Behavioral Ecology Advance Access published June 22, 2016

Behavioral Ecology

The official journal of the ISBE International Society for Behavioral Ecology

Behavioral Ecology (2016), 00(00), 1–10. doi:10.1093/beheco/arw091

Original Article

Beak color dynamically signals changes in fasting status and parasite loads in king penguins

Quentin Schull,[a,b] F. Stephen Dobson,[c] Antoine Stier,[a,b] Jean-Patrice Robin,[a,b] Pierre Bize,[d] and Vincent A. Viblanc[a,b]

[a]Université de Strasbourg, 23 rue Becquerel, 67087 Strasbourg, France, [b]Département d'Ecologie, Physiologie et Ethologie, CNRS, IPHC, UMR 7178, 23 rue Becquerel, 67087 Strasbourg, France, [c]Department of Biological Sciences, Auburn University, 311 Funchess Hall, Auburn, AL 36849, USA, and [d]Institute of Biological and Environmental Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, AB24 2TZ, Scotland, UK

Received 13 January 2016; revised 18 May 2016; accepted 20 May 2016.

Dynamic ornamental signals that vary over minutes, hours or weeks can yield continuous information on individual condition (e.g., energy reserves or immune status), and may therefore be under strong social and/or sexual selection. In vertebrates, the coloration of the integument is often viewed as a dynamic ornament, which in birds can be apparent in the beak. King penguins (*Aptenodytes patagonicus*) are monomorphic seabirds that possess conspicuous yellow–orange (YO) and ultraviolet (UV) beak spots that are used by both males and females in mate choice. We studied the dynamicity of beak spot sexual traits, and to what extent they reflected changes in individual condition in fasting king penguins and in penguins treated with an anti-parasitic drug. We also describe the maturation of this colorful ornament during the yearly catastrophic moult. On a time-scale of days to weeks, beak spot coloration changed in response to fasting and experimental changes in parasite load. Beak spot UV brightness decreased over a 10-day fast in breeding birds. For birds caught during courtship and held in captivity YO chroma decreased after a 24-day fast. Birds that were treated with an anti-parasitic solution showed an increase in UV coloration after parasite removal. Altogether, our results show that beak spot coloration is a dynamic ornament that reflects multiple dimensions of changes in individual condition in breeding-fasting penguins.

Key words: **dynamic ornament, fasting, honest signal, king penguin, parasites, sexual selection.**

INTRODUCTION

Darwin's theory of sexual selection has been central to evolutionary biology, providing scientists with a framework for understanding mechanisms that might lead to the evolution of individuals selecting mates that produce fitter offspring (Darwin 1871). When assessing mate or competitor condition, animals often rely on ornamental signals that are costly to produce and/or maintain, and are therefore expected to honestly reflect individual quality (Zahavi 1975; Grafen 1990; Cotton et al. 2004; Walther and Clayton 2004). Mates may use such ornaments to assess the direct and/or indirect fitness benefits (e.g., paternal care, genetic benefits; Møller and Thornhill 1998; Mays and Hill 2004; Fromhage et al. 2009) that arise from mating with partners able to bear their cost.

In species where interactions with mates and/or social competitors occur repeatedly, there should be strong selection for dynamic signals that allow continuous tracking of changes in individual condition over extended periods of time (Velando et al. 2006; Ardia et al. 2010; Rosenthal et al. 2012). Dynamic changes in integument coloration have been reported in fish and amphibians (Sköld et al. 2013), reptiles (Weiss 2002), mammals (Stephen et al. 2009), and birds (Velando et al. 2006; Ardia et al. 2010). In birds, studies have suggested that beak coloration may serve as a dynamic signal of individual condition (Blount et al. 2003; Faivre, Grégoire, et al. 2003; Navarro et al. 2010; Rosenthal et al. 2012). In contrast to feathers that are replaced only during moult and constitute an inert (nonvascularized) tissue, the beak is a vascularized part of the integument (Lucas and Stettenheim 1972). Thus, rapid changes in beak coloration may reflect more dynamic changes than feathers in the deposition or mobilization of pigments (e.g., carotenoids; Alonso-Alvarez et al. 2004), or rearrangement of local microstructures (e.g., keratin; Dresp and Langley 2006) linked to modifications in individual condition over time.

One important trade-off shaping the evolution of honest signals is between sexual ornaments and immune function, and by extension resistance to parasites (Hamilton and Zuk 1982). Differential allocation of pigments to ornaments or immune function is thought

Address correspondence to Q. Schull. E-mail: quentin.schull@gmail.com.

to act as a constraint, so that only high quality individuals are able to concurrently invest pigments into both colored ornaments and efficient immune defences (Blount et al. 2003; Faivre, Préault, et al. 2003; Aguilera and Amat 2007). In addition, pigment-based coloration appears to change rapidly under stressful conditions or immune stimulation (Faivre, Grégoire, et al. 2003; Rosenthal et al. 2012), raising questions about the potential role of pigmented ornaments in reflecting rapid changes in body condition and energy depletion. In addition to pigmented ornaments, structural colors such as ultraviolet (UV) may also provide information on individual quality. For instance, inter-individual variation in integument UV coloration has been linked to inter-individual variation in body condition in several species (Bize et al. 2006; Jacot and Kempenaers 2007; Dobson et al. 2008; Viblanc et al. 2016). However, because of the structural nature of UV colors, it is unclear whether this trait is labile and reflects intra-individual changes in condition over short to long time periods.

We studied the dynamicity of beak coloration in king penguins (*Aptenodytes patagonicus*), and tested whether it responded to ecological factors (parasites, long-term fasting) that might produce changes in beak coloration. Both male and female king penguins display colorful yellow–orange (YO) beak spots that also reflect UV (Dresp et al. 2005; Jouventin et al. 2005). In particular, beak UV appears to be an important signal of individual quality used in mutual mate choice. For instance, experimental studies have shown that reducing beak UV brightness decreases the pairing likelihood in both males and females (Nolan et al. 2010). Further, beak UV is associated with indices of condition. Beak UV brightness was positively correlated to body condition in breeding males, but negatively correlated in breeding females (Dobson et al. 2008; Viblanc et al. 2016). Beak UV hue is negatively related to total oxidative damage in breeding females but not in males, and is negatively related to individuals' responsiveness to acute stress in both sexes (Viblanc et al. 2016).

UV coloration of penguin beaks is structural, resulting from the reflection of light off stacks of elongated lamellae in the horny layer of the beak, forming a photonic microstructure that reflects light in the UV to violet wavelengths (Dresp and Langley 2006). Removing 17% of the beak spot upper-layer results in a decrease of 10% in maximum reflectance. When the horn thickness is reduced by 38% the UV reflectance disappears but the YO orange reflectance remains (Dresp et al. 2005). The remaining reflectance (starting after 450 nm) is that of the YO beak color, likely caused by carotenoid pigments, assimilated through diet, that are only present in the deeper parts of the beak (McGraw et al. 2007). Indeed, YO beak colors appear to be constrained by the availability of environmental resources, birds displaying higher YO beak hue in good years (Keddar, Couchoux, et al. 2015). Because previous studies of beak spot coloration in this species have relied on measures of beak coloration taken on different individuals at a single point in time, it remains unclear whether beak coloration may signal changes in bird condition during breeding, and what underlying factors might trigger short-term changes in coloration. Here, we used repeated measures on breeding adult king penguins to investigate changes in beak coloration focusing on 2 original aspects of variation in beak color. First, we examined the development of beak coloration following moult. King penguins (and probably the closely related emperor penguin, which has similar UV and YO beak spots; Jouventin et al. 2005b) entirely renew the colored keratin superficial layer on both sides of the beak at the end of feather moult (see Supplementary Material ESM1), a feature that appears unique in birds. Renewed beak spots are present by the time birds return to land after the post-moult foraging trip at sea, and are displaying for mates on the beach adjacent to the breeding colony.

Second, we studied variation in beak spot coloration in response to physiological constraints (fasting and parasite load) of key importance to king penguins during reproduction. King penguin fast on-land, and face repetitive long-term fasting periods (up to 3–5 weeks for the male during the first shift of incubation) (Groscolas and Robin 2001). As many colonial animals, king penguins must also cope with parasites. Fasting and parasite resistance are thus 2 essential aspects of reproduction. Breeding birds will abandon reproduction if their energy reserves are critically depleted (Groscolas and Robin, 2001), and strong ectoparasite loads are known to diminish the health of adults and their offspring (Gauthier-Clerc et al. 1998; Mangin et al. 2003; Bize et al., unpublished data), and as pathogen vectors (Gauthier-Clerc et al. 1999) may be responsible for disease transmission. As bi-parental investment is an obligate condition for reproductive success, ornamental signals that dynamically reflect individual energetic reserves or parasite loads should be of importance for assessing partner condition throughout the season, and allow birds to adjust their reproductive effort accordingly.

METHODS

Study species

We studied king penguins in the "Baie du Marin" colony on Possession Island, Crozet Archipelago (46°25′ S, 51°45′ E) during the breeding season (November to March) in 2011–2012, 2012–2013 and 2014–2015. King penguins are long-lived seabirds with a unique breeding cycle. After having moulted and replenished their energy stores at sea, males and females will court and establish a breeding territory during a period of ca. 11 days before the female lays a single egg (Weimerskirch et al. 1992). Pairing takes place after ritualized interactions with several potential partners that include calling and exposing colored ornaments (including the beak spot) to tentative partners in stereotyped postures (sky-pointing of the head in unison with a potential partner; Jouventin 1982). Once the egg is laid, males take charge of the first incubation shift and must continue what is already a prolonged fasting period. The female relieves them some 16 days later, and males then return at sea to forage (Weimerskirch et al. 1992). Alternated incubation continues until the egg hatches on average 54 days later (Stonehouse 1960). Birds followed in these studies were at least 3–4 years old, the age at first reproduction in king penguins (Stonehouse 1960), but their exact age in unknown.

Measures of beak spot coloration

Colors reflected by the beak spot were measured using a portable JAZ spectrophotometer (Ocean Optics Inc., Dunedin, FL, USA) with a spectral resolution of 0.3 nm across the spectral range of 320–700 nm. The JAZ contains a pulsed-xenon light and was calibrated against a white standard (Ocean Optics Spectralon). Measures were repeated 3 times across each beak spot (in the YO region) using a 200-µm fiber-optic probe with a 90° angle window. The obtained spectra were smoothed and averaged using an R script adapted from Montgomerie (Montgomerie 2008) before calculating mean brightness, hue, and chroma (see below) over the spectral range 320–700 nm, which corresponds to the full range of spectral sensitivity in birds (Cuthill 2006). The reflectance spectrum of king penguin beak spots is composed of a peak in the UV-violet region and a plateau in the YO region of the spectrum (Figure 2).

For the UV part of the spectrum, the average wavelength of maximum reflectance (i.e., the peak maxima) is around 390 nm, but may reach as far as 420 nm when hydrated (Dresp and Langley 2006). Whereas, Keddar and colleagues split the entire spectrum into 2 parts (before and after 499 nm) in order to separate UV and YO domains (Keddar et al. 2013; Keddar, Jouventin, et al. 2015), the UV peak of birds in our study usually extended beyond 450 nm (see Figure 2), ending at around 490 nm (which also corresponded to the start of the YO beak color). Thus, to avoid missing a part of light/information emitted by the microstructure for the UV signal and to avoid integrating light in the YO domain that actually came from a structural rather than pigment based coloration, we calculated color variables separately over those 2 regions: 320–490 nm for UV and 491–700 nm YO colors. The spectral intensity of the beak spot, mean UV-violet brightness ($UV_{brightness}$) and mean YO brightness ($YO_{brightness}$) were calculated by averaging reflectance over wavelengths 320–490 nm and 491–700 nm, respectively (Montgomerie 2006). Hue is a measure of color appearance (e.g., "blue," "yellow," etc.). For the YO plateau portion of the spectrum, YO hue (YO_{hue}) was calculated as the wavelength at which the reflectance was halfway between its maximum and minimum (Keddar et al. 2013). For the UV-violet peak, UV hue (UV_{hue}) was calculated as the wavelength of maximum reflectance between 320 and 490 nm. Finally, chroma is a measure of color purity and was calculated within the region of interest (UV_{chroma} and YO_{chroma}) as the difference between maximum and minimum reflectance over the mean reflectance for that particular region (formula S_8; Hill and McGraw 2006, p. 108). Repeatability of the color measurements were high (between 0.70 and 0.91) and are given in Supplementary Material ESM 1. Correlations between those spectral parameters are presented in Figure 1a (see also Viblanc et al. 2016). Briefly, the correlations between UV and YO color parameters were very low, consistent with the fact that those colors in the king penguin are produced by 2 different mechanisms (structural for the UV and pigmentary for YO colors; see Dresp et al. 2005). For UV colors, brightness, hue and chroma parameters were not strongly associated and therefore we considered them separately in further analyses. For YO colors however, those parameters were highly correlated. Thus, we chose to focus YO_{chroma} for the following reasons. First, it has been argued that the signal with the highest among-individual variance also contains the most information (Dale 2006). YO_{chroma} was the parameter the most correlated with both brightness and hue, and also that presenting the highest Coefficient of Variation (Figure 1b). Second, YO_{chroma} has been shown to directly reflect ornament pigment concentration in several species (Saks et al. 2003; McGraw and Gregory 2004).

Changes in beak spot coloration following the moult

For adult king penguins, beak spots are renewed each year at the end of the period of moult of the entire plumage (Supplementary Material ESM 2). Moulting of feathers and beak spots occurs before the start of the breeding season in November-January and the whole process takes ca. 32 days (Groscolas and Cherel 1992). To study the maturation of the new beak spot following the moult process, 25 moulting males were caught shortly before the end of moult and kept captive in a pen. Birds were checked daily for moult completion, and the coloration of their beak spot was measured before the moult started and after the moult was completed. Seventeen birds were kept captive for an extra 2 days, (5 were released because they were close to reaching a critical body mass, that is, phase 3 of fasting; Groscolas and Robin 2001) and the coloration of the beak spot was measured a second time. All birds were released and departed the breeding colony to forage at sea. Before being released, birds were identified by marking them on the breast with a unique letter/number combination using a non-toxic human hair dye (Franck Provost, blue-black 2.1). The beach was walked every day to search for birds that returned to the colony to start displaying for a mate after having being at sea to feed and replenish their body reserves. Eighteen of the 25 followed birds were caught on the beach within a few hours after returning from their foraging trip and beak measurements were taken a final time. At the end of the moult, 3 individuals presented no reflectance signal whatsoever in the UV part of the beak (completely flat spectrum), but had a classical YO reflectance spectrum, clearly due to a lack of maturation of the UV component. These 3 individuals were not taken into account in the analyses of UV color parameters, explaining the variation of the sample size between UV and YO color analyses (i.e., $N = 22$ vs. 25).

We studied changes in beak coloration following the moult by specifying beak color variables (hue, chroma and brightness) as dependent variables in separate Linear Mixed Models (LMMs). The time period of color measurement was entered as a discrete ordinal variable with 3 levels: the day the old beak spots were shed (day 0), 2 days later (day 2), and the day birds returned from their post-moult foraging trip at sea (to begin courtship). Bird ID was entered as a random effect to account for repeated measurements on individuals. Correlations between color parameters before moult and after their post-moult foraging trip (courtship) were investigate using Spearman's rank test on 16 birds for which we had both measurements.

Changes in beak coloration during fasting

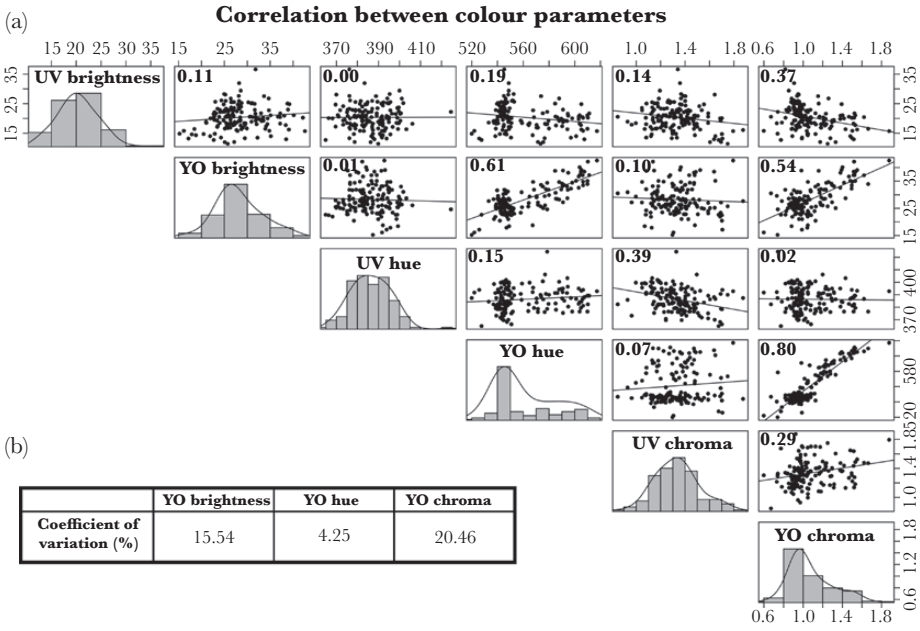
We investigated the effect of fasting on beak spot coloration using either breeding penguins that were naturally fasting while incubating their egg or captive penguins that were forced to fast. We ran 3 different studies that covered different fasting and duration periods for this species.

In a first study performed in 2011–2012, we studied 22 males and 22 females during the third and fourth incubation shift, respectively. We measured beak spots directly in the colony while birds were incubating their eggs. Birds were measured on their second day of incubation and again 6 days later (incubation day 8). We waited 2 days before the first measure to insure that individuals had settled on their eggs, thus avoiding any risk of breeding abandonment. Those birds had all just returned from their foraging trip before taking their incubation shift, and thus this study covers changes in beak coloration during the first days of fasting.

In a second study performed in 2014–2015, we investigated changes in beak coloration of 36 breeding males during their first incubation shift. Beak coloration was measured on the third day after the start of incubation and again 10 days later, that is, on day 13 of incubation. Males do not return at sea to feed between the period of courtship display and their first incubation shift, and thus they had already endured at least 10 days of fasting before our first measurement of coloration. Hence, this study covers changes in beak coloration during the second half of a long (>20 days) fasting period.

Finally, in a third study performed in 2013–2014 and 2014–2015, we caught 20 males during courtship on land (10 birds each year) and kept them captive in a pen. These individuals experienced a forced fasting period (0–24 days) covering the natural fasting periods of our first (2–8 days) and second (ca. 13–23 days) studies presented above.

(a)



(b)

	YO brightness	YO hue	YO chroma
Coefficient of variation (%)	15.54	4.25	20.46

Figure 1

(a) Pairwise correlation plots between the different color parameters in adult king penguin (*Aptenodytes patagonicus*) beak spots. Spearman correlation coefficients are provided (the slope of the best fit line shows positive or negative association) and the distribution of the each color parameter is presented. Note that UV and YO color parameters are weakly correlated (b) Coefficient of variation of the different YO color parameters. We compiled first measurements of the 181 individuals (36 where in courtship and 145 at the beginning of their breeding shift).

Such prolonged fasting periods are well within the natural range of fasting observed in this species. In these birds, we measured body mass and beak spot coloration every 6 days to investigate changes in color as fasting progressed. On release, all birds were observed departing at sea to feed and subsequently seen returning at the colony to breed.

Within each experiment, monitored birds were all caught on same day of the same breeding shift and thus had comparable breeding status. Changes in beak coloration during fasting were investigated by specifying beak color variables (hue, chroma and brightness) as dependent variables in separate LMMs. The time period of color measurement was entered as a discrete ordinal variable (e.g., day 2 and day 8 in the first study). Bird sex was entered as a fixed factor in the models, and for the first experiment (the only one where both sexes were monitored), we considered the interaction of sex and time. However, as the interaction was not significant, we removed it from the final model. In addition, we accounted for body girth, a proxy for body condition (Viblanc et al. 2012), as a covariate in the models. Again however, as body girth never significantly affected color parameters, we removed it from the final models. Bird ID was entered as a random effect to account for repeated measurements on individuals. In the third study, captive birds were measured in 2 different years (2013–2014 and 2014–2015), and thus we entered the year as random factor to control for potential year effects on coloration (Keddar, Couchoux, et al. 2015).

Response of beak coloration to experimental parasite removal

In 2011–2012, we investigated the effects of seabird parasite loads on beak coloration by experimentally removing parasites in an experimental group of 20 breeding pairs using the anti-parasitic solution Eprinex Pour-On®. This solution is commonly used in cattle and poultry and known to remove a large spectrum of ecto- and endo-parasites including worms, lice, ticks, mange mites, and grubs (Shoop et al. 1996). A control group of 20 pairs was treated with a solution of propylene glycol, which is the solvent used in Eprinex Pour-On®. We obtained information on changes in beak spot coloration in 27 treated birds (14 males, 13 females) and 33 control birds (16 males, 17 females). To control for possible confounding effects of breeding timing and localization in the colony on inter-individual variation in beak spot coloration, we applied our treatments so that treated and control pairs did not differ in their breeding onset (all were early breeders) or where they were breeding in the colony. The anti-parasitic and control solutions were deposited on the skin of the birds just below the feathers at the base of the neck at the beginning of the first and third incubation shift in males and at the beginning of the second and fourth incubation shift in females. The efficiency of the anti-parasitic treatment was controlled by counting tick loads (*Ixodes uriae*) on a small part of the body (the head) of monitored birds. At the start of the treatments (i.e., beginning of shift one of males and shift 2 of females), there was no difference in the number of ticks on the head of birds treated with the anti-parasitic versus sham solutions (mean ± standard error [SE] = 2.26 ticks ± 0.62 vs. 1.39 ticks ± 0.59; Wilcoxon's test: chi square = 0.21, df = 1, $P = 0.64$). The Eprinex Pour-On® solution was efficient at removing parasites as reflected by lower tick loads after treatment on the head of birds treated with the anti-parasitic versus sham solutions (0.05 ticks ± 0.64 vs. 3.15 ticks ± 0.62; Wilcoxon's test: chi square = 27.23, df = 1, $P < 0.001$). Effects of our treatments on changes in beak spot coloration were measured during shifts 3 and 4 for males and females, respectively, by taking a first measure of beak coloration on the second day after the start of incubation and again 6 days later, that is, on day 8 of incubation.

Changes in beak coloration in response to our treatments were investigated by specifying beak color variables (hue, chroma and brightness) as dependent variables in separate LMMs. The effect of the experimental anti-parasitic treatment on beak coloration

was tested from the significance of the interaction between treatment (anti-parasitic vs. sham) and time period (day 3 vs. day 8) in the model. Sex was added as a fixed factor in the model to test for potential sex differences in beak coloration variation and its interaction with treatment was also considered. Again, body girth was added as a covariate in the model and its interaction with treatment and period considered, but as it never showed any significant effect on color parameters, it was removed from the final models. Bird ID was entered as a random effect to account for repeated measurements on individuals.

Statistics

Statistical analyses were run with R v.3.1.1 (R development core team). F-statistics for fixed effects (tests of differences from zero), the total number of observations (n) and corresponding number of individuals (N) are given. Effects were considered significant for $P < 0.05$. When appropriate, significant differences between groups were assessed using Tukey's Honest Significant Difference (HSD) tests for least square means. We insured residuals followed a normal distribution using qqplots (opposing theoretical Quantiles to Sample Quantiles).

Ethical statement

All experiments were approved by an independent ethics committee (Comité d'éthique Midi-Pyrénées pour l'expérimentation animale) commissioned by the French Polar Institute and comply with the current laws of France. Authorizations to enter the breeding colony and handle the birds were provided by the "Terres Australes et Antarctiques Françaises" (permit n°2010–65 issued on the 3 September 2010, n°2011–96 issued on 14 October 2011, n°2012–116 issued on 29 October 2012, n°2013–72 issued on 29 October 2013 and n°2014–127 issued on 15 October 2014).

RESULTS

Changes in beak spot coloration following the moult

Birds showed pronounced changes in the coloration of their beak spot following the moult. The new beak spot appeared particularly pinkish and changed from day to day (Figure 2). $UV_{brightness}$ of the new beak decreased by 14% within the first 2 days of shedding the old beak spot, and continued to decrease by another 23% during the post-moult foraging trip at sea (LMM; F =79.21, df = 2, $P < 0.001$, $n = 55$, $N = 22$). Freshly moulted birds exhibited a rapid decrease in the UV_{hue} of their new beak spot (by 20.7 nm on average) within the first 2 days of shedding the old beak spot (Figure 2). UV_{hue} continued to decrease during the post-moult foraging trip (another 10.7 nm on average), albeit less substantially (F = 30.20, df = 2, $P < 0.001$, $n = 55$, $N = 22$). We observed increases both in UV_{chroma} (+19% within 2 days post-moult and another 23% by the time the birds returned for courtship; F = 51.04, df = 2, $P < 0.001$,

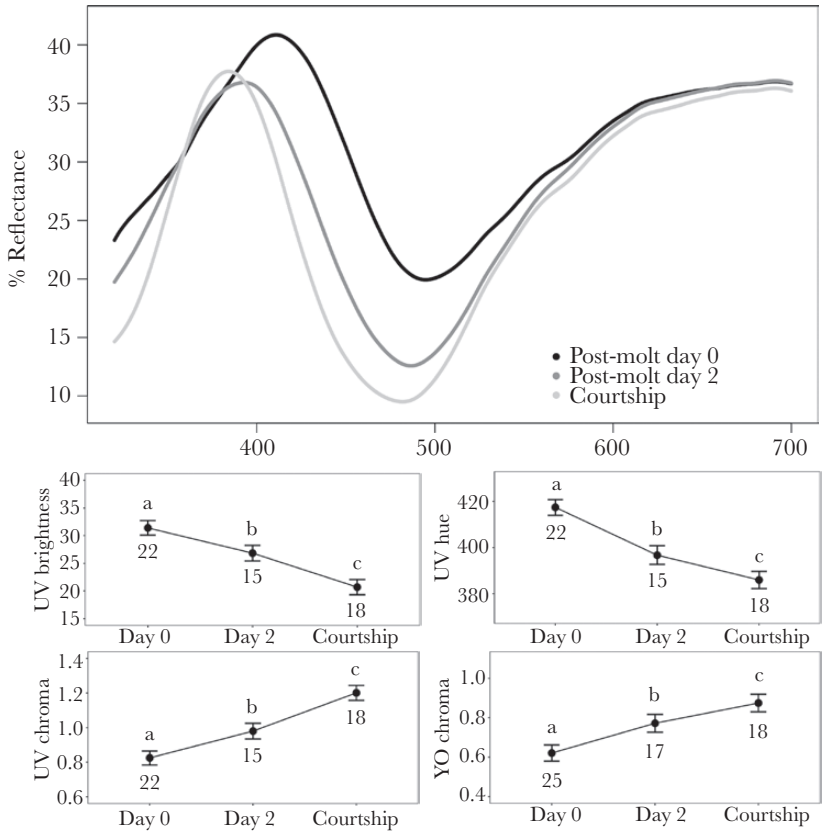


Figure 2
Changes in the beak coloration of adult king penguins (*Aptenodytes patagonicus*) following the moult. Average changes in raw spectral data over all birds are presented for illustrative purposes. Beak color was measured on the day the old beak spot was shed (day 0), 2 days later (day 2), and after birds returned from their post-moult foraging trip for breeding (courtship). Changes in brightness, hue and chroma were assessed using LMMs, with bird ID specified as a random variable. Least-Square means ± SE are presented. Values not sharing a common letter are significantly different at $P < 0.05$. Sample sizes are given below the means.

$n = 55$, $N = 22$) and YO_{chroma} (+24% within 2 days post-moult, and another 13% by courtship; $F = 23.88$, df = 2, $P < 0.001$, $n = 60$, $N = 25$).

When comparing color parameters before the moult and after birds came back from their post-moult foraging trip, UV_{hue} was significantly correlated (Spearman's rank correlation; $\rho = 0.64$, $S = 246.7$, $P = 0.008$, $n = 30$, $N = 15$, Figure 3). Other parameters, however, remained uncorrelated ($-0.36 < \rho < 0.38$, $422 < S < 992$, $0.148 < P < 0.51$, $n = 30$, $N = 15$).

Changes in beak spot coloration during fasting

In breeding penguins naturally fasting in the colony, we found no significant changes in beak spot coloration during the first days of fasting (between day 2 and 8 of males in shift 3 and females in shift 4; LMMs; $0.01 < F < 0.51$, $0.48 < P < 0.98$, $n = 88$, $N = 44$). In contrast, we found a significant 6% decrease in $UV_{brightness}$ in breeding male penguins at the end of a long natural fasting period (between day 13 and 23 of the first incubation shift) (LMM; $F = 7.90$, $P < 0.01$, $n = 72$, $N = 36$, Figure 4) and no significant changes for the other color parameters (LMMs; $0.16 < F < 2.54$, $0.12 < P < 0.69$, $n = 72$, $N = 36$).

In captive birds that fasted up to a similar body mass of that naturally occurring at partner relief (24 days), fasting duration did not affect UV beak coloration (brightness, hue or chroma; LMMs: $0.92 < F < 2.04$, df = 4, $0.10 < P < 0.46$, $n = 91$, $N = 20$). In contrast, YO_{chroma} decreased ($F = 4.23$, df = 4, $P < 0.05$) with increased fasting (Figure 5), and showed a strong significant decrease at the end of the fasting period (Tukey's HSD: $-3.63 < z < -2.77$, $0.003 < P < 0.044$, Supplementary Material ESM 3).

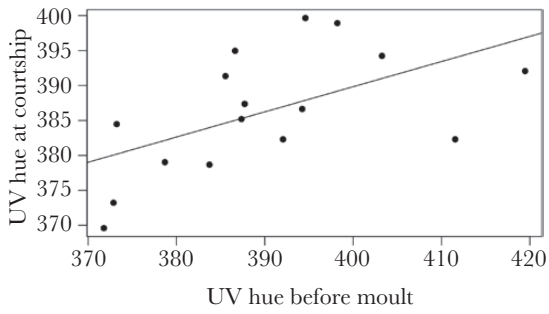


Figure 3
Correlation between beak UV_{hue} before king penguins (*Aptenodytes patagonicus*) moult and after the post-moult foraging trip at the time the birds come to the breeding colony for courtship. Correlation were assessed using Spearman's rank correlation test ($\rho = 0.64$; $S = 246.7$; $P = 0.008$).

Response of beak coloration to experimental parasite removal

Six days after the experimental treatments (Eprinex® or sham), birds treated with the anti-parasitic solution showed significant increases in beak $UV_{brightness}$ and UV_{hue}, and a decrease in UV_{chroma} compared with before the treatments (Table 1; Figure 6). Birds did not show significant changes in beak coloration after having received the control treatment (Tukey's HSD; $0.80 < P < 0.99$, $n = 120$, $N = 60$). Bird sex, whether considered independently or in interaction with treatments and/or time period, never significantly affected beak coloration ($0.12 < P < 0.96$ $n = 120$, $N = 60$). The effect of sex was removed from the final models presented in Table 1.

DISCUSSION

Dynamic ornamental signals can provide continuous information on individual condition, and are expected to be under strong sexual and social selection (e.g., Velando et al. 2006). In birds, beak coloration has been proposed to function as a dynamic ornament (Faivre, Grégoire, et al. 2003; Navarro et al. 2010; Rosenthal et al. 2012) as indeed appears to be the case in our species. King penguins showed a rapid maturation of beak coloration following the moult and we found dynamic changes in coloration in response to changes in individual condition (fasting status and parasite load) in breeding birds on the scale of a single breeding season.

Changes in beak spot coloration following the moult

Although studies on avian moult are numerous, there is virtually no information on moulted structures other than feathers (King and Murphy 1990). Horn, claw and beak material are thought to grow continuously in response to wear, but king penguins appear to be an exception in that the entire horny material of their beak spot is shed every year at the end of the moult, while the rest of the black beak horn is not. Thus, beak spots are replaced each time these long-lived seabirds breed, most of the time attracting a new breeding partner (Olsson 1998; Bried et al. 1999). After the moult, beak spot coloration becomes less bright, changing to a deeper (decrease in hue) and purer UV color (Figure 2, Supplementary Material ESM4). As previously mentioned, the UV coloration of penguin beaks is structural, resulting from the reflection of stacks of elongated lamellae (multiple layers of doubly folded membranes) in the horny layer of the beak (Dresp and Langley 2006). The distance (in nm) separating those double-folds is responsible for UV_{hue}, that is, the lattice dimension of the photonic crystals (Dresp and Langley 2006). This photonic property is explained by Bragg's law, with $\lambda\ max = n2d\ sin\theta$, where $\lambda\ max$ is the peak wavelength of reflected

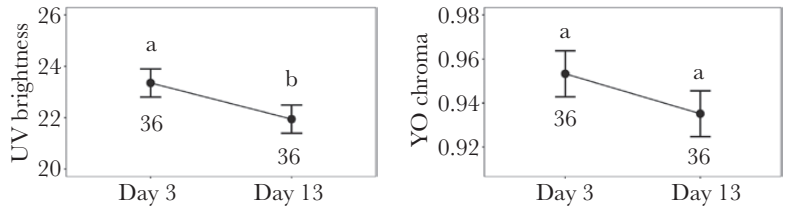


Figure 4
Changes in beak $UV_{brightness}$ and YO_{chroma} for 36 male king penguins (*Aptenodytes patagonicus*) that were measured on day 3 of their first incubation shift and on day 13 after having experienced a 10-day fast while breeding in the colony. Changes in brightness were assessed using LMMs, with bird ID specified as a random variable. Least-Square (LS) means ± SE are presented. Values not sharing a common letter are significantly different for $P < 0.05$. The sample sizes are given below the means.

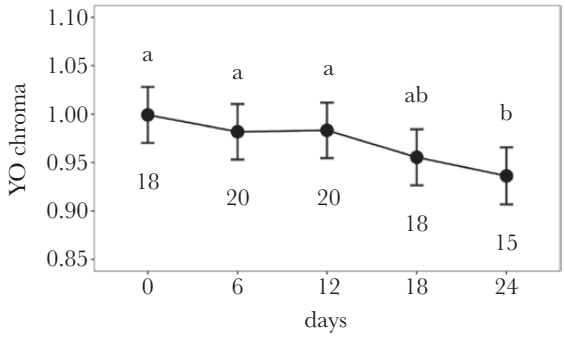


Figure 5
Changes in yellow–orange beak chroma (YO_{chroma}) in 20 male king penguins (*Aptenodytes patagonicus*) that endured a prolonged fast in captivity. Changes in chroma were assessed using LMMs, with bird ID and year specified as random variables.

Table 1
Mixed model estimates (±SE) for the effects of an anti-parasitic treatment on beak coloration in breeding king penguins (*Aptenodytes patagonicus*)

	Term	Estimate ± SE	*t* Ratio	$P > \|t\|$
$UV_{brightness}$	Intercept	18.56 ± 0.57	32.99	<0.001*
	Period[Post-treatment]	−0.52 ± 0.66	−0.78	0.437
	Treatment [T]	−0.23 ± 0.84	−0.27	0.787
	Period × Treatment	4. 68 ± 0.98	4.79	<0.001*
UV_{hue}	Intercept	388.65 ± 1.51	256.57	<0.001*
	Period[Post-treatment]	0.12 ± 1.10	0.11	0.915
	Treatment [T]	−1.34 ± 2.26	−0.61	0.54
	Period × Treatment	4.15 ± 1.62	2.56	<0.001*
UV_{chroma}	Intercept	1.33 ± 0.03	46.21	<0.001*
	Period[Post-treatment]	−0.02 ± 0.04	−0.43	0.666
	Treatment [T]	0.08 ± 0.04	1.87	0.064
	Period × Treatment	−0.12 ± 0.05	−2.20	0.032*
YO_{chroma}	Intercept	1.16 ± 0.04	28.27	<0.001*
	Period[Post-treatment]	−0.01 ± 0.06	−0.13	0.898
	Treatment [T]	0.13 ± 0.06	2.16	0.032*
	Period × Treatment	−0.10 ± 0.08	−1.249	0.217

The control level for the treatment factor is tested against the treatment level [T]. The time period [post-treatment] is tested against the [pre-treatment] level. Bird ID were entered as a random factor in the model to account for repeated measures on the individual. Significant values for $P < 0.05$ are indicated by an asterisk (*). Note the significant interaction terms revealing different treatment effects on pre- and post-treatment measures of treated vs. control birds. $n = 120$ observations, $N = 60$ birds.

light, n is the average refractive index of the tissue, d is the separation of the layers (lattice dimension), and θ is the angle of incidence of the light (Bragg 1915). Thus, our results suggest that as the beak matures, there is a decrease in the distance between the doubly folded membrane structures that compose the upper-layer of the beak. Simultaneously, although the magnitude of change is weaker, the YO color of the beak also becomes purer as chroma increases. These latter color changes occur as the bird prepares to mate, over a period of 13–20 days and are likely explained by the deposition of carotenoid pigments in the deeper layers of beak spots (higher chroma), as is the case in many bird species (Saks et al. 2003; McGraw and Gregory 2004). Interestingly, comparing colors before and at courtship for the same birds, UV_{hue} appeared strongly correlated (Figure 3). This suggests that UV_{hue} (the distance between doubly-folded membranes) is partly influenced by genetic or developmental factors. However, there is considerable scope for environmental influences as well (i.e., time since the last moult, time spent at sea during the pas year, time spent preening, etc.).

Beak spot coloration and fasting

Nutritional status and body condition are important information that may be used by conspecifics both in reproductive and social contexts. In the Alpine swift and the European starling for instance, parents adapt their feeding effort to the UV coloration of chick skin, which is positively correlated with body mass and structural size, and chick coloration is used by parents to adapt their feeding behavior to the intensity of the reflected color of their chicks (Bize et al. 2006). In the same way, in blue-footed boobies, rapid experimental changes in male foot integument coloration (reflecting nutritional status) elicited rapid adjustments in female reproductive strategies. Specifically, females reduced their allocation of energy to their broods, laying smaller eggs compared to controls (Velando et al. 2006). In our study, male beak coloration appeared to reflect changes in nutritional status. Over 24 fasting days, captive males showed a progressive decline in YO_{chroma} but no change in UV coloration. However, when fasting was prolonged for even longer periods in colonial conditions (over >24 days including 11–15 days of courtship followed by 13 days of incubation; Figure 4), $UV_{brightness}$ appeared to decrease. Those results suggests that UV and YO coloration may signal fasting status on different time-scales. A progressive decrease in YO_{chroma} in fasting birds may reflect a re-allocation of beak pigments to antioxidant defences, in line with the hypothesis of costly signalization by (limited) carotenoid-dependent structures (e.g., in birds: Alonso-Alvarez et al. 2004; in fish: Pike et al. 2010; but see Cote et al. 2010 in reptiles). This suggestion is consistent with our recent data suggesting that plasmatic anti-oxidant defences indeed increase over the course of fasting (Q.S., V.A.V., A.S., J-P.R., and P.B.; unpublished data). YO colors are often produced by exogenous carotenoid pigments, which are acquired from the diet and may reflect yearly environmental forage conditions (Linville and Breitwisch 1997; McGraw et al. 2009; Slagsvold and Lifjeld 2009). In king penguins, similar mechanisms might explain variation in YO color production between years (i.e., higher YO color in years of high resource availability; Keddar, Couchoux, et al. 2015). Decreases in $UV_{brightness}$ only at advanced fasting stages may suggest a cost for UV maintenance and the inability to maintain high UV reflectance when energy is critically limiting. For instance, preening and associated comfort behavior (keeping the beak clean) is generally costly in birds (Walther and Clayton 2004), including king penguins (Viblanc et al. 2011), and reducing those behaviors may allow substantial savings at advanced stages of fasting. Whether such behavioral changes linked to fasting status might indirectly affect the maintenance of beak coloration remains to be tested. Furthermore, as captive birds and free-living breeders also differed in their social environmental and breeding status, we cannot exclude that such differences also affected changes in beak coloration differently between the 2 groups. Further investigations are needed to clarify how beak spot dynamics are conditioned by the rate of nutrient reserves mobilization and availability of dietary antioxidants such as carotenoids. This could be achieved by concomitantly following plasma carotenoid availability and changes in beak coloration over the course of fasting.

Beak spot coloration and parasites

Parasites drain resources (including pigments and nutrients) from their hosts, which might otherwise be partly allocated to producing ornaments. Furthermore, in fighting parasites, hosts also mount immune responses that divert resources from other functions such as ornamentation (Rosenthal et al. 2012; Velando et al. 2014). Regardless of the underlying mechanism, parasitism should

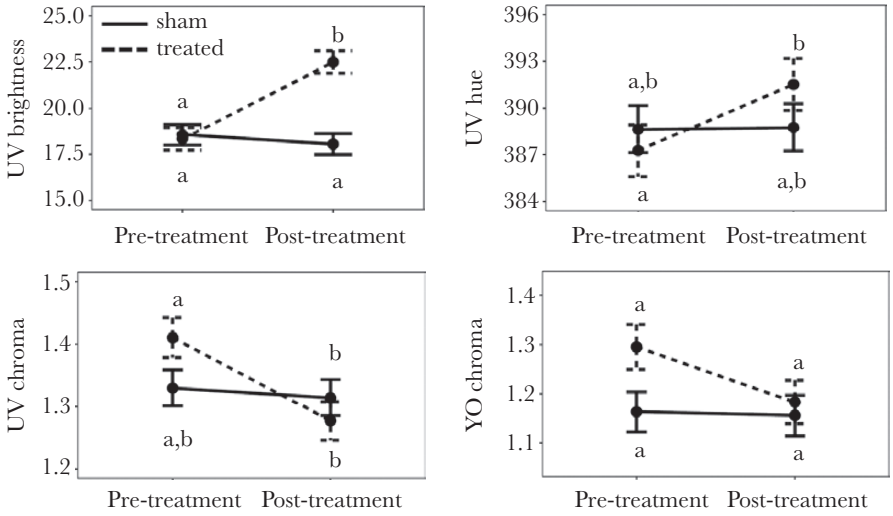


Figure 6
Interactive effects of an experimental anti-parasitic treatment on the beak coloration of adult kings penguins (*Aptenodytes patagonicus*) freely incubating in the colony. Changes in brightness, hue and chroma were assessed using LMMs, with bird ID specified as a random variable, the treatment (treated vs. control), the time period (pre-treatment vs. post-treatment) and the interaction between those factors specified as independent variables. Least-Square (LS) means are presented. Values not sharing a common letter are significantly different for $P < 0.05$.

affect ornamental signals (e.g., parasite-mediated sexual selection; Hamilton and Zuk 1982). Consistent with this hypothesis, we found that removing parasites from males and females during incubation produced significant changes in the UV component of beak coloration. While not influencing YO colors, removing parasites resulted in beak spots of higher $UV_{brightness}$, higher UV_{hue} and lower $UV_{saturation}$ (Figure 6). Birds relieved of parasites should have a less stimulated immune system, and may therefore invest more into beak coloration. Accordingly, several studies in birds have highlighted strong links between UV ornamentation and parasite load (Hõrak et al. 2001; Mougeot et al. 2005) or in a broader context immuno-competence (Griggio et al. 2010).

Here again, the independent changes we observe in UV or YO coloration support previous findings that in king penguins, UV and YO colors of the beak are produced by distinctly different mechanisms (Dresp and Langley 2006), and appear to change over the course of the breeding season. Indeed, YO carotenoid-based coloration is produced by pigments embedded in the deeper beak layers and was relatively unaffected in our anti-parasitic treatment. In contrast, changes in beak UV suggest structural modifications. UV reflectance depends on the thickness of the upper beak layer. This layer is composed of doubly folded membrane structures that result from the differentiation of basal cells into dead keratin (as is the case for skin renewal; Dresp and Langley 2006). An increase in UV reflectance following parasite removal suggests either an increase in cell division or a reorganization of those structures. However, whether the thickness of the upper beak layer can be increased after the moult (i.e., complete renewal of the structure), or whether it can be remodeled is currently unknown and requires further investigation. Moreover, ectoparasites are known disease vectors, as for instance Lyme disease carried by ticks (Gauthier-Clerc et al. 1999), which would not be cured by our anti-parasitic treatment and could limit the effect of our experimental treatment. Whereas other proximal mechanisms are likely, our results point to parasitism as an important influence on structural based UV color signalization in the king penguin.

CONCLUDING REMARKS

The beak coloration of king penguins is highly flexible, with both components of coloration (UV and YO) modified separately. Both beak UV hue and chroma, and YO chroma appear to have a maturation process, with an associated decline in beak spot brightness that continues through pre-breeding moult, subsequent feeding at sea, return to the breeding grounds, and mating. Importantly, due to its dynamicity, beak coloration may serve as an important signal of short-term changes in individual condition over the course of a single breeding season. This supports the idea that the information conveyed by sexual ornaments is not restricted to the single time period when mate choice occurs (e.g., Velando et al. 2006; Ardia et al. 2010).

Previous studies have found that higher beak $UV_{brightness}$ is associated with greater mating prospects (Nolan et al. 2010) and different body condition in males and females (Dobson et al. 2008; Viblanc et al. 2016). Higher beak UV_{hue} is negatively related with hormonal stress responses and oxidative damages in females (Viblanc et al. 2016). In line with these studies, our current results highlighted positive associations between $UV_{brightness}$ and YO_{chroma} and fasting, that is, a decrease in beak coloration with a decrease in individual body condition. On the contrary, when parasites were removed during breeding, the condition of the birds increased, as well as $UV_{brightness}$. The next step is to focus on how such variations might be perceived by the mate at the time it takes over egg or chick-guarding duties, how beak coloration may help parents coordinate their efforts throughout the breeding season, and how dynamic changes in beak coloration may be perceived by social conspecifics.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

FUNDING

This research was funded by the French Polar Institute (IPEV–Research Program 119) and the French National Centre for

Scientific Research (CNRS-INEE). Field logistic support was provided by Terres Australes et Antarctiques Françaises. Q.S. was funded by a doctoral fellowship from the Ministère Français de l'Education Supérieur et de la Recherche.

We thank all over-wintering assistants: Benoit Gineste, Sylvia Pardonnet, Laureline Durand, Emilie Lefol and Hédi Saadaoui for field work and Emilio Rojas for helpful discussion on the analyses. We apologize to our stick insect (*Carausius morosus*) for bearing with VAV's inquisitive curiosity during our debates on color ornaments in king penguins. We sincerely thank the editor and 2 anonymous reviewers for their helpful comments on a previous version of the paper.

Data accessibility: The data related to this publication are archived at the Dryad Digital Repository DOI:10.5061/dryad.5j40q. Analyses reported in this article can be reproduced using the data provided by Schull et al. (2016).

Handling editor: Marc Thery

REFERENCES

Aguilera E, Amat JA. 2007. Carotenoids, immune response and the expression of sexual ornaments in male greenfinches (Carduelis chloris). Naturwissenschaften. 94:895–902.

Alonso-Alvarez C, Bertrand S, Devevey G, Gaillard M, Prost J, Faivre B, Sorci G. 2004. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. Am Nat. 164:651–659.

Ardia DR, Broughton DR, Gleicher MJ. 2010. Short-term exposure to testosterone propionate leads to rapid bill color and dominance changes in zebra finches. Horm Behav. 58:526–532.

Bize P, Piault R, Moureau B, Heeb P. 2006. A UV signal of offspring condition mediates context-dependent parental favouritism. Proc Biol Sci. 273:2063–2068.

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

Bragg WH. 1915. Bakerian lecture: X-rays and crystal structure. Philos. Trans. R. Soc. London. Ser A. 215:253–274.

Bried J, Jeguet F, Jouventin P. 1999. Why do Aptenodytes penguins have high divorce rates? Auk. 116:504–512.

Cote J, Meylan S, Clobert J, Voituron Y. 2010. Carotenoid-based coloration, oxidative stress and corticosterone in common lizards. J Exp Biol. 213:2116–2124.

Cotton S, Fowler K, Pomiankowski A. 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? Proc Biol Sci. 271:771–783.

Cuthill IC. 2006. Color perception. In: Hill GE, McGraw KJ, editors. Bird coloration, volume 1: mechanisms and measurements. Cambridge (MA): Harvard University Press. p. 3–40.

Dale J. 2006. Intraspecific variation in coloration. In: Hill GE, McGraw KJ, editors. Bird coloration, vol. 2: function and evolution. Cambridge (MA): Harvard University Press. p. 36–86.

Darwin C. 1871. The descent of man, and selection in relation to sex. London: Murray.

Dobson FS, Nolan PM, Nicolaus M, Bajzak C, Coquel A-S, Jouventin P. 2008. Comparison of Color and Body Condition Between Early and Late Breeding King Penguins. Ethology. 114:925–933.

Dresp B, Jouventin P, Langley K. 2005. Ultraviolet reflecting photonic microstructures in the King Penguin beak. Biol Lett. 1:310–313.

Dresp B, Langley K. 2006. Fine structural dependence of ultraviolet reflections in the King Penguin beak horn. Anat Rec A Discov Mol Cell Evol Biol. 288:213–222.

Faivre B, Grégoire A, Préault M, Cézilly F, Sorci G. 2003. Immune activation rapidly mirrored in a secondary sexual trait. Science. 300:103.

Faivre B, Préault M, Salvadori F, Théry M, Gaillard M, Cézilly F. 2003. Bill colour and immunocompetence in the European blackbird. Anim Behav. 65:1125–1131.

Fromhage L, Kokko H, Reid JM. 2009. Evolution of mate choice for genome-wide heterozygosity. Evolution. 63:684–694.

Gauthier-Clerc M, Clerquin Y, Handrich Y. 1998. Hyperinfestation by ticks Ixodes uria: a possible cause of death in adult king penguins, a long-lived seabird. Colon Waterbirds. 21:229–233.

Gauthier-Clerc M, Jaulhac B, Frenot Y, Bachelard C, Monteil H, Le Maho Y, Handrich Y. 1999. Prevalence of Borrelia burgdorferi (the Lyme disease agent) antibodies in king penguin Aptenodytes patagonicus in Crozet Archipelago. Polar Biol. 22:141–143.

Grafen A. 1990. Biological signals as handicaps. J Theor Biol. 144:517–546.

Griggio M, Zanollo V, Hoi H. 2010. UV plumage color is an honest signal of quality in male budgerigars. Ecol Res. 25:77–82.

Groscolas R, Cherel Y. 1992. How to molt while fasting in the cold: the metabolic and hormonal adaptations of emperor and king penguins. Ornis Scand. 23:328–334.

Groscolas R, Robin J. 2001. Long-term fasting and re-feeding in penguins. Comp Biochem Physiol Part A Mol Integr Physiol. 128:643–653.

Hamilton WD, Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites? Science. 218:384–387.

Hill GE, McGraw KJ, editors. 2006. Bird coloration, vol. 1, mechanisms and measurements. Cambridge (MA): Harvard University Press.

Hõrak P, Ots I, Vellau H, Spottiswoode C, Møller AP. 2001. Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. Oecologia. 126:166–173.

Jacot A, Kempenaers B. 2007. Effects of nestling condition on UV plumage traits in blue tits: An experimental approach. Behav Ecol. 18:34–40.

Jouventin P. 1982. Visual and vocal signals in penguins, their evolution and adaptive characters. Adv Ethol. 24:1–149.

Jouventin P, Nolan PM, Örnborg J, Dobson FS. 2005a. Ultraviolet beak spots in king and emperor penguins. Condor. 107:144.

Jouventin P, Nolan PM, Örnborg J, Dobson FS. 2005b. Ultraviolet Beak Spots in King and Emperor Penguins. Condor. 107:144.

Keddar I, Andris M, Bonadonna F, Dobson FS. 2013. Male-Biased Mate Competition in King Penguin Trio Parades. Ethology. 119:389–396.

Keddar I, Couchoux C, Jouventin P, Dobson FS. 2015. Variation of mutual colour ornaments of king penguins in response to winter resource availability. Behavior. 152:1679–1700.

Keddar I, Jouventin P, Dobson FS. 2015. Color ornaments and territory position in king penguins. Behav Processes. 119:32–37.

King JR, Murphy ME. 1990. Estimates of the Mass of Structures Other than Plumage Produced during Molt by White-Crowned Sparrows. Condor. 92:839.

Linville SU, Breitwisch R. 1997. Carotenoid Availability and Plumage Coloration in a Wild Population of Northern Cardinals. Auk. 114:796–800.

Lucas AM, Stettenheim PR. 1972. Avian Anatomy—Integument. Agriculture Handbook 362, Washington, DC: U.S. Dept. Agric.

Mangin S, Gauthier-clerc M, Frenot Y, Gendner J, Maho Le Y. 2003. Ticks Ixodes uriae and the breeding performance of a colonial seabird, king penguin Aptenodytes patagonicus. J Avian Biol. 34:30–34.

Mays HL Jr, Hill GE. 2004. Choosing mates: good genes versus genes that are a good fit. Trends Ecol Evol. 19:554–559.

McGraw KJ, Gregory AJ. 2004. Carotenoid pigments in male American goldfinches: What is the optimal biochemical strategy for becoming colourful? Biol J Linn Soc. 83:273–280.

McGraw KJ, Massaro M, Rivers TJ, Mattern T. 2009. Annual, sexual, size- and condition-related variation in the colour and fluorescent pigment content of yellow crest-feathers in Snares Penguins (Eudyptes robustus). Emu. 109:93.

McGraw KJ, Toomey MB, Nolan PM, Morehouse NI, Massaro M, Jouventin P. 2007. A description of unique fluorescent yellow pigments in penguin feathers. Pigment Cell Res. 20:301–304.

Møller AP, Thornhill R. 1998. Male parental care, differential parental investment by females and sexual selection. Anim Behav. 55:1507–1515.

Montgomerie R. 2006. Analyzing colors. In: Hill GE, McGraw KJ, editors. Bird Coloration. I. Mechanisms and Measurements. Cambridge (MA): Harvard University Press. p. 90–147.

Montgomerie R. 2008. RCLR. Kingston (Canada): Queen's Univ.

Mougeot F, Redpath SM, Leckie F. 2005. Ultra-violet reflectance of male and female red grouse, *Lagopus lagopus scoticus*: sexual ornaments reflect nematode parasite intensity. J Av Biol. 36:203–209.

Navarro C, Pérez-Contreras T, Avilés JM, Mcgraw KJ, Soler JJ. 2010. Beak colour reflects circulating carotenoid and vitamin A levels in spotless starlings (Sturnus unicolor). Behav Ecol Sociobiol. 64:1057–1067.

Nolan PM, Dobson FS, Nicolaus M, Karels TJ, McGraw KJ, Jouventin P. 2010. Mutual mate choice for colorful traits in king penguins. Ethology. 116:635–644.
Olsson O. 1998. Divorce in King Penguins: asynchrony, expensive fat storing and ideal free mate choice. Oikos. 83:574–581.
Pike TW, Blount JD, Metcalfe NB, Lindström J. 2010. Dietary carotenoid availability and reproductive effort influence the age-related decline in performance. Behav Ecol. 21:1048–1053.
Rosenthal MF, Murphy TG, Darling N, Tarvin KA. 2012. Ornamental bill color rapidly signals changing condition. J Avian Biol. 43:553–564.
Saks L, McGraw K, Hõrak P. 2003. How feather colour reflects its carotenoid content. Funct Ecol. 17:555–561.
Schull Q, Dobson FS, Stier A, Robin J, Bize P, Viblanc VA. 2016. Data from: Beak colour dynamically signals changes in fasting status and parasite loads in king penguins. Behav Ecol. doi:http://dx.doi.org/10.5061/dryad.5j40q.
Shoop WL, Egerton JR, Eary CH, Haines HW, Michael BF, Mrozik H, Eskola P, Fisher MH, Slayton L, Ostlind DA, et al. 1996. Eprinomectin: a novel avermectin for use as a topical endectocide for cattle. Int J Parasitol. 26:1237–1242.
Sköld NH, Aspengren S, Wallin M. 2013. Rapid color change in fish and amphibians - function, regulation, and emerging applications. Pigment Cell Melanoma Res. 26:29–38.
Slagsvold T, Lifjeld JT. 2009. Variation in plumage colour of the Great tit Parus major in relation to habitat, season and food. J Zool. 206:321–328.
Stephen ID, Coetzee V, Law Smith M, Perrett DI. 2009. Skin blood perfusion and oxygenation colour affect perceived human health. PLoS One. 4:e5083.
Stonehouse B. 1960. The king penguin, Aptenodytes patagonica of South Georgia Falkland Islands Dependencies Survey. Sci Rep. 23:1–81.
Velando A, Beamonte-Barrientos R, Torres R. 2006. Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. Oecologia. 149:535–542.
Velando A, Beamonte-Barrientos R, Torres R. 2014. Enhanced male coloration after immune challenge increases reproductive potential. J Evol Biol. 27:1582–1589.
Viblanc VA, Bize P, Criscuolo F, Le Vaillant M, Saraux C, Pardonnet S, Gineste B, Kauffmann M, Prud'homme O, Handrich Y, et al. 2012. Body girth as an alternative to body mass for establishing condition indexes in field studies: a validation in the king penguin. Physiol Biochem Zool. 85:533–542.
Viblanc VA, Dobson FS, Stier A, Schull Q, Saraux C, Gineste B, Pardonnet S, Kauffmann M, Robin J-P, Bize P. 2016. Mutually honest? Physiological "qualities" signalled by colour ornaments in monomorphic king penguins. Biol J Linn Soc. 118:200–214.
Viblanc VA, Mathien A, Saraux C, Viera VM, Groscolas R. 2011. It costs to be clean and fit: energetics of comfort behavior in breeding-fasting penguins. PLoS One. 6:e21110.
Walther BA, Clayton DH. 2004. Elaborate ornaments are costly to maintain: evidence for high maintenance handicaps. Behav Ecol. 16:89–95.
Weimerskirch H, Stahlt JC, Jouventin P. 1992. The breeding biology and population dynamics of King Penguins Aptenodytes patagonica on the Crozet Islands. Ibis (Lond. 1859). 134:107–117.
Weiss SL. 2002. Reproductive signals of female lizards: pattern of trait expression and male response. Ethology 108:793–813.
Zahavi A. 1975. Mate selection-a selection for a handicap. J Theor Biol. 53:205–214.