1 **Mutually honest? Physiological 'qualities' signaled by color ornaments in**

2 **monomorphic king penguins**

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17

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19 Designed the study: VAV, FSD and PB. Did the fieldwork: BG, MK, SP, JPR and PB. Did

20 the lab work: VAV, AS, QS. Analyzed the data: VAV, CS and PB. Wrote the paper: VAV.

21 All authors contributed to its revision.

22

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24 ornament, oxidative stress, sexual selection, UV-signals

25

26   **ABSTRACT**

27   In species for which successful reproduction relies strongly on shared and substantial parental

28   investment by males and females, mate choice is expected to be important to the fitness of

29   both sexes. Reciprocal selection may then favor the evolution of morphological signals

30   providing mutual information on the condition/quality of tentative partners. However,

31   because males and females often have differing physiological constraints, it is unclear which

32   proximate physiological pathways guarantee the honesty of male and female signals in

33   similarly ornamented species. We used the monomorphic king penguin (*Aptenodytes*

34   *patagonicus*) as a model to investigate the physiological qualities signaled by color and

35   morphological ornaments known to be under sexual selection (coloration of the beak spots

36   and size of auricular feather patches). In both sexes of this slow-breeding seabird, we

37   investigated the links between ornaments and multiple indices of individual quality; including

38   body condition, immunity, stress and energy status. In both sexes, individual innate immunity,

39   resting metabolic rate, and the ability to mount a stress response in answer to an acute

40   disturbance (capture) were similarly signaled by various aspects of beak coloration or

41   auricular patch size. However, we also reveal interesting and contrasting relationships

42   between males and females in how ornaments may signal individual quality. Body condition

43   and oxidative stress status were signaled by beak coloration, though in opposite directions for

44   the sexes. Over an exhaustive set of physiological variables, several suggestive patterns

45   indicated conveyance of honest information about mate quality in this monomorphic species.

46   However, sex-specific patterns suggest that monomorphic ornaments may signal different

47   information concerning body mass and oxidative balance of males and females, at least in

48   king penguins.

49

50

INTRODUCTION

The evolutionary explanation for conspicuous and similar ornaments in both sexes (i.e. in sexually monomorphic ornamented species) has been a long-standing quandary in evolutionary biology (reviewed by Kraaijeveld et al. 2007). Two main hypotheses have been proposed to explain mutual ornamentation. The first suggests that female ornaments are non-functional, but arise as a by-product of genetic correlations between the sexes (Lande 1980, Price 1996). The second, mutual selection, suggests that functional ornaments may result from selection on their expression in both sexes. Processes that may select for both male and female ornaments include mimicry to conceal sexual identity (Burley 1981), mutual sexual selection for high quality partners (Hooper and Miller 2008), or social competition over non-mate resources in both sexes (West-Eberhard 1979, Tobias et al. 2012). As pointed out by Kraaijeveld et al. (2007), these processes are not mutually exclusive, as traits may be used in several contexts, for instance both in contests over resources (either mates or non-mate resources) and mate choice (Berglund et al. 1996).

Mutual sexual selection is expected when variance in reproductive success is similar between males and females, and when mate quality is an important predictor of variation in male and female success (Trivers 1972, Clutton-Brock and Vincent 1991), such as in slow breeding seabirds (e.g. Velando et al. 2001). Where both sexes should be choosy in their pairing preferences, ornaments may be favored because they assist the individual expressing them in acquiring a high quality mate, whereas preferences for ornaments may do the same for receivers (Johnstone et al. 1996, Kokko and Johnstone 2002, Hooper and Miller 2008). Furthermore, mating systems with extended mate-sampling periods are expected to lead to reduced mutual ornamentation ("dull monomorphism"; Badyaev and Qvarnström 2002, Badyaev and Hill 2003), whereas mating systems with short mate-sampling periods should favor extravagant "bright" monomorphism (Fitzpatrick 1994). However, because males and

76   females often differ in physiological constraints, the aspects of individual quality signaled and

77   of interest to receivers may differ between the sexes (Alvarez et al. 2005, Lopez et al. 2008).

78   For instance, in goldfinches (*Spinus tristis*), monomorphic bill coloration is correlated with

79   acquired immunity in females but not males, probably linked to the different functional roles

80   of beak coloration in male and female social communication (Kelly et al. 2012).

81   King penguins (*Aptenodytes patagonicus*) are monomorphic seabirds, where both

82   sexes experience a highly energy demanding breeding cycle (Groscolas and Robin 2001) and

83   cooperate for as long as 14 months to successfully raise a single chick (Stonehouse 1960).

84   Both males and females display conspicuous color ornaments including auricular feather

85   patches that only reflect yellow-orange colors, a breast feather patch that reflects yellow to

86   rusty-brown colors (Pincemy et al. 2009), and keratin beak spots on their lower mandibles

87   that reflect yellow-orange *and* UV color (Jouventin et al. 2005). Although it has been

88   previously demonstrated that feather and beak spot colorations are used in mate choice

89   (Pincemy et al. 2009, Nolan et al. 2010), little is known on the information carried by those

90   ornaments. We tested whether the ornaments of king penguins convey similar information in

91   both sexes in order to determine whether the condition-dependence of ornamental features

92   occurs only in one sex, suggesting that selection operates primarily in that sex and that

93   monomorphism is the outcome of genetic correlation between the sexes; or whether

94   condition-dependence occurs in both sexes (though not necessarily on the same ornaments

95   nor related to the same qualities) supporting the idea of mutual sexual selection. We aimed at

96   providing an extensive list of quality measures choosing key mediators of vertebrate life

97   histories expected to exhibit important associations with fitness. Those included body

98   condition, immune status, energy expenditure, hormonal stress status, hormonal and heart rate

99   stress responsiveness, and oxidative status (e.g. Norris and Evans 2000, Monaghan et al.

100   2009).

101   Because beak UV is important to pairing decisions for both male and female king

102   penguins (Nolan et al. 2010), we expected it to reflect information on individual quality in

103   both sexes. In contrast, larger auricular patches are more important to females during mate

104   choice (Pincemy et al. 2009, Dobson et al. 2011), but have also been positively linked to

105   social aggressiveness in both sexes (Viera et al. 2008). Thus, we expected auricular patch size

106   to yield information on male quality, or non-exclusively to signal male and female abilities to

107   cope with their aggressive colonial environment, including via physiological stress responses

108   (e.g. Parker et al. 2002, Bortolotti et al. 2009). Social competition has been suggested to favor

109   the evolution of ornaments as 'badges of status' that are used in alternative contexts to mate

110   choice (West-Eberhard 1979, Kraaijeveld et al. 2007). King penguins are known to

111   aggressively compete over breeding sites, and thus colored ornaments might convey

112   information about social dominance or aggressiveness (Viera et al. 2008, Keddar et al.

113   2015a). Specifically, given that males perform the first and longest reproductive fast of the

114   breeding cycle (typically 1-mo. including courtship and incubation; Stonehouse 1960),

115   information on body condition should be more important to females. We predicted that

116   ornamental features should be associated with body condition, especially in males. In

117   contrast, information relating to immunity should be particularly relevant to both sexes in this

118   species, since ticks (*Ixodes uriae*) are prevalent in king penguin colonies and detrimentally

119   affect adult and offspring fitness (Mangin et al. 2003, Bize P., Schull Q., Pardonnet S.,

120   Handrich Y., Criscuolo F., Viblanc V.A., Robin J.P., unpubl. data). Finally, stress status

121   (including oxidative stress; von Schantz et al. 1999) in relation to mate choice (e.g. parental

122   breeding quality; Angelier and Chastel 2009) or social territory acquisition should be

123   mutually important to males and females, and associated with ornamental traits in both sexes.

124

125   **METHODS**

Field site and study species

This study was conducted in the king penguin colony of *La Baie du Marin* (Possession Island, Crozet Archipelago; 46°25'S, 51°45'E) during the 2011-2012 breeding season (Dec.–Mar.). After an initial courtship period (~15 days), male and female penguins alternate periods fasting on-land and foraging at sea during incubation and chick-brooding (Stonehouse 1960). Hatching occurs after approximately 54-days and both parents alternate feeding and guarding duties on-land during most of the austral summer.

In early November (breeding onset), we captured 31 penguin pairs and marked them with non-permanent animal dye (Porcimark; Kruuse, Langeskov, Denmark) and plastic flipper-bands. Because of logistical constraints, all birds were caught after courtship, and had already undergone the mate choice and the pairing processes. We assumed that ornaments at mate choice were correlated with the moment at which we measured them, after birds had paired (see below). Accordingly, the size of the ear patch is determined at molt and beak measures at the start of breeding showed little within-individual variation compared to between individual variation (Schull Q., Viblanc V.A., Dobson F.S., Bize P., unpubl. data). Males (*N* = 31) were tagged during the first incubation shift, shortly after the female had departed to feed at sea. Females (*N* = 30) were tagged upon return from their foraging trip. Birds were observed daily from a distance, during the entire breeding season (Nov.–Mar.), to monitor their breeding status and determine sex-specific breeding shifts. All plastic flipper-bands were removed at the end of the study.

Morphometric measures

Flipper (± 1 mm) and beak length (± 0.1 mm) were measured using a solid metal ruler and dial calipers (Stonehouse 1960). Body girth (thoracic circumference) was measured (± 1 mm) with a flexible tape-ruler just below the upper articulation of the flippers to the body (Viblanc

151 et al. 2012a). Birds were measured at the onset of incubation shift 2 for females and

152 incubation shift 3 for males, to insure that both males and females had experienced similar

153 minimal fasting durations (2-3 days) on land.

154

155 **Ornament measures**

156 Standardized measures of the width and height of the right and left auricular feather patches

157 were performed using a flexible tape-ruler (see online supporting information S1). Left and

158 right distances were averaged and the surface of the patch was calculated as *width x height*

159 (mm^2).

160 Reflectance measurements of the beak spot were obtained using a portable JAZ

161 spectrophotometer (Ocean Optics Inc., Dunedin, FL, USA) with a spectral resolution of 0.3

162 nm across the spectral range 320-700 nm. The spectrophotometer contained a pulsed-xenon

163 light module and was calibrated against a white Spectralon standard. All measures were

164 performed using a 200 μm fiber probe with a 90° angle window. Measures were repeated 3

165 times across each bill plate (in the orange region from bill tip to base) and spectra were

166 averaged using an R script adapted from Montgomerie (2008). From spectral data, we

167 calculated tri-stimulus color variables: mean brightness, hue and chroma. We considered the

168 spectral range 320-700 nm, given the range of spectral sensitivity in birds (Cuthill 2006). The

169 reflectance of king penguin beak spots is characterized by a bimodal pattern including a

170 reflectance peak in UV and a peak/plateau in the yellow-orange (YO) portion of the spectrum

171 (see Fig. 1). Thus, we calculated color variables over wavelength sub-regions of interest. For

172 yellow-orange colors, we focused on the 500-700 nm portion of the spectrum. For the UV

173 peak, we focused on the range 320-450 nm. Although this region extends beyond UV

174 coloration *per se*, the choice was deliberate to account for the UV peak of king penguin beak

175 spots in its entirety (Jouventin et al. 2005). Mean brightness is a measure of spectral intensity

176 of the ornament, and yellow-orange and UV mean brightness were calculated by averaging

177 reflectance over wavelengths 500-700 nm and 320-450 nm, respectively (Montgomerie 2006).

178 Hue is a measure of color appearance (e.g. 'blue', 'yellow', etc.). For the YO plateau portion

179 of the spectrum, it was calculated as the wavelength at which the reflectance was halfway

180 between its maximum and minimum (Keddar et al. 2013). For the UV peak, hue was

181 calculated as the wavelength of maximum reflectance between 320 and 450 nm. Finally,

182 chroma is a measure of color purity and was calculated as the difference between maximum

183 and minimum reflectance over the mean reflectance for that particular region (formula S_8;

184 Montgomerie 2006).

185

186 **Body condition**

187 We used a principal component analysis to calculate a structural size index (SSI), which

188 explained 86% of the variation in beak size and flipper length (SSI = 0.95 x flipper + 0.31 x

189 beak). We then regressed body girth on this SSI ($F_{1,59}$ = 18.87, $P < 0.001$, $R^2 = 0.24$) and used

190 the residuals as an index of body condition. This method yields condition indices very similar

191 to classical mass/size regressions (correlation, r = 0.92; Viblanc et al. 2012a), but is more

192 practical than weighing birds within the breeding colony.

193

194 **Immunity measures**

195 Immune status was assessed from blood samples collected during the second incubation shift

196 of males and females. Blood (1 mL) was collected within 3-minutes of capture (see stress

197 protocol below) from the marginal flipper vein using a 0.7*40 mm, 22G needle fitted to a 5

198 mL heparinized syringe. Within 10 min of sampling, blood was centrifuged at 3000xg for 5

199 min separating plasma and blood cells. Samples were kept at -18°C until the end of the day

200 before being transferred at -80°C until lab-analyses. Constitutive innate humoral immunity

201   was determined using the hemolysis-hemagglutination assay described for birds (including

202   seabirds) by (Matson et al. 2005). This assay evaluates natural antibody (NAb) levels and

203   associated complement activation potential in plasma. Briefly, NAbs are innate non-specific

204   antibodies encoded by the germ line that react with virtually any antigen. They are naturally

205   present in antigen-naïve individuals, form a large portion of serum immunoglobulin, and

206   initiate the complement enzyme cascade that ends in cell lysis (Matson et al. 2005). We

207   exposed 25µL of penguin plasma (serially diluted from 1 to 1/1024) to 25µL of a 1% rabbit

208   blood cell suspension and scored lysis (Lysis titers) and agglutination (NAb titers) for each

209   sample. All assays were run on the same day and scored by the same observer (AS). Within

210   and among-assay variation was 2.4% and 7.5% for lysis, and 3.0% and 4.1% for agglutination

211   titers, respectively.

212

213   **Resting metabolic rate**

214   An estimate of bird's resting metabolic rate was obtained by measuring their daily resting

215   heart rate (rHR). The conversion of HR to VO_2 (the classic measure of metabolic rate) using

216   previously established calibrations is complicated by various issues including error

217   measurement (for a discussion see Green 2011). Thus, we used raw HR data as a qualitative

218   rather than quantitative index of metabolic rate in king penguins (Viblanc et al. 2014). We

219   attached external HR-loggers (Polar® RS800 and RS800CX, Polar Electro Oy, Kempele,

220   Finland) to breeding birds on the 6th day of their second incubation shift (shift 3 for males, N

221   = 26; shift 4 for females, N =24). Details on logger attachment, technology and accuracy of

222   HR measurement are provided elsewhere (Groscolas et al. 2010). Birds' HR was recorded for

223   48 hours (until day 8 of their incubation shift) at a rate of 1 value every 5 or 2 seconds

224   (depending on the logger model and memory). HR typically recovered to resting levels within

225   30 minutes of the initial capture stress (Viblanc et al. 2012b). We thus systematically

226   discarded the first 60 minutes of each recording to avoid confounding our calculations with

227   handling stress. We calculated daily rHR using moving averages to determine the 10

228   consecutive minutes where HR was lowest over 12-h periods. Daily rHR values were highly

229   repeatable (r = 0.95; Lessels & Boag 1987) and were averaged (Viblanc et al. 2014).

230

231   **Stress status**

232   We assessed penguins' stress status by measuring plasma total corticosterone (CORT), the

233   main glucocorticoid stress hormone in birds. We determined both basal total CORT levels

234   and acute total CORT increase to a standardized capture stress on the 8th day of second

235   incubation shift, at the same time that HR-loggers were removed. The capture stress was a

236   standardized approach starting > 25m away from the bird, before hooding and capturing it. At

237   the start of the approach, the experimenter insured that the bird was resting. The time at which

238   it became vigilant to the approaching experimenter was considered T_0 and a first blood

239   sample (as previously described) was made within the following 3-5 minutes. In king

240   penguins, plasma CORT levels do not significantly increase due to a capture-handling stress

241   within this time period (Ménard 1998). After initial blood sampling, the experimenter loosely

242   maintained the bird captive for 30 min and performed a second blood-sample at T_{30}.

243   Concentrations of plasma CORT were measured in duplicate using a quantitative competitive

244   sandwich enzyme immunoassay technique according to guidelines provided by the

245   manufacturer (ELISA Corsticosterone kit, Enzo Life Sciences, Farmingdale, NY, USA, ADI-

246   900-097). Kit sensitivity was 27.0 pg/mL, intra- and inter-assay variation were 7.6% and

247   13.3%, respectively. The CORT response to acute stress was calculated as $100*(CORT_{30} -$

248     $CORT_0)/CORT_0$.

249   During the standardized capture protocol we also measured HR response. We defined

250   the initial resting HR (HR_i) as the HR at the moment preceding a rapid constant increase in

251 HR due to the approaching experimenter (Viblanc et al. 2012b). Maximal HR (HR_{max}) in

252 response to the capture corresponded to the maximal HR achieved in the 3 minutes following

253 the onset of the stress. The maximum increase in HR was then calculated as $100*(HR_{max} -$

254 $HR_i)/HR_i$. HR-loggers were removed at the end of the stress.

255

256 **Oxidative status**

257 On the 8th day of the second incubation shift, we determined plasma oxidative status as

258 previously described for king penguins (Geiger et al. 2012). The antioxidant capacity of

259 penguin's plasma (OXY) and its concentration of reactive oxygen metabolites (ROM; a

260 measure of exposure to oxidative stress) were respectively measured using commercially

261 available OXY adsorbent and dROM kits (Diacron International srl, Grosseto, Italy). Intra-

262 and inter-assay variation was 7.4% and 7.0% for OXY, and 6.4% and 7.9% for ROM.

263

264 **Data analyses**

265 Analyses were performed using R v.3.0.2. All individuals only appeared once in the data set

266 and we had no repeated measures. First, we investigated male and female dimorphism by

267 considering the effect of sex on structural size, beak color variables and auricular patch

268 surface in linear models. For auricular patch surface, we also considered sexual dimorphism

269 controlling for structural size (specified as a covariate in the analysis). We then investigated

270 whether ornaments reflected physiological variables (*i.e.* could the birds "predict"

271 physiological quality from the ornaments) by running separate models for each physiological

272 trait and specifying beak color traits (hue, chroma and brightness) and auricular patch size as

273 predictor variables in our models. Sex was included as a cofactor in the analyses and its

274 interactions with beak coloration variables and auricular patch size were considered. The area

275 of the colony in which the bird was sampled (close to the beach or further up the valley) was

276   fixed as a cofactor in all analyses to account for known colony-related differences in parasites

277   and stress responses (Viblanc et al. 2012b). Independent variables were standardized prior to

278   analyses, so that model estimates were comparable (Schielzeth 2010). We used multi-model

279   inference with Akaike's Information Criterion corrected for small sample size to identify the

280   best model (AICc and AIC weights) for each physiological parameter considered ('dredge'

281   package in R; Bartoń 2015). We retained the most parsimonious model within potential

282   candidates (ΔAICc < 2). Models were compared using Maximum Likelihood. Because most

283   color variables were correlated to some extent (see online supporting information S2), we

284   insured collinearity was not an issue before performing model selection in our analyses. We

285   checked for variance inflation factors (VIFs) in the full model (suggested cut-off = 5; Zuur et

286   al. 2007). Yellow hue was the only variable which appeared problematic in all models, with

287    $7.2 < VIF < 9.4$. Thus, we removed it from all analyses, and subsequent collinearity was low

288    $(1.2 < VIFs < 5.2)$. Due to sampling and slight variations in success of laboratory analyses,

289   sample sizes varied across physiological measures. Diagnostic plots and the Shapiro-Wilk

290   normality test were used to inspect model residuals for normality and potential outliers. When

291   necessary (i.e. for resting HR and the acute CORT response), data were transformed prior to

292   analyses using Box-Cox power transformations (Viblanc et al. 2012b) to insure residual

293   normality. For each model, we calculated effect sizes (ES, Hedges' unbiased d and z-

294   transformed r) and their associated 95% confidence intervals based on respective t-statistics

295   using equations 10, 11, 14, 15, 17 and 19 from (Nakagawa and Cuthill 2007). We use the

296   benchmarks $r = 0.1, 0.3, 0.5$ and $d = 0.2, 0.5, 0.8$, to discuss small, medium and large effect

297   sizes (Nakagawa and Cuthill 2007).

298

299   **RESULTS**

300   **Male and female dimorphism in sexual ornaments**

301 Males were slightly but significantly larger than females (3-4% for flipper and beak,

302 respectively; Fig2; online supporting information S3), and had significantly larger auricular

303 patches (14%), even when accounting for structural size as a covariate in the model (Fig. 2).

304 Sexes did not differ significantly in terms of ornamental colors, except for UV chroma, which

305 was slightly higher in males (Fig. 2).

306

307 **Body condition and ornaments**

308 The most parsimonious model explaining body condition in breeding birds with the lowest

309 AICc and highest AIC weight retained beak UV brightness, yellow orange chroma, and their

310 interactions with sex as important factors (Table 1, see online supplementary material S4).

311 Patterns of association between beak UV brightness, yellow-orange chroma, and body

312 condition were different in males and females (Fig. 3, Table 1). Beak UV brightness was

313 weakly positively ($Zr = +0.29$; $CI_{95} = [-0.00, 0.58]$) related to body condition in males, but

314 moderately negatively in females ($Zr = -0.51$; $CI_{95} = [-0.22, -0.80]$) (Fig. 3A). Beak yellow-

315 orange chroma was moderately positively related to body condition in females ($Zr = +0.53$;

316 $CI_{95} = [0.24, 0.82]$), but not in males ($Zr = -0.06$; $CI_{95} = [-0.35, 0.23]$) (Fig. 3B).

317

318 **Oxidative status and ornaments**

319 UV hue, sex and their interaction were selected by AICc as important variables related to

320 ROM levels (Table 2, online supplementary material S5). In females, beak UV hue was

321 strongly negatively related to ROM levels ($Zr = -0.59$; $CI_{95} = [-0.20, -0.99]$), whereas the

322 association was positive in males, though the effect was weak as CI barely overlapped zero

323 ($Zr = +0.37$; $CI_{95} = [-0.02, 0.77]$) (Fig. 4). In contrast, OXY levels were not related to beak

324 coloration or auricular patch surface, *i.e.* only the intercept was retained in the best model

325 (online supplementary material S6).

326

Immunity and ornaments

The most parsimonious model retained YO beak chroma as a feature explaining variation in

lysis scores in both sexes, but no sex interaction (Table 3, online supplementary material S7).

YO chroma was weakly negatively ($\mathcal{Z}r = -0.24$; $CI_{95} = [-0.54, 0.05]$) related to lysis titers

(Fig. 5A). NAb titers were moderately negatively ($\mathcal{Z}r = -0.42$; $CI_{95} = [-0.72, -0.12]$) related to

patch surface in both sexes (again, no sex interaction) (Table 4, online supplementary material

S8) (see Fig. 5B).

Resting metabolic rate and ornaments

Model selection retained UV brightness as a variable related to daily resting HR, but no sex

interaction (Table 5, online supplementary material S9). UV brightness was moderately

positively ($\mathcal{Z}r = +0.35$; $CI_{95} = [0.05, 0.66]$) associated with daily resting HR levels (Fig. 6).

Stress and ornaments

Beak and patch ornaments did not relate to basal total CORT levels, as the best and most

parsimonious model only retained colony area as an important factor explaining CORT levels

($d_{unbiased} = +0.94$; $CI_{95} = [0.29, 1.59]$, see online supplementary material S10). Birds breeding

further up the valley had significantly higher basal CORT (3.56 ± 0.35 ng.mL^{-1}) levels than

birds breeding close to the beaches (2.15 ± 0.23 ng.mL^{-1}). For the birds' acute CORT

response to a standardized 30-min capture, model selection retained UV hue as a variable

explaining variation in the CORT response, but no sex interaction (Table 6; see online

supplementary material S11). UV hue ($\mathcal{Z}r = -0.37$; $CI_{95} = [-0.69, -0.06]$) was moderately

negatively related to the acute CORT response (Fig. 7). Finally, birds' HR response to capture

did not appear to be related to beak or auricular patch ornaments. Indeed, the best and most

351 parsimonious model only retained colony area as an important factor explaining variation in

352 birds' acute HR response to stress (d_{unbiased} = +0.59; CI_{95} = [-0.09, 1.26]; see online

353 supplementary material S12). Birds breeding up the valley had slightly higher HR responses

354 to captures (132.6 ± 8.1 %) than birds breeding close to the beaches (113.8 ± 11.6 %).

355

356 **DISCUSSION**

357 The two main hypotheses proposed to explain the evolution of elaborate ornamentation in

358 males and females are the 'genetic correlation' and the 'mutual selection' hypotheses

359 (Kraaijeveld et al. 2007). The former proposes that showy ornaments are functional in males,

360 but evolve as non-functional by-products of genetic correlations between the sexes in females

361 (Lande 1980). Selection then operates in males and the condition-dependence of ornaments

362 should be primarily related to the male sex. The latter proposes that ornaments are functional

363 in both sexes, evolving as honest signals of individual quality related to sexual or other, not

364 mutually-exclusive, forms of social selection (e.g. social competitiveness for breeding sites)

365 (Johnstone et al. 1996, Kokko and Johnstone 2002, Hooper and Miller 2008, Tobias et al.

366 2012). Although the genetic correlation hypothesis predicts that ornaments should convey

367 information mostly in males, the mutual selection hypothesis predicts that ornament should

368 convey information in both sexes.

369 In agreement with the mutual selection hypothesis, in king penguins we found that the

370 showy ornaments used in mate choice were related to various aspects of physiological quality

371 in both sexes. Successful breeding in this species involves obligate bi-parental care over an

372 extended 14-mo. period (Stonehouse 1960). Adults experience high annual divorce rates (up

373 to 81%; Olsson 1998) and courting birds encounter prospective mates at a high rate. Such

374 conditions provide scope for mutual choosiness (Johnstone et al. 1996, Kokko and Johnstone

375 2002) and are indeed expected to favor the evolution of ornamental signals reflecting

376   individual quality in both sexes (Kraaijeveld 2003, Kraaijeveld et al. 2007). However, we also

377   found that not all facets of physiological quality were similarly related to ornamentation in

378   both sexes, suggesting that mutual ornamentation may be maintained by varying selective

379   pressures in males and females (e.g. Murphy 2007).

380

381   **Mutual ornamentation and immunity**

382   One important cost of colonial breeding is parasitism (Mangin 2003). The

383   immunocompetence hypothesis predicts that, given limited resources (energy, nutrients,

384   protein), trade-offs occur between energy allocations to immunity or to the production and

385   maintenance of ornamentation (Saino et al. 1997, Verhulst et al. 1999). Consistently, we

386   found weak to moderate negative associations between measures of innate immunity and

387   ornamental features in both sexes. Lysis and NAb titers were negatively related to YO beak

388   chroma and auricular patch surface respectively suggesting that investing into larger auricular

389   patches and more YO beaks may incur a cost in terms of immunity. Interestingly, Nolan et al.

390   (2006) previously documented a link between the PHA skin test and breast coloration in

391   males, although they failed to detect an association with beak coloration or auricular patch

392   size. Unlike the PHA-test that measures a wide range of immune responses involving both

393   innate and acquired immunity (Tella et al. 2008), NAb titers reflect a well-defined component

394   of the innate immune response not induced by an experimental infection (Matson et al. 2005).

395   These findings support the notion that different ornaments may signal different components

396   of immunity in breeding birds (Kelly et al. 2012).

397

398   **Mutual ornamentation and body condition**

399   Acquiring information on body condition should be especially important to mate choice in

400   breeding seabirds that undergo extended periods of fasting while caring for the egg or chick

401 (Groscolas and Robin 2001). Surprisingly, we found that body condition was related to beak

402 spot coloration differently in males and females. Better body condition was associated with

403 lower UV brightness and higher YO chroma (both strong effects) in females, but higher UV

404 brightness (moderate effect) in males. These results are consistent with previous findings of

405 lower UV brightness for females in better body condition (Dobson et al. 2008), but at odds

406 with the idea that mutual selection for high UV reflectance occurs in both sexes (Nolan et al.

407 2010, Keddar et al. 2015b). One explanation is that males and females use beak spot signals

408 differently. As males have to endure the longest reproductive fast (Stonehouse 1960),

409 including courtship and the first incubation shift, choosing mates of high body condition

410 should be especially important for females. In females, poor body condition to an extent could

411 reflect greater investments into reproduction to the detriment of self-maintenance, which

412 should be favored by males. In females, body condition was negatively associated with

413 increasing UV brightness but positively associated with increasing YO chroma, raising

414 questions about the interactions between carotenoid and structural signals (Shawkey and Hill

415 2005, Mougeot et al. 2007, Dugas and McGraw 2011). For instance, in red grouse (Mougeot

416 et al. 2007) and nestling house sparrows (Dugas and McGraw 2011), carotenoid pigments

417 appear to act as a mask, decreasing UV reflectance in soft structures. There is some

418 suggestion that carotenoid pigments are also found in the beak of king penguins (see McGraw

419 et al. 2007), and similar interactions might explain the opposite relationships we find for beak

420 YO chroma and UV brightness. Further, only high condition females may have been able to

421 allocate carotenoid pigments to their beak spots to function as signals (Blount et al. 2003,

422 Mougeot et al. 2010).

423

424 **Mutual ornamentation and metabolic rate**

425 We found that beak UV brightness was positively (medium effect size) associated with

426 resting HR levels (a proxy for resting metabolic rate; Viblanc et al. 2014) in both sexes. High

427 resting metabolic rates may reflect increased capacities to engage in a suite of challenging

428 activities such as foraging, caring for the young or competing for ressources, and might be

429 honestly reflected by color ornaments (Biro and Stamps 2010, Kelly et al. 2012). The links

430 between UV coloration and metabolic rate may lie within the energy costs of

431 producing/maintaining structural colors (Siefferman and Hill 2005, Doutrelant et al. 2012).

432 For example, Siefferman and Hill (2005) showed that experimentally reducing the energy cost

433 of reproduction by reducing brood size in bluebirds (*Sialia sialis*) allowed males to increase

434 their investment into plumage UV in the subsequent year. Rather than a long-term energy

435 trade-off between competing functions (conserving energy for ornament production vs.

436 expanding it for current reproduction), our results suggest possible indirect metabolic costs,

437 such as keeping the beak clean, for UV maintenance.

438

439 **Mutual ornamentation and stress**

440 Glucocorticoid hormones (GC) play key roles in mediating physiological trade-offs and

441 energy allocation, and baseline GC levels have been suggested to ensure signal honesty

442 (Husak and Moore 2008, Weiss et al. 2013). Whereas we found no link between baseline

443 CORT and ornaments in our study, UV hue was moderately and negatively associated with

444 the birds' CORT response to acute stress ($Zr = -0.37$; $CI_{95} = [-0.69, -0.06]$). Birds with more

445 UV hued beaks mounted a greater stress response to capture. Because stress responses are

446 energy costly, this is consistent with the idea that the ability to mount stress responses while

447 fasting is reflected in ornamentation, which may be particularly relevant in the context of

448 colonial breeding during exposure to overt social aggressiveness (Côté 2000). In contrast, we

449 did not observe a link between ornaments and the acute HR response to stress, suggesting that

450   HPA and sympathetic stress pathways may be modulated and signaled independently in

451   breeding birds (e.g. Nephew et al. 2003). We found that birds up the valley mounted slightly

452   higher HR responses to capture, and had higher baseline CORT levels than birds breeding

453   close to the beach. These results suggest two alternatives: that birds breeding close to the

454   beach might have habituated to chronic human disturbance (Viblanc et al. 2012b), and that

455   birds up the valley may have been more exposed to parasites (Bize P., Schull Q., Pardonnet

456   S., Handrich Y., Criscuolo F., Viblanc V.A., Robin J.P., unpubl. data), Manipulating

457   circulating CORT levels in breeding birds may allow further exploration of the interplay

458   between ornamentation, glucocorticoids, and cardiovascular function. For instance, chronic

459   experimental increases in baseline stress levels (via CORT implants) have been shown to

460   negatively affect UV and orange-red reflectance in female striped plateau lizards (*Sceloporus*

461   *virgatus*) (Weiss et al. 2013).

462

463   **Mutual ornamentation and oxidative stress**

464   We observed sex-related differences in UV advertising for oxidative stress. In females, lower

465   UV hue (i.e., hue more strongly embedded in the peak UV wavelengths) was strongly and

466   positively associated with higher pro-oxidant levels (higher ROM but not higher OXY levels),

467   whereas the opposite occurred in males (a moderate effect and the CI overlapped zero). This

468   result was surprising for a structural color, as links between ornamentation and oxidative

469   status are expected for yellow-orange colors, because of the allocation trade-off of carotenoid

470   pigments to either anti-oxidant or ornamental functions (von Schantz et al. 1999, Mougeot et

471   al. 2010). However, the interplay between UV and yellow-orange color reflectance might also

472   convey information on carotenoid availability (Jacot et al. 2010). Carotenoids absorb

473   wavelengths of short to medium wavelengths (400-515 nm), and greater deposition of

474   carotenoids in feathers has been experimentally shown to cause a shift in the UV peak to

475 shorter wavelengths in great tits (Jacot et al. 2010). The precise link between carotenoid

476 concentration and beak reflectance both in UV and YO wavelengths remains to be determined

477 in king penguins. But our result may suggest that females depositing more carotenoids in their

478 beak suffered from greater oxidative stress, highlighting a trade-off between pigment

479 allocation to anti-oxidant defenses or beak coloration. The exhaustive measurement of

480 oxidative status of breeding birds requires supplementary markers of oxidative damage and

481 antioxidant defense (*e.g.* lipid peroxidation, antioxidant enzymatic activity), and preferentially

482 in different tissues (Selman et al. 2012). However, our results add to the evidence that

483 condition-dependent UV signals indeed occur in many bird species (Keyser and Hill 2000,

484 Bize et al. 2006, Mougeot et al. 2010), likely in interaction with carotenoid signaling.

485

486 **Conclusion**

487 Taken together our results suggest that monomorphic ornamentation reflects several aspects

488 of physiological quality in king penguins, supporting the mutual selection hypothesis.

489 Interestingly, the qualities signaled by mutual ornamentation may nonetheless differ (in fact

490 be opposite) between the sexes, likely due to physiological differences and varying selection

491 pressures. Because we collected the physiological and ornamental measures only at only one

492 point in time, it remains to be explored if some of those traits are dynamic (e.g. beak

493 coloration: Faivre et al. 2003, Pham et al. 2014) and whether birds may use them for short-

494 term behavioral decisions. The further study of monomorphic species should shed new

495 insights on the maintenance, information and costs of sexual signals.

496

507 **FIGURE CAPTIONS**

508 **Fig 1. Reflectance curve obtained from the beak spot of a breeding king penguin**

509 **(*Aptenodytes patagonicus*).** Note the typical bi-modal pattern with a UV-peak around 380-

510 390 nm and a yellow-orange plateau from 500-700 nm.

511 **Fig 2. Effect sizes and 95% confidence intervals for ornamental and structural size**

512 **dimorphism between king penguin males and females.** Effect sizes and 95% CI were

513 calculated after Nakagawa & Cuthill 2007. Effects are considered significant if their 95% CI

514 does not overlap zero.

515 **Fig 3. Relationships between beak coloration and body condition in breeding king**

516 **penguins.** Relationships are given for (A) beak UV brightness, and (B) beak yellow-orange

517 chroma. Females are depicted by open circles and a full line, males by filled circles and a

518 dashed line. The right panel provides effect sizes and 95% CI calculated after Nakagawa &

519 Cuthill 2007. Effects are considered significant if their 95% CI does not overlap zero.

520 **Fig 4. Relationship between beak coloration and standardized plasma concentration of**

521 **reactive oxygen metabolites [ROM] in breeding king penguins.** Females are depicted by

522 open circles and a full line, males by filled circles and a dashed line. The lower panel provides

523 effect sizes and 95% CI calculated after Nakagawa & Cuthill 2007. Effects are considered

524 significant if their 95% CI does not overlap zero.

525 **Fig 5. Relationship between beak coloration, auricular patch surface and innate**

526 **immunity in breeding king penguins.** Relationships are given for (A) plasma lysis titers and

527 yellow-orange chroma, and (B) plasma Nab titers and auricular patch surface. On the left

528 panel, males are depicted by filled circles, females by open circles. The right panel provides

529 effect sizes and 95% CI calculated after Nakagawa & Cuthill 2007. Effects are considered

530 significant if their 95% CI does not overlap zero.

Fig 6. Relationship between beak UV brightness and daily resting HR levels (bpm) in breeding king penguins. On the left panel, males are depicted by filled circles, females by open circles. The right panel provides effect sizes and 95% CI calculated after Nakagawa & Cuthill 2007. Effects are considered significant if their 95% CI does not overlap zero.

Fig 7. Relationship between the relative corticosterone increase in response to a standardized 30 minute capture and beak UV hue in breeding king penguins. On the left panels, males are depicted by filled circles, females by open circles. The right panel provides effect sizes and 95% CI calculated after Nakagawa & Cuthill 2007. Effects are considered significant if their 95% CI does not overlap zero.

REFERENCES

Alvarez F, Sanchez C, Angulo S. 2005. The frontal shield of the moorhen: sex differences and relationship with body condition. *Ethology Ecology & Evolution* **17**: 135-148.

Angelier F, Chastel O. 2009. Stress, prolactin and parental investment in birds: A review. *General and Comparative Endocrinology* **163**: 142-148.

Badyaev AV, Hill GE. 2003. Avian sexual dichromatism in relation to phylogeny and ecology. *Annual Review of Ecology and Systematics* **34**: 27-49.

Badyaev AV, Qvarnström A. 2002. Putting sexual traits into the context of an organism: a life history perspective in studies of sexual selection. *Auk* **119:** 301-310.

Bartoń K. 2015. MuMln: multi-model inference. R package, version 1.13.14. Available at: http://CRAN.R-project.org/package=MuMIn.

Berglund A, Bisazza A, Pilastro A. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biological Journal of the Linnean Society* **58**:385-399.

Bize P, Piault R, Moureau B, Heeb P. 2006. A UV signal of offspring condition mediates context-dependent parental favouritism. *Proceedings of the Royal Society B Biological Sciences* **273**:2063-2068.

Biro PA, Stamps JA. 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution* **25**: 653-659.

Blount JD, Metcalfe NB, Birkhead TR, Surai, PF. 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300**:125-127.

Bortolotti GR, Mougeot F, Martinez-Padilla J, Webster LMI, Piertney SB. 2009. Physiological stress mediates the honesty of social signals. *Plos One* **4**: e4983.

Burley N. 1981. The evolution of sexual indistinguishability. Pages 121-137 *in* R. D. Alexander and D. W. Tinkle, editors. Natural selection and social behaviour: recent research and new theory. Chiron Press, New York.

569 **Clutton-Brock TH, Vincent ACJ. 1991.** Potential reproductive rates and the
570 operation of sexual selection. *Quarterly Review of Biology* **67**:437-456.
571 **Côté SD. 2000.** Aggressiveness in king penguins in relation to reproductive status
572 and territory location. *Animal Behaviour* **59**:813-821.
573 **Cuthill IC. 2006.** Color perception. Pages 3-40 *in* G. E. Hill and K. J. McGraw, editors.
574 Bird coloration, volume 1: Mechanisms and measurements. Harvard University Press,
575 Cambridge, MA.
576 **Dobson FS, Couchoux C, Jouventin P. 2011.** Sexual Selection on a Coloured
577 Ornament in King Penguins. *Ethology* **117**:872-879.
578 **Dobson FS, Nolan PM, Nicolaus M, Bajzak C, Coquel A.-S., Jouventin P. 2008.**
579 Comparison of color and body condition between early and late breeding king penguins.
580 *Ethology* **114**: 925-933.
581 **Doutrelant C, Gregoire A, Midamegbe A, Lambrechts M, Perret P. 2012.** Female
582 plumage coloration is sensitive to the cost of reproduction. An experiment in blue tits.
583 *Journal of Animal Ecology* **81**:87-96.
584 **Dugas MB, McGraw KJ. 2011.** Proximate correlates of carotenoid-based mouth
585 coloration in nestling house sparrows. *Condor* **113**:691-700.
586 **Faivre B, Gregoire A, Preault M, Cezilly F, Sorci G. 2003.** Immune activation
587 rapidly mirrored in a secondary sexual trait. *Science* **300**:103-103.
588 **Fitzpatrick S. 1994.** Colourful migratory birds: evidence for a mechanism other than
589 parasite resistance for maintenance of 'good genes' sexual selection. *Proceedings of the
590 Royal Society B Biological Sciences* **257**:155-160.
591 **Geiger S, Le Vaillant M, Lebard T, Reichert S, Stier A, Le Maho Y, Criscuolo F.
592 2012.** Catching-up but telomere loss: half-opening the black box of growth and ageing
593 trade-off in wild king penguin chicks. *Molecular Ecology* **21**:1500-1510.
594 **Green JA. 2011.** The heart rate method for estimating metabolic rate: Review and
595 recommendations. *Comparative Biochemistry and Physiology A Molecular & Integrative
596 Physiology* **158**:287-304.
597 **Groscolas R, Robin JP. 2001.** Long-term fasting and re-feeding in penguins.
598 *Comparative Biochemistry and Physiology A Molecular & Integrative Physiology* **128**:645-
599 655.
600 **Groscolas R, Viera VM, Guerin N, Handrich Y, Côté SD. 2010.** Heart rate as a
601 predictor of energy expenditure in undisturbed fasting and incubating penguins. *Journal
602 of Experimental Biology* **213**:153-160.
603 **Hill GE, McGraw KJ. 2006.** Bird coloration, volume 1: mechanisms and
604 measurements. Harvard University Press, Cambridge, MA.
605 **Hooper PL, Miller GF. 2008**. Mutual mate choice can drive costly signaling even
606 under perfect monogamy. *Adaptive Behavior* **16**:53-70.
607 **Husak JF, Moore IT.** 2008. Stress hormones and mate choice. *Trends in Ecology &
608 Evolution* **23**:532-534.
609 **Jacot A, Romero-Diaz C, Tschirren B, Richner H, Fitze PS. 2010**. Dissecting
610 carotenoid from structural components of carotenoid-based coloration: a field
611 experiment with Great Tits (*Parus major*). *American Naturalist* **176**: 55-62.
612 **Johnstone RA, Reynolds JD, Deutsch JC. 1996.** Mutual mate choice and sex
613 differences in choosiness. *Evolution* **50**:1382-1391.
614 **Jouventin P, Nolan PM, Ornborg J, Dobson FS. 2005.** Ultraviolet beak spots in King
615 and Emperor penguins. *Condor* **107**:144-150.
616 **Keddar I, Jouventin P, Dobson FS. 2015a.** Color ornaments and territory position in
617 king penguins. *Behavioural Processes* **119**:32-37.

618   **Keddar I, Altmeyer S, Couchoux C, Jouventin P, Dobson FS. 2015b.** Mate choice
619   and colored beak spots of king penguins. *Ethology* **in press**.
620   **Keddar I, Andris M, Bonadonna F, Dobson FS. 2013.** Male-Biased Mate
621   Competition in King Penguin Trio Parades. *Ethology* **119**:389-396.
622   **Kelly RJ, Murphy TG, Tarvin KA, Burness G. 2012.** Carotenoid-based ornaments of
623   female and male American goldfinches (*Spinus tristis*) show sex-specific correlations
624   with immune function and metabolic rate. *Physiological and Biochemical Zoology*
625   **85**:348-363.
626   **Keyser AJ, Hill GE. 2000.** Structurally based plumage coloration is an honest signal
627   of quality in male blue grosbeaks. *Behavioral Ecology* **11**:202-209.
628   **Kokko H, Johnstone RA. 2002.** Why is mutual mate choice not the norm?
629   Operational sex ratios, sex roles and the evolution of sexually dimorphic and
630   monomorphic signalling. *Philosophical Transactions of the Royal Society of London Series*
631   *B Biological Sciences* **357**:319-330.
632   **Kraaijeveld K. 2003.** Degree of mutual ornamentation in birds is related to divorce
633   rate. *Proceedings of the Royal Society B Biological Sciences* **270**:1785-1791.
634   **Kraaijeveld K, Kraaijeveld-Smit FJL, Komdeur J. 2007.** The evolution of mutual
635   ornamentation. *Animal Behaviour* **74**:657-677.
636   **Lande R. 1980.** Sexual dimorphism, sexual selection, and adaptation in polygenic
637   characters. *Evolution* **34**:292-305.
638   **Lopez G, Figuerola J, Soriguer R. 2008.** Carotenoid-based masks in the European
639   goldfinch *Carduelis carduelis* reflect different information in males and females. *Ardea*
640   **96**:233-242.
641   **Mangin S, Gauthier-Clerc M, Frenot Y, Gendner J.-P., Le Maho Y. 2003.** *Ticks Ixodes*
642   *uriae* and the breeding performance of a colonial seabird, king penguin Aptenodytes
643   patagonicus. *Journal of Avian Biology* **34**:30-34.
644   **Matson KD, Ricklefs RE, Klasing KC. 2005.** A hemolysis-hemagglutination assay for
645   characterizing constitutive innate humoral immunity in wild and domestic birds.
646   *Developmental and Comparative Immunology* **29**:275-286.
647   **McGraw KJ, Toomey MB, Nolan PM, Morehouse NI, Massaro M, Jouventin P.**
648   **2007.** A description of unique fluorescent yellow pigments in penguin feathers. *Pigment*
649   *Cell Research* **20**:301-304.
650   **Ménard JJ. 1998.** Conséquences hormonales et métaboliques du stress de contention
651   chez le manchot royal (*Aptenodytes patagonicus*). Université Paul Sabatier, Toulouse,
652   France.
653   **Monaghan P, Metcalfe NB, Torres R. 2009.** Oxidative stress as a mediator of life
654   history trade-offs: mechanisms, measurements and interpretation. *Ecology Letters*
655   **12**:75-92.
656   **Montgomerie R. 2006.** Analyzing colors. Pages 90-147 *in* G. E. Hill and K. J. McGraw,
657   editors. Bird coloration, Volume 1: Mechanisms and measurements. Harvard University
658   Press, Cambridge, MA.
659   **Montgomerie R. 2008.** RCLR. Queen's University, Kingston, Canada.
660   **Mougeot F, Martinez-Padilla J, Blount JD, Perez-Rodriguez L, Webster LMI,**
661   **Piertney SB. 2010.** Oxidative stress and the effect of parasites on a carotenoid-based
662   ornament. *Journal of Experimental Biology* **213**:400-407.
663   **Mougeot F, Martinez-Padilla J, Perez-Rodriguez L, Bortolotti GR. 2007.**
664   Carotenoid-based colouration and ultraviolet reflectance of the sexual ornaments of
665   grouse. *Behavioral Ecology and Sociobiology* **61**:741-751.

666   **Murphy TG. 2007.** Racketed tail of the male and female turquoise-browed momot:
667 male but not female tail length correlates with pairing success, performance, and
668 reproductive success. *Behavioral Ecology and Sociobiology* **61**:911-918.
669   **Nakagawa S., Cuthill IC. 2007.** Effect size, confidence interval and statistical
670 significance: a practical guide for biologists. *Biological Reviews* **82**:591-605.
671   **Nephew BC, Kahn SA, Romero LM, 2003.** Heart rate and behavior are regulated
672 independently of corticosterone following diverse acute stressors. *General and*
673 *Comparative Endocrinology* **132**: 172-180.
674   **Nolan PM, Dobson FS, Dresp B, Jouventin P. 2006.** Immunocompetence is signalled
675 by ornamental colour in king penguins, *Aptenodytes patagonicus. Evolutionary Ecology*
676 *Research* **8**:1-8.
677   **Nolan PM, Dobson FS, Nicolaus M, Karels TJ, McGraw KJ, Jouventin P. 2010.**
678 Mutual mate choice for colorful traits in king penguins. *Ethology* **116**:635-644.
679   **Norris K, Evans MR. 2000.** Ecological immunology: life history trade-offs and
680 immune defense in birds. *Behavioral Ecology* **11**:19-26.
681   **Olsson O. 1998.** Divorce in king penguins: asynchrony, expensive fat storing and
682 ideal free mate choice. *Oikos* **83**:574-581.
683   **Parker TH, Knapp R, Rosenfield JA. 2002.** Social mediation of sexually selected
684 ornamentation and steroid hormone levels in male junglefowl. *Animal Behaviour*
685 **64**:291-298.
686   **Pham TT, Queller PS, Tarvin KA, Murphy TG. 2014.** Honesty of a dynamic female
687 aggressive status signal: baseline testosterone relates to bill color in female American
688 goldfinches. *Journal of Avian Biology* **45**:22-28.
689   **Pincemy G, Dobson FS, Jouventin P. 2009.** Experiments on colour ornaments and
690 mate choice in king penguins. *Animal Behaviour* **78**:1247-1253.
691   **Price DK. 1996.** Sexual selection, selection load and quantative genetics of zebra
692 finch bill colour. *Proceedings of the Royal Society B Biological Sciences* **263**:217-221.
693   **Saino N, Bolzern AM, Møller AP. 1997.** Immuno-competence, ornamentation and
694 viability of male barn swallows (*Hirundo rustica*). *Proceedings of the National Academy of*
695 *Sciences of the United States of America* **94**:579-585.
696   **Schielzeth H. 2010.** Simple means to improve the interpretability of regression
697 coefficients. *Methods in Ecology and Evolution* **1**:103-113.
698   **Selman C, Blount JD, Nussey DH, Speakman JR. 2012.** Oxidative damage, ageing,
699 and life-history evolution: where now? *Trends in Ecology & Evolution* **27**: 570-577.
700   **Shawkey MD, Hill GE. 2005.** Carotenoids need structural colors to shine. *Biology*
701 *Letters* **1**:121-124.
702   **Siefferman L, Hill GE. 2005.** Male eastern bluebirds trade future ornamentation for
703 current reproductive investment. *Biology Letters* **1**:208-211.
704   **Stonehouse B. 1960.** The king penguin *Aptenodytes patagonicus* of South Georgia I.
705 Breeding behavior and development. *Falkland Island Dependency Survey Scientific Report*
706 **23**:1-83.
707   **Tella JL, Lemus JA, Carrete M, Blanco G. 2008.** The PHA Test Reflects Acquired T-
708 Cell Mediated Immunocompetence in Birds. *Plos One* **3**: e3295.
709   **Tobias JA, Montgomerie R, Lyon BE. 2012**. The evolution of female ornaments and
710 weaponry: social selection, sexual selection and ecological competition. *Philosophical*
711 *Transactions of the Royal Society B Biological Sciences* **367**:2274-2293.
712   **Trivers RL. 1972.** Parental investment and sexual selection. Pages 136-179 *in* B.
713 Campbell, editor. Sexual selection and the descent of man. Aldine, Chicago.

714 **Velando A, Lessells CM, Márquez JC. 2001.** The function of female and male
715 ornaments in the Inca Tern: evidence for links between ornament expression and both
716 adult condition and reproductive performance. *Journal of Avian Biology* **32**:311-318.
717 **Verhulst S, Dieleman SJ, Parmentier HK. 1999.** A tradeoff between
718 immunocompetence and sexual ornamentation in domestic fowl. *Proceedings of the*
719 *National Academy of Sciences of the United States of America* **96**:4478-4481.
720 **Viblanc VA, Bize P, Criscuolo F, Le Vaillant M, Saraux C, Pardonnet S, Gineste B,**
721 **Kauffmann M, Prud'homme O, Handrich Y, Massemin S, Groscolas R, Robin JP.**
722 **2012a.** Body girth as an alternative to body mass for establishing condition indexes in
723 field studies: a validation in the king penguin. *Physiological and Biochemical Zoology*
724 **85**:533-542.
725 **Viblanc VA, Smith AD, Gineste B, Groscolas R. 2012b.** Coping with continuous
726 human disturbance in the wild: insights from penguin heart rate response to various
727 stressors. *BMC Ecology* **12**:10.
728 **Viblanc VA, Saraux C, Malosse N, Groscolas R. 2014.** Energetic adjustments in
729 freely breeding-fasting king penguins: does colony density matter? *Functional Ecology*
730 **28**:621-631.
731 **Viera VM, Nolan PM, Côté SD, Jouventin P, Groscolas R. 2008.** Is territory defence
732 related to plumage ornaments in the king penguin Aptenodytes patagonicus? *Ethology*
733 **114**:146-153.
734 **von Schantz T, Bensch S, Grahn M, Hasselquist D, Wittzell H. 1999.** Good genes,
735 oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society*
736 *B Biological Sciences* **266**:1-12.
737 **Weiss SL, Mulligan EE, Wilson DS, Kabelik D. 2013.** Effect of stress on female-
738 specific ornamentation. *Journal of Experimental Biology* **216**:2641-2647.
739 **West-Eberhard MJ. 1979.** Sexual Selection, Social Competition, and Evolution.
740 *Proceedings of the American Philosophical Society* **123**:222-234.
741 **Zuur AF, Ieno EN, Smith GM. 2007.** Analysing ecological data. Springer, New York.
742

743

TABLES

	Estimate	Std. Error	t value
Intercept	-2.00	0.53	-3.73
Sex[M]	2.46	0.55	4.45
UV brightness	-1.59	0.45	-3.51
YO chroma	1.71	0.47	3.61
Colony area [A1]	0.82	0.58	1.41
Sex[M]*UV brightness	2.34	0.59	3.99
Sex[M]*YO chroma	-1.85	0.59	-3.11

Table 1. Model estimates for the influence of beak color variables on body condition in breeding king penguin (*Aptenodytes patagonicus*). The sex effect is given in reference to the female level [F]. The colony area effect is given in reference to area [A2]. See Fig. 3 for effect sizes with 95% CI.

	Estimate	Std. Error	t value
Intercept	2.43	0.20	12.10
Sex[M]	0.18	0.27	0.66
UV hue	-0.50	0.16	-3.20
Sex[M]*UV hue	0.93	0.27	3.48

Table 2. Model estimates for the influence of beak UV hue on plasma reactive oxygen metabolite levels in breeding king penguin (*Aptenodytes patagonicus*). The sex effect is given in reference to the female level [F]. See Fig. 4 for effect sizes with 95% CI.

	Estimate	Std. Error	t value
Intercept	3.33	0.14	23.13
YO chroma	-0.15	0.09	-1.70
Colony area [A1]	-0.65	0.18	-3.55

Table 3. Model estimates for the influence of beak YO chroma on plasma lysis titers in breeding king penguin (*Aptenodytes patagonicus*). The colony area effect is given in reference to area [A2]. See Fig. 5A for effect sizes with 95% CI.

	Estimate	Std. Error	t value
Intercept	5.46	0.24	22.32
Patch surface	-0.47	0.16	-2.94
Colony area [A1]	-0.85	0.31	-2.70

Table 4. Model estimates for the influence of auricular patch surface on plasma NAb titers in breeding king penguin (*Aptenodytes patagonicus*). The colony area effect is given in reference to area [A2]. See Fig. 5B for effect sizes with 95% CI.

	Estimate	Std. Error	t value
Intercept	5.97	0.07	83.36
UV brightness	0.11	0.05	2.40
Colony area [A1]	-0.07	0.09	-0.72

Table 5. Model estimates for the influence of UV brightness on daily resting heart rate in breeding king penguin (*Aptenodytes patagonicus*). The colony area effect is given in reference to area [A2]. See Fig. 6 for effect sizes with 95% CI.

	Estimate	Std. Error	t value
Intercept	2.42	0.24	10.25
UV hue	-0.36	0.15	-2.45
Colony area [A1]	-1.16	0.31	-3.74

Table 6. Model estimates for the influence of beak UV hue on the acute relative increase in plasma total corticosterone levels in response to a standardized 30-min capture in breeding king penguin (*Aptenodytes patagonicus*). The colony area effect is given in reference to area [A2]. See Fig. 7 for effect sizes with 95% CI.

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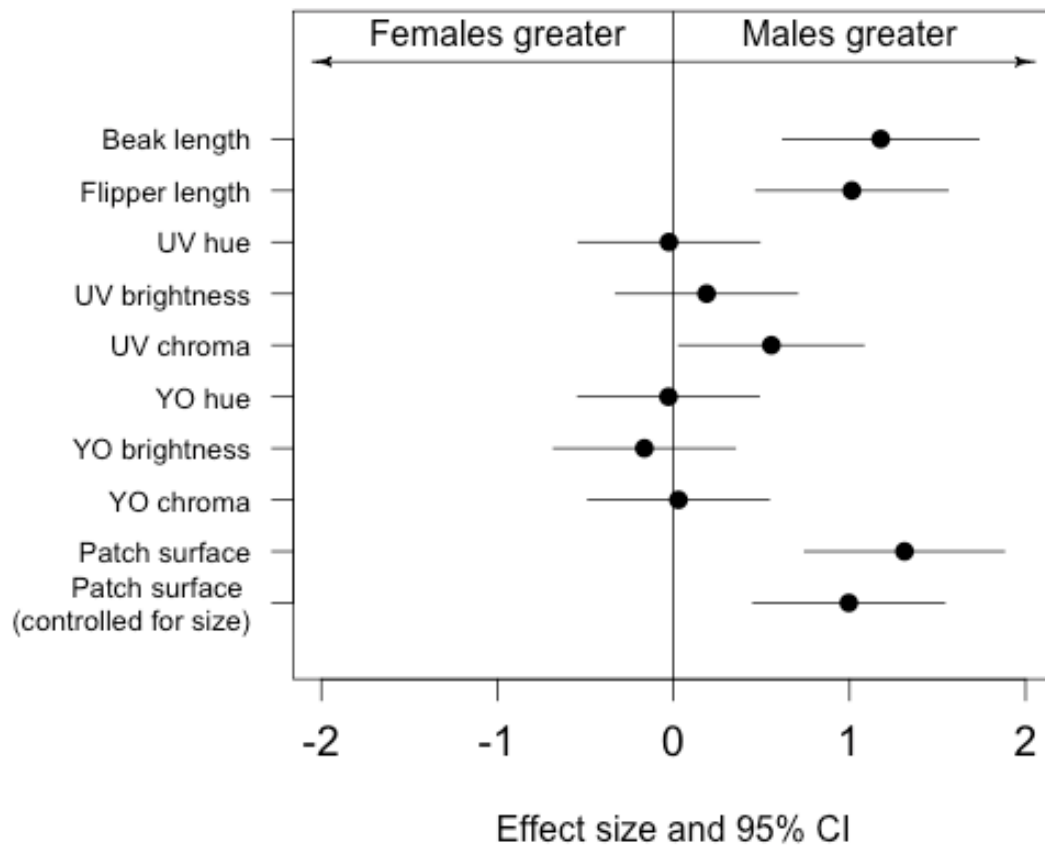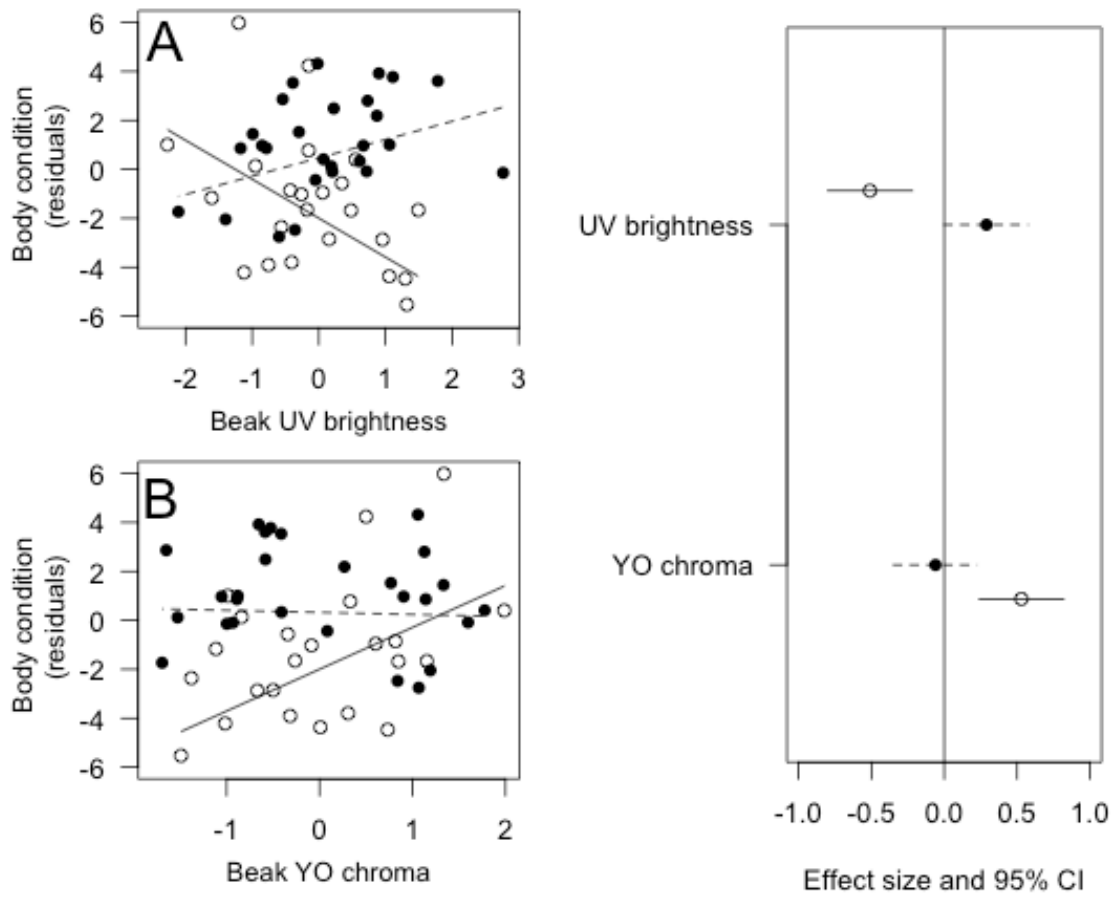
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3   **Fig 1.**

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Fig 2.

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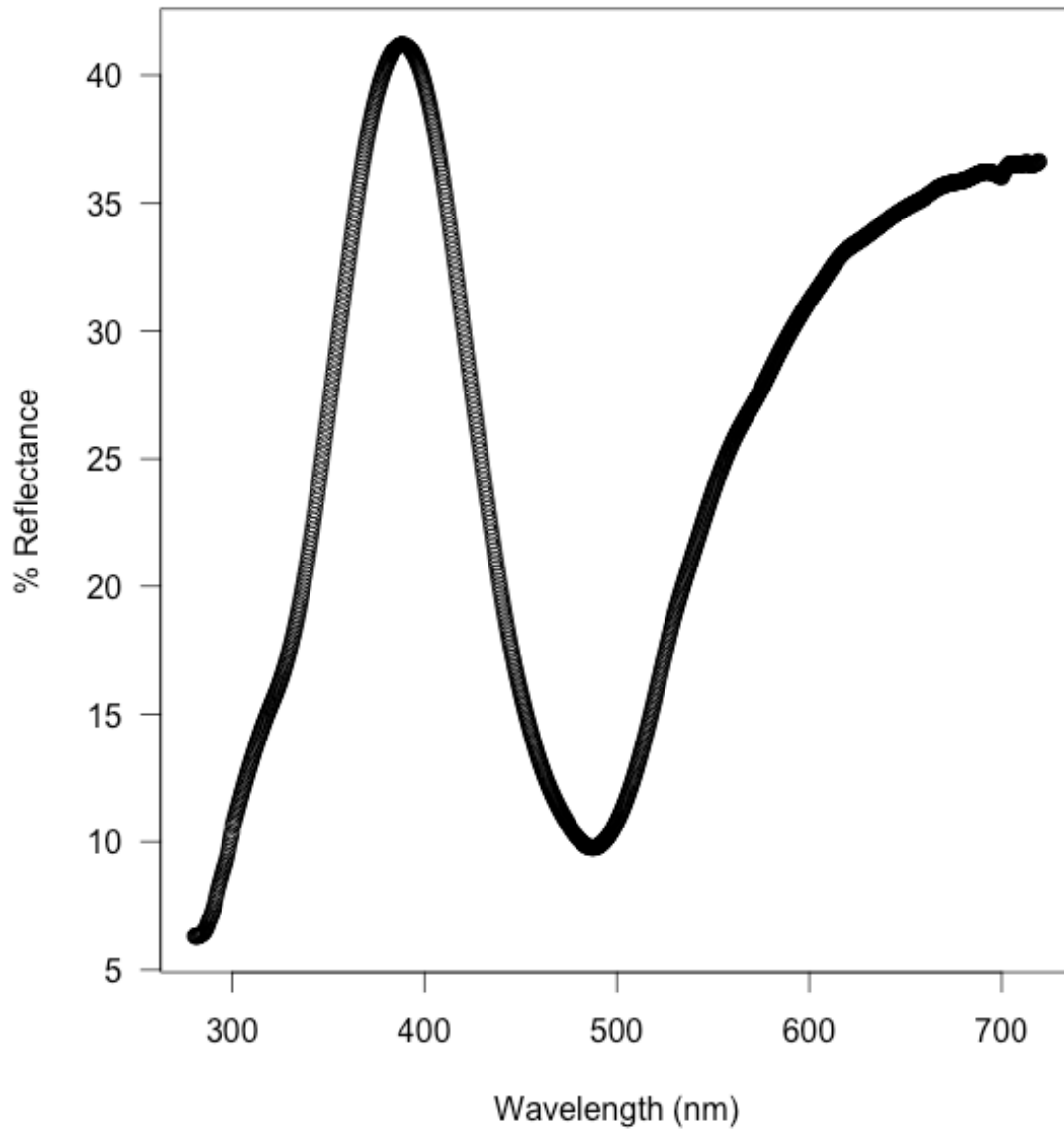
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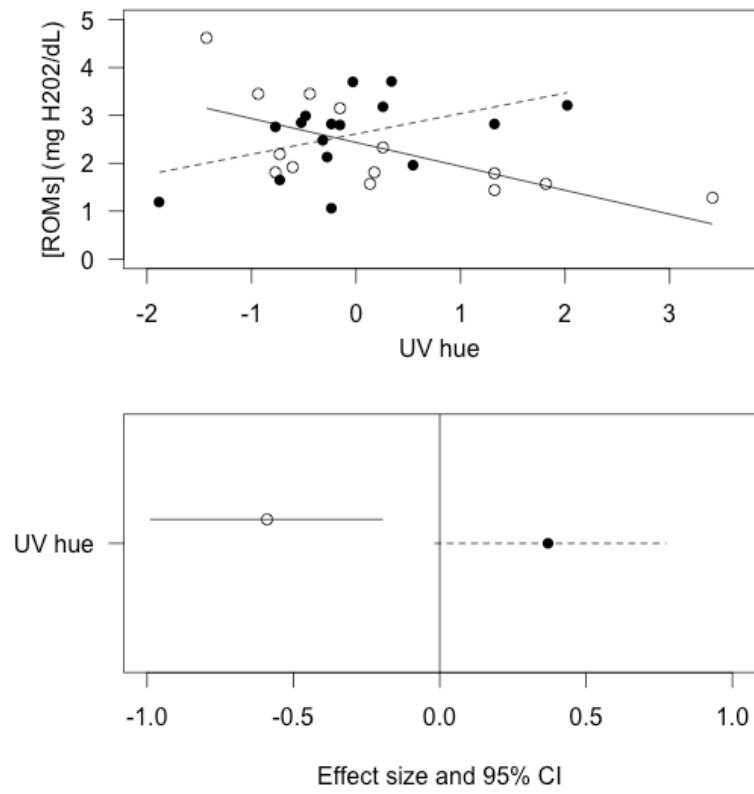12

13 **Fig 3.**

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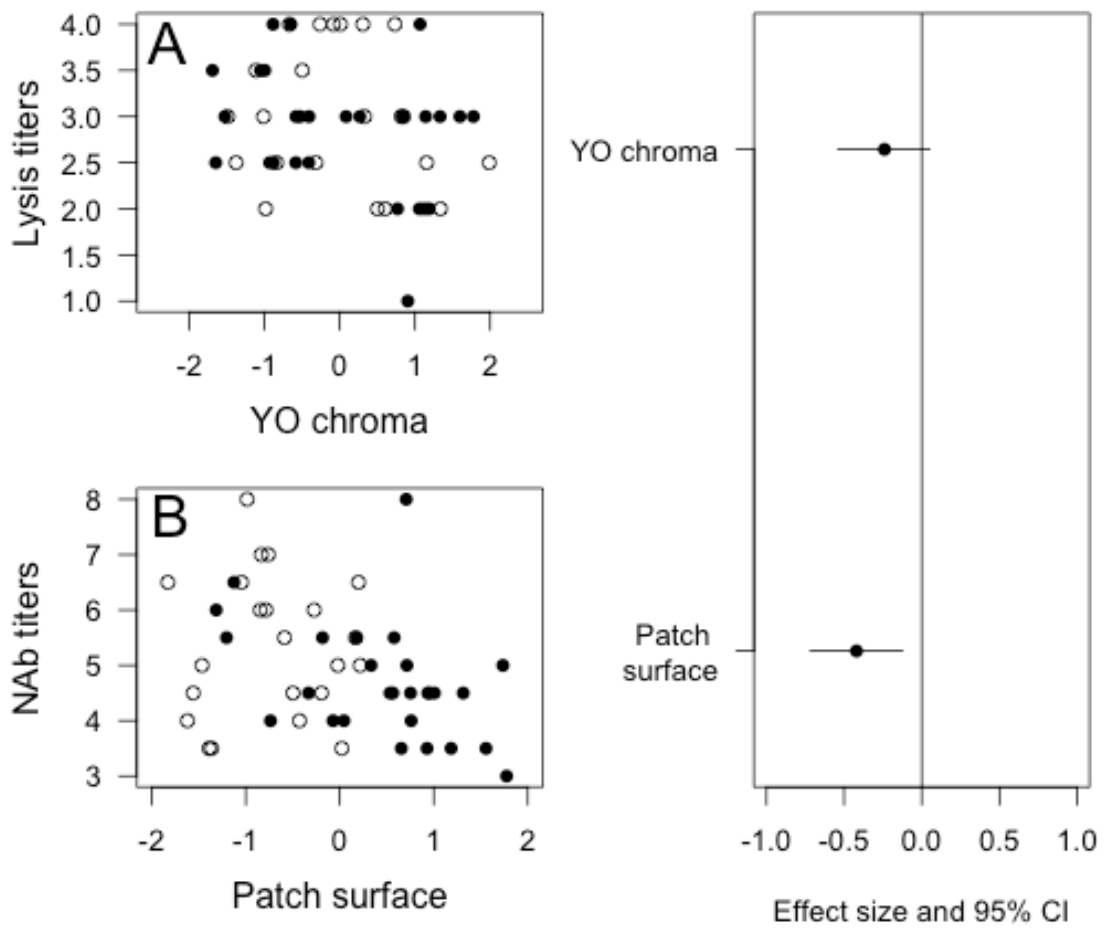
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22 **Fig 4.**

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27   **Fig 5.**

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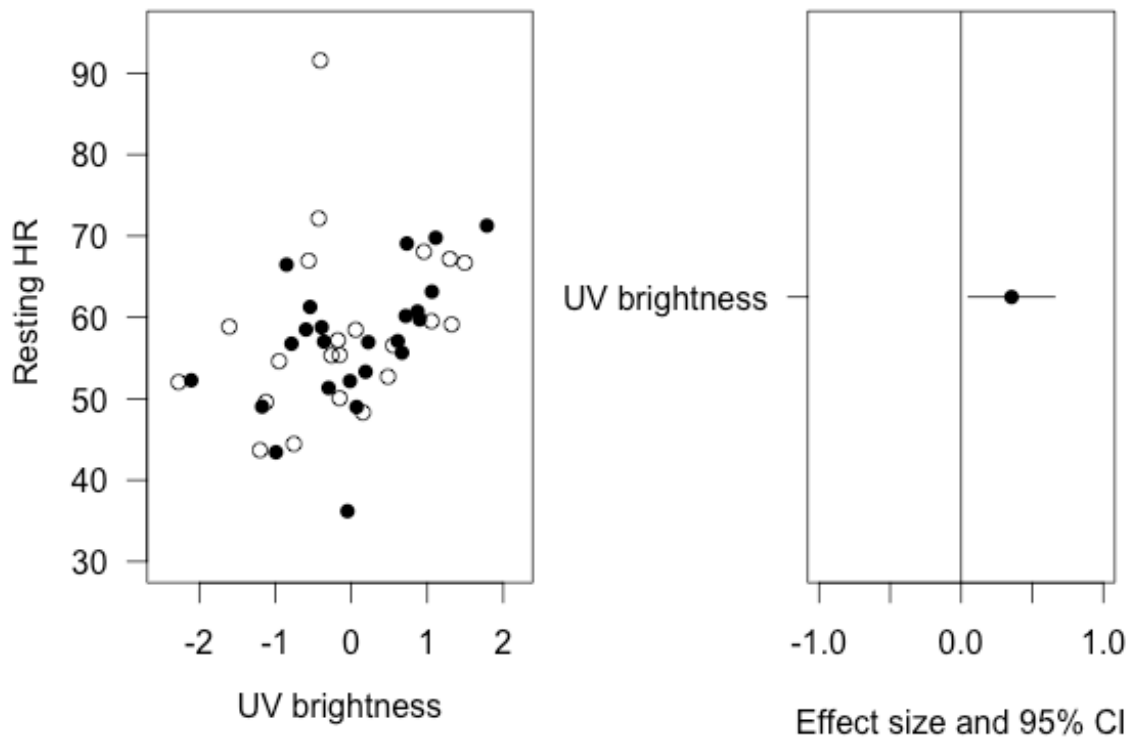
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39 **Fig 6.**

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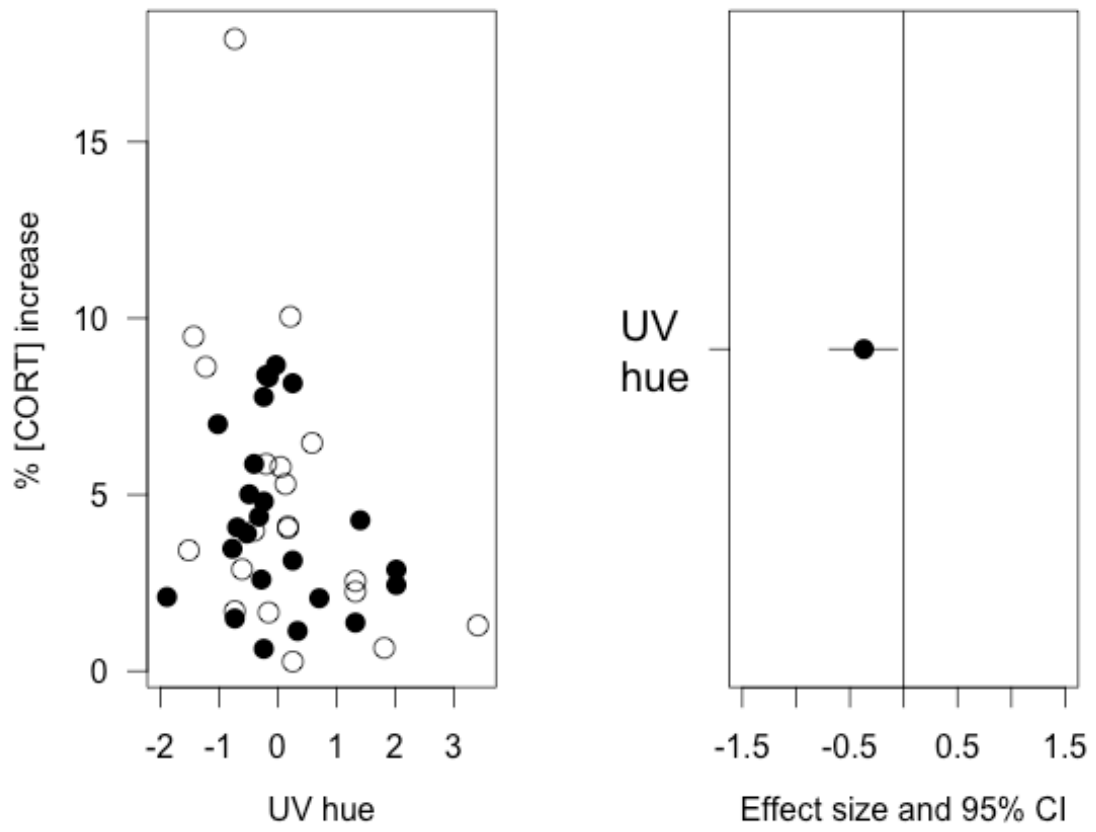
Fig 7.

Online supporting information S1:

Standardized measures of the auricular patches of breeding king penguin (*Aptenodytes patagonicus*). The head of the bird was held such that its beak rested on the shoulder opposite to the side of the body where the auricular patch was measured (Fig. 1A). A virtual line was pictured along the side of the auricular patch closest to the eye (line 1; Fig 1A). Then, a second perpendicular line reaching the most distant point of the circle (diameter) was pictured (line 2; Fig 1A), and the width of the auricular patch was measured (distance A; Fig 1B). From the center of distance A (line 3; Fig 1A), the height of the auricular patch was measured at a 90° angle (distance B; Fig 1C).
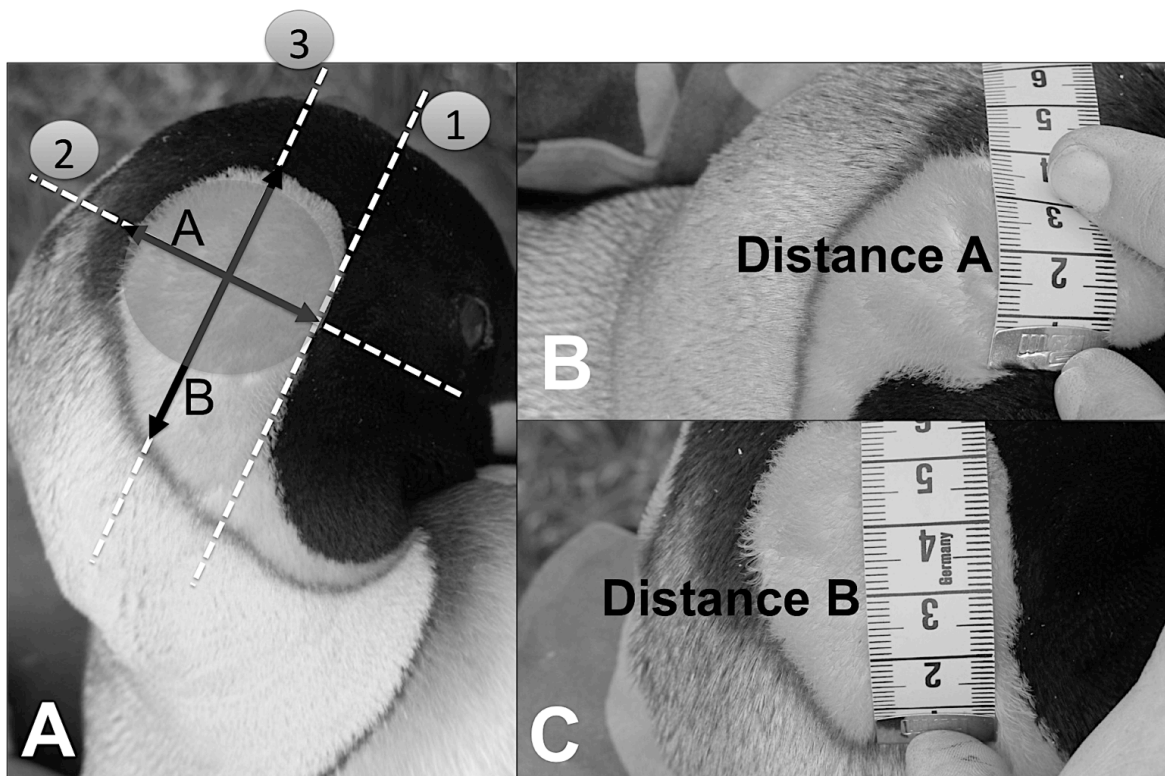
Online supporting information S2:

Correlation matrix for the ultraviolet (UV) and yellow-orange (YO) beak coloration measures (hue, brightness and chroma), and auricular patch surface, of breeding king penguins (*Aptenodytes patagonicus*). The upper right panel presents the standardized data, the lower left panel the correlation value (colors from light grey to black representing weak to strong correlations).
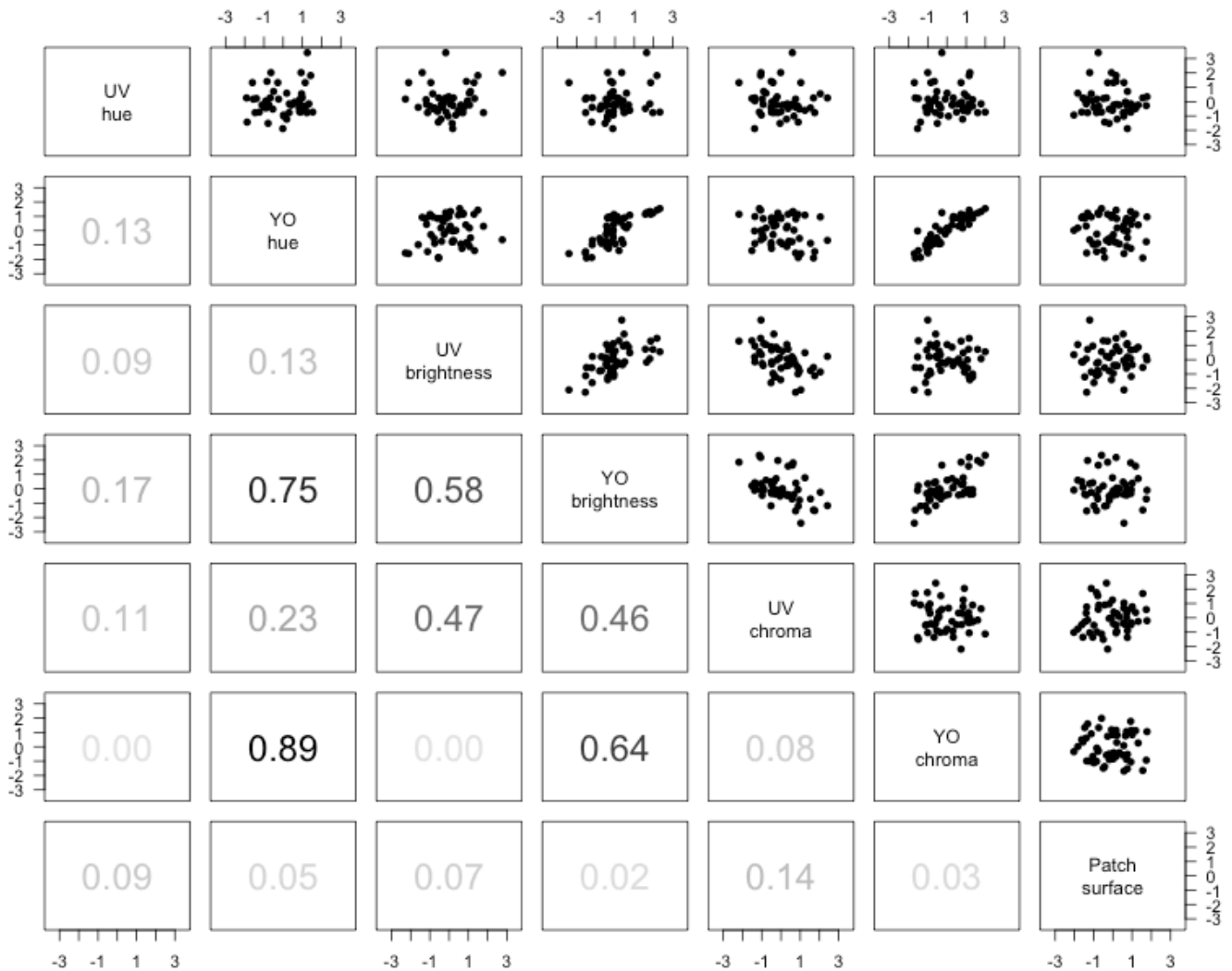
Online supporting information S3:

Summary statistics of the structural size and ornamental data of breeding king penguin (*Aptenodytes patagonicus*) used in the study. Sample size is indicated in parentheses and varies across measures due to constraints during field sampling. The average percent difference between males and females is given. Beak mean brightness, hue and chroma were calculated both across the UV-blue peak and yellow-orange regions characteristic of king penguin beak spots (Jouventin et al. 2004).

	Variable	FEMALES Mean ± SD	MALES Mean ± SD	% Difference males - females
Structural size	Flipper length	317.4 ± 9.5 (30)	327.1 ± 9.6 (31)	3%
	Beak Length	124.2 ± 3.8 (30)	129.7 ± 5.4 (31)	4%
Ornaments	Beak UV-blue (320-450 nm) Mean brightness (% reflectance)	18.1 ± 3.4 (23)	18.8 ± 3.6 (27)	4%
	Hue (nm)	388.5 ± 9.9 (23)	388.3 ± 7.8 (27)	-0%
	Chroma (ratio, % reflectance)	1.26 ± 0.17 (23)	1.35 ± 0.14 (27)	7%
	Beak yellow-orange (550-625 nm) Mean brightness	31.1 ± 6.7 (23)	30.1 ± 5.6 (27)	-3%
	Hue	575.4 ± 26.8 (23)	574.7 ± 24.0 (27)	-0%
	Chroma	1.13 ± 0.22 (23)	1.14 ± 0.25 (27)	1%
	Auricular patch Surface (mm²)	1744.0 ± 185.0 (30)	1995.0 ± 198.3 (31)	14%

Online supporting information S4:

Model selection for the effects of beak coloration and auricular patch surface on body condition (residuals, see Methods) in breeding king penguin (*Aptenodytes patagonicus*). Colony area in which the bird was sampled was fixed in all models to account for known differences in parasite loads and stress responses that may affect body condition. All models with a ΔAICc <2 compared to the best model are presented. The most parsimonious model retained is indicated in bold.

#	Model	R^2	F	df	logLik	AICc	delta	weight
1	UV brightness + YO chroma + patch surface + sex + sex*UV brightness + sex*YO chroma + sex* patch surface + colony area	0.58	7.06	10	-97.06	219.77	0.00	0.45
2	**UV brightness + YO chroma + sex + sex*UV brightness + sex*YO chroma + colony area**	**0.52**	**7.75**	**8**	**-100.38**	**220.27**	**0.50**	**0.35**
3	UV brightness + UV chroma + YO chroma + sex + sex*UV brightness + sex*YO chroma + sex* UV chroma + colony area	0.57	6.67	10	-97.88	221.41	1.64	0.20

Online supporting information S5:

Model selection for the effects of beak coloration and auricular patch surface on plasma reactive oxygen metabolite (ROM) levels in breeding king penguin (*Aptenodytes patagonicus*). Colony area was not included in this analyses as ROM levels were only determined for birds in one location of the colony. All models with a ΔAICc <2 compared to the best model are presented. The most parsimonious model retained is indicated in bold.

#	Model	R^2	F	df	logLik	AICc	delta	weight
1	**UV hue + sex + sex*UV hue**	**0.37**	**5.04**	**5**	**-31.34**	**75.20**	**0.00**	**0.71**
2	UV hue + YO chroma + sex + sex*UV hue + sex*YO chroma	0.46	4.1	7	-28.94	77.00	1.78	0.29

Online supporting information S6:

Model selection for the effects beak coloration and auricular patch surface on plasma antioxidant capacity (OXY) in breeding king penguin (*Aptenodytes patagonicus*). Colony area was not included in this analyses as OXY levels were only determined for birds in one location of the colony. All models with a ΔAICc <2 compared to the best model are presented. The most parsimonious model retained is indicated in bold.

#	Model	R^2	F	df	logLik	AICc	delta	weight
1	**Intercept only**	**0.00**		**2**	**-146.00**	**296.30**	**0.00**	**0.70**
2	UV hue	0.02	0.76	3	-145.60	298.00	1.66	0.30

Online supporting information S7:

Model selection for the effects of beak coloration and auricular patch surface on plasma lysis titers in breeding king penguin (*Aptenodytes patagonicus*). Colony area in which the bird was sampled was fixed in all models to account for known differences in parasite loads and stress responses that may affect immune status. All models with a ΔAICc <2 compared to the best model are presented. The most parsimonious model retained is indicated in bold.

#	Model	R^2	F	df	logLik	AICc	delta	weight
1	YO brightness + YO chroma + colony area	0.33	7.26	5	-42.87	97.1	0.00	0.29
2	UV chroma + YO chroma + colony area	0.31	6.68	5	-43.52	98.4	1.29	0.15
3	UV brightness + YO brightness + YO chroma + colony area	0.34	5.61	6	-42.44	98.9	1.74	0.12
4	**YO chroma + colony area**	**0.26**	**8.20**	**4**	**-45.07**	**99.0**	**1.91**	**0.11**
5	YO brightness + YO chroma + patch surface + colony area	0.33	5.55	6	-42.54	99.1	1.94	0.11
6	YO brightness + YO chroma + UV hue + colony area	0.33	5.54	6	-42.56	99.1	1.97	0.11
7	YO brightness + YO chroma + UV chroma + colony area	0.33	5.53	6	-42.57	99.1	2.00	0.11

Online supporting information S8:

Model selection for the effects of beak coloration and auricular patch surface on plasma NAb titers in breeding king penguin (*Aptenodytes patagonicus*). Colony area in which the bird was sampled was fixed in all models to account for known differences in parasite loads and stress responses that may affect immune status. All models with a ΔAICc <2 compared to the best model are presented. The most parsimonious model retained is indicated in bold.

#	Model	R^2	F	df	logLik	AICc	delta	weight
1	**Patch surface + colony area**	**0.24**	**7.31**	**4**	**-71.14**	**151.2**	**0.00**	**0.68**
2	Patch surface + UV hue + colony area	0.26	5.15	5	-70.67	152.7	1.54	0.32

Online supporting information S9:

Model selection for the effects of beak coloration and auricular patch surface on daily resting heart rate in breeding king penguin (*Aptenodytes patagonicus*). Colony area in which the bird was sampled was fixed in all models to account for known differences in parasite loads and stress responses that may affect energy expenditure. All models with a ΔAICc <2 compared to the best model are presented. The most parsimonious model retained is indicated in bold.

#	Model	R^2	F	df	logLik	AICc	delta	weight
1	**UV brightness + colony area**	**0.14**	**3.61**	**4**	**-9.84**	**28.6**	**0.00**	**0.71**
2	UV brightness + sex + colony area	0.15	2.62	5	-9.47	30.4	1.78	0.29

Online supporting information S10:

Model selection for the effects of beak coloration and auricular patch surface on baseline plasma total corticosterone levels in breeding king penguin (*Aptenodytes patagonicus*). Colony area in which the bird was sampled was fixed in all models to account for known differences in parasite loads and stress responses that may affect corticosterone levels. All models with a ΔAICc <2 compared to the best model are presented. The most parsimonious model retained is indicated in bold.

#	Model	R^2	F	df	logLik	AICc	delta	weight
1	Patch surface + sex + colony area	0.29	5.54	5	-76.26	164.1	0.00	0.23
2	Patch surface + sex + UV chroma + colony area	0.33	4.73	6	-75.21	164.7	0.59	0.17
3	Sex + colony area	0.23	6.28	4	-78.03	165.1	0.99	0.14
4	Patch surface + sex + UV brightness + colony area	0.32	4.56	6	-75.46	165.2	1.10	0.13
5	UV chroma + sex + colony area	0.27	5.02	5	-76.88	165.3	1.24	0.12
6	**Colony area**	**0.18**	**9.32**	**3**	**-79.50**	**165.6**	**1.50**	**0.11**
7	UV brightness + sex + colony area	0.26	4.79	5	-77.16	165.9	1.80	0.09

Online supporting information S11:

Model selection for the effects of beak coloration and auricular patch surface on the relative corticosterone increase in response to a standardized 30 minute capture in breeding king penguin (*Aptenodytes patagonicus*). Colony area in which the bird was sampled was fixed in all models to account for known differences in parasite loads and stress responses. All models with a ΔAICc <2 compared to the best model are presented. The most parsimonious model retained is indicated in bold.

#	Model	R^2	F	df	logLik	AICc	delta	weight
1	UV chroma + UV hue + YO brightness + colony area	0.43	7.46	6	-57.96	130.2	0.00	0.24
2	UV choma + UV brightness + UV hue + YO brightness +colony area	0.46	6.47	7	-56.91	130.9	0.75	0.16
3	**UV hue + colony area**	**0.35**	**11.05**	**4**	**-60.98**	**131.0**	**0.79**	**0.16**
4	UV hue + YO chroma + colony area	0.38	8.13	5	-59.99	131.6	1.37	0.12
5	UV chroma + UV hue + YO chroma + colony area	0.41	6.90	6	-58.69	131.7	1.47	0.11
6	UV chroma + UV hue + colony area	0.38	8.07	5	-60.05	131.7	1.49	0.11
7	UV brightness + UV hue + YO brightness + colony area	0.41	6.79	6	-58.83	131.9	1.74	0.10

Online supporting information S12:

Model selection for the effects of beak coloration and auricular patch surface on the relative heart rate increase in response to a standardized capture in breeding king penguin (*Aptenodytes patagonicus*). Colony area in which the bird was sampled was fixed in all models to account for known differences in parasite loads and stress responses. All models with a ΔAICc <2 compared to the best model are presented. The most parsimonious model retained is indicated in bold.

#	Model	R^2	F	df	logLik	AICc	delta	weight
1	Patch surface + colony area	0.11	2.27	4	-193.8	396.9	0.00	0.44
2	**Colony area**	**0.05**	**1.87**	**3**	**-195.2**	**397.1**	**0.20**	**0.40**
3	UV hue + colony area	0.07	1.26	4	-194.8	398.9	2.00	0.16