sampling period, and females reluctant to
165 become broody were represented for a longer duration. Dynamic sex ratios and differential periods of
166 female receptivity are both characteristic of wild populations (Collias et al. 1966), and should not affect
167 the relations between male phenotype and either mating or reproductive success.

168

169 In seasons 3 and 4, we observed each group for one 40-minute session per day over a 12-day period,
170 at approximately the same times each day (0705-1115 hours or 1620-1920 hours AET). During a group's
171 daily session, two of the three males were observed simultaneously by one of two observers (XJN or
172 DRW) assigned to them at random. Each male was observed on eight of the 12 days (selected at
173 random), for a total of 320 minutes. Observers sat on either side of the coop, which was located in the
174 centre of one end of the aviary, and scored behaviour using JWatcher™ software (version 1.0) on a

175 Macintosh laptop computer. At least one hen in each group was laying eggs during the 12-day
176 observation period, but no hen became broody until after data collection for her group was complete.
177

178 During each focal session, we recorded the number of individual crows and the number of bouts
179 (defined by intervening silences not exceeding five seconds) of aerial alarm calls, ground alarm calls and
180 food calls produced by each focal male. Occasionally, males produce food calls in response to inedible
181 objects (Gyger & Marler 1988) and alarm calls in response to innocuous species (Gyger et al. 1987). We
182 could not always identify the item eliciting a food calling bout, or the perceived threat to which a male
183 alarm called, but we commonly observed a genuine food item or threat in the vicinity of a vocalizing
184 male. In addition to vocalizations, we also scored bouts of courtship waltzing and wingflapping (Kruijt
185 1964). As waltzing can also be an aggressive display towards other males (Kruijt 1964), we considered
186 only those bouts in which a female was within one metre of the focal male and no rival males were
187 within this radius. We scored male-male interactions involving a focal animal as a 'win' if the focal male
188 displaced the other male and a 'loss' if he was displaced by him. Our criteria for displacements required
189 that the two males were within one metre of each other, and that movement of one (defined by taking
190 at least one step away) occurred within one second of movement by the other. All males within a group
191 interacted at least once. Finally, we estimated each male's reproductive success by scoring all
192 copulations, defined as the male grasping the back of a female's neck with his mandibles and mounting
193 her with both feet.

194
195 Following data collection, we converted each male's total number of crows, ground alarm calls,
196 aerial alarm calls, food calls, courtship waltzes, wingflaps, and copulations observed during all
197 observation sessions into average rates/hour to facilitate comparisons across periods of unequal
198 duration. As social status may affect a male's behaviour and mating success (Collias et al. 1966), we
199 calculated a dominance score for each male using Kalinoski's (1975) Frequency Success Index (FSI),
200 which is the most appropriate measure for this system (Bayly et al. 2006). FSI is calculated by

201 subtracting an individual's losses from its wins, and dividing the difference by the total number of
202 interactions in the group. The result for each male ranges between -1 (most subordinate) and +1
203 (most dominant).

204

205 **Morphological Measurements**

206

207 Immediately following each group's observation period, we captured each male, measured his body
208 weight (accuracy: ± 10 g) using a Pesola™ spring scale and a cloth bag and took a digital photograph in
209 right side profile (Canon EOS 300 digital camera; 6.5 megapixels resolution). For consistency with
210 previous mate-choice studies, we measured the maximum length of the comb. We then measured the
211 size of all head and facial ornaments from the digital images (Fig. 1a) using NIH ImageJ software
212 (versions 1.62 and 1.33u), calibrated on a scale that had been placed beside each male's head.
213 Specifically, we measured the total red surface area of the comb, wattle, ear lappet, and red facial skin
214 (accuracy: ± 1 mm²), thereby estimating the size of the ornament in two of its three dimensions.

215

216 Comb and feather colour have also been shown to affect mate choice, although previous
217 measurements of colour (Zuk et al. 1990b, c) have used subjective techniques such as the Munsell
218 system, which has been criticized for its reliance upon a perceptual model of human vision (Bennett et
219 al. 1994). During season 3 ($N_{\text{males}} = 31$), we used a USB2000 Miniature Fiber Optic Spectrometer (Ocean
220 Optics, Inc., Dunedin, FL, USA) to measure the reflectance spectra of each male's comb and hackle
221 feathers at four randomly selected locations on each structure (Fig. 1). Reflectance was measured
222 relative to a white WS-1 diffuse reflectance standard (reflectivity: > 99%; wavelength range: 200-1100
223 nm) using a two-fibre probe. Illumination was provided by a MINI-D2T miniature deuterium tungsten
224 light source (Ocean Optics, Inc; peak-to-peak stability: 0.3% from 200-850 nm). Measurements were
225 taken using OOIBase32 spectrometer operating software at 0.37-nm increments between 350 and 700
226 nm, which corresponds to the complete spectral sensitivity of the fowl visual system (Prescott &

227 Wathes 1999). Finally, we calculated a median reflectance spectrum from each male's four
228 measurements for the comb and for the hackle feathers. We then standardized the reflectance spectra,
229 such that for each structure the highest median reflectance value received a score of one and the
230 lowest a score of zero.

231

232 **Paternity Analysis**

233

234 We conducted a paternity analysis in season 3 on a subset of seven groups (21 males and 21
235 females). A total of 97 eggs laid during the 12-day observation periods were collected and incubated at
236 38.3°C and 85% relative humidity. Embryonic development was stopped by chilling at 72 hours and all
237 tissue was dissected and placed into 70% ethanol. At the end of each observation period, we used a 21-
238 gauge needle to draw approximately 1 ml of blood from the brachial vein of every adult in the social
239 group. All samples were stored at -20°C.

240

241 DNA was extracted from 42 adults and 71 embryos (26 eggs were not fertilized) using a proteinase
242 K/salting out method (Sunnucks & Hales 1996). For all samples, microsatellite loci were amplified using
243 approximately 50 ng of genomic DNA in 50- μ l reactions using the procedures outlined in Curley &
244 Gillings (2004). Polymerase chain reactions (PCRs) contained 2 mM MgCl₂, 200 μ M of each dNTP, 20
245 μ g/ml RNaseA, 0.5 μ M of each primer, and 0.15 units of Red Hot DNA polymerase (Advanced
246 Biotechnologies) in the buffer supplied with the enzyme. Amplifications were made with a Hybaid
247 Omne cycler and PCR conditions were 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 s,
248 48-62°C for 30 s (see Appendix A for details), and 72°C for 90 s, with a final extension at 72°C for 5
249 minutes. An aliquot of each PCR was electrophoresed on 2% agarose and stained with ethidium
250 bromide to confirm amplification. PCRs were then diluted 1:10 in sterile water and analyzed on a
251 3130xl Genetic Analyzer (Applied Biosystems). Primer sets with nonoverlapping allele sizes and
252 different fluorochrome labels were pooled for analysis to minimize time and costs. Allele sizes were

253 measured using GeneMapper (version 4.0) software (Applied Biosystems) relative to internal LIZ™-
254 labeled GeneMapper 500 size standards from Applied Biosystems.

255

256 Genotype matching was done manually using a first-principles approach because each group was a
257 closed system in which the genotypes of all offspring and potential parents were known. We
258 constructed a 3 x 3 matrix for each embryo, in which the columns and rows represented the genotypes
259 of potential fathers and potential mothers, respectively. At each locus, the embryo's genotype was
260 examined and all parental combinations that violated the Mendelian assumption that each parent had
261 donated one randomly-selected allele to the embryo were eliminated from the matrix. Remaining cells
262 in the matrix were assigned an equal probability, such that the sum of all remaining cells was one. Each
263 adult's probability of parentage was equal to the sum of his or her respective row or column. An
264 identified parent thus obtained a score of one and an excluded parent a score of zero. This procedure
265 was repeated for every embryo in the group. Each male's probability of paternity was summed across
266 all embryos within his group; this total provided an individual estimate of a male's cumulative
267 reproductive success. Note that this score does not reflect inter-female variation in reproductive
268 success (see Appendix B for details of both inter-male and inter-female variation), and hence should be
269 used cautiously when considering the precise mechanisms of sexual selection that might underlie the
270 observed variation in male reproductive success. Nevertheless, cumulative reproductive success is the
271 most appropriate measure for our purpose because selection for traits that affect male reproductive
272 success, whether it acts through male-male competition or female choice, will be dependent on a
273 male's overall fitness, as opposed to the number of females that contribute to it.

274

275 **Statistical Analysis**

276

277 We examined 10 potential predictors of male mating frequency ($N_{\text{males}} = 64$) and reproductive
278 success ($N_{\text{males}} = 21$), including dominance (FSI), body weight, ornamentation (total red area of the

279 ornaments and comb length), and the rates of crowing, ground alarm calling, aerial alarm calling, food
280 calling, courtship waltzing, and wingflapping (see Table 1 for descriptive statistics). Each male
281 contributed only one observation to each dataset to preserve independence (Machlis et al. 1985).
282 Because independent variables were numerous and inter-correlated (see Table 2 for correlation
283 matrix), we first performed a principal components analysis with varimax rotation to reduce the 10
284 independent variables in each dataset to three orthogonal factors (Table 3), thereby simplifying the
285 datasets for exploration. We used multiple regression analysis to assess the statistical significance of
286 each factor as a predictor of male mating and reproductive success.

287

288 Using the original data, we also tested the statistical significance ($\alpha = 0.05$) of the 10 independent
289 variables using a multiple regression model and a forward stepwise selection procedure ($P \leq 0.05$ to
290 add, $P \geq 0.10$ to remove). Residuals derived from a preliminary version of the model predicting mating
291 success were not normally distributed, so we used a \log_{10} -transformation (one-sample
292 Kolmogorov-Smirnov test of normality: $P > 0.05$ following transformation; Chatterjee et al. 2000). We
293 also assessed the fit of each predictor variable by independently regressing it against each dependent
294 variable and examining the residuals. Weight, when regressed against mating success, violated the
295 assumption of normality, but was improved by a \log_{10} -transformation (one-sample
296 Kolmogorov-Smirnov test of normality: $P > 0.05$ following transformation). Transformed variables were
297 used in all analyses, including the principal components analysis.

298

299 We tested predictor variables for possible multicollinearity by examining variance inflation factors
300 (VIF; Chatterjee et al. 2000). VIFs greater than ten indicate potential problems associated with
301 multicollinearity (Chatterjee et al. 2000); our greatest VIF was 2.01 in the model predicting mating
302 success (Table 4) and 5.30 in the model predicting reproductive success (Table 5). Our final models
303 complied with all of the assumptions of linear regression (Chatterjee et al. 2000).

304

305 The reflectance properties of combs and feathers have not previously been tested for their effects
306 on female mate choice in fowl. The potentially salient region(s) of the colour spectrum therefore
307 remain unknown. For each character (comb and hackle feathers), we compared the reflectance spectra
308 of the 10 males with the highest copulation rates to those of the 10 males with the lowest copulation
309 rates in season 3. Interquartile ranges were plotted for each group of males and areas along the
310 spectrum where these failed to overlap were considered to be statistically different from each other. A
311 separate analysis of colour relative to paternity was unnecessary, because the assignment of males to
312 groups was identical to that based upon mating frequency.

313

314

RESULTS

315

316 The factor analysis reduced the original 10 variables in each dataset to three orthogonal factors, which
317 together accounted for 69.8% (mating success dataset, $N = 64$) and 79.2% (reproductive success
318 dataset, $N = 21$) of the original variation. Factor loading scores presented in Table 3 show that, for both
319 datasets, factor one best explained dominance, behaviours related to dominance (crows, wingflaps),
320 and referential signalling (ground alarm calls, aerial alarm calls, food calls). Factor two best explained
321 the traits related to sexual advertisement, including courtship waltzing, total red area of the ornaments,
322 and comb length. Factor three best explained body weight. Only factors one and three explained a
323 significant amount of the variation in mating (multiple regression analyses: $F_{3,60} = 19.93$, $P < 0.01$,
324 $R^2_{\text{adjusted}} = 0.47$; factor 1: $t = 7.42$, $P < 0.01$; factor 2: $t = 0.10$, $P = 0.92$; factor 3: $t = -2.19$, $P = 0.03$) and
325 reproductive success ($F_{3,17} = 6.29$, $P < 0.01$, $R^2_{\text{adjusted}} = 0.44$; factor 1: $t = 3.21$, $P < 0.01$; factor 2: $t = -1.94$,
326 $P = 0.07$; factor 3: $t = -2.20$, $P = 0.04$) (Fig. 2).

327

328 The multiple linear regression model and forward stepwise selection procedure accounted for a
329 significant amount of the variation observed in both mating frequency ($F_{3,60} = 24.93$, $P < 0.01$,
330 $R^2_{\text{adjusted}} = 0.53$) and reproductive success ($F_{2,18} = 7.83$, $P < 0.01$, $R^2_{\text{adjusted}} = 0.41$). Mating success was

331 predicted by aerial alarm calls, dominance, and (inversely) by body weight (Table 4), while reproductive
332 success was predicted by ground alarm calls and (inversely) by the total red area of the ornaments
333 (Table 5). Although several of the included variables were intercorrelated (Table 2), their high partial
334 correlation coefficients (Tables 4 & 5) show that they account for considerable variation in mating and
335 reproductive success, even after controlling for the effects of the other predictors. With the exception
336 of aerial alarm calls (test for equality of slopes (Zar 1996): $P < 0.05$), regressions of mating and
337 reproductive success on any given trait were remarkably concordant (Fig. 2) and statistically
338 indistinguishable (all $P > 0.1$), suggesting that mating and reproductive success related similarly to the
339 predictor variables. Mating frequency did not, however, predict reproductive success directly (simple
340 linear regression analysis: $t_{19} = 0.721$, $P = 0.48$).

341
342 Finally, we could detect no differences in the colour of either combs or hackle feathers between
343 males that were highly successful in mating and those that were not (Figs 1b & c). For each structure,
344 the inter-quartile bands of the two groups overlapped considerably across the entire spectrum to which
345 fowl are sensitive (350-700 nm), despite significant differences in their rates of copulation (lowest-
346 mating males, $X \pm SE$: 0.02 ± 0.01 copulations/hour; highest-mating males: 0.38 ± 0.04
347 copulations/hour; unpaired t -test: $t_{18} = 9.47$, $P < 0.01$). These reflectance characteristics hence provide
348 no sensory basis for either female mate choice or opponent assessment.

349

350

DISCUSSION

351

352 Mating and reproductive success were directly related to dominance, as would be expected given the
353 pervasive importance of social status in this system (Schjelderup-Ebbe 1935). Surprisingly, production
354 of referential signals was also important. The best predictor of both mating and reproductive success
355 was the rate at which males produced antipredator alarm calls. This is the first such demonstration in
356 any species. The relations between alarm calling and mating / reproductive success persisted even after

357 controlling for the effects of dominance. They were also quite specific to potentially costly alarm signals
358 (Marler 1955; Alatalo & Helle 1990; Wood et al. 2000). Food calling, crowing, and wingflapping
359 predicted mating and reproductive success, but only to the extent that these attributes were associated
360 with social status (see also Pizzari 2003). Overall, behavioural aspects of male phenotype accounted for
361 almost half of the total observed variation in mating and reproductive success. Well-documented
362 post-copulatory mechanisms (Pizzari & Birkhead 2000) likely account for much of the remainder.

363

364 In marked contrast to previous experimental mate choice studies (reviewed in Parker & Ligon 2003),
365 we found no significant relation between mating frequency and male ornament size, while the relation
366 between ornament size and reproductive success was negative. In these previous tests, females had
367 been given between 30 and 120 minutes to observe and mate with either of two males. Males could
368 not physically interact with each other, so females had no information about relative dominance (Zuk et
369 al. 1990a). Females were also initially unfamiliar with the males. They hence had insufficient time to
370 assess traits that require integration over hours or days, such as individual differences in rates of
371 facultative signalling (Sullivan 1990). Female preference for male ornamentation might therefore be a
372 secondary assessment strategy used by females when primary cues, such as dominance and signalling
373 behaviour (Sullivan 1990; Zuk et al. 1992), are unavailable. In nature, females sometimes encounter
374 unfamiliar males from other groups (Collias et al. 1966). During such transient encounters they may rely
375 on the size and condition of a male's ornamentation, which provides an instantaneous 'snapshot' of his
376 underlying quality, resistance to parasites, and prospect as an extragroup mate (Sullivan 1990; Zuk et al.
377 1990b).

378

379 Male morphology may have been unimportant in our study because female preferences for it were
380 obscured by the effects of male-male competition (e.g. Petersson et al. 1999), a factor excluded in
381 experimental choice tests. Alternatively, our inability to detect a role for ornaments in particular may
382 have been due to morphological differences between our birds (Fig. 1a) and red junglefowl, which have

383 a more pronounced 'row' comb. Note, however, that variation in comb length among the males used in
384 our social groups (coefficient of variation: 14.0%) was substantially greater than that in previous studies
385 that have revealed a role for ornament size (coefficient of variation among 48 males in 1987: 9.2%; Zuk
386 et al. 1990c). Hens hence failed to express a preference, even though they had ample perceptual
387 information with which to do so. Further experimentation will be necessary to better understand the
388 relative importance of cue availability, assessment time, conflict between intersexual and intrasexual
389 selection, and strain morphology in this system.

390

391 Mating frequency failed to predict reproductive success, a finding which probably reflects
392 well-documented post-copulatory mechanisms (see also Bilcik & Estevez 2005). Fowl are highly
393 promiscuous and exhibit cryptic female choice, female sperm storage, differential sperm allocation, and
394 sperm competition (Brillard 1993; Ligon & Zwartjes 1995a; Pizzari & Birkhead 2000; Froman et al. 2002;
395 Pizzari et al. 2003). All of these mechanisms can act to de-couple male mating from reproductive
396 success, which might explain why ornamentation was unrelated to mating frequency (Table 4), but
397 inversely related to reproductive success (Table 5) in the present study. Using natural social groups,
398 Bilcik & Estevez (2005) showed that a male's comb size did not predict how often females would solicit
399 matings from him, but was positively related to his probability of mating forcibly (Bilcik & Estevez
400 2005). It is thus possible that females may respond to forced copulations by large-combed males by
401 ejecting their sperm hence reducing their paternity.

402

403 The evolution of alarm calling is a classic problem in behavioural biology. Signallers risk predation by
404 warning conspecifics of impending danger (Alatalo & Helle 1990), yet obtain no obvious benefits in
405 return. Kin selection (Maynard Smith 1965) has often been invoked as a potential explanation. In
406 Belding's ground squirrels (*Spermophilus beldingi*), for example, females are significantly more likely to
407 alarm call if their offspring are within view (Sherman 1977). Similar phenomena have been described in
408 round-tailed ground squirrels (*S. tereticaudus*), Sonoma chipmunks (*Eutamias sonomae*), black-tailed

409 and Gunnison's prairie dogs (*Cynomys ludovicianus*; *C. gunnisoni*), yellow-bellied marmots (*Marmota*
410 *flaviventris*), and Siberian jays (*Perisoreus infaustus*), suggesting that female alarm calling functions to
411 warn descendent, and potentially non-descendent, kin (Dunford 1977; Smith 1978; Hoogland 1983;
412 Hoogland 1996; Blumstein et al. 1997; Griesser & Ekman 2004).

413

414 Males of these species also produce alarm calls, but the reason for this is less clear (Dunford 1977;
415 Sherman 1977; Smith 1978; Hoogland 1983; Hoogland 1996; Blumstein et al. 1997; Griesser & Ekman
416 2004). Males are not philopatric, so warning non-descendent kin is unlikely. In addition, multiple mating
417 by females diminishes a male's certainty of paternity and, consequently, the direct benefits he might
418 obtain from warning her young (Hare et al. 2004). Males could selectively warn offspring, but this
419 would require either that they recognize their own young using a phenotypic marker, or that they
420 remain resident in their offspring's natal territory so that a spatiotemporal rule has the same functional
421 effect. As previous studies (Dunford 1977; Sherman 1977; Smith 1978; Hoogland 1983; Hoogland 1996;
422 Blumstein et al. 1997; Griesser & Ekman 2004) have not established paternity, it remains unknown
423 whether the presence of descendent kin affects male alarm calling effort. More generally, a link
424 between male mating success and alarm calling effort has not previously been reported for any species.

425

426 The strong predictive relation between male alarm calling and reproductive success apparent in our
427 study offers a new insight into the evolution of this signal. Male alarm calling provides females with
428 protection from predators (Kruijt 1964). In addition, the risk associated with alarm calling (Marler 1955;
429 Alatalo & Helle 1990; Wood et al. 2000) may advertise the male's ability to shun predators, since only
430 individuals best able to evade attack should be able to increase their conspicuousness with impunity.
431 Alarm calling in fowl is testosterone-dependent (Gyger et al. 1988), and high levels of testosterone are
432 known to impose significant physiological costs by compromising immune function (Zuk et al. 1995a),
433 so high rates of alarm calling may also reflect superior health and resistance to parasites. Our findings

434 are thus consistent with the idea that male alarm calling is a sexually-selected trait that has evolved via
435 female choice (Zahavi 1975; Hamilton & Zuk 1982).

436
437 It is also possible that alarm calling reflects judicious investment in mates and prospective offspring
438 by males that have achieved recent mating success. Male dunnocks (*Prunella modularis*), for example,
439 adjust their chick-feeding effort according to the proportion of matings obtained (Davies et al. 1992).
440 Similarly, male willow tits (*Parus montanus*) increase their rate of alarm calling when their mate is
441 within sight (Hogstad 1995). In many avian species, mating is associated with elevated testosterone
442 titre (Moore 1982). This provides a possible androgen mechanism by which the production of alarm
443 calls and mating / reproductive success might be linked. The male investment and female choice
444 models outlined here are not, of course, mutually exclusive. Further experiments are needed to
445 elucidate the causal relationship(s) between alarm calling and mating / reproductive success in fowl.

446
447 In conclusion, fowl are one of the best studied examples of sexual selection, yet the context in which
448 this work has been done has often been artificial (Sullivan 1990). Under naturalistic social conditions,
449 and with a more extended sampling period, male reproductive success was not positively related to
450 ornamentation. Instead, our results show that referential signalling and dominance both predict male
451 mating and reproductive success, and that the best predictor among those examined is a male's rate of
452 antipredator alarm signalling.

453

Acknowledgments

454
455
456 This research adhered to the Animal Behavior Society's *Guidelines for the Use of Animals in Research*
457 and was conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals*
458 *for Scientific Purposes* (NHMRC, 1997). All procedures were approved under Macquarie University AEC
459 protocols 99002 and 2006/025. We thank N. Lambert, W. McTegg, and R. Miller for bird care, P. Taylor
460 and A. Taylor for assistance with the statistical analysis, M. Holley and P. Worden for assistance with
461 the paternity analysis, and A. Cockburn, J. Hare, and several anonymous reviewers for comments on
462 previous drafts of the manuscript. This research was supported by a Sigma Xi Grant-in-Aid-of-Research
463 and Newton Society Sponsorship to KLB and by an Australian Research Council Discovery-Project Grant
464 to CSE.

References

- 466
467
468 **Alatalo, R. V. & Helle, P.** 1990. Alarm calling by individual willow tits, *Parus montanus*. *Animal*
469 *Behaviour*, **40**, 437-442. doi: 10.1016/S0003-3472(05)80523-8.
- 470 **Altmann, J.** 1974. Observational study of behavior: sampling methods. *Behaviour*, **49**, 227-267.
- 471 **Andersson, M.** 1994. *Sexual selection*. New Jersey: Princeton University Press.
- 472 **Bayly, K. L., Evans, C. S. & Taylor, A.** 2006. Measuring social structure: a comparison of eight
473 dominance indices. *Behavioural Processes*, **73**, 1-12. doi: 10.1016/j.beproc.2006.01.011.
- 474 **Bennett, A. T. D., Cuthill, I. C. & Norris, K. J.** 1994. Sexual selection and the mismeasure of color.
475 *American Naturalist*, **144**, 848-860. doi: 10.1086/285711.
- 476 **Bilcik, B. & Estevez, I.** 2005. Impact of male-male competition and morphological traits on mating
477 strategies and reproductive success in broiler breeders. *Applied Animal Behaviour Science*, **92**
478 307-323.

- 479 **Blumstein, D. T., Steinmetz, J., Armitage, K. B. & Daniel, J. C.** 1997. Alarm calling in yellow-bellied
480 marmots: II. The importance of direct fitness. *Animal Behaviour*, **53**, 173-184. doi:
481 10.1006/anbe.1996.0286.
- 482 **Brillard, J. P.** 1993. Sperm storage and transport following natural mating and artificial insemination.
483 *Poultry Science*, **72**, 923-928.
- 484 **Candolin, U.** 2003. The use of multiple cues in mate choice. *Biological Reviews*, **78**, 575-595.
- 485 **Chappell, M. A., Zuk, M., Johnsen, T. S. & Kwan, T. H.** 1997. Mate choice and aerobic capacity in red
486 junglefowl. *Behaviour*, **134**, 511-529.
- 487 **Chatterjee, S., Hadi, A. S. & Price, B.** 2000. *Regression analysis by example*. 3rd edn. New York: John
488 Wiley & Sons, Inc.
- 489 **Cheng, K. M. & Burns, J. T.** 1988. Dominance relationship and mating behavior of domestic cocks-a
490 model to study mate-guarding and sperm competition in birds. *Condor*, **90**, 697-704. doi:
491 10.2307/1368360.
- 492 **Collias, N. E.** 1987. The vocal repertoire of the red junglefowl: a spectrographic classification and the
493 code of communication. *Condor*, **89**, 510-524. doi: 10.2307/1368641.
- 494 **Collias, N. E., Collias, E. C., Hunsaker, D. & Minning, L.** 1966. Locality fixation, mobility and social
495 organization within an unconfined population of red jungle fowl. *Animal Behaviour*, **14**, 550-559.
496 doi: 10.1016/S0003-3472(66)80059-3.
- 497 **Curley, B. G. & Gillings, M. R.** 2004. Isolation of highly polymorphic microsatellite loci from the
498 temperate damselfish *Parma microlepis*. *Molecular Ecology Notes*, **4**, 551-553. doi:
499 10.1111/j.1471-8286.2004.00729.x.
- 500 **Darwin, C. R.** 1871. *The Descent of Man, and Selection in Relation to Sex*. Princeton: Princeton
501 University Press.
- 502 **Davies, N. B., Hatchwell, B. J., Robson, T. & Burke, T.** 1992. Paternity and parental effort in dunnocks
503 *Prunella modularis*: how good are male chick-feeding rules? *Animal Behaviour*, **43**, 729-745. doi:
504 10.1016/S0003-3472(05)80197-6.

505 **Dunford, C.** 1977. Kin selection for ground squirrel alarm calls. *American Naturalist*, **111**, 782-785. doi:
506 10.1086/283206.

507 **Evans, C. S.** 1997. Referential signals. *Perspectives in Ethology*, **12**, 99-143.

508 **Evans, C. S. & Evans, L.** 1999. Chicken food calls are functionally referential. *Animal Behaviour*, **58**, 307-
509 319. doi: 10.1006/anbe.1999.1143.

510 **Evans, C. S., Evans, L. & Marler, P.** 1993. On the meaning of alarm calls: functional reference in an avian
511 vocal system. *Animal Behaviour*, **46**, 23-38. doi: 10.1006/anbe.1993.1158.

512 **Froman, D. P., Pizzari, T., Feltmann, A. J., Castillo-Juarez, H. & Birkhead, T. R.** 2002. Sperm mobility:
513 mechanisms of fertilizing efficiency, genetic variation and phenotypic relationship with male status
514 in the domestic fowl, *Gallus gallus domesticus*. *Proceedings of the Royal Society of London, Series B*,
515 **269**, 607-612. doi: 10.1098/rspb.2001.1925.

516 **Griesser, M. & Ekman, J.** 2004. Nepotistic alarm calling in the Siberian jay, *Perisoreus infaustus*. *Animal*
517 *Behaviour*, **67**, 933-939. doi: 10.1016/j.anbehav.2003.09.005.

518 **Guhl, A. M. & Warren, D. C.** 1946. Number of offspring sired by cockerels related to social dominance
519 in chickens. *Poultry Science*, **25**, 460-472.

520 **Guhl A. M., Collias, N. E. & Allee, W. C.** 1945. Mating behavior and the social hierarchy in small flocks of
521 white leghorns. *Physiological Zoology*, **18**, 365-390.

522 **Gyger, M., Karakashian, S. J., Dufty, A. M. Jr. & Marler, P.** 1988. Alarm signals in birds: the role of
523 testosterone. *Hormones and Behaviour*, **22**, 305-314.

524 **Gyger, M. & Marler, P.** 1988. Food calling in the domestic fowl, *Gallus gallus*: The role of external
525 referents and deception. *Animal Behaviour*, **36**, 358-365.

526 **Gyger, M., Marler, P. & Pickert, R.** 1987. Semantics of an avian alarm call system: The male domestic
527 fowl, *Gallus domesticus*. *Behaviour*, **102**, 15-40.

528 **Hagelin, J. C.** 2002. The kinds of traits involved in male-male competition: a comparison of plumage,
529 behavior, and body size in quail. *Behavioral Ecology*, **13**, 32-41. doi: 10.1006/anbe.2000.1618.

- 530 **Hare, J. F., Todd, G. & Untereiner, W. A.** 2004. Multiple mating results in multiple paternity in
531 Richardson's ground squirrels (*Spermophilus richardsonii*). *Canadian Field-Naturalist*, **118**, 90-94.
- 532 **Hamilton, W. D. & Zuk, M.** 1982. Heritable true fitness and bright birds: a role for parasites? *Science*,
533 **218**, 384-387. doi: 10.1126/science.7123238.
- 534 **Hogstad, O.** 1995. Alarm calling by willow tits, *Parus montanus*, as mate investment. *Animal Behaviour*,
535 **49**, 221-225. doi: 10.1016/0003-3472(95)80170-7.
- 536 **Hoogland, J. L.** 1983. Nepotism and alarm calling in the black-tailed prairie dog (*Cynomys ludovicianus*).
537 *Animal Behaviour*, **31**, 472-479. doi: 10.1016/S0003-3472(83)80068-2.
- 538 **Hoogland, J. L.** 1996. Why do Gunnison's prairie dogs give anti-predator calls?
539 *Animal Behaviour*, **51**, 871-880. doi: 10.1006/anbe.1996.0091.
- 540 **Horn, A. G. & Leonard, M. L. & Weary, D. M.** 1995. Oxygen consumption during crowing by roosters:
541 talk is cheap. *Animal Behaviour*, **50**, 1171-1175.
- 542 **Johnsen, T. S., Zuk, M. & Fessler, E. A.** 2001. Social dominance, male behaviour and mating in mixed-
543 sex flocks of red jungle fowl. *Behaviour*, **138**, 1-18. doi: 10.1163/156853901750077754.
- 544 **Kalinoski, R.** 1975. Intra- and interspecific aggression in house finches and house sparrows. *Condor*, **77**,
545 375-384. doi: 10.2307/1366086.
- 546 **Karakashian, S. J., Gyger, M. & Marler, P.** 1988. Audience effect on alarm calling in chickens (*Gallus*
547 *gallus*). *Journal of Comparative Psychology*, **102**, 129-135. doi: 10.1037/0735-7036.102.2.129.
- 548 **Kratzer, D. D. & Craig, J. V.** 1980. Mating behavior of cockerels: effects of social status, group size and
549 group density. *Applied Animal Ethology*, **6**, 49-62. doi: 10.1016/0304-3762(80)90093-0.
- 550 **Kruijt, J. P.** 1964. Ontogeny of social behaviour in Burmese red junglefowl, (*Gallus gallus spadiceus*).
551 *Behaviour*, **Supplement 12**, 1-201.
- 552 **Leonard, M. L. & Horn, A. G.** 1995. Crowing in relation to social status in roosters. *Animal Behaviour*,
553 **49**, 1283-1290.
- 554 **Ligon, J. D. & Zwartjes, P. W.** 1995a. Female red junglefowl choose to mate with multiple males. *Animal*
555 *Behaviour*, **49**, 127-135. doi: 10.1016/0003-3472(95)80160-X.

556 **Ligon, J. D. & Zwartjes, P. W.** 1995b. Ornate plumage of male red junglefowl does not influence mate
557 choice by females. *Animal Behaviour*, **49**,117-125. doi: 10.1016/0003-3472(95)80159-6.

558 **Ligon, J. D., Kimball, R. & Merola-Zwartjes, M.** 1998. Mate choice by female red junglefowl: the issues
559 of multiple ornaments and fluctuating asymmetry. *Animal Behaviour*, **55**, 41-50. doi:
560 10.1006/anbe.1997.0582.

561 **Machlis, L., Dodd, P. W. D. & Fentress, J. C.** 1985. The pooling fallacy: problems arising when
562 individuals contribute more than one observation to the data set. *Zeitschrift für Tierpsychologie*, **68**,
563 201-214.

564 **Marler, P.** 1955. Characteristics of some animal calls. *Nature*, **176**, 6-8. doi: 10.1038/176006a0.

565 **Maynard Smith, J.** 1965. The evolution of alarm calls. *American Naturalist*, **99**, 59-63.

566 **Moore, M. C.** 1982. Hormonal response of free-living male white-crowned sparrows to experimental
567 manipulation of female sexual behavior. *Hormones and Behaviour*, **16**, 323-329.

568 **Moore, A. J. & Moore, P. J.** 1999. Balancing sexual selection through opposing mate choice and male
569 competition. *Proceedings of the Royal Society of London, Series B*, **266**, 711-716. doi:
570 10.1098/rspb.1999.0694.

571 **Mulder, R. A. & Magrath, M. J. L.** 1994. Timing of prenuptial molt as a sexually selected indicator of
572 male quality in superb fairy-wrens (*Malurus cyaneus*). *Behavioral Ecology*, **5**, 393-400. doi:
573 10.1093/beheco/5.4.393.

574 **Parker, T. H. & Ligon, J. D.** 2003. Female mating preferences in red junglefowl: a meta-analysis.
575 *Ethology Ecology & Evolution*, **15**, 63-72.

576 **Petersson, E., Järvi, T., Olsén, H., Mayer, I. & Hedenskog, M.** 1999. Male-male competition and female
577 choice in brown trout. *Animal Behaviour*, **57**, 777-783.

578 **Pizzari, T.** 2001. Indirect partner choice through manipulation of male behaviour by female fowl, *Gallus*
579 *gallus domesticus*. *Proceedings of the Royal Society of London, Series B*, **268**, 181-186. doi:
580 10.1098/rspb.2000.1348.

581 **Pizzari, T.** 2003. Food, vigilance, and sperm: the role of male direct benefits in the evolution of female
582 preference in a polygamous bird. *Behavioral Ecology*, **14**, 593-601. doi: 10.1093/beheco/arg048.

583 **Pizzari, T. & Birkhead, T. R.** 2000. Female feral fowl eject sperm of subdominant males. *Nature*, **405**,
584 787-789. doi: 10.1038/35015558.

585 **Pizzari, T., Cornwallis, C. K., Løvlie, H., Jakobsson, S. & Birkhead, T. R.** 2003. Sophisticated sperm
586 allocation in male fowl. *Nature*, **426**, 70-74. doi: 10.1038/nature02004.

587 **Prescott, N. B. & Wathes, C. M.** 1999. Spectral sensitivity of the domestic fowl (*Gallus g. domesticus*).
588 *British Poultry Science*, **40**, 332-339.

589 **Qvarnström, A. & Forsgren, E.** 1998. Should females prefer dominant males? *Trends in Ecology &*
590 *Evolution*, **13**, 498-501. doi: 10.1016/S0169-5347(98)01513-4.

591 **Sherman, P. W.** 1977. Nepotism and the evolution of alarm calls. *Science*, **197**, 1246-1253. doi:
592 10.1126/science.197.4310.1246.

593 **Schjelderup-Ebbe, T.** 1935. Social behaviour of birds. In: A Handbook of Social Psychology (Ed. by C.
594 Murchison), pp. 947-972. New York: Russell and Russell.

595 **Smith, S. F.** 1978. Alarm calls, their origin and use in *Eutamias sonomae*. *Journal of Mammalogy*, **59**,
596 888-893. doi: 10.2307/1380172.

597 **Sullivan, M. S.** 1990. Assessing female choice for mates when the males' characters vary during the
598 sampling period. *Animal Behaviour*, **40**, 780-782. doi: 10.1016/S0003-3472(05)80709-2.

599 **Sullivan, M. S.** 1994. Mate choice as an information gathering process under time constraint:
600 implications for behaviour and signal design. *Animal Behaviour*, **47**, 141-151. doi:
601 10.1006/anbe.1994.1016.

602 **Sunnucks, P. & Hales, D. F.** 1996. Numerous transposed sequences of mitochondrial cytochrome
603 oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*,
604 **13**, 510-524.

605 **Wood, S. R., Sanderson, K. J. & Evans, C. S.** 2000. Perception of terrestrial and aerial alarm calls by
606 honeyeaters and falcons. *Australian Journal of Zoology*, **48**, 127-134. doi: 10.1071/ZO99020.

607 **Zahavi, A.** 1975. Mate selection-a selection for a handicap. *Journal of Theoretical Biology*, **53**, 205-214.
608 doi: 10.1016/0022-5193(75)90111-3.

609 **Zar, J.H.** 1996. *Biostatistical Analysis*. 3rd edn. New Jersey: Prentice-Hall, Inc.

610 **Zuk, M., Johnson, K., Thornhill, R. & Ligon, J. D.** 1990a. Mechanisms of female choice in red jungle
611 fowl. *Evolution*, **44**, 477-485. doi: 10.2307/2409430.

612 **Zuk, M., Thornhill, R. & Ligon, J. D.** 1990b. Parasites and mate choice in red jungle fowl. *American*
613 *Zoologist*, **30**, 235-244. doi: 10.1093/icb/30.2.235.

614 **Zuk, M., Thornhill, R., Ligon, J. D., Johnson, K., Austad, S., Ligon, S. H., Thornhill, N. W. & Costin, C.**
615 1990c. The role of male ornaments and courtship behavior in female mate choice of red jungle fowl.
616 *American Naturalist*, **136**, 459-473. doi: 10.1086/285107.

617 **Zuk, M., Ligon, J. D. & Thornhill, R.** 1992. Effects of experimental manipulation of male secondary sex
618 characters on female mate preference in red jungle fowl. *Animal Behaviour*, **44**, 999-1006. doi:
619 10.1016/S0003-3472(05)80312-4.

620 **Zuk, M., Johnsen, T. S. & Maclarty, T.** 1995a. Endocrine-immune interactions, ornaments and mate
621 choice in red jungle fowl. *Proceedings of the Royal Society of London, Series B*, **260**, 205-210. doi:
622 10.1098/rspb.1995.0081.

623 **Zuk, M., Popma, S. L. & Johnsen, T. S.** 1995b. Male courtship displays, ornaments and female mate
624 choice in captive red jungle fowl. *Behaviour*, **132**, 821-836.

673 **Table 1.** Descriptive statistics for 10 potential predictors of male mating frequency ($N = 64$) and
 674 reproductive success ($N = 21$)

675	Variable	Mean	SE	Minimum	Maximum	CV (%)
676	Dependent					
677	Matings	0.19	0.03	0.00	0.75	106.3
678	Paternity	3.38	0.68	0.17	10.33	92.5
679	Independent					
680	Crows	11.17	1.11	0.00	35.44	79.2
681	Ground alarm calls	1.73	0.34	0.00	16.36	155.3
682	Aerial alarm calls	3.97	0.40	0.19	14.32	79.6
683	Food calls	1.14	0.16	0.00	5.44	112.9
684	Courtship waltzes	1.79	0.21	0.00	8.25	94.7
685	Wingflaps	6.69	0.46	0.25	15.75	54.6
686	Dominance (FSI)	0.00	0.08	-0.89	1.00	62.1
687	Weight (kg)	1.10	0.02	0.76	1.48	14.1
688	Ornament area (cm ²)	27.23	0.60	17.39	40.22	17.6
689	Comb length (cm)	6.83	0.12	4.72	8.94	14.0
690						

691 Note: SE: standard error; CV: coefficient of variation. The coefficient of variation for FSI was calculated
 692 using FSI + 1 to avoid division by zero. Data are presented prior to transformation. Matings,
 693 vocalizations, and visual displays are expressed as rates per hour.

4 **Table 2.** Correlations among 10 potential predictors of male mating success

5

6 Variable	Crow	Ground 7 alarm	Aerial alarm	Food call	Waltz	Wingflap	Dominance	Weight	Ornament area	Comb length
8 Crow	.	0.23	0.00*	0.01*	0.00*	0.00*	0.00*	0.62	0.15	0.07
9 Ground alarm	0.15	.	0.00*	0.00*	0.84	0.01*	0.00*	0.02*	0.04*	0.22
0 Aerial alarm	0.53*	0.55*	.	0.00*	0.01*	0.00*	0.00*	0.26	0.18	0.25
1 Food call	0.32*	0.53*	0.48*	.	0.99	0.01*	0.00*	0.20	0.26	0.23
2 Waltz	0.37*	0.03	0.35*	0.00	.	0.01*	0.27	0.02*	0.01*	0.01*
3 Wingflap	0.52*	0.32*	0.50*	0.34*	0.35*	.	0.00*	0.03*	0.02*	0.06
4 Dominance	0.55*	0.50*	0.61*	0.40*	0.14	0.62*	.	0.76	0.93	0.74
5 Weight	-0.06	-0.30*	-0.14	-0.16	0.30*	0.27*	-0.04	.	0.00*	0.00*
6 Ornament area	0.18	-0.25*	0.17	-0.14	0.34*	0.29*	-0.01	0.46*	.	0.00*
7 Comb length	0.23	-0.16	0.15	-0.15	0.33*	0.24	-0.04	0.36*	0.82*	.

8

9 Note: Pairwise Pearson correlation coefficients are presented below the diagonal and *P*-values above the diagonal (2-tailed, *N* = 64, statistical significance

0 indicated by an asterisk ($\alpha = 0.05$). Weight has been \log_{10} -transformed. All vocalizations and visual displays are based upon rates per hour.

711 **Table 3.** Principal components analysis of male morphology and behaviour

712		<i>N</i> = 64 males			<i>N</i> = 21 males		
713	Variable	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
714	Crow	0.64	0.42	-0.05	0.78	0.40	-0.18
715	Ground alarm	0.73	-0.24	-0.19	0.94	0.06	-0.05
716	Aerial alarm	0.82	0.29	-0.15	0.75	0.54	-0.07
717	Food call	0.70	-0.19	-0.07	0.52	-0.03	0.29
718	Waltz	0.26	0.55	0.31	0.40	0.70	-0.29
719	Wingflap	0.69	0.25	0.48	0.83	0.23	0.35
720	Dominance	0.83	-0.01	0.17	0.82	-0.09	0.15
721	Weight	-0.18	0.26	0.89	0.14	0.18	0.91
722	Ornament area	-0.08	0.88	0.23	0.03	0.94	0.23
723	Comb length	-0.07	0.90	0.08	-0.01	0.93	0.20
724							
725	% variance	33.7	23.4	12.7	38.7	27.8	12.7

726 Note: Factor loading scores are presented for the complete dataset (*N* = 64 males) and for a subset of
 727 the data in which paternity is known (*N* = 21 males). Each factor is comprised of a linear combination of
 728 the 10 variables. The square of each factor loading score represents the proportion of variance in the
 729 relevant measure predicted by that factor. Weight was log₁₀-transformed in the *N* = 64 dataset. All
 730 vocalizations and visual displays are based upon rates per hour. Orthogonal rotation method: varimax
 731
 732

733 **Table 4.** Coefficients table for predictors of male mating success

734	Included:	<i>B</i>	SE	<i>Beta</i>	<i>t</i>	<i>P</i>	<i>r</i>	VIF
735	Intercept	0.81	0.29		2.77	0.01		
736	Aerial alarm	0.01	0.00	0.42	3.79	0.00	0.44	1.64
737	Dominance	0.04	0.01	0.34	3.13	0.00	0.38	1.61
738	Weight	-0.26	0.10	-0.23	-2.67	0.01	-0.33	1.03
739	Excluded:				<i>t</i>	<i>P</i>	<i>r</i>	VIF
740	Crow				-1.61	0.11	-0.20	1.57
741	Food call				1.27	0.21	0.08	1.35
742	Waltz				-0.69	0.49	0.16	1.36
743	Alarm				0.59	0.56	-0.09	1.67
744	Wingflap				0.26	0.80	0.03	2.01
745	Ornament area				-0.21	0.84	-0.03	1.42
746	Comb length				0.16	0.88	0.02	1.26
747								

748 Note: *B*: Unstandardized coefficient; SE: standard error; *Beta*: Standardized coefficient; *r*: partial
 749 correlation coefficient; VIF = variance inflation factor. Variables were entered into a multiple regression
 750 model using a forward stepwise procedure ($P \leq 0.05$ to add, $P \geq 0.10$ to remove; $N = 64$). Mating success
 751 and weight were \log_{10} -transformed.

752 **Table 5.** Coefficients table for predictors of male reproductive success

753	Included:	<i>B</i>	SE	<i>Beta</i>	<i>t</i>	<i>P</i>	<i>r</i>	VIF
754	Intercept	8.97	2.96		3.03	0.01		
755	Ground alarm	2.27	0.75	0.52	3.04	0.01	0.58	1.00
756	Ornament area	0.00	0.00	-0.46	-2.64	0.02	-0.52	1.00
757	Excluded:				<i>t</i>	<i>P</i>	<i>r</i>	VIF
758	Weight				-1.60	0.13	0.19	1.15
759	Wingflap				1.27	0.22	0.18	3.14
760	Food call				-1.14	0.27	-0.27	1.31
761	Waltz				1.02	0.32	0.24	2.13
762	Comb length				0.87	0.40	0.29	5.30
763	Dominance				0.81	0.43	0.19	1.88
764	Crow				0.79	0.44	-0.36	2.90
765	Aerial alarm				0.74	0.47	0.21	4.12
766								

767 Note: *B*: Unstandardized coefficient; SE: standard error; *Beta*: Standardized coefficient; *r*: partial
 768 correlation coefficient; VIF = variance inflation factor. Variables were entered into a multiple regression
 769 model using a forward stepwise procedure ($P \leq 0.05$ to add, $P \geq 0.10$ to remove; $N = 21$).

770 **Figure Captions**

771

772 **Figure 1.** Feather and ornament colour do not predict male mating success. Shown (a) are the hackle
773 feathers and four fleshy red ornaments, including the comb, wattle, ear lappet, and red facial skin.
774 Standardized reflectance of the comb (b) and hackle feathers (c) is presented on the ordinate (0-1) and
775 wavelength on the abscissa (350-700 nm). For each character (comb and hackle feathers), interquartile
776 ranges are plotted for the 10 males from season 3 with the lowest mating success (yellow) and the 10
777 males with the highest mating success (red). The interquartile ranges of the two groups overlap
778 (orange) considerably across the full range visible to females.

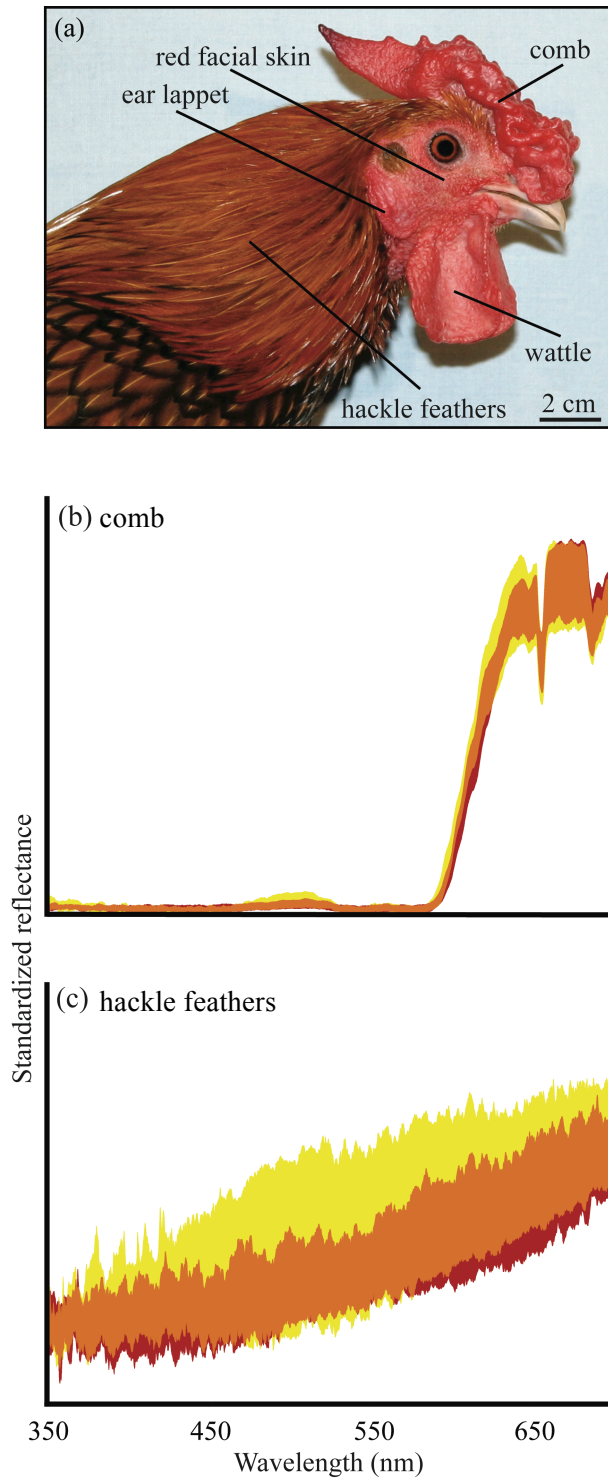
779

780 **Figure 2.** Behaviour predicts both mating frequency (black filled circles, $N = 64$) and reproductive
781 success (open red circles, $N = 21$) in naturalistic social groups. Abscissa represent three orthogonal
782 principal components and ten original variables. Each factor is followed immediately by the variables(s)
783 from which it was derived. Factor loading scores are presented in Table 3. Data are expressed as
784 standard deviates to facilitate comparisons across variables. R -squared values, statistical significance (*
785 $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.0001$), and trendlines fitted using the least squares method are
786 presented on each graph. The slopes of the regression lines for mating and reproductive success differ
787 statistically only for aerial alarm calls (t -test: $t = 2.32$, $P < 0.05$; all other $P > 0.1$).

788

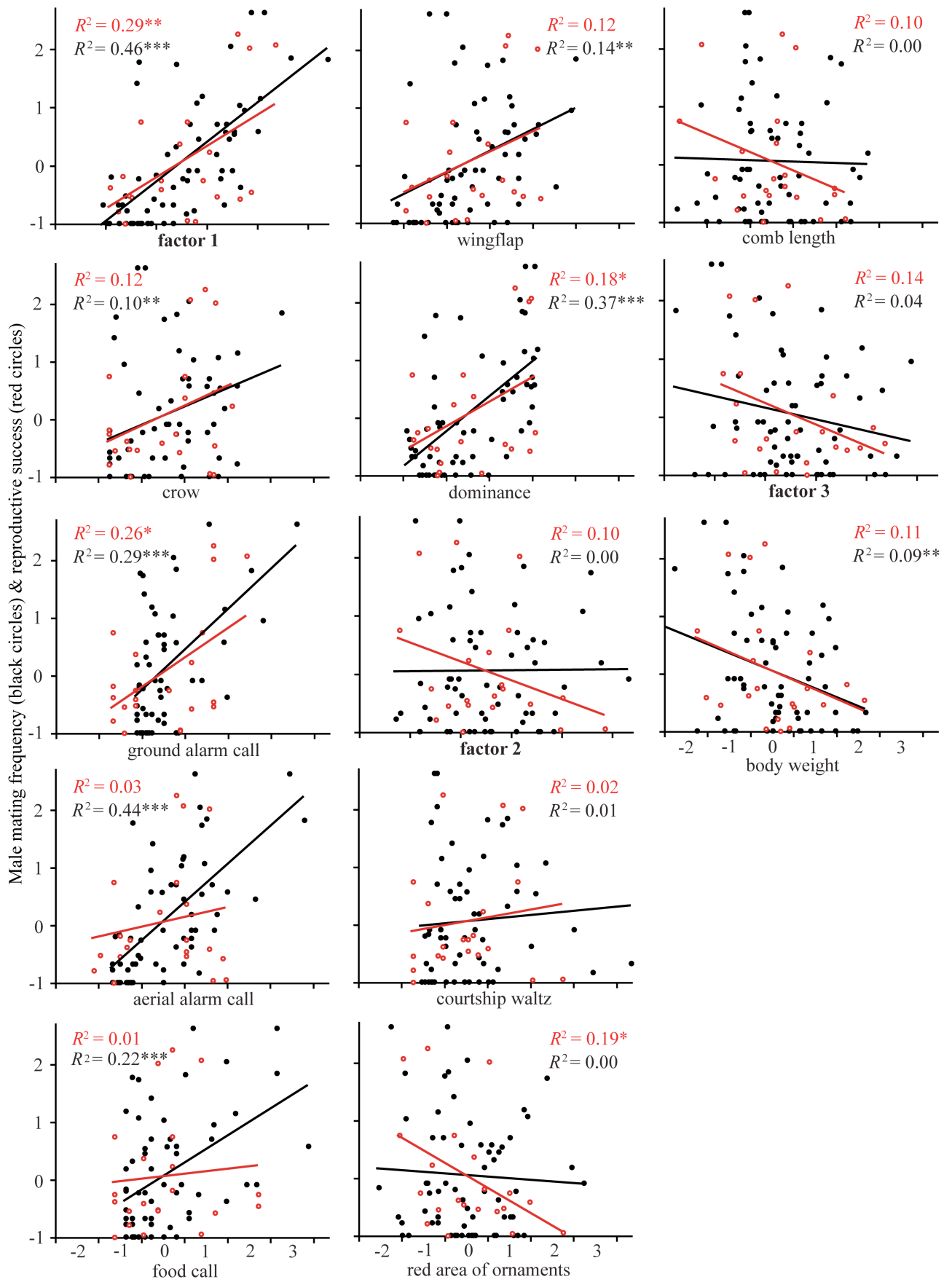
789 **Figure 1.**

790



791

792



5 **Appendix A.** Microsatellite loci used to establish paternity in seven groups of fowl

6

7

Locus*	Primer sequences (5'-3') [†]	Repeat	T _a °C	Size range	k	H _o
8 ADL0176	F: tetTTGTGGATTCTGGTGGTAGC	(GT) ₁₂	48	180-200	4	0.07
9 (G01598)	R: TTCTCCCGTAACACTCGTCA					
0 ADL268	F: famCTCCACCCCTCTCAGAACTA	(GT) ₁₂	48	108-112	2	0.26
1 (G01688)	R: CAACTTCCCATCTACCTACT					
2 LEI0192	F: famTGCCAGAGCTTCAGTCTGT	(CTTT) ₁₂	58	254-266	2	0.58
3 (Z83797)	R: GTCATTACTGTTATGTTTATTGC					
4 LEI0221	F: nedCCTTTATCCACTCTTCATGCAC	(CTTT) ₂₁	62	205-211	2	0.49
5 (Z83791)	R: TGCATAAATTCATGGGTAAGC					
6 LEI0243	F: petTTCAAATCTGTCACTGGAAAGG	(GAAA) ₂₆	62	189-205	4	0.48
7 (Z94843)	R: CAGGGTGCATGTGTATCATACC					
8 LEI0258	F: famCACGCAGCAGAACTTGGTAAGG	((CTTT) ₂ CCTT) ₁₈	54	251-307	2	0.48
9 (DQ239559)	R: AGCTGTGCTCAGTCCTCAGTGC					

0

1 * Locus name and GenBank accession number

2 † Primers were 5' end labeled with the indicated fluorochrome

3 Shown are the locus name, primer sequences, repeat motif, annealing temperature (T_a °C), observed size range, number of observed alleles (k), and observed

4 heterozygosity (H_o). All adults (21 males, 21 females) and embryos (N = 71) were genotyped for all loci, except ADL268, where N_{embryos} = 58.

645 **Appendix B.** Individual reproductive success in seven groups of fowl

646	Group	Embryos	Male	Female 1	Female 2	Female 3
647						
648	1	2	1	0.17	0.00	0.00
649			2	0.17	0.17	1.17
650			3	0.17	0.17	0.00
651	2	14	1	0.33	0.00	1.67
652			2	0.00	2.00	8.33
653			3	0.33	0.67	0.67
654	3	4	1	0.00	0.14	0.14
655			2	0.00	0.81	0.81
656			3	0.81	0.64	0.64
657	4	9	1	0.00	0.93	0.93
658			2	0.42	1.02	1.27
659			3	1.08	1.08	2.27
660	5	21	1	2.92	0.47	2.22
661			2	4.67	0.42	4.67
662			3	2.92	0.47	2.22
663	6	9	1	0.00	0.00	2.50
664			2	1.50	0.00	1.00
665			3	3.50	0.00	0.50
666	7	12	1	0.25	0.17	1.17
667			2	0.25	2.42	6.92
668			3	0.00	0.17	0.67
669						

670 Males and females are arranged arbitrarily within their respective groups.

671 Values represent the estimated reproductive success for each parental combination. See text for details

672 of how estimates were derived.