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10 **Artificial enhancement of an extended phenotype signal increases**

11 **investment in courtship by three-spine sticklebacks**

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41   Interactions between the components of a multiple-signal sexual display can be

42   complex, and previous work has shown that alteration of one component can

43   lead to changed investment in either the altered or other display components.

44   Extended phenotype signals (non-bodily structures that carry a signalling

45   function) provide an ideal means to manipulate signal quality in a non-invasive

46   manner, to investigate investment patterns in display components. We make 3

47   predictions as to how males might alter investment in display components in

48   response to artificial enhancement of an extended phenotype signal, and test

49   those predictions using three-spine sticklebacks (*Gasterosteus aculeatus*). We

50   demonstrate that the addition of brightly coloured ornaments (threads) to the

51   nests of male sticklebacks leads to increased investment in both courtship of

52   females and nest construction. In a field experiment, males offered coloured

53   threads spent increased time engaged in interactions with females, and in the

54   laboratory, they built nests that were neater and more compact (better quality),

55   relative to males offered dull threads. Our findings support a hypothesis based

56   on resource budgeting, and suggest that resources saved by having an artificially

57   enhanced nest are reallocated to other courtship behaviours. The study provides

58   a framework for investigating the interaction between signal components, and

59   demonstrates that manipulation of extended phenotype signals can provide

60   insight into the ways in which animals balance investment in interacting signal

61   components in sexual displays.

62

65

66 Sexual displays can be complex, involving multiple signal components, often

67 across different sensory modalities (Candolin 2003; Hebets & Papaj 2005). For

68 instance brightly coloured ornamentation may be combined with vocalisations

69 or courtship displays . Multiple traits may convey similar ("redundant" or

70 "backup signals") or different ("multiple messages") information about

71 underlying male quality, or interact to enhance the information content of the

72 signals ("emergent messages", "signal enhancers" and "amplifiers").

73 Alternatively, different signals may contain information for different receivers

74 ("multiple receivers"), or may represent adaptation to fluctuating environments

75 or dynamic variation in selection pressures (see reviews by Candolin 2003;

76 Hebets & Papaj 2005; Bro-Jørgensen 2010). Although it has been argued that

77 multiple ornaments may be only weakly condition-dependent (Møller &

78 Pomiankowski 1993), other studies support the idea that multiple signals are

79 honest indicators of underlying male quality (Candolin 2003; van Doorn &

80 Weissing 2004) and that males invest optimally in signalling (Andersson 1982;

81 Delcourt & Rundle 2011).

82

83 The interaction between signal components is likely to be complex (Candolin

84 2003), making it difficult to predict how animals might respond to changes in

85 their signal quality. For example, activation of the immune system in birds can

86 reduce the behavioural display component of a signal, but the reduction is lower

87 in individuals expressing plumage-based signals indicative of high quality

88 (Garamszegi 2004; Loyau et al. 2005). Experimental manipulation of individual

89 signal components is perhaps challenging, particularly where signals are

90 correlated (Candolin 2003), but can provide insight into how traits interact to

91   convey information to a receiver. Wolf spiders (*Schizocosa* spp) use a

92   combination of visual and vibratory (seismic) signals in courtship. When placed

93   on a substrate that does not allow for the transmission of vibratory signals

94   (granite), males increase investment in the visual component of courtship

95   display (Gordon & Uetz 2011) allowing them to maintain courtship success

96   (Hebets & Papaj 2005).

97

98   "Extended phenotype signals" are non-bodily structures (such as nests, burrows

99   and bowers) built by males that can act to inform mate choice (Schaedelin &

100   Taborsky 2009). Females prefer to mate with males with particular signal

101   characteristics that indicate either male quality or enhance the survival of eggs

102   (birds: Hansell 2005, fiddler crabs *Uca annulipes*: Backwell & Passmore 1996,

103   bowerbirds: Borgia 1995; Humphries & Ruxton 1999; Madden 2003). Evidence

104   suggests that many extended phenotype signals are condition dependent, and

105   honestly signal builder quality (e.g. Barber et al. 2001; Soler et al. 2001; Olsson et

106   al. 2009). Thus, extended phenotype signals provide an ideal means to

107   experimentally manipulate signal quality without potentially confounding direct

108   physical or physiological impacts on signaller behaviour (Schaedelin & Taborsky

109   2009; Schaedelin & Taborsky 2010; Sergio et al. 2011). This allows for

110   investigation into how male investment in display components is influenced by

111   manipulation of the extended phenotype signal.

112

113   When an extended phenotype signal is experimentally manipulated, males may

114   either alter their investment in the manipulated trait, or they may alter

115   investment in alternative components of their display. In black wheatear

116   *Oenanthe leucura* (Soler et al. 1996) and the Lake Tanganyika cichlid

117   *Cyathopharynx furcifer* (Schaedelin & Taborsky 2006), males compensated for

118   alteration to their nests and mating craters respectively through increased

119   investment in building behaviour and rapid reconstruction of the signals to their

120   original dimensions. In contrast, satin bowerbirds (*Ptilonorhynchus violaceus*)

121   increased investment in bower construction when bower decorations were

122   experimentally removed (Bravery & Goldizen 2007), and barn swallows

123   (*Hirundo rustica*) with experimentally enhanced tail lengths reduced nest-

124   building effort (Soler et al. 1998). Here, we investigate how male three-spine

125   sticklebacks (*Gasterosteus aculeatus*) alter investment in behaviour and nest

126   construction, in response to artificial enhancement of the quality of their nest,

127   which acts as an extended phenotype signal in this species.

128

129   In sticklebacks, the males build nests from sediment and plant material, and are

130   solely responsible for parental care (Van Iersel 1953). The nests are held

131   together with a kidney-secreted protein called spiggin (Jakobsson et al. 1999),

132   and are known to have a courtship signalling function (von Frisch 1974; Östlund-

133   Nilsson 2001; Barber et al. 2001; Östlund-Nilsson & Holmlund 2003). Females

134   are first alerted to the presence of males via olfactory cues (Mclennan 2003),

135   after which the male uses his courtship display to lead females to the nest

136   (Candolin 1997); thus, nest inspection by females occurs late in the courtship

137   sequence. Nest quality, measured as neatness and compactness, increases with

138   male quality and immunological function (Östlund-Nilsson 2001; Barber et al.

139   2001), and so nests act as an honest signal of male quality. Males may also

140   'decorate' their nest with algae of contrasting colours or artificial materials

141 provided experimentally (such as threads, foil sticks and sequins), and females

142 prefer males with nests decorated with brightly coloured objects over

143 undecorated nests (Östlund-Nilsson & Holmlund 2003). The provision of brightly

144 coloured objects therefore provides a simple experimental means of

145 manipulating perceived nest quality.

146

147 Here, we manipulate nest quality by providing male sticklebacks with brightly

148 coloured cotton threads. In a field experiment, we investigate behavioural

149 investment in nest-building, female courtship, male-male aggression and other

150 fitness-related behaviours in response to nest enhancement (relative to a

151 control). In a complementary laboratory study, we investigate investment in nest

152 construction by analysing nest quality. We test three hypotheses linking nest

153 quality to behaviour:

154   *1. Decreased investment hypothesis*: When one trait (here, the nest) is

155   enhanced, investment in other aspects of courtship could be reduced so

156   that the overall level of signalling remains the same, and honestly

157   indicates male quality. This may explain the reduction in nest building

158   effort by male barn swallows with enhanced tail lengths (Soler et al.

159   1998), and may be particularly relevant when female preferences are

160   based on the simultaneous, combined effect of multiple cues (Lehtonen et

161   al. 2007, Lancaster et al. 2009). This hypothesis predicts that stickleback

162   males with enhanced nests will decrease the time invested in courtship

163   behaviour and decrease nest quality relative to males with control nests.

164   *2. Resource budgeting hypothesis*: If males have a limited resource (e.g.

165   energy, time) budget to allocate to mate attraction, we predict that

166   increasing the quality of one trait could allow for increased investment in

167   other aspects of mate attraction, as lower resource allocation to the

168   enhanced trait is needed. This hypothesis predicts that stickleback males

169   with enhanced nests will increase the time spent courting females, and

170   increase nest quality, relative to males with control nests.

171   *3. Alternative allocation hypothesis*: As an alternative to hypothesis 2, male

172   resources could be allocated to other behaviours outside the mate-

173   attraction sphere, such as foraging or resting. This hypothesis is perhaps

174   particularly relevant to species where males provide parental care, and

175   resources must be allocated to, or retained for, continued investment in

176   offspring provisioning or survival (Kokko et al. 2002). This hypothesis

177   predicts that stickleback males with enhanced nests will increase the time

178   spent resting and/or foraging relative to males with control nests, while

179   time spent courting and nest quality do not differ between enhanced and

180   control nests.

181

182   METHODS

183   *Study system*

184   The three-spined stickleback (*Gasterosteus aculeatus*) is a small shoaling fish,

185   native to freshwater, brackish and marine habitats in the northern temperate

186   and arctic region. In the breeding season (May-July in the UK) males leave their

187   groups and develop bright nuptial colouration of red throat and lips and bright

188   blue irises. Males establish small territories in shallow water (<1m), in which

189   they construct a nest from plant material and spiggin. This nest, his breeding

190   colouration and a courtship dance attract females to lay their eggs in his nest,

191   which he will then fertilise and care for alone (Van Iersel 1953; Wootton 1984).

192

193   *Field experiment*

194   Our field experiment was carried out in an artificial brackish fishing pond in

195   Saltfleet, Lincolnshire, UK (53º 25.2' N, 0º, 11.4' E; OS Explorer 283 map grid

196   reference 459939), measuring approximately 115 x 40m, between April and July

197   2009. The pond was created in 1980 and has had a resident stickleback

198   population since 1981 (local fishermen, pers. comm.). The pond is characterised

199   by wide, shallow silty banks providing both suitable stickleback nesting habitat

200   and areas from which to observe nesting sticklebacks.

201

202   In total, 50 nests were identified as being suitable for study. These were nests

203   made by males whose entire territory could be identified from the bank,

204   positioned less than 1m from the shore, and at a depth of 30cm or less, allowing

205   reliable observations to be made from the bank. Nests were required to be

206   almost complete (defined by the presence of a visible nest entrance; Barber et al.

207   2001; Rushbrook et al. 2008), yet still under construction (defined by males

208   carrying and adding material to the nest, and so the nest would be unlikely to

209   contain eggs; Van Iersel 1953).

210

211   Following nest selection, the observers positioned themselves on the bank close

212   to the nest and allowed a 20-minute acclimatisation period, allowing the male to

213   return to normal behaviour following any disturbance. Males were observed

214   during this time to allow for identification of the position of territory boundaries.

215   Behavioural observations were then made for a further 20 minutes. We used a

216   point-sampling approach to record the behaviour in which a male was engaged,

217   and the presence and sex of other sticklebacks within his territory every 30

218   seconds for the 20 minute period. The following behaviours were noted: building

219   the nest, staying motionless in the water (i.e. not engaged in any other

220   behaviour), foraging, fanning the nest (either caring for eggs or used as a

221   courtship signal indicating ability to care for eggs; Candolin 1997; Ishikawa &

222   Mori 2000) and engaging in courtship interactions with any females in the

223   territory. We also recorded the total amount of time (in seconds) that the male

224   spent engaging in aggressive interactions with other males, and the total time the

225   focal male spent gluing his nest.

226

227   At the end of the first observation period (we refer to this as 'stage 1'), 600

228   cotton threads (2cm in length) were placed in the focal male's territory. Males

229   were randomly allocated to either the 'control' treatment or the 'colour'

230   treatment. Control males received 600 threads in colours similar to those of

231   natural nesting materials already in use (light sandy brown, light grey brown,

232   dark brown, dark grey and black; Anchor brand colour codes 373, 393, 382, 401,

233   403 respectively). Colour males received 600 threads in novel colours not

234   observed in any natural nests (yellow, green, blue, red and white; DMC

235   Corporation brand colour codes 3821, 699, 498, 796, BLANC respectively).

236   Thread colours were chosen subjectively (i.e. based on human vision). Each male

237   received 120 threads of each of the 5 colours. Focal males were then left for 24

238   hours (±2 hours) after which the behavioural observations above were repeated

239 (stage 2). In total, behavioural data were collected for 10 colour and 16 control

240 nests, with stage 1 and stage 2 data for each nest (trial).

241

242 Focal males were caught immediately after the completion of stage 2

243 observations, using a long-handled hand net, and photographed in a water-filled

244 Perspex box (55 x 47 x 67mm) with a scale bar using a digital SLR camera (Nikon

245 D90 with Sigma 105mm F2.8 EX DG lens) and portable lighting equipment (2 x

246 80w portable lights with 50w halogen bulbs) inside a light cube. White balance

247 was calibrated before each photograph. After photographing, males were

248 released to a neighbouring pond. Photographs were used to measure male body

249 length and to assess nuptial colouration following Boughman (2007). Nuptial

250 colouration in sticklebacks consists of a red throat and belly, and a blue eye (Van

251 Iersel 1953). The extent and intensity of red colouration were scored on a 0-5

252 scale, where 0 is no redness present and 5 is the greatest area covered or

253 intensity within the observed population. Intensity of eye blueness was also

254 scored on a 0-5 scale. Three independent observers scored each male and the

255 mean score was used in subsequent analysis. Humans and sticklebacks have

256 been found to rank red and blue colouration in a similar way (Rowe et al. 2006),

257 so this method of assessing male colouration is appropriate. Focal nests were

258 removed from the water, dried and dismantled to assess thread incorporation.

259 No nests were found to contain eggs, ensuring that males were still in the

260 courtship phase (Van Iersel 1953) and females could not use the presence of

261 eggs in their mate choice decisions (Goldschmidt et al. 1993). Additionally, no

262 control nests contained coloured threads, or *vice versa*.

263

Laboratory experiment

265 Sticklebacks were obtained from 3 freshwater sites in West Yorkshire (Balne

266 Beck; SE 317 218, Wortley Beck; SE 259 323 and Wyke Beck; SE 279 376)

267 between May and July 2010. At least 40 sticklebacks in breeding colouration

268 were captured at each site using hand-held sweep nets, hand seine nets and

269 bottle traps. Traps were made from 2l plastic bottles with the neck end cut and

270 reversed into the body of the bottle. Traps were baited with bloodworm,

271 weighted with stones, and placed in the river at 10:00, then checked regularly

272 throughout the collection period (10:00 – 15:00). Any sticklebacks found in the

273 traps were removed to an aerated container with river water before being

274 transferred back to the laboratory at 15:00.

275

276 Populations were initially housed in mixed gender tanks (30x60x30cm) held at

277 21°C and on a 16:8 light:dark cycle. Any fish showing signs of ill health were

278 removed. For each population, 18 gravid females with a standard body length of

279 between 40 and 56mm were removed to a separate holding tank (30x60x30cm),

280 filled to a depth of 25cm with aerated water containing 30g of Aquarium salt and

281 6ml of Stress Coat (Rushbrook & Barber 2008), and furnished with an activated

282 carbon filter, airstone, several artificial plants and terracotta plant-pot refuges,

283 and a gravel substrate. These females were used as "presentation females" to

284 stimulate nest building in the focal males (Braithwaite & Barber 2000l

285 Rushbrook & Barber 2008; Rushbrook et al. 2008).

286

287 18 males from each population (16 for Wyke Beck), showing typical breeding

288 colouration, were removed to individual nesting tanks (46.7x30.5x17.5cm), filled

289 to a depth of 8cm with water treated with 10g Aquarium salt and 2ml Stress

290 Coat. The tanks were partitioned with a transparent, perforated (approximately

291 150 equally spaced 10x2mm slits) partition 10cm from one end. The larger

292 'male' compartment contained a single artificial *Eugeria* plant, a plastic nesting

293 dish (15cm in diameter and 5cm deep) containing 150g fine sand, and 200 black

294 threads (7cm long; Candolin 1997; Braithwaite & Barber 2000; Barber et al.

295 2001; Rushbrook & Barber 2008; Heuschele et al. 2009). The nesting dish was

296 positioned close to the partition between the two compartments. The water was

297 aerated via an airstone located in the small 'female' compartment. Brown

298 cardboard was placed underneath and around each tank to minimise

299 disturbance and prevent male-male interaction between neighbouring tanks.

300 Individual males were placed into the larger compartment, and the smaller

301 compartment was used to present females to males daily for 14 days or until

302 nests were complete (see below). On each day, females (from the same

303 population) were randomly assigned to male tanks to ensure all males were

304 exposed to females of varying gravidity (Rushbrook et al. 2008), and placed into

305 the smaller compartment for a 20-minute period between 10:00 and 12:00

306 (Braithwaite & Barber 2000). All fish were fed defrosted frozen bloodworm

307 following the completion of female presentation each day.

308

309 Nest development was observed daily after female presentations were complete.

310 Once at least 75% of the black threads had been incorporated into the nest,

311 males were provided with 50 additional threads from either a colour (ten

312 strands each of; yellow, green, blue, red and white) or control treatment (ten

313 strands each of; light sandy brown, light grey brown, dark brown, dark grey and

314   black). Males were allocated to each treatment alternately to control for any

315   effects of latency to begin building on nest structure. Once nests were complete

316   with a defined entrance, and males had been observed creeping through the nest

317   (Barber et al. 2001; Rushbrook et al. 2008), nesting dishes were removed from

318   the tank to allow for the nest to be photographed for analysis. Any males that had

319   not completed their nests within the 14-day period were excluded from the

320   analysis.

321

322   Males were photographed using the same procedure as for the field experiment.

323   Images were scored for redness, extent of red colouration and eye colour by 4

324   independent observers, and mean scores calculated. Nests were photographed in

325   situ from directly above, with a scale bar placed close to the nest. After

326   photographing, nests were dried at room temperature until a consistent weight

327   was achieved and then dismantled. The total amount of substratum deposited on

328   top of the nest was weighed to the nearest 0.0001g, and the total number of

329   threads used ($N_{threads}$) was counted. At the end of the study, fish were returned to

330   their population of origin.

331

332   Nest images were analysed using ImageJ to assess: 1) the number of thread ends

333   not fixed into the nest (N_{ends}), 2) the total nest area (A_{tot} in mm^2, defined as the

334   minimum area polygon containing all threads) and 3) the bulk area (A_{bulk}, in

335   mm^2, defined as the total nest area where no substratum could be seen below the

336   nest). We then calculated 'neatness' and 'compactness' indices following Barber

337   et al. (2001) and Rushbrook et al. (2008). Nest compactness was defined as the

338   bulk area of the nest divided by the total area (A_{bulk}/A_{tot}), and neatness measures

339   the proportion of available thread ends (i.e. 2 x $N_{threads}$) that are anchored into

340   the nest or covered by substratum. Neatness was calculated as 1-

341   [$N_{ends}/2(N_{threads})$].

342

343   *Statistical analysis*

344   All data were analysed using R version 2.13.0 (R Development Core Team 2011).

345   P-values were corrected for multiple comparisons within an experiment

346   (field/laboratory) using the Benjamini & Hochberg (1995) procedure for false

347   discovery rate control. Both returned and adjusted p-values are reported here.

348   Descriptive statistics are presented as mean ± S.D.

349

350   For the field experiment, we used principal component analysis (PCA) to reduce

351   the three male colour variables (extent, redness and blueness) to a single

352   significant component explaining 74.35% of the variation. This component

353   loaded strongly on to each of the colour variables (extent: -0.5105; redness: -

354   0.571 and blueness: -0.643) and was extracted as 'male colour'. To test for

355   differences between treatments in the proportion of threads used, a generalised

356   linear model with quasibinomial errors (to account for overdispersion) was

357   used. Male body length and male colour conformed to the assumptions of

358   normality and homogeneity of variance (checked via plotting of residuals), and

359   differences between treatments in these variables were analysed using general

360   linear models.

361

362   PCA on the behavioural data revealed no clear correlations between

363   combinations of variables, so each was analysed separately. We used generalised

364   linear mixed effects modelling approaches using the *lme4* package in R (Bates et

365   al. 2011). We investigated the effect of stage (1 or 2) and treatment (control or

366   colour threads), and their interaction, on each of the behavioural measures,

367   using binomial error distribution as appropriate for proportion data. Nest (trial)

368   ID was added as a random effect to allow for a repeated-measures analysis of

369   stage, assuming that the male present on a nest during stage 2 was the same

370   nest-owner as was present during stage 1. We note that it is possible that nest

371   takeovers (Kraak et al. 2000, Lehtonen & Wong 2009) occurred during the

372   course of our study, and that nest owner identity was not the same between the

373   two stages. However, during over 50 hours of observation of nests, we never

374   observed a nest takeover; males were observed to be of similar size and colour

375   during stage 1 and stage 2, and often clearly identifiable by other marks. Other

376   studies have also found nest takeovers to be a rare occurrence (Kraak et al 2000;

377   see Discussion). Exploratory modelling using generalised linear models revealed

378   a tendency for overdispersion in the data, and an observation-level random

379   effect was added to account for this (Bates et al. 2011). In each case, the addition

380   of this random effect either increased or had no significant impact on the fit of

381   the model in comparison to a model without this effect, judged using the Akaike

382   Information Criterion (AIC). Non-significant interaction terms were removed as

383   part of model simplification (Crawley 2007), and only the minimum adequate

384   models are presented here. Full models can be found in the Appendix (table A1).

385

386   For the laboratory experiment, we again used PCA to reduce the three male

387   colour variables to a single significant component explaining 65.69% of the

388   variation. This component loaded strongly on extent (-0.644) and redness (-

389   0.681) and more weakly on eye colour (-0.348). PC1 was extracted and is again

390   referred to as 'male colour'. PCA was also used to reduce the three nest variables

391   (neatness, compactness and deposited substratum) to a single measure of nest

392   quality. Nest PC1 explained 78.03% of the variation in nest structure and loaded

393   strongly on all three variables (compactness: -0.602; neatness: -0.531; deposited

394   substratum: 0.596). Thus, negative values of nest PC1 indicate nests that are

395   neater, more compact and have little deposited substratum (we define these as

396   'good quality', as previous work has demonstrated that nest quality, measured as

397   neatness and compactness, correlates with male quality and physiological state

398   (Barber et al. 2001), while positive values indicate nests that are less neat, less

399   compact and have more deposited substratum ('poor quality').

400

401   We used general linear models to confirm that males offered colour and control

402   threads were matched in terms of colour and body size. A linear mixed-effect

403   model was used to investigate the effect of treatment and male colour, and their

404   interaction, on nest structure. To control for potential differences in nest

405   construction between populations, population of origin was added as a random

406   effect. There was no significant effect of male body size or its interactions on nest

407   structure, so this was removed from the analysis, and only the minimum

408   adequate model is presented here. The full model can be found in the Appendix

409   (table A2).

410

411   RESULTS

412   *Field experiment*

413   48% (24/50) nests observed and offered threads were no longer present after

414   24 hours (7/17 colour nests and 17/33 control nests disappeared). Exact causes

415   could not be determined, but may include predation, male-male competition

416   such as the destruction of nests and theft of nesting material (Li & Owings 1978;

417   Mori 1995), and disturbance (dogs and ducks were both frequently observed in

418   the shallow water). In total, behavioural data for both stages were available for

419   10 colour and 16 control nests. Males were successfully captured from 10 colour

420   nests and 9 control nests, and nests removed for 10 colour and 15 control nests.

421   There were no significant differences between colour and control nests in the

422   proportion of threads used (colour: 0.007 ± 0.007; control: 0.015 ± 0.017; $t_{1,24}$ =

423   1.293, returned P = 0.209, adjusted P = 0.401), male body colour (colour: 0.154 ±

424   1.844; control: -0.170 ± 1.984; $F_{1,17}$ = 0.136, returned P = 0.717, adjusted P =

425   0.8246), male body length (colour: 52.5 ± 4.0mm; control: 51.4 ± 1.9mm; $F_{1,17}$ =

426   0.527, returned P = 0.478, adjusted P = 0.647), or in the proportion of nest losses

427   (binomial test, returned P = 0.471, adjusted P = 0.676).

428

429   The minimum adequate models for the behavioural analysis can be found in

430   table 1. Of specific interest are significant interactions between stage and

431   treatment, indicating that a change in behaviour resulting from the addition of

432   threads differed between treatments. An effect of stage alone indicates an effect

433   of adding threads per se, or changes in behaviour as a result of nests being 24

434   hours older. We consider first the behaviour of the male when not interacting

435   with conspecifics. We found a significant negative effect of stage on the

436   proportion of time spent building (table 1, figure 1a) and a positive effect of

437   stage on the proportion of time spent motionless (table 1, figure 1b), but no

438 effect of treatment, and no interaction. The reduction in building and increase in

439 time spent motionless suggest that nest-building was nearing completion by

440 stage 2. There was no significant effect of either treatment or stage, or their

441 interaction, on male foraging, fanning or gluing behaviour (table 1).

442

443 There was no change in the proportion of observations where another male or a

444 female was present in the territory in response to either treatment or stage

445 (table 1). However, there was a significant decrease in the proportion of time

446 where the male was engaged in an aggressive interaction with another male

447 during stage 2 (table 1, figure 1c), and a significant interaction effect on the

448 proportion of observations in which the male was engaged in an interaction with

449 a female (table 1, figure 1d). While males with control threads experienced a

450 drop in female interactions, males with coloured threads in their nests

451 experienced an increase between the first and second stages (figure 1d).

452 Additionally, we investigated male-female interactions as a proportion of

453 observations where females were present on the territory (male-female

454 interactions/females present), and found an identical pattern (table 1).

455

456 *Laboratory experiment*

457 In total, 19/52 males built complete nests within the 14-day period (10 control

458 and 9 colour). A further 25 failed to complete nest building, 3 died and a further

459 5 were removed from the experiment and treated for fungal infection. Males

460 offered colour and control threads were matched in terms of male body colour

461 (colour: -0.286 ± 1.384; control: 0.258 ± 1.443; GLM: $F_{1,17}$ = 0.6997, returned P =

462 0.415, adjusted P = 0.415) and body size (colour: 42.3 ± 1.4mm ; control: 44.1 ±

463   3.1mm; GLM: $F_{1,17}$ = 2.4377, returned P = 0.137, adjusted P = 0.183). We found a

464   significant effect of both male colour (linear mixed-effects model: t_{14} = 2.5616,

465   returned P = 0.023, adjusted P = 0.045) and thread colour treatment (t_{14} =

466   3.5870, returned P = 0.003, adjusted P = 0.012) on nest structure, after removal

467   of non-significant interaction terms (see methods for details of model

468   simplification). Males offered coloured threads completed nests that were neater

469   and more compact, with less deposited substratum, than males offered control

470   threads (figure 2). Nest neatness and compactness also increased with increasing

471   male brightness (figure 2), independently of thread colour treatment.

472

473   DISCUSSION

474   The incorporation of coloured decorations into male stickleback nests has

475   previously been found to enhance their attractiveness to females (Östlund-

476   Nilsson & Holmlund 2003). We found that allowing males to add brightly

477   coloured threads to their nests also increased investment in other aspects of

478   courtship. In the field experiment, males increased the amount of time spent

479   interacting with females, both overall and with respect to the number of females

480   present on their territory (figure 1d), but did not change their allocation of time

481   to other behaviours. In the laboratory experiment, males offered coloured

482   threads to build with constructed nests that were neater and more compact (i.e.

483   of higher quality; Barber et al. 2001; Östlund-Nilsson 2001) than those built by

484   males offered only control threads (figure 2). An increased investment in other

485   aspects of courtship fits with our resource budgeting hypothesis (hypothesis 2),

486   meaning the resources required to make an 'attractive' nest are allocated instead

487   to courtship behaviour.

488

489 By enhancing the attractiveness of nests (Östlund-Nilsson & Holmlund 2003),

490 males are able to alter their investment in mate courtship behaviour and other

491 aspects of nest construction to maximise their mating success. Although nest

492 construction and parental behaviour are energetically costly in sticklebacks

493 (construction: Wootton 1984; Candolin & Voigt 2001a; Rushbrook & Barber

494 2006; Rushbrook et al. 2010, parental behaviour: Smith & Wootton 1999) and

495 carried out by the male alone (Van Iersel 1953), we found no evidence to suggest

496 that males were re-allocating time or energy to other activities such as foraging

497 (hypothesis 3: alternative allocation hypothesis) or maintaining an overall level

498 of courtship display (hypothesis 1: decreased investment hypothesis).

499

500 How males might alter investment in alternative display components may

501 depend on the sequence by which females assess traits before making a mating

502 decision. Traits may be assessed sequentially, where one trait (or combination of

503 traits) acts as an "alerting" trait (Candolin 2003) to attract the attention of the

504 female, and another informs the final mating decision. For example, white bones

505 on the bower of the spotted bowerbird (*Chlamydera maculata*; Borgia 1995) and

506 the sand hood constructed by fiddler crabs (*Uca terpsichores*; Christy & Backwell

507 2006) may serve to draw female attention to the male. In our study, we

508 manipulated the quality of a trait assessed late in the courtship sequence.

509 Enhancement of the alerting trait, however, could lead to a further prediction. A

510 "female response hypothesis" predicts that if females are first attracted to a male

511 by an enhanced trait, males could respond to increased female interest by

512 increasing investment in later stages of courtship. Both zebra finch (*Taeniopygia*

513   *guttata*; Royle & Pike 2010) and stickleback (Rowland 2000) males are known to

514   increase courtship in response to perceived interest from females.

515

516   Previous studies manipulating signal quality suggest that the interaction

517   between signal components can be complex. We assumed in our hypotheses and

518   experiment that females used the traits under consideration in mate choice, and

519   continued to do so, but this may not necessarily be the case. Zuk et al. (1992)

520   manipulated the phenotypic traits of male jungle fowl (*Gallus gallus*), and found

521   that this had no effect on courtship rates relative to un-manipulated males.

522   Females, however, ignored the manipulated characteristics and based their

523   choice on other traits that were previously less important (Zuk et al. 1992). In

524   the Lake Malawi cichlid *Nyassachromis microcephalus*, mating craters built on

525   rock ledges (enhancing their apparent size) attract increased female interest

526   relative to craters built on the sandy lake floor, but this is not reflected in

527   increased mating success for ledge-building males (Martin 2010), as craters built

528   on ledges are an unreliable signal of male quality. In sand gobies (*Pomatoschistus*

529   *minutus*) nest quality is not well correlated with male quality, and may not act to

530   inform female mate choice (Lehtonen & Wong 2009). These studies suggest an

531   alternative motivation for males to increase investment in other aspects of

532   courtship in response to nest ornamentation: If females perceive an ornamented

533   nest as an unreliable signal, males may need to increase courtship to secure

534   matings. The fact that female sticklebacks have previously been found to prefer

535   decorated nests (Östlund-Nilsson & Holmlund 2003), and the female is led to the

536   nest by the courting male (Candolin 1997), however, makes this suggestion

537   unlikely in our particular system.

538

The ability of a male to use a particular trait may be influenced by environmental

conditions, providing a further mechanism by which the quality of a trait can be

manipulated, and investment in display components can be explored. Male

courtship behaviour is often plastic, and males are known to adjust investment

in aspects of courtship in response to environmental conditions that affect signal

transmission (Hebets & Papaj 2005; Gordon & Uetz 2011). Studies of

environmental effects on signal transmission often focus on the degradation of

the signal, resulting in a compensatory increase in better-transmitted signals.

Lizards increase the speed of visual displays where the background is visually

noisy and signals less easily detectable (Ord et al. 2007; Peters et al. 2007). Wolf

spiders (*Schizocosa ocreata*) used significantly more visual courtship signals on

substrates that attenuated seismic (vibration) signals (Gordon & Uetz 2011), and

sticklebacks increase investment in sexual displays (Candolin et al. 2007) and

rely more on olfactory cues (Heuschele et al. 2009) in turbid waters where

visibility is reduced. In guppies, males compensate for a reduction in the efficacy

of visual signalling under low light conditions by switching from sigmoid

courtship displays to 'sneaky' mating (Chapman et al. 2009).

There may be other explanations for our results, which we touch on briefly here.

We did not observe nest takeovers (Kraak et al. 2000, Lehtonen & Wong 2009),

during our field study, but the possibility that they occurred remains. If this is the

case, males observed during the 2nd observation period may not have been the

original nest-owner, instead being higher-quality males who were able to invest

more in courtship display. This would require that takeovers occurred more

563   commonly on colour nests, and we would predict that males that were successful

564   in these contests were larger (Candolin & Voigt 2001b) and redder (Bakker &

565   Sevenster 1983; Milinski & Bakker 1990; Candolin 1999) than unsuccessful

566   males. We found no difference between treatments in male size or body colour;

567   and no greater increase in aggression towards males with coloured threads,

568   suggesting that takeovers, if they occurred, did not occur more frequently on

569   colour nests. Males in the laboratory experiment had no physical, visual or

570   olfactory contact with other males, and so nest-takeovers cannot explain the

571   changes in nest structure in response to artificial ornamentation. The increased

572   conspicuousness of colour nests may also have increased their vulnerability to

573   predation, potentially causing males to invest more in courtship to rapidly

574   attract a female, but we found no evidence that nest losses were higher for

575   coloured nests, and previous work links increased risk with decreased, not

576   increased, courtship behaviours in this species (Sargent & Gebler 1980; Candolin

577   & Voigt 1998), as courtship generally increases vulnerability to predation

578   (Magnhagen 1991).

579

580   In conclusion, males may increase investment in signals that form part of a multi-

581   component display for a variety of reasons. A reduction in the efficacy of

582   transmission of a particular signal can lead to compensatory investment in

583   signals utilizing other sensory modalities (Candolin et al. 2007; Chapman et al.

584   2009; Heuschele et al. 2009; Gordon & Uetz 2011), increased female interest may

585   encourage males to increase investment to secure a mating (Royle & Pike 2010),

586   or as we demonstrate here, artificial enhancement of one trait may lead to

587   increased investment in other display components. The interactions between the

588 components of multiple signals may be complex, and extended phenotype signals

589 provide a non-invasive method of manipulating these components, providing an

590 opportunity to investigate how multiple display components interact with and

591 inform female choice. We demonstrate here that utilising an extended phenotype

592 signal in this way can provide insight into the mechanisms by which animals

593 balance investment in interacting signalling components in sexual displays.

594

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606

607 REFERENCES

608 **Andersson, M.** 1982. Sexual selection, natural selection and quality
609 advertisement. *Biological Journal Of The Linnean Society*, **17**, 375–393.

610 **Backwell, P. R. Y. & Passmore, N. I.** 1996. Time constraints and multiple choice
611 criteria in the sampling behaviour and mate choice of the fiddler crab, *Uca*
612 *annulipes. Behavioral Ecology and Sociobiology*, **38**, 407–416.

613 **Bakker, T. C. M. & Sevenster, P.** 1983. Determinants of dominance in male
614 sticklebacks (*Gasterosteus aculeatus* L.). *Behaviour*, **86**, 55–71.

615   **Barber, I., Nairn, D. & Huntingford, F. A.** 2001. Nests as ornaments: revealing
616   construction by male sticklebacks. *Behavioral Ecology*, **12**, 390–396.

617   **Bates, D, Maechler, M & Bolker, B**. 2011. lme4: Linear mixed-effects models
618   using S4 classes. R package version 0.999375-39. http://CRAN.R-
619   project.org/package=lme4

620   **Benjamini, Y. & Hochberg, Y.** 1995. Controlling the false discovery rate: a
621   practical and powerful approach to multiple testing. *Journal of the Royal*
622   *Statistical Society Series B-Methodological*, **57**, 289–300.

623   **Borgia, G.** 1995. Complex male display and female choice in the spotted
624   bowerbird: specialized functions for different bower decorations. *Animal*
625   *Behaviour*, **49**, 1291–1301.

626   **Boughman, J. W.** 2007. Condition-dependent expression of red colour differs
627   between stickleback species. *Journal of Evolutionary Biology*, **20**, 1577–1590.

628   **Braithwaite, V. A. & Barber, I.** 2000. Limitations to colour-based sexual
629   preferences in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behavioral*
630   *Ecology and Sociobiology*, **47**, 413–416.

631   **Bravery, B. D. & Goldizen, A. W.** 2007. Male satin bowerbirds (*Ptilonorhynchus*
632   *violaceus*) compensate for sexual signal loss by enhancing multiple display
633   features. *Naturwissenschaften*, **94**, 473–476.

634   **Bro-Jørgensen, J.** 2010. Dynamics of multiple signalling systems: animal
635   communication in a world in flux. *Trends In Ecology & Evolution*, **25**, 292–300.

636   **Candolin, U.** 1997. Predation risk affects courtship and attractiveness of
637   competing threespine stickleback males. *Behavioral Ecology and Sociobiology*, **41**,
638   81–87.

639   **Candolin, U.** 1999. Male-male competition facilitates female choice in
640   sticklebacks. *Proceedings of The Royal Society B-Biological Sciences*, **266**, 785–
641   789.

642   **Candolin, U.** 2003. The use of multiple cues in mate choice. *Biological Reviews*,
643   **78**, 575–595.

644   **Candolin, U. & Voigt, H.-R.** 1998. Predator-induced nest site preference: safe
645   nests allow courtship in sticklebacks. *Animal Behaviour*, **56**, 1205–1211.

646   **Candolin, U. & Voigt, H.-R.** 2001a. No effect of a parasite on reproduction in
647   stickleback males: a laboratory artefact? *Parasitology*, **122**, 457–464.

648   **Candolin, U. & Voigt, H.-R.** 2001b. Correlation between male size and territory
649   quality: consequence of male competition or predation susceptibility? *Oikos*, **95**,
650   225–230.

651   **Candolin, U., Salesto, T. & Evers, M.** 2007. Changed environmental conditions

652   weaken sexual selection in sticklebacks. *Journal of Evolutionary Biology*, **20**, 233–
653   239.

654   **Chapman, B. B., Morrell, L. J. & Krause, J.** 2009. Plasticity in male courtship
655   behaviour as a function of light intensity in guppies. *Behavioral Ecology and*
656   *Sociobiology*, **63**, 1757–1763.

657   **Christy, J. H. & Backwell, P. R. Y.** 2006. No preference for exaggerated courtship
658   signals in a sensory trap. *Animal Behaviour*, **71**, 1239–1246.

659   **Crawley, M.J.** 2007. *The R Book*. Wiley.

660   **Delcourt, M. & Rundle, H. D.** 2011. Condition dependence of a multicomponent
661   sexual display trait in Drosophila serrata. *The American Naturalist*, **177**, 812–
662   823.

663   **Garamszegi, L. Z.** 2004. Immune challenge mediates vocal communication in a
664   passerine bird: an experiment. *Behavioral Ecology*, **15**, 148–157.

665   **Goldschmidt, T., Bakker, T. C. M. & Feuth-de Bruijn, E.** 1993. Selective copying
666   in mate choice of female sticklebacks. *Animal Behaviour*, **45**, 541–547.

667   **Gordon, S. D. & Uetz, G. W.** 2011. Multimodal communication of wolf spiders on
668   different substrates: evidence for behavioural plasticity. *Animal Behaviour*, **81**,
669   367–375.

670   **Hansell, M.** 2005. *Animal architecture.* Oxford University Press.

671   **Hebets, E. A. & Papaj, D. R.** 2005. Complex signal function: developing a
672   framework of testable hypotheses. *Behavioral Ecology and Sociobiology*, **57**, 197–
673   214.

674   **Heuschele, J., Mannerla, M., Gienapp, P. & Candolin, U.** 2009. Environment-
675   dependent use of mate choice cues in sticklebacks. *Behavioral Ecology*, **20**, 1223–
676   1227.

677   **Humphries, S. & Ruxton, G. D.** 1999. Bower-building: coevolution of display
678   traits in response to the costs of female choice? *Ecology Letters*, **2**, 404–413.

679   **Ishikawa, M. & Mori, S.** 2000. Mating success and male courtship behaviors in
680   three populations of the threespine stickleback. *Behaviour*, **137**, 1065–1080.

681   **Jakobsson, S., Borg, B., Haux, C. & Hyllner, S. J.** 1999. An 11-ketotestosterone
682   induced kidney-secreted protein: the nest building glue from male three-spined
683   stickleback, *Gasterosteus aculeatus. Fish Physiology and Biochemistry*, **20**, 79–85.

684   **Kokko, H., Brooks, R., McNamara, J.M. & Houston, A.I.** 2002. The sexual
685   selection continuum. *Proceedings of the Royal Society of London* B 269:1331–
686   1340.

687   **Kraak, S. B. M., Bakker, T. C. M. & Hočevar, S.** 2000. Stickleback males,

688 especially large and red ones, are more likely to nest concealed in macrophytes.
689 *Behaviour*, **137**, 907–919.

690 **Lancaster, L. T., Hipsley, C. A. & Sinervo, B.** 2009. Female choice for optimal
691 combinations of multiple male display traits increases offspring survival.
692 *Behavioral Ecology*, **20**, 993–999.

693 **Lehtonen, T. K., Rintakoski, S. & Lindstrom, K.** 2007. Mate preference for
694 multiple cues: interplay between male and nest size in the sand goby,
695 *Pomatoschistus minutus. Behavioral Ecology*, **18**, 696–700.

696 **Lehtonen, T. K. & Wong, B. B.** 2009. Should females prefer males with elaborate
697 nests? *Behavioral Ecology*, **20**, 1015–1019.

698 **Li, S. K. & Owings, D. H.** 1978. Sexual selection in the three-spined stickleback: ii.
699 nest raiding during the courtship phase. *Behaviour*, **64**, 298–304.

700 **Loyau, A., Saint Jalme, M., Cagniant, C. & Sorci, G.** 2005. Multiple sexual
701 advertisements honestly reflect health status in peacocks (Pavo cristatus).
702 *Behavioral Ecology and Sociobiology*, **58**, 552–557.

703 **Madden, J. R.** 2003. Bower decorations are good predictors of mating success in
704 the spotted bowerbird. *Behavioral Ecology and Sociobiology*, **53**, 269–277.

705 **Magnhagen, C.** 1991. Predation risk as a cost of reproduction. *Trends In Ecology*
706 *& Evolution*, **6**, 183–186.

707 **Martin, C. H.** 2010. Unexploited females and unreliable signals of male quality in
708 a Malawi cichlid bower polymorphism. *Behavioral Ecology*, **21**, 1195–1202.

709 **Mclennan, D. A.** 2003. The importance of olfactory signals in the gasterosteid
710 mating system: sticklebacks go multimodal. *Biological Journal Of The Linnean*
711 *Society*, **80**, 555–572.

712 **Milinski, M. & Bakker, T. C. M.** 1990. Female sticklebacks use male coloration in
713 mate choice and hence avoid parasitized males. *Nature*, **344**, 330–333.

714 **Mori, S.** 1995. Factors associated with and fitness effects of nest-raiding in the
715 three-spined stickleback, *Gasterosteus aculeatus*, in a natural situation.
716 *Behaviour*, **132**, 1011–1023.

717 **Møller, A. P. & Pomiankowski, A.** 1993. Why have birds got multiple sexual
718 ornaments? *Behavioral Ecology and Sociobiology*, **32**, 167–176.

719 **Olsson, K. H., Kvarnemo, C. & Svensson, O.** 2009. Relative costs of courtship
720 behaviours in nest-building sand gobies. *Animal Behaviour*, **77**, 541–546.

721 **Ord, T. J., Peters, R. A., Clucas, B. & Stamps, J. A.** 2007. Lizards speed up visual
722 displays in noisy motion habitats. *Proceedings of The Royal Society B-Biological*
723 *Sciences*, **274**, 1057–1062.

724 **Östlund-Nilsson, S.** 2001. Fifteen-spined stickleback (*Spinachia spinachia*)
725 females prefer males with more secretional threads in their nests: an honest-
726 condition display by males. *Behavioral Ecology and Sociobiology*, **50**, 263–269.

727 **Östlund-Nilsson, S. & Holmlund, M.** 2003. The artistic three-spined stickleback
728 (*Gasterosteous aculeatus*). *Behavioral Ecology and Sociobiology*, **53**, 214–220.

729 **Peters, R. A., Hemmi, J. M. & Zeil, J.** 2007. Signaling against the wind: Modifying
730 motion-signal structure in response to increased noise. *Current Biology*, **17**,
731 1231–1234.

732 **R Development Core Team.** 2011. R: A language and environment for statistical
733 computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-
734 900051-07-0, URL http://www.R-project.org/.

735 **Rowe, M., Baube, C. & Phillips, J.** 2006. Trying to see red through stickleback
736 photoreceptors: Functional substitution of receptor sensitivities. *Ethology*, **112**,
737 218–229.

738 **Rowland, W. J.** 2000. Habituation and development of response specificity to a
739 sign stimulus: male preference for female courtship posture in stickleback.
740 *Animal Behaviour*, **60**, 63–68.

741 **Royle, N. J. & Pike, T. W.** 2010. Social feedback and attractiveness in zebra
742 finches. *Behavioral Ecology and Sociobiology*, **64**, 2015–2020.

743 **Rushbrook, B. J. & Barber, I.** 2006. Nesting, courtship and kidney hypertrophy
744 in *Schistocephalus*-infected male three-spined stickleback from an upland lake.
745 *Journal of Fish Biology*, **69**, 870–882.

746 **Rushbrook, B. J. & Barber, I.** 2008. A comparison of nest building by three-
747 spined sticklebacks *Gasterosteus aculeatus* from still and flowing waters. *Journal
748 of Fish Biology*, **73**, 746–752.

749 **Rushbrook, B. J., Dingemanse, N. J. & Barber, I.** 2008. Repeatability in nest
750 construction by male three-spined sticklebacks. *Animal Behaviour*, **75**, 547–553.

751 **Rushbrook, B. J., Head, M. L., Katsiadaki, I. & Barber, I.** 2010. Flow regime
752 affects building behaviour and nest structure in sticklebacks. *Behavioral Ecology
753 and Sociobiology*, **64**, 1927–1935.

754 **Sargent, R. C. & Gebler, J. B.** 1980. Effects of nest site concealment on hatching
755 success, reproductive success, and paternal behavior of the threespine
756 stickleback, *Gasterosteus aculeatus*. *Behavioral Ecology and Sociobiology*, **7**, 137–
757 142.

758 **Schaedelin, F. C. & Taborsky, M.** 2006. Mating craters of *Cyathopharynx furcifer*
759 (Cichlidae) are individually specific, extended phenotypes. *Animal Behaviour*, **72**,
760 753–761.

761 **Schaedelin, F. C. & Taborsky, M.** 2009. Extended phenotypes as signals.

762   *Biological Reviews Of The Cambridge Philosophical Society*, **84**, 293–313.

763   **Schaedelin, F. C. & Taborsky, M.** 2010. Female choice of a non-bodily ornament:
764   an experimental study of cichlid sand craters in *Cyathopharynx furcifer*.
765   *Behavioral Ecology and Sociobiology*, **64**, 1437–1447.

766   **Sergio, F., Blas, J., Blanco, G., Tanferna, A., Lopez, L., Lemus, J. A. & Hiraldo, F.**
767   2011. Raptor nest decorations are a reliable threat against conspecifics. *Science*,
768   **331**, 327–330.

769   **Smith, C. & Wootton, R. J.** 1999. Parental energy expenditure of the male three-
770   spined stickleback. *Journal of Fish Biology*, **54**, 1132–1136.

771   **Soler, J. J., Cuervo, J. J., Møller, A. P. & De Lope, F.** 1998. Nest building is a
772   sexually selected behaviour in the barn swallow. *Animal Behaviour*, **56**, 1435–
773   1442.

774   **Soler, J. J., de Neve, L., Martinez, J. G. & Soler, M.** 2001. Nest size affects clutch
775   size and the start of incubation in magpies: an experimental study. In: *Behavioral*
776   *Ecology*, Vol 12 pp. 301–307.

777   **Soler, M., Soler, J. J., Møller, A. P., Moreno, J. & Linden, M.** 1996. The functional
778   significance of sexual display: Stone carrying in the black wheatear. *Animal*
779   *Behaviour*, **51**, 247–254.

780   **van Doorn, G. S. & Weissing, F. J.** 2004. The evolution of female preferences for
781   multiple indicators of quality. *The American Naturalist*, **164**, 173–186.

782   **Van Iersel, J. J. A.** 1953. An analysis of the parental behaviour of the male three-
783   spined stickleback (*Gasterosteus aculeatus* L.). *Behaviour Supplement*, 1–159.

784   **von Frisch, K.** 1974. *Animal architecture.* Harcourt.

785   **Wootton, R. J.** 1984. *A functional biology of sticklebacks.* Berkeley: University of
786   Californa Press.

787   **Zuk, M., Ligon, J. D. & Thornhill, R.** 1992. Effects of experimental manipulation
788   of male secondary sex characters on female mate preference in red jungle fowl.
789   *Animal Behaviour*, **44**, 999–1006.

790

Artificial enhancement of an extended phenotype signal increases

investment in courtship

793   **Appendix**

794   **Table A1**: Results of the generalised linear mixed effects modelling analysis for

795   the field (behaviour) data, before the removal of non-significant interaction

796   terms.

Behaviour	Fixed effect	Estimate	Standard Error	*Z* value	*P* value
Building	(Intercept)	-1.3515	0.1881		
	Stage	-0.3566	0.1978	-1.803	0.072
	Treatment	0.0884	0.3021	0.293	0.770
	Interaction	-0.4226	0.3254	-1.299	0.194
Motionless	(Intercept)	-1.6426	0.2049		
	Stage	0.8598	0.2651	3.243	0.001
	Treatment	0.1808	0.3279	0.551	0.581
	Interaction	-0.0067	0.4265	-0.016	0.987
Foraging	(Intercept)	-2.1053	0.1966		
	Stage	-0.2238	0.1800	-1.243	0.214
	Treatment	-0.4336	0.3320	-1.306	0.192
	Interaction	-0.1490	0.3300	-0.452	0.651
Fanning	(Intercept)	-3.1898	0.4294		
	Stage	0.1226	0.3360	0.365	0.715
	Treatment	-0.6599	0.7138	-0.924	0.355
	Interaction	1.2764	0.5575	2.289	0.022
Gluing	(Intercept)	-3.5177	0.3407		
	Stage	-0.7858	0.3900	-2.015	0.044
	Treatment	-0.6625	0.5533	-1.198	0.231
	Interaction	0.3545	0.6360	0.557	0.577
Male presence	(Intercept)	-3.2587	0.2693		
	Stage	-0.2256	0.3795	-0.594	0.552
	Treatment	0.8450	0.3958	2.135	0.033
	Interaction	-0.9800	0.5935	-1.651	0.099
Female presence	(Intercept)	-4.0507	0.4653		
	Stage	-0.2493	0.5666	-0.440	0.660
	Treatment	0.1435	0.7410	0.194	0.846
	Interaction	0.6232	0.8672	0.719	0.472
Male-male	(Intercept)	-4.3067	0.2192		

aggression	Stage	-0.4567	0.2473	-1.847	0.065
	Treatment	0.2408	0.3499	0.688	0.491
	Interaction	-0.1798	0.3930	-0.457	0.647

797 In all cases, N = 52 observations from 26 individuals. In each case, the intercept is

798 the predicted value for control nests before the addition of threads. Minimum

799 adequate models (after model simplification via removal of non-significant

800 interactions) are presented in the main document. Behaviours where the

801 interaction term was significant following FDR control are not shown here. In

802 one case (fanning) the significant interaction term was rendered non-significant

803 following FDR control, and so was removed from the final model.

804

805

806 **Table A2:** Results of the linear mixed effects modelling analysis of the laboratory

807 (nest construction) data, before the removal of non-significant interaction terms.

808 The minimum adequate model is presented in the main document.

Fixed effect	Value	Standard Error	DF	*t* value	*P* value
(Intercept)	-0.8773	0.5063			
Treatment	1.7072	0.4362	13	3.9137	0.002
Male colour	0.8268	0.2579	13	3.2060	0.007
Interaction	-0.7169	0.4068	13	-1.7623	0.102

809

810 TABLES

811 **Table 1:** Results of the generalised linear mixed effects modelling analysis of the

812 field data.

813

Behaviour	Fixed effect	Estimate	Standard Error	Z value	Returned P	Adjusted P
Building	(Intercept)	-1.2791	0.1796			
	Stage	-0.5153	0.1614	-3.193	**0.001**	**0.009**
	Treatment	-0.1014	0.2644	-0.384	0.701	0.829
Motionless	(Intercept)	-1.6412	0.1856			
	Stage	0.8572	0.2077	4.128	**<0.001**	**<0.001**
	Treatment	0.1772	0.2416	0.733	0.463	0.669
Foraging	(Intercept)	-2.085	0.1909			
	Stage	-0.2687	0.1508	-1.782	0.075	0.177
	Treatment	-0.4991	0.2998	-1.665	0.096	0.208
Fanning	(Intercept)	-3.4865	0.4312			
	Stage	0.6207	0.3043	2.040	**0.041**	0.135
	Treatment	0.0691	0.6328	0.109	0.913	0.989
Gluing	(Intercept)	-3.5823	0.3214			
	Stage	-0.6537	0.3104	-2.106	**0.035**	0.131
	Treatment	-0.4919	0.4602	-1.069	0.285	0.495
Male	(Intercept)	-3.0997	0.2461			

presence	Stage	-0.631	0.3106	-2.032	**0.042**	0.122
	Treatment	0.4414	0.3116	1.416	0.157	0.313
Female presence	(Intercept)	-4.1934	0.4391			
	Stage	0.0112	0.4386	0.026	0.980	0.980
	Treatment	0.4599	0.6015	0.765	0.444	0.679
Male-male aggression	(Intercept)	-4.2732	0.2065			
	Stage	-0.5282	0.1932	-2.734	**0.006**	**0.034**
	Treatment	0.1556	0.2958	0.526	0.599	0.741
Male-female interactions	(Intercept)	-4.9660	0.5962			
	Stage	-1.6897	0.6715	-2.517	0.012	0.051
	Treatment	0.0319	0.9228	0.035	0.972	1.000
	Interaction	2.6992	0.8168	3.305	**<0.001**	**0.008**
Male-female interactions, controlling for female presence	(Intercept)	-0.3658	0.6183			
	Stage	1.3379	0.7495	1.785	0.074	0.193
	Treatment	0.7740	0.8176	0.947	0.344	0.559
	Interaction	-3.9049	1.1552	-3.380	**<0.001**	**0.009**

814 *P*-values are presented for both before ('returned *P*') and after ('adjusted *P*')

815 controlling for multiple testing using the Benjamini & Hochberg (1995)

816 procedure for false discovery rate control. Significant *P*-values are presented in

817 bold font. In all cases N = 52 observations from 26 individuals. In each case, the

818 intercept is the predicted value for control nests before the addition of threads.
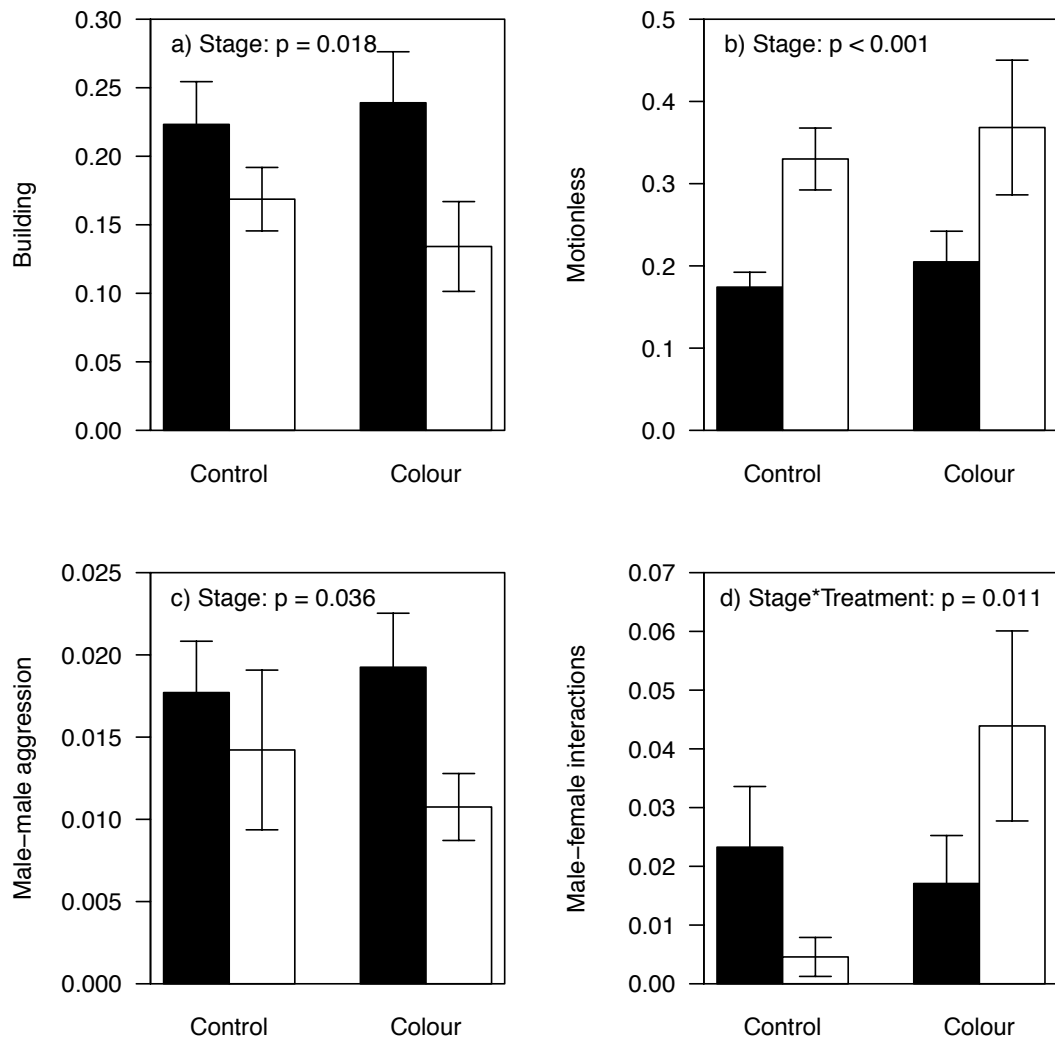
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Figure 1: Mean (± 1 S.E.) proportion of time where the focal male was **a**) building (point samples), **b**) motionless (point samples), **c)** engaged in an aggressive interaction with another male (time) and **d)** interacting with a female (point samples), for colour and control nests, during stage 1 (filled bars) and stage 2 (open bars). FDR-adjusted *P*-values are presented for significant effects.

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Figure 2: The effect of male colour and treatment on nest structure. Open symbols (data) and dashed lines (model predictions) are nests with coloured threads. Closed symbols and solid lines are nests with control threads. Note that the x and y axes are reversed, such that male colour increases from left to right along the x axis, and nest structure becomes neater and more compact towards the top of the y axis.
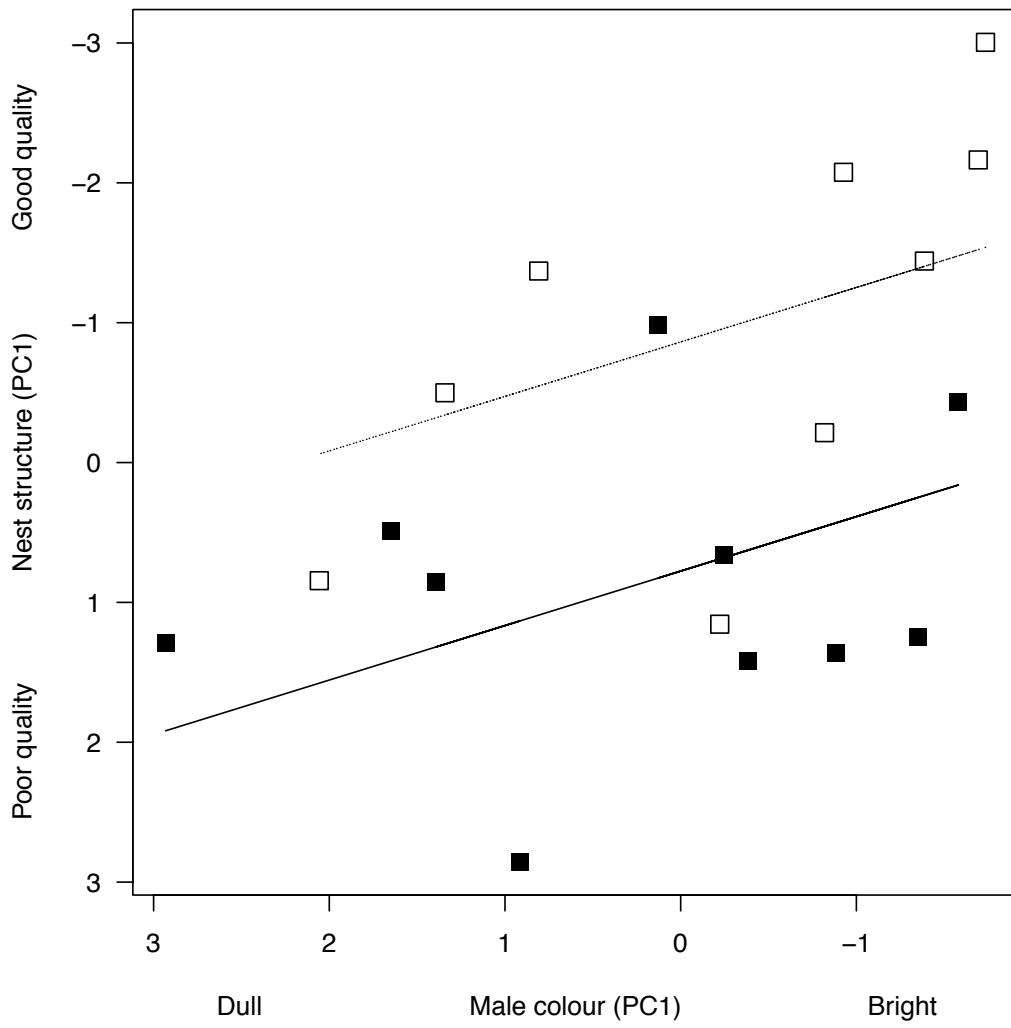
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835   Figure 1

a) Stage: p = 0.018

b) Stage: p < 0.001

c) Stage: p = 0.036

d) Stage*Treatment: p = 0.011

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837

838   Figure 2

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