



## Mutually honest? Physiological ‘qualities’ signalled by colour ornaments in monomorphic king penguins

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Mate choice is expected to be important for the fitness of both sexes for species in which successful reproduction relies strongly on shared and substantial parental investment by males and females. Reciprocal selection may then favour the evolution of morphological signals providing mutual information on the condition/quality of tentative partners. However, because males and females often have differing physiological constraints, it is unclear which proximate physiological pathways guarantee the honesty of male and female signals in similarly ornamented species. We used the monomorphic king penguin (*Aptenodytes patagonicus*) as a model to investigate the physiological qualities signalled by colour and morphological ornaments known to be under sexual selection (coloration of the beak spots and size of auricular feather patches). In both sexes of this slow-breeding seabird, we investigated the links between ornaments and multiple indices of individual quality; including body condition, immunity, stress and energy status. In both sexes, individual innate immunity, resting metabolic rate, and the ability to mount a stress response in answer to an acute disturbance (capture) were similarly signalled by various aspects of beak coloration or auricular patch size. However, we also reveal interesting and contrasting relationships between males and females in how ornaments may signal individual quality. Body condition and oxidative stress status were signalled by beak coloration, although in opposite directions for the sexes. Over an exhaustive set of physiological variables, several suggestive patterns indicated the conveyance of honest information about mate quality in this monomorphic species. However, sex-specific patterns suggested that monomorphic ornaments may signal different information concerning body mass and oxidative balance of males and females, at least in king penguins. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, 118, 200–214.

**KEYWORDS:** body condition – king penguin – monomorphic seabird – mutual mate choice – ornament – oxidative stress – sexual selection – ultra-violet signals.

### 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, Kraaijeveld-Smit & Komdeur, 2007). Two main hypotheses have been proposed to explain mutual ornamentation. The first suggests that female ornaments are non-functional, but arise as a

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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 & Miller, 2008), or social competition over non-mate resources in both sexes (West-Eberhard, 1979; Tobias, Montgomerie & Lyon, 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, Bisazza & Pilastro, 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 & Vincent, 1991), such as in slow-breeding seabirds (e.g. Velando, Lessells & Márquez, 2001). Where both sexes should be choosy in their pairing preferences, ornaments may be favoured because they assist the individual expressing them in acquiring a high quality mate, whereas preferences for ornaments may do the same for receivers (Johnstone, Reynolds & Deutsch, 1996; Kokko & Johnstone, 2002; Hooper & Miller, 2008). Furthermore, mating systems with extended mate-sampling periods are expected to lead to reduced mutual ornamentation ('dull monomorphism'; Badyaev & Qvarnström, 2002; Badyaev & Hill, 2003), whereas mating systems with short mate-sampling periods should favour extravagant 'bright' monomorphism (Fitzpatrick, 1994). However, because males and females often differ in physiological constraints, the aspects of individual quality signalled and of interest to receivers may differ between the sexes (Alvarez, Sanchez & Angulo, 2005; Lopez, Figuerola & Soriguer, 2008). For instance, in goldfinches (*Spinus tristis*), monomorphic bill coloration is correlated with acquired immunity in females but not males, probably linked to the different functional roles of beak coloration in male and female social communication (Kelly *et al.*, 2012).

King penguins (*Aptenodytes patagonicus*) are monomorphic seabirds, where both sexes experience a highly energy demanding breeding cycle (Groscolas & Robin, 2001) and cooperate for as long as 14 months to successfully raise a single chick (Stonehouse, 1960). Both males and females display conspicuous colour ornaments including auricular feather patches that only reflect yellow-orange colours, a breast feather patch that reflects yellow to rusty-brown colours (Pincemy, Dobson & Jouventin,

2009), and keratin beak spots on their lower mandibles that reflect yellow-orange and UV colour (Jouventin *et al.*, 2005). Although it has been previously demonstrated that feather and beak spot colorations are used in mate choice (Pincemy *et al.*, 2009; Nolan *et al.*, 2010), few facts are known on the information carried by those ornaments. We tested whether the ornaments of king penguins convey similar information in both sexes in order to determine whether the condition dependence of ornamental features occurs only in one sex, suggesting that selection operates primarily in that sex and that monomorphism is the outcome of genetic correlation between the sexes; or whether condition dependence occurs in both sexes (though not necessarily on the same ornaments nor related to the same qualities) supporting the idea of mutual sexual selection. We aimed at providing an extensive list of quality measures choosing key mediators of vertebrate life histories expected to exhibit important associations with fitness. Those included body condition, immune status, energy expenditure, hormonal stress status, hormonal and heart rate stress responsiveness, and oxidative status (e.g. Norris & Evans, 2000; Monaghan, Metcalfe & Torres, 2009).

Because beak UV is important to pairing decisions for both male and female king penguins (Nolan *et al.*, 2010), we expected it to reflect information on individual quality in both sexes. In contrast, larger auricular patches are more important to females during mate choice (Pincemy *et al.*, 2009; Dobson, Couchoux & Jouventin, 2011), but have also been positively linked to social aggressiveness in both sexes (Viera *et al.*, 2008). Thus, we expected auricular patch size to yield information on male quality, or non-exclusively to signal male and female abilities to cope with their aggressive colonial environment, including via physiological stress responses (e.g. Parker, Knapp & Rosenfield, 2002; Bortolotti *et al.*, 2009). Social competition has been suggested to favour the evolution of ornaments as 'badges of status' that are used in alternative contexts to mate choice (West-Eberhard, 1979; Kraaijeveld *et al.*, 2007). King penguins are known to aggressively compete over breeding sites, and thus coloured ornaments might convey information about social dominance or aggressiveness (Viera *et al.*, 2008; Keddar, Jouventin & Dobson, 2015a). Specifically, given that males perform the first and longest reproductive fast of the breeding cycle (typically 1-month including courtship and incubation; Stonehouse, 1960), information on body condition should be more important to females. We predicted that ornamental features should be associated with body condition, especially in males. In contrast, information relating to immunity should be particularly relevant to both sexes in

this species, as ticks (*Ixodes uriae*) are prevalent in king penguin colonies and detrimentally affect adult and offspring fitness (Mangin *et al.*, 2003; P. Bize, Q. Schull, S. Pardouret, Y. Handrich, F. Criscuolo, V.A. Viblanc, J.P. Robin, unpubl. data). Finally, stress status (including oxidative stress; von Schantz *et al.*, 1999) in relation to mate choice (e.g. parental breeding quality; Angelier & Chastel, 2009) or social territory acquisition should be mutually important to males and females, and associated with ornamental traits in both sexes.

## 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 with between individual variation (Q. Schull, V.A. Viblanc, F.S. Dobson, P. Bize, 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 (November–March) 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

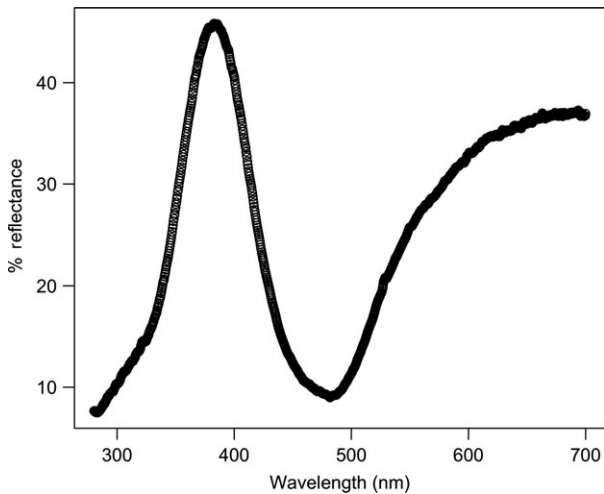
Flipper ( $\pm 1$  mm) and beak length ( $\pm 0.1$  mm) were measured using a solid metal ruler and dial calipers (Stonehouse, 1960). Body girth (thoracic circumfer-

ence) was measured ( $\pm 1$  mm) with a flexible tape-ruler just below the upper articulation of the flippers to the body (Viblanc *et al.*, 2012a). Birds were measured at the onset of incubation shift 2 for females and incubation shift 3 for males, to insure that both males and females had experienced similar minimal fasting durations (2–3 days) on land.

### ORNAMENT MEASURES

Standardized measures of the width and height of the right and left auricular feather patches were performed using a flexible tape-ruler (see online Fig. S1). Left and right distances were averaged and the surface of the patch was calculated as  $width \times height$  (mm<sup>2</sup>).

Reflectance measurements of the beak spot were obtained using a portable JAZ spectrophotometer (Ocean Optics Inc., Dunedin, FL, USA) with a spectral resolution of 0.3 nm across the spectral range 320–700 nm. The spectrophotometer contained a pulsed-xenon light module and was calibrated against a white Spectralon standard. All measures were performed using a 200  $\mu$ m fiber probe with a 90° angle window. Measures were repeated three times across each bill plate (in the orange region from bill tip to base) and spectra were averaged using an R script adapted from Montgomerie (2008). From spectral data, we calculated tri-stimulus colour variables: mean brightness, hue and chroma. We considered the spectral range 320–700 nm, given the range of spectral sensitivity in birds (Cuthill, 2006). The reflectance of king penguin beak spots is characterized by a bi-modal pattern including a reflectance peak in UV and a peak/plateau in the yellow-orange (YO) portion of the spectrum (see Fig. 1). Thus, we calculated colour variables over wavelength sub-regions of interest. For yellow-orange colours, we focused on the 500–700 nm portion of the spectrum. For the UV peak, we focused on the range 320–450 nm. Although this region extends beyond UV coloration *per se*, the choice was deliberate to account for the UV peak of king penguin beak spots in its entirety (Jouventin *et al.*, 2005). Mean brightness is a measure of spectral intensity of the ornament, and yellow-orange and UV mean brightness were calculated by averaging reflectance over wavelengths 500–700 nm and 320–450 nm, respectively (Montgomerie, 2006). Hue is a measure of colour appearance (e.g. 'blue', 'yellow', etc.). For the YO plateau portion of the spectrum, it was calculated as the wavelength at which the reflectance was halfway between its maximum and minimum (Keddar *et al.*, 2013). For the UV peak, hue was calculated as the wavelength of maximum reflectance between 320 and 450 nm. Finally, chroma is a measure of colour purity and was calculated as the difference between maximum and



**Figure 1.** Reflectance curve obtained from the beak spot of a breeding king penguin (*Aptenodytes patagonicus*). Note the typical bi-modal pattern with a UV peak around 380–390 nm and a yellow-orange plateau from 500 to 700 nm.

minimum reflectance over the mean reflectance for that particular region (formula  $S_g$ ; Montgomerie, 2006).

#### BODY CONDITION

We used a principal component analysis to calculate a structural size index (SSI), which explained 86% of the variation in beak size and flipper length ( $SSI = 0.95 \times \text{flipper} + 0.31 \times \text{beak}$ ). We then regressed body girth on this SSI ( $F_{1,59} = 18.87$ ,  $P < 0.001$ ,  $R^2 = 0.24$ ) and used the residuals as an index of body condition. This method yields condition indices very similar to classical mass/size regressions (correlation,  $r = 0.92$ ; Viblanc *et al.*, 2012a), but is more practical than weighing birds within the breeding colony.

#### IMMUNITY MEASURES

Immune status was assessed from blood samples collected during the second incubation shift of males and females. Blood (1 mL) was collected within 3 min of capture (see stress protocol below) from the marginal flipper vein using a 0.7\*40 mm, 22G needle fitted to a 5 mL heparinized syringe. Within 10 min of sampling, blood was centrifuged at 3000  $g$  for 5 min separating plasma and blood cells. Samples were kept at  $-18^\circ\text{C}$  until the end of the day before being transferred at  $-80^\circ\text{C}$  until lab-analyses. Constitutive innate humoral immunity was determined using the hemolysis-hemagglutination assay described for birds (including seabirds) by (Matson, Ricklefs & Klasing,

2005). This assay evaluates natural antibody (NAb) levels and associated complement activation potential in plasma. Briefly, NAbs are innate non-specific antibodies encoded by the germ line that react with virtually any antigen. They are naturally present in antigen-naïve individuals, form a large portion of serum immunoglobulin, and initiate the complement enzyme cascade that ends in cell lysis (Matson *et al.*, 2005). We exposed 25  $\mu\text{L}$  of penguin plasma (serially diluted from 1 to 1/1024) to 25  $\mu\text{L}$  of a 1% rabbit blood cell suspension and scored lysis (lysis titres) and agglutination (NAb titres) for each sample. All assays were run on the same day and scored by the same observer (AS). Within and among-assay variation was 2.4 and 7.5% for lysis, and 3.0 and 4.1% for agglutination titres, respectively.

#### RESTING METABOLIC RATE

An estimate of bird's resting metabolic rate was obtained by measuring their daily resting heart rate (rHR). The conversion of HR to  $\text{VO}_2$  (the classic measure of metabolic rate) using previously established calibrations is complicated by various issues including error measurement (for a discussion see Green, 2011). Thus, we used raw HR data as a qualitative rather than quantitative index of metabolic rate in king penguins (Viblanc *et al.*, 2014). We attached external HR-loggers (Polar<sup>®</sup> RS800 and RS800CX, Polar Electro Oy, Kempele, Finland) to breeding birds on the 6th day of their second incubation shift (shift 3 for males,  $N = 26$ ; shift 4 for females,  $N = 24$ ). Details on logger attachment, technology and accuracy of HR measurement are provided elsewhere (Groscolas *et al.*, 2010). Birds' HR was recorded for 48 h (until day 8 of their incubation shift) at a rate of 1 value every 5 or 2 s (depending on the logger model and memory). HR typically recovered to resting levels within 30 min of the initial capture stress (Viblanc *et al.*, 2012b). We thus systematically discarded the first 60 min of each recording to avoid confounding our calculations with handling stress. We calculated daily rHR using moving averages to determine the 10 consecutive minutes where HR was lowest over 12-h periods. Daily rHR values were highly repeatable ( $r = 0.95$ ; Lessels & Boag 1987) and were averaged (Viblanc *et al.*, 2014).

#### STRESS STATUS

We assessed penguins' stress status by measuring plasma total corticosterone (CORT), the main glucocorticoid stress hormone in birds. We determined both basal total CORT levels and acute total CORT increase to a standardized capture stress on the 8th day of second incubation shift, at the same time that



HR-loggers were removed. The capture stress was a standardized approach starting > 25 m away from the bird, before hooding and capturing it. At the start of the approach, the experimenter insured that the bird was resting. The time at which it became vigilant to the approaching experimenter was considered  $T_0$  and a first blood sample (as previously described) was made within the following 3–5 min. In king penguins, plasma CORT levels do not significantly increase due to a capture-handling stress within this time period (Ménard, 1998). After initial blood sampling, the experimenter loosely maintained the bird captive for 30 min and performed a second blood sample at  $T_{30}$ . Concentrations of plasma CORT were measured in duplicate using a quantitative competitive sandwich enzyme immunoassay technique according to guidelines provided by the manufacturer (ELISA Corsticosterone kit, Enzo Life Sciences, Farmingdale, NY, USA). Kit sensitivity was  $27.0 \text{ pg mL}^{-1}$ , intra- and inter-assay variation were 7.6 and 13.3%, respectively. The CORT response to acute stress was calculated as  $100 \times (\text{CORT}_{30} - \text{CORT}_0) / \text{CORT}_0$ .

During the standardized capture protocol we also measured HR response. We defined the initial resting HR ( $\text{HR}_i$ ) as the HR at the moment preceding a rapid constant increase in HR due to the approaching experimenter (Viplanc *et al.*, 2012b). Maximal HR ( $\text{HR}_{\text{max}}$ ) in response to the capture corresponded to the maximal HR achieved in the 3 min following the onset of the stress. The maximum increase in HR was then calculated as  $100 \times (\text{HR}_{\text{max}} - \text{HR}_i) / \text{HR}_i$ . HR-loggers were removed at the end of the stress.

#### OXIDATIVE STATUS

On the 8th day of the second incubation shift, we determined plasma oxidative status as previously described for king penguins (Geiger *et al.*, 2012). The anti-oxidant capacity of penguin's plasma (OXY) and its concentration of reactive oxygen metabolites (ROM; a measure of exposure to oxidative stress) were respectively measured using commercially available OXY adsorbent and dROM kits (Diacron International srl, Grosseto, Italy). Intra- and inter-assay variation was 7.4 and 7.0% for OXY, and 6.4 and 7.9% for ROM.

#### DATA ANALYSES

Analyses were performed using R v.3.0.2. All individuals only appeared once in the data set and we had no repeated measures. First, we investigated male and female dimorphism by considering the effect of sex on structural size, beak colour variables and auricular patch surface in linear models. For auricular patch surface, we also considered sexual dimorphism controlling for structural size (specified as a covariate in the

analysis). We then investigated whether ornaments reflected physiological variables (i.e. could the birds 'predict' physiological quality from the ornaments) by running separate models for each physiological trait and specifying beak colour traits (hue, chroma and brightness) and auricular patch size as predictor variables in our models. Sex was included as a cofactor in the analyses and its interactions with beak coloration variables and auricular patch size were considered. The area of the colony in which the bird was sampled (close to the beach or further up the valley) was fixed as a cofactor in all analyses to account for known colony-related differences in parasites and stress responses (Viplanc *et al.*, 2012b). Independent variables were standardized prior to analyses, so that model estimates were comparable (Schielzeth, 2010). We used multi-model inference with Akaike's Information Criterion corrected for small sample size to identify the best model (AICc and AIC weights) for each physiological parameter considered ('dredge' package in R; Barton, 2015). We retained the most parsimonious model within potential candidates ( $\Delta\text{AICc} < 2$ ). Models were compared using Maximum Likelihood. Because most colour variables were correlated to some extent (see Fig. S2), we insured collinearity was not an issue before performing model selection in our analyses. We checked for variance inflation factors (VIFs) in the full model (suggested cut-off = 5; Zuur, Ieno & Smith, 2007). Yellow hue was the only variable which appeared problematic in all models, with  $7.2 < \text{VIF} < 9.4$ . Thus, we removed it from all analyses, and subsequent collinearity was low ( $1.2 < \text{VIFs} < 5.2$ ). Due to sampling and slight variations in success of laboratory analyses, sample sizes varied across physiological measures. Diagnostic plots and the Shapiro–Wilk normality test were used to inspect model residuals for normality and potential outliers. When necessary (i.e. for resting HR and the acute CORT response), data were transformed prior to analyses using Box-Cox power transformations (Viplanc *et al.*, 2012b) to insure residual normality. For each model, we calculated effect sizes (ES, Hedges' unbiased  $d$  and  $z$ -transformed  $r$ ) and their associated 95% confidence intervals based on respective  $t$ -statistics using equations 10, 11, 14, 15, 17 and 19 from (Nakagawa & Cuthill, 2007). We use the benchmarks  $r = 0.1, 0.3, 0.5$  and  $d = 0.2, 0.5, 0.8$ , to discuss small, medium and large effect sizes (Nakagawa & Cuthill, 2007).

## RESULTS

### MALE AND FEMALE DIMORPHISM IN SEXUAL ORNAMENTS

Males were slightly but significantly larger than females (3–4% for flipper and beak, respectively;

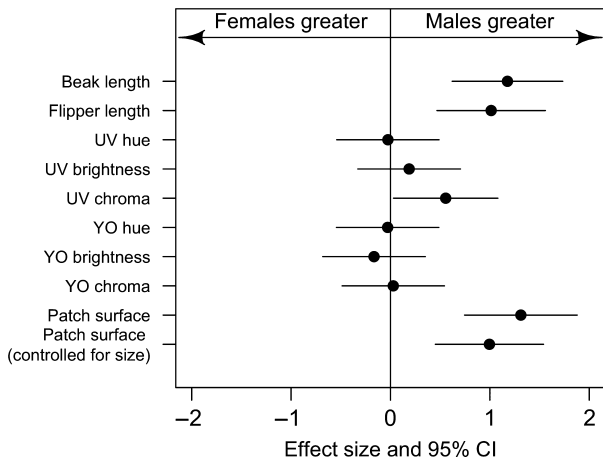
Fig. 2; Table S1), and had significantly larger auricular patches (14%), even when accounting for structural size as a covariate in the model (Fig. 2). Sexes did not differ significantly in terms of ornamental colours, except for UV chroma, which was slightly higher in males (Fig. 2).

BODY CONDITION AND ORNAMENTS

The most parsimonious model explaining body condition in breeding birds with the lowest AICc and highest AIC weight retained beak UV brightness, yellow-orange chroma, and their interactions with sex as important factors (Table 1, see Table S2). Patterns of association between beak UV brightness, yellow-orange chroma, and body condition were different in males and females (Fig. 3, Table 1). Beak UV brightness was weakly positively ( $Zr = +0.29$ ;  $CI_{95} = [+0.00, 0.59]$ ) related to body condition in males, but moderately negatively in females ( $Zr = -0.51$ ;  $CI_{95} = [-0.22, -0.80]$ ) (Fig. 3A). Beak yellow-orange chroma was moderately positively related to body condition in females ( $Zr = +0.53$ ;  $CI_{95} = [0.24, 0.82]$ ), but not in males ( $Zr = -0.06$ ;  $CI_{95} = [-0.35, 0.23]$ ) (Fig. 3B).

OXIDATIVE STATUS AND ORNAMENTS

UV hue, sex and their interaction were selected by AICc as important variables related to ROM levels (Table 2, Table S3). In females, beak UV hue was strongly negatively related to ROM levels



**Figure 2.** Effect sizes and 95% confidence intervals for ornamental and structural size dimorphism between king penguin males and females. Effect sizes and 95% CI were calculated after Nakagawa & Cuthill, 2007. Effects are considered significant if their 95% CI does not overlap zero.

**Table 1.** Model estimates for the influence of beak colour 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	SE	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

( $Zr = -0.59$ ;  $CI_{95} = [-0.20, -0.99]$ ), whereas the association was positive in males, though the effect was weak as CI barely overlapped zero ( $Zr = +0.37$ ;  $CI_{95} = [-0.02, 0.77]$ ) (Fig. 4). In contrast, OXY levels were not related to beak coloration or auricular patch surface, i.e. only the intercept was retained in the best model (Table S4).

IMMUNITY AND ORNAMENTS

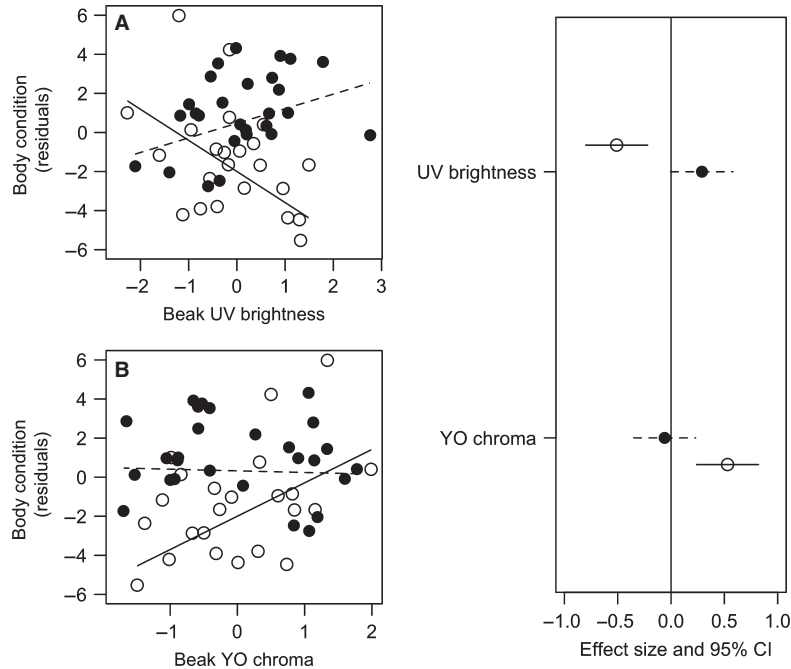
The most parsimonious model retained YO beak chroma as a feature explaining variation in lysis scores in both sexes, but no sex interaction (Table 3, Table S5). YO chroma was weakly negatively ( $Zr = -0.24$ ;  $CI_{95} = [-0.54, 0.05]$ ) related to lysis titres (Fig. 5A). NAb titres were moderately negatively ( $Zr = -0.42$ ;  $CI_{95} = [-0.72, -0.12]$ ) related to patch surface in both sexes (again, no sex interaction) (Table 4, Table S6) (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 (Tables 5 and S7). UV brightness was moderately positively ( $Zr = +0.35$ ;  $CI_{95} = [0.05, 0.66]$ ) 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 Table S8). Birds breeding further up the valley had significantly higher basal CORT ( $3.56 \pm 0.35 \text{ ng mL}^{-1}$ ) levels than birds breeding close to the beaches ( $2.15 \pm 0.23 \text{ ng mL}^{-1}$ ).



**Figure 3.** Relationships between beak coloration and body condition in breeding king penguins. Relationships are given for (A) beak UV brightness, and (B) beak yellow-orange chroma. Females are depicted by open circles and a full line, males by filled circles and a dashed line. 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.

**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

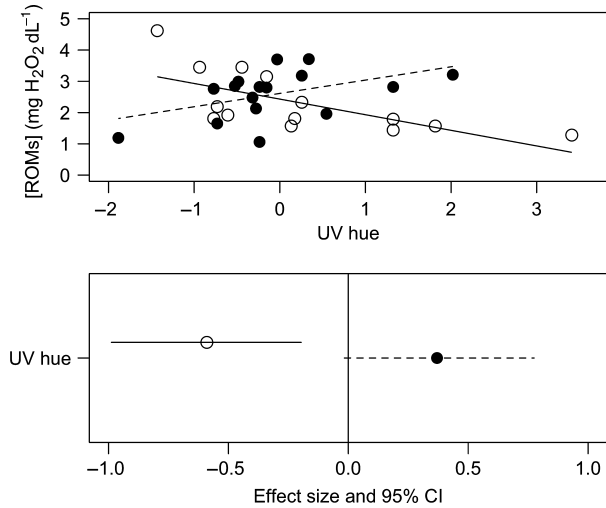
	Estimate	SE	<i>t</i> -value
Intercept	2.43	0.20	12.10
Sex[M]	0.18	0.27	0.66
UV hue	-0.50	0.16	-3.20
Sex[M]*UV hue	0.93	0.27	3.48

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 Table S9). UV hue ( $Z_r = -0.37$ ;  $CI_{95} = [-0.69, -0.06]$ ) 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 parsimonious model only retained colony area as an important factor explaining variation in birds' acute HR response to stress ( $d_{\text{unbiased}} = +0.59$ ;  $CI_{95} = [-0.09, 1.26]$ ; see Table S10). Birds breeding up the valley had slightly higher HR responses to

captures ( $132.6 \pm 8.1\%$ ) than birds breeding close to the beaches ( $113.8 \pm 11.6\%$ ).

## DISCUSSION

The two main hypotheses proposed to explain the evolution of elaborate ornamentation in males and females are the 'genetic correlation' and the 'mutual selection' hypotheses (Kraaijeveld *et al.*, 2007). The former proposes that showy ornaments are functional in males, but evolve as non-functional by-products of genetic correlations between the sexes in females (Lande, 1980). Selection then operates in males and the condition-dependence of ornaments should be primarily related to the male sex. The latter proposes that ornaments are functional in both sexes, evolving as honest signals of individual quality related to sexual or other, not mutually exclusive, forms of social selection (e.g. social competitiveness for breeding sites) (Johnstone *et al.*, 1996; Kokko & Johnstone, 2002; Hooper & Miller, 2008; Tobias *et al.*, 2012). Although the genetic correlation hypothesis predicts that ornaments should convey information mostly in males, the mutual selection hypothesis predicts that ornaments should convey information in both sexes.



**Figure 4.** Relationship between beak coloration and standardized plasma concentration of reactive oxygen metabolites [ROM] in breeding king penguins. Females are depicted by open circles and a full line, males by filled circles and a dashed line. The lower panel provides effect sizes and 95% CI calculated after Nakagawa & Cuthill, 2007. Effects are considered significant if their 95% CI does not overlap zero.

**Table 3.** Model estimates for the influence of beak YO chroma on plasma lysis titres 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	SE	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

In agreement with the mutual selection hypothesis, in king penguins we found that the showy ornaments used in mate choice were related to various aspects of physiological quality in both sexes. Successful breeding in this species involves obligate biparental care over an extended 14-month period (Stonehouse, 1960). Adults experience high annual divorce rates (up to 81%; Olsson, 1998) and courting birds encounter prospective mates at a high rate. Such conditions provide scope for mutual choosiness (Johnstone *et al.*, 1996; Kokko & Johnstone, 2002) and are indeed expected to favour the evolution of ornamental signals reflecting individual quality in both sexes (Kraaijeveld, 2003; Kraaijeveld *et al.*, 2007). However, we also found that not all facets of physiological quality were similarly related to

ornamentation in both sexes, suggesting that mutual ornamentation may be maintained by varying selective pressures in males and females (e.g. Murphy, 2007).

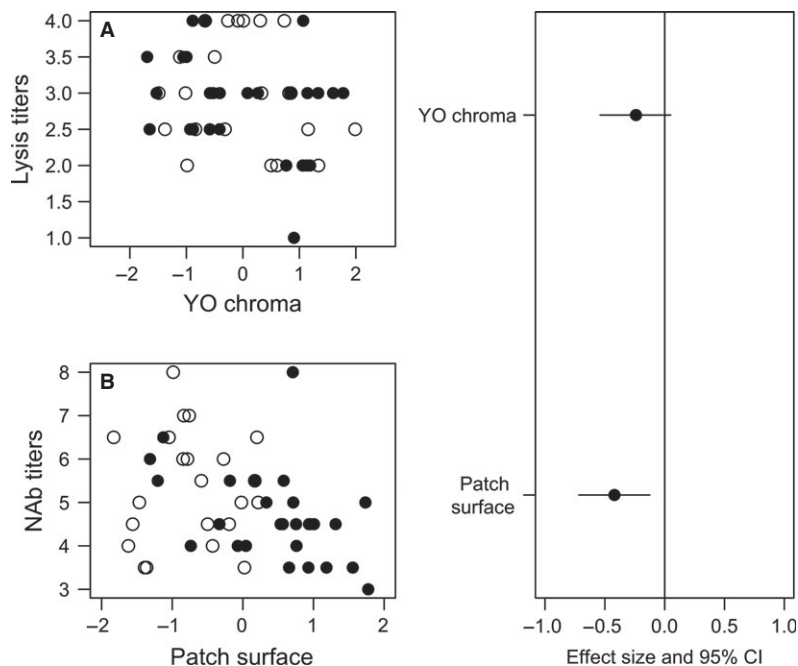
MUTUAL ORNAMENTATION AND IMMUNITY

One important cost of colonial breeding is parasitism (Mangin *et al.*, 2003). The immunocompetence hypothesis predicts that, given limited resources (energy, nutrients, protein), trade-offs occur between energy allocations to immunity or to the production and maintenance of ornamentation (Saino, Bolzern & Møller, 1997; Verhulst, Dieleman & Parmentier, 1999). Consistently, we found weak to moderate negative associations between measures of innate immunity and ornamental features in both sexes. Lysis and NAb titres were negatively related to YO beak chroma and auricular patch surface respectively suggesting that investing into larger auricular patches and more YO beaks may incur a cost in terms of immunity. Interestingly, Nolan *et al.* (2006) previously documented a link between the PHA skin test and breast coloration in males, although they failed to detect an association with beak coloration or auricular patch size. Unlike the PHA test that measures a wide range of immune responses involving both innate and acquired immunity (Tella *et al.*, 2008), NAb titres reflect a well defined component of the innate immune response not induced by an experimental infection (Matson *et al.*, 2005). These findings support the notion that different ornaments may signal different components of immunity in breeding birds (Kelly *et al.*, 2012).

MUTUAL ORNAMENTATION AND BODY CONDITION

Acquiring information on body condition should be especially important to mate choice in breeding seabirds that undergo extended periods of fasting while caring for the egg or chick (Groscolas & Robin, 2001). Surprisingly, we found that body condition was related to beak spot coloration differently in males and females. Better body condition was associated with lower UV brightness and higher YO chroma (both strong effects) in females, but higher UV brightness (moderate effect) in males. These results are consistent with previous findings of lower UV brightness for females in better body condition (Dobson *et al.*, 2008), but at odds with the idea that mutual selection for high UV reflectance occurs in both sexes (Nolan *et al.*, 2010; Keddar *et al.*, 2015b). One explanation is that males and females use beak spot signals differently. As males have to endure the longest reproductive fast (Stonehouse, 1960), including courtship and the first incubation shift,





**Figure 5.** Relationship between beak coloration, auricular patch surface and innate immunity in breeding king penguins. Relationships are given for (A) plasma lysis titres and yellow-orange chroma, and (B) plasma NAb titres and auricular patch surface. 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.

**Table 4.** Model estimates for the influence of auricular patch surface on plasma NAb titres 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	SE	<i>t</i> -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

choosing mates of high body condition should be especially important for females. In females, poor body condition to an extent could reflect greater investments into reproduction to the detriment of self-maintenance, which should be favoured by males. In females, body condition was negatively associated with increasing UV brightness but positively associated with increasing YO chroma, raising questions about the interactions between carotenoid and structural signals (Shawkey & Hill, 2005; Mougeot *et al.*, 2007; Dugas & McGraw, 2011). For instance, in red grouse (Mougeot *et al.*, 2007) and nestling house sparrows (Dugas & McGraw, 2011), carotenoid pigments appear to act as a mask,

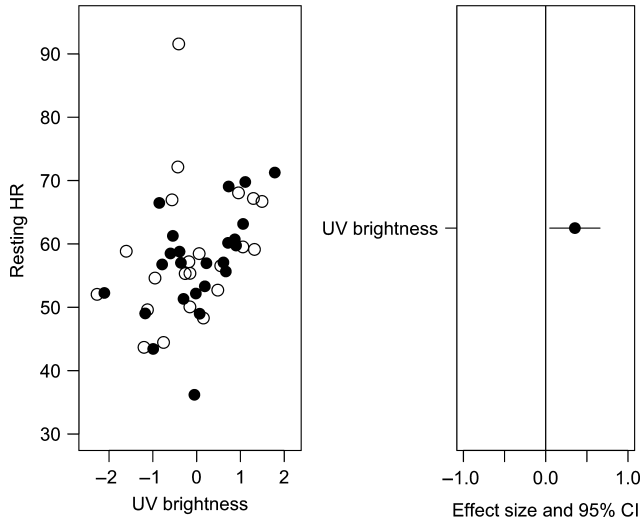
**Table 5.** Model estimates for the influence of UV brightness on daily resting heart rate in breeding king penguin (*Aptenodytes patagonicus*). The colony area effect is given in reference to area [A2]. See Fig. 6 for effect sizes with 95% CI

	Estimate	SE	<i>t</i> -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

decreasing UV reflectance in soft structures. There is some suggestion that carotenoid pigments are also found in the beak of king penguins (see McGraw *et al.*, 2007), and similar interactions might explain the opposite relationships we find for beak YO chroma and UV brightness. Further, only high condition females may have been able to allocate carotenoid pigments to their beak spots to function as signals (Blount *et al.*, 2003; Mougeot *et al.*, 2010).

#### MUTUAL ORNAMENTATION AND METABOLIC RATE

We found that beak UV brightness was positively (medium effect size) associated with resting HR levels

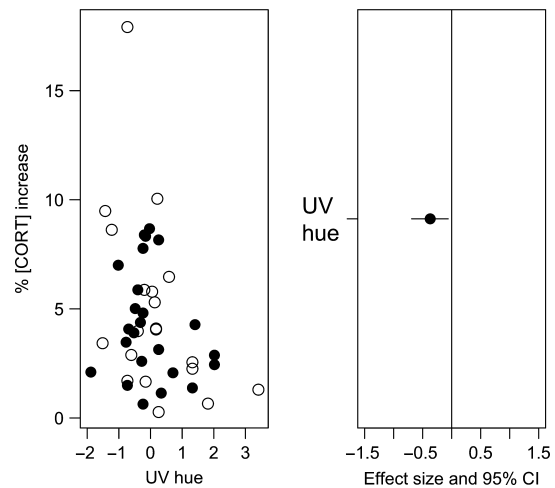


**Figure 6.** Relationship between beak UV brightness and daily resting HR levels (bpm) in breeding king penguins. On the left panel, males are depicted by filled circles, females by open circles. The right panel provides effect sizes and 95% CI calculated after Nakagawa & Cuthill, 2007. Effects are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

**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

	Estimate	SE	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

(a proxy for resting metabolic rate; Viblanc *et al.*, 2014) in both sexes. High resting metabolic rates may reflect increased capacities to engage in a suite of challenging activities such as foraging, caring for the young or competing for resources, and might be honestly reflected by colour ornaments (Biro & Stamps, 2010; Kelly *et al.*, 2012). The links between UV coloration and metabolic rate may lie within the energy costs of producing/maintaining structural colours (Siefferman & Hill, 2005; Doutrelant *et al.*, 2012). For example, Siefferman & Hill (2005) showed that experimentally reducing the energy cost of reproduction by reducing brood size in bluebirds (*Sialia sialis*) allowed males to increase their investment into plumage UV in the subsequent year. Rather than a long-term energy trade-off between competing functions (conserving energy for ornament production vs. expanding it for current reproduction), our results suggest possible indirect metabolic costs, such as keeping the beak clean, for UV maintenance.



**Figure 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.

MUTUAL ORNAMENTATION AND STRESS

Glucocorticoid hormones (GC) play key roles in mediating physiological trade-offs and energy allocation, and baseline GC levels have been suggested to ensure signal honesty (Husak & Moore, 2008; Weiss *et al.*, 2013). Whereas we found no link between baseline CORT and ornaments in our study, UV hue

was moderately and negatively associated with the birds' CORT response to acute stress ( $Zr = -0.37$ ;  $CI_{95} = [-0.69, -0.06]$ ). Birds with more UV hued beaks mounted a greater stress response to capture. Because stress responses are energy costly, this is consistent with the idea that the ability to mount stress responses while fasting is reflected in ornamentation, which may be particularly relevant in the context of colonial breeding during exposure to overt social aggressiveness (Côté, 2000). In contrast, we did not observe a link between ornaments and the acute HR response to stress, suggesting that HPA and sympathetic stress pathways may be modulated and signalled independently in breeding birds (e.g. Nephew, Kahn & Romero, 2003). We found that birds up the valley mounted slightly higher HR responses to capture, and had higher baseline CORT levels than birds breeding close to the beach. These results suggest two alternatives: that birds breeding close to the beach might have habituated to chronic human disturbance (Viblanc *et al.*, 2012b), and that birds up the valley may have been more exposed to parasites (P. Bize, Q. Schull, S. Pardouret, Y. Handrich, F. Criscuolo, V.A. Viblanc, J.P. Robin, unpubl. data), Manipulating circulating CORT levels in breeding birds may allow further exploration of the interplay between ornamentation, glucocorticoids, and cardiovascular function. For instance, chronic experimental increases in baseline stress levels (via CORT implants) have been shown to negatively affect UV and orange-red reflectance in female striped plateau lizards (*Sceloporus virgatus*) (Weiss *et al.*, 2013).

#### MUTUAL ORNAMENTATION AND OXIDATIVE STRESS

We observed sex-related differences in UV advertising for oxidative stress. In females, lower UV hue (i.e., hue more strongly embedded in the peak UV wavelengths) was strongly and positively associated with higher pro-oxidant levels (higher ROM but not higher OXY levels), whereas the opposite occurred in males (a moderate effect and the CI overlapped zero). This result was surprising for a structural colour, as links between ornamentation and oxidative status are expected for yellow-orange colours, because of the allocation trade-off of carotenoid pigments to either anti-oxidant or ornamental functions (von Schantz *et al.*, 1999; Mougeot *et al.*, 2010). However, the interplay between UV and yellow-orange colour reflectance might also convey information on carotenoid availability (Jacot *et al.*, 2010). Carotenoids absorb wavelengths of short to medium wavelengths (400–515 nm), and greater deposition of carotenoids in feathers has been experimentally shown to cause a shift in the UV peak to shorter wavelengths in great

tits (Jacot *et al.*, 2010). The precise link between carotenoid concentration and beak reflectance both in UV and YO wavelengths remains to be determined in king penguins. But our result may suggest that females depositing more carotenoids in their beak suffered from greater oxidative stress, highlighting a trade-off between pigment allocation to anti-oxidant defences or beak coloration. The exhaustive measurement of oxidative status of breeding birds requires supplementary markers of oxidative damage and anti-oxidant defence (e.g. lipid peroxidation, anti-oxidant enzymatic activity), and preferentially in different tissues (Selman *et al.*, 2012). However, our results add to the evidence that condition-dependent UV signals indeed occur in many bird species (Keyser & Hill, 2000; Bize *et al.*, 2006; Mougeot *et al.*, 2010), likely in interaction with carotenoid signalling.

#### CONCLUSION

Taken together our results suggest that monomorphic ornamentation reflects several aspects of physiological quality in king penguins, supporting the mutual selection hypothesis. Interestingly, the qualities signalled by mutual ornamentation may nonetheless differ (in fact be opposite) between the sexes, likely due to physiological differences and varying selection pressures. Because we collected the physiological and ornamental measures only at only one point in time, it remains to be explored if some of those traits are dynamic (e.g. beak coloration: Faivre *et al.*, 2003; Pham *et al.*, 2014) and whether birds may use them for short-term behavioural decisions. The further study of monomorphic species should shed new insights on the maintenance, information and costs of sexual signals.

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## AUTHOR CONTRIBUTIONS

Designed the study: VAV, FSD and PB. Did the field-work: BG, MK, SP, JPR and PB. Did the lab work: VAV, AS, QS. Analysed the data: VAV, CS and PB. Wrote the paper: VAV. All authors contributed to its revision.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Standardized measures of the auricular patches of breeding king penguin (*Aptenodytes patagonicus*).

**Figure 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*).

**Table S1.** Summary statistics of the structural size and ornamental data of breeding king penguin (*Aptenodytes patagonicus*) used in the study.

**Table S2.** Model selection for the effects of beak coloration and auricular patch surface on body condition (residuals, see Methods) in breeding king penguin (*Aptenodytes patagonicus*).

**Table S3.** 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*).

**Table S4.** Model selection for the effects beak coloration and auricular patch surface on plasma anti-oxidant capacity (OXY) in breeding king penguin (*Aptenodytes patagonicus*).

**Table S5.** Model selection for the effects of beak coloration and auricular patch surface on plasma lysis titres in breeding king penguin (*Aptenodytes patagonicus*).

**Table S6.** Model selection for the effects of beak coloration and auricular patch surface on plasma NAb titres in breeding king penguin (*Aptenodytes patagonicus*).

**Table S7.** Model selection for the effects of beak coloration and auricular patch surface on daily resting heart rate in breeding king penguin (*Aptenodytes patagonicus*).

**Table S8.** Model selection for the effects of beak coloration and auricular patch surface on baseline plasma total corticosterone levels in breeding king penguin (*Aptenodytes patagonicus*).

**Table S9.** Model selection for the effects of beak coloration and auricular patch surface on the relative corticosterone increase in response to a standardized 30 min capture in breeding king penguin (*Aptenodytes patagonicus*).

**Table S10.** 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*).