

# Beak colouration as a possible sexual ornament in gentoo penguins: sexual dichromatism and relationship to body condition

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**Abstract** Gentoo penguins (*Pygoscelis papua*) have conspicuous red beak spots, the function of which is currently unknown. We hypothesized that beak spots might be sexual ornaments and investigated sexual dichromatism, assortative mating and the possible relationship between beak spot colouration and body condition. Beak colouration was measured with a portable spectroradiometer in 19 breeding pairs of gentoo penguin. Body mass and body mass relative to structural body size were used as estimates of body condition. We found that beak spots were sexually dichromatic, as they were more UV in males and more violet in females, but males and females did not mate assortatively in relation to beak spot colouration. Body condition was strongly related to red colouration in males, with individuals in good condition having redder beaks and individuals in poor condition more orange beaks. The beaks of males in good condition were also brighter. Body condition was not significantly related to beak spot colouration in females, so females might show red beak spots because of genetic correlation with the male trait. These results suggest that the red colour of the beak spot has the potential to be a secondary sexual character in males. Interpretation of the sexual dichromatism in the UV colour will require further knowledge of the capability of gentoo penguins to discriminate small differences in UV wavelengths. In any case, experi-

mental manipulation of beak colouration will be needed to ascertain the role of this trait.

**Keywords** Body mass relative to body size · Carotenoids · *Pygoscelis papua* · Spectroradiometry · Ultraviolet

## Introduction

Striking colours are present in many animal species, and numerous studies have dealt with their function and evolution (Andersson 1994). Examples range from orange body spots in guppies (Brooks and Endler 2001) to the colourful dewlap of anole lizards (Rand and Williams 1970), to red and blue facial markings of male mandrills (Setchell and Dixson 2001). Birds have particularly attracted the attention of researchers interested in colouration, because of the wide variety of both colours and colour patterns, very often being bold and brilliant (Hill and McGraw 2006a, b). Colour evolution may be favoured by both natural and sexual selection and the significance of avian colouration may be as diverse as the colours themselves. For example, several viability-related explanations for bird colours have been suggested, including protection from ultraviolet (UV) radiation, thermoregulation, concealment and predator deception (review in Bortolotti 2006). Visual communication is of capital importance in birds, so it is not a surprise that colour patterns often signal conspecifics. For example, coloured ornaments may influence mate choice, making individuals attractive to potential partners (review in Hill 2006). These signals may also occur in a context of intrasexual competition for mates or competition for other resources, such as food and territory (review in Senar 2006).

Despite the considerable research on avian colouration (reviewed by Hill and McGraw 2006a, b), the functions of

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colour patches in many bird species are still unknown. One of these species is the gentoo penguin (*Pygoscelis papua*), a seabird of the Spheniscidae family with black and white plumage and a conspicuous red beak spot on each side of the beak. Gentoo penguins are socially monogamous colonial birds that breed in the Antarctic and sub-Antarctic islands (Williams 1995). Both sexes share parental duties (Bost and Clobert 1992) and have similar colouration. Very little information is available about the beak spot in this species, but it reflects very little light in the UV wavelengths (Jouventin et al. 2005; Meyer-Rochow and Shimoyama 2008). The colour is affected by carotenoid intake (Jouventin et al. 2007), which suggests that the red colour is produced at least in part by this kind of pigment. This suggestion has been recently confirmed, because astaxanthin, the main carotenoid present in krill (Yamaguchi et al. 1983), is also the most abundant pigment in the beak spot of gentoo penguins (Barbosa et al., unpublished data). Carotenoids are acquired in the diet (they cannot be synthesized by animals) and might indicate foraging efficiency, but they are involved in physiological functions such as immunocompetence and detoxification, and might also indicate health (reviews in Møller et al. 2000; Hill 2002; McGraw 2006). As the beak colouration in gentoo penguins is based on carotenoids, this trait could potentially signal individual quality and thus play a role in sexual selection (Hill 2006).

The function of colourful head patches has been studied in two other penguin species, and current knowledge suggests that these colours might be involved in mate acquisition. It has been shown that saturation of the yellow eye colour and head plumage of yellow-eyed penguins (*Megadyptes antipodes*) are related to breeding success, body condition and age (Massaro et al. 2003). Therefore, yellow eyes and head stripes could be used as cues to assess individual quality and might be important in mate choice. In king penguins (*Aptenodytes patagonicus*), the size of the auricular feather patches is related to the ability to defend territory (Viera et al. 2008) and influences the time males need to find a mate (Jouventin et al. 2008). Moreover, males that breed early in the season have more reddish (less yellow) breast feathers, better body condition and more reproductive success than later breeding males (Olsson 1996; Dobson et al. 2008). Yellow feather colours in these two species are not due to carotenoids, as previously assumed, but are probably caused by another group of pigments, the pterines (McGraw et al. 2004, 2007).

King penguins also have a colourful (orange) beak spot, but contrary to gentoo penguins, their beak spot strongly reflects UV wavelengths (Jouventin et al. 2005). UV reflectance can be exclusively structural, i.e. the result of the microstructure of the reflecting material, although some pigments may produce additional UV reflectance (Prum 2006). The microstructure of the beak spot of king pen-

guins has been well studied (Dresp et al. 2005). Contrary to humans, birds studied to date can see UV light, although some species are able to see shorter wavelengths than others (Ödeen and Håstad 2003). The only two species of penguins studied so far for UV sensitivity, the Adelie penguin (*Pygoscelis adeliae*) and the Humboldt penguin (*Spheniscus humboldti*), are unable to see very short UV-wavelength reflectance, and this is probably true for all penguins (Ödeen and Håstad 2003). However, according to studies in bird species with similar retinal photoreceptors, penguins should be considerably sensitive to wavelengths between 315 and 400 nm (Hart et al. 1999), and the UV reflectance peak of the king penguin beak is clearly within this range (Dresp et al. 2005; Jouventin et al. 2005). Recent studies have shown that king penguin beak spots change colour (mainly UV) with age (Nicolaus et al. 2007). Moreover, males breeding early in the season are in better condition and their beak spots reflect more UV light than late-breeding males (Dobson et al. 2008). Therefore, the colour of the beak spot in this species might be signalling sexual maturity and physical condition, potentially very important pieces of information in the context of sexual selection.

As shown above, current knowledge of the gentoo penguin and other species suggests that colourful head patches in penguins might play a role in sexual selection. We investigated the hypothesis that the red gentoo penguin beak spot is a sexually selected character (an ornament) signalling individual quality. The study consisted of preliminary observations that might provide clues to assess the potential of the beak spot as a sexual character, although experimental manipulation would be needed to ascertain the role of this trait. First, we investigated the relationship between colouration and condition, because some sexual selection models (e.g. the handicap principle) predict that the expression of secondary sexual characters is condition-dependent (Zahavi 1975; Iwasa et al. 1991). Ornament elaboration is indeed related to condition in a large number of species (Andersson 1994). We studied beak colouration in relation to two closely related estimates of body condition, body mass and body mass relative to structural body size. Body mass relative to body size represents underlying energy reserves and is the most commonly used estimate of condition in birds (e.g. Brown 1996; Green 2001). However, the advantage of using relative instead of absolute body mass as an index of condition is not always clear (Schamber et al. 2009), so both indices were used. Finding a relationship between beak spot colouration and body condition would not demonstrate that the beak spot is an ornament, because, as stated above, only experimental manipulation could confirm this point. Nevertheless, a relationship between colouration and condition would indicate beak spot potential as a phenotypic quality signal, information that might be used by conspecifics in sexual selection processes such as mate choice.

Second, we investigated whether the red beak spot of gentoo penguins is sexually dimorphic. Although sexual dimorphism may arise due to natural non-sexual selection (e.g. the niche segregation hypothesis; Darwin 1871), more often it arises because of sexual selection (Andersson 1994). In fact, sexual dimorphism has often been used to identify secondary sexual characters in birds (e.g. Oakes 1992; Höglund and Sillén-Tullberg 1994). Both male and female gentoo penguins have red beak spots, and sexual dimorphism in the expression of the trait might be produced by different sexual selection mechanisms. Owing to genetic correlation between the sexes, a trait can be expressed in both sexes even if it is only selected in one of them (Lande 1980; Muma and Weatherhead 1989). In this case, different selection pressures in males and females weaken the genetic correlation between the sexes, giving rise to sexual dimorphism (Lande and Arnold 1985). Alternatively, a trait can be the direct target of selection in both sexes, and mutual mate choice has been described in some bird species (Jones and Hunter 1993; Amundsen et al. 1997). Mutual mate choice may account for sexually monomorphic ornaments (Jones and Hunter 1993), but any difference between sexes in the intensity of sexual selection and/or in the cost of expressing the trait would also lead to sexual dimorphism (Andersson 1994). We investigated whether the beak spot showed sexual dichromatism, a kind of dimorphism particularly linked to the intensity of sexual selection (Owens and Hartley 1998).

Finally, we also investigated whether males and females mated assortatively in relation to beak spot colouration. Assortative mating is defined as non-random mating in relation to a phenotypic trait (Burley 1983) and can be predicted if the trait reliably indicates individual quality in both sexes (Møller 1993). Identifying the mechanism giving rise to assortative mating is often difficult, because assortative mating may be produced not only by mutual mate choice (Bortolotti and Iko 1992), but also by a relationship between the trait of interest and other characteristics such as age (Reid 1988; Jouventin et al. 1999). Moreover, assortative mating in relation to age may be caused by active mate choice, but might also be a passive effect due to age-related time of arrival at the breeding grounds. Experimental manipulation and control of confounding effects will be crucial to determine the particular mechanism responsible for assortative mating.

## Materials and methods

The study was carried out in the Stranger Point penguin colony on Potter Peninsula, King George Island, South Shetland Islands, Antarctica (62°15'S, 58°37'W), in January 2007. A total of 19 pairs of breeding gentoo penguins

were captured using a long-handled net at their nests during chick rearing. The position of nests within subcolonies was not recorded, because subcolonies were lax and small and most nests could be considered peripheral. Moreover, previous studies on another Antarctic population of gentoo penguins found no significant relationship between subcolony size and reproductive success (Quintana and Cirelli 2000), suggesting that position of nests within subcolonies did not have a strong effect on reproductive performance (but see Barbosa et al. 1997 for different results in chinstrap penguins). Adults feeding or brooding a chick were assumed to be the parents of that chick. When possible, both members of a pair were caught simultaneously, although more often only one adult was present at the nest and the other parent had to be caught on a different day (up to 4 days later). Families with very small chicks (approximately less than 1-week old) or very large (approximately more than 3 weeks old) were avoided, the first because newly hatched chicks are more sensitive to disturbance, and the latter because large chicks do not stay in the nest, making the location of chicks and identification of parents more difficult. Only pairs with two chicks were included in the study because this is the modal brood size for the species (Williams 1995). When adults were captured, their chicks were taken from the nest and kept safe from predation. Adults and chicks were marked with waterproof paint to allow individual identification and avoid recapture. When adults had been measured and sampled, chicks were replaced in the nest and adults released in the proximity. After release, all adults immediately resumed care of the chicks.

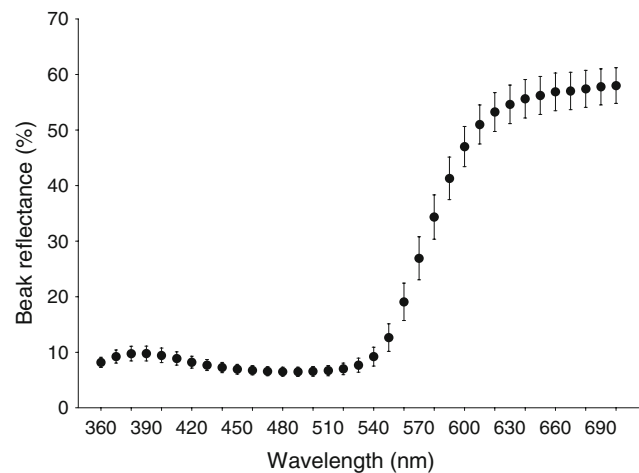
Adult gentoo penguins were weighed (to the nearest 50 g with a Pesola spring balance), and flipper length, and beak length and height were measured (the flipper to the nearest 0.1 cm with a ruler and the beak to the nearest 0.01 mm with a digital calliper). All these measurements were taken by the same person (M. J. P.) to avoid inter-observer variability. Flipper length was not measured in one individual. Multivariate size estimates are better than univariate ones in birds (Freeman and Jackson 1990) and, therefore, as in other penguin studies (e.g., Forero et al. 2001), the first factor of a principal components analysis including flipper length, beak length and beak height was used as an estimate of structural body size. This body size estimate was strongly correlated with the three length measurements ( $r \geq 0.840$  in the three cases). Body mass relative to body size was estimated as the residuals from regressing weight on our structural size estimate (Schulte-Hostedde et al. 2005). When comparing body mass relative to body size between the sexes, all individuals were analysed together ( $F_{1,35} = 62.26$ ,  $P < 0.0001$ ). However, when checking the relationship between body mass relative to body size and colour parameters, analyses were done separately for males

( $F_{1,17} = 9.86$ ,  $P = 0.0060$ ) and females ( $F_{1,16} = 12.56$ ,  $P = 0.0027$ ).

A blood sample was taken from each individual in a 75- $\mu$ l heparinized capillary tube after pricking a foot vein with a needle. Afterwards, the sample was frozen for molecular sex determination, which was done in the Molecular Ecology Laboratory at Doñana Biological Station (Seville, Spain) by polymerase chain reaction amplification of CHD-1 gene fragments, following the methods of Griffiths et al. (1998).

Beak colouration was quantified in terms of reflectance spectra using a Konica Minolta Sensing CM-2600d spectroradiometer that measures reflectance at 10-nm intervals between 360 and 700 nm. Reflectance is defined as the ratio of the total amount of radiation reflected by a surface to the total amount of radiation incident on the surface, and has a value between 0 and 1. Four measurements of the red spot were taken for each individual, two on the right-hand side of the beak and two on the left-hand side. The measurements were referenced to a white standard (CM-A145, Konica Minolta Sensing) and to a dark standard (CM-A32, Konica Minolta Sensing), and calibrated before measuring each bird. All spectroradiometric measurements were taken by the same person (J. J. C.). Mean reflectance for each wavelength was calculated for every individual.

Reflectance was used to assess a number of colour variables following Montgomerie (2006). Total brightness is a measure of the total amount of light reflected by a unit of surface, and was estimated by adding up all reflectance between 360 and 700 nm. High brightness indicates that colour is relatively lighter. As the beak spot of gentoo penguins reflects strongly in red wavelengths (Fig. 1) and appears red to human observers, we were especially interested in this colour, and two red-related variables were calculated. Chroma is a measure of colour purity (saturation), and red chroma was estimated by adding up the reflectance in the red band (630–700 nm) and dividing by total reflectance (360–700 nm). Hue indicates the wavelength that most influences colouration, and, in this case, was calculated as the midpoint between two consecutive wavelengths with the widest difference in reflectance. This variable was termed as the red hue, and the higher it was, the redder the colour; in contrast, the lower it was, the less red the colour (more orange). Interestingly, the beak spot showed a small reflectance peak in UV wavelengths (Fig. 1), so chroma and hue were also investigated in this band. UV chroma was estimated by adding up reflectance in the UV band (360–400 nm) and dividing by total reflectance (360–700 nm). UV hue was defined as the wavelength with the most reflectance in the UV band. Reflectance at 360 nm was always less than at 370 nm, and so we are confident that the UV peak was always within our wavelength range. Between-individual variation was larger than within-individual vari-



**Fig. 1** Mean ( $\pm$ SD) red beak spot reflectance spectra between 360 and 700 nm in 19 breeding pairs of gentoo penguin ( $n = 38$ )

ation for the five colour variables (ANOVA,  $F_{37,114} \geq 3.40$ ,  $P < 0.001$ ; repeatability following Lessells and Boag (1987),  $r \geq 0.376$  in the five cases).

All variables conformed to a normal distribution (Kolmogorov–Smirnov test,  $P > 0.20$ ) with the exception of UV and red hues, which we were unable to transform to a normal distribution. Sexual dimorphism was tested within breeding pairs using paired  $t$  and Wilcoxon tests for normally and non-normally distributed variables, respectively. The relative difference between sexes in percentage [ $100 \text{ (male - female) / (male + female)}$ ] (Storer's index; Storer 1966) was calculated to express sexual dimorphism in body mass and colour parameters. Assortative mating and relationships between colour variables were tested using Pearson and Spearman rank order correlations for normally and non-normally distributed variables, respectively. Any possible relationship between estimates of condition and colouration was explored separately in males and females using the Akaike information criterion (AIC). The model with the lowest AIC was considered the most parsimonious and plausible (Burnham and Anderson 2002). However, models do not differ in plausibility if they do not differ by at least two AIC units (Burnham and Anderson 2002), and we assumed that, other things being equal, the model with the fewest variables would be the most parsimonious. Therefore, of all the models differing by less than two AIC units from the one with the lowest AIC, the model that included the fewest variables was chosen as the most parsimonious. Once the best (most parsimonious) model explaining variation in condition had been found, Generalized Linear Model (GLM) included in the Statistica V8.0 software (StatSoft Inc. 2007) with the identity link function was performed, including only the variables from the best model. Body condition estimates (body mass or body mass relative to body size) were entered in the model as normally

**Table 1** Mean (SD) estimates of condition and beak spot colouration in male and female gentoo penguins

Variable	Males	Females	<i>n</i>	<i>t</i>	<i>z</i>	<i>P</i>	<i>r</i>	Storer's index (%)
Body mass (kg)	5.782 (0.537)	4.829 (0.553)	19	4.55	–	<0.001	0.731	17.96
Body mass relative to body size	0.036 (0.435)	–0.064 (0.443)	18	0.59	–	0.56	0.142	–
Brightness	884.96 (74.17)	891.20 (44.92)	19	–0.30	–	0.77	0.070	–0.70
Red chroma	0.514 (0.019)	0.509 (0.019)	19	1.01	–	0.33	0.231	0.98
UV chroma	0.053 (0.005)	0.052 (0.004)	19	0.71	–	0.48	0.166	1.90
Red hue (nm)	569.74 (8.41)	570.26 (9.05)	19	–	0.25	0.80	0.059	–0.09
UV hue (nm)	383.68 (4.96)	388.42 (6.02)	19	–	2.29	0.022	0.526	–1.23

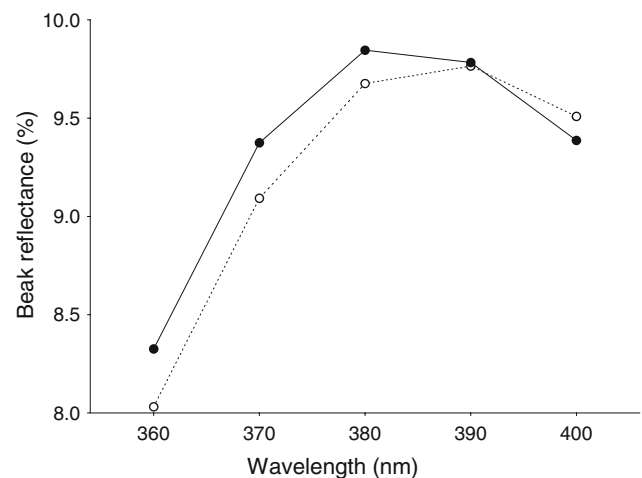
Differences between sexes were tested within breeding pairs using paired *t* or Wilcoxon tests. Effect sizes (Pearson's *r*) of these tests and sexual dimorphism (Storer's index) are also shown. See text for definition and calculation of variables

distributed dependent variables, and colour measurements as covariates. GLZ was preferred over the general linear model (GLM) because its normality and homoscedasticity requirements are less restrictive. All statistical tests were two-tailed with a 0.05 significance level.

Multiple statistical tests performed in this study increased the risk of incurring a Type I error. The Bonferroni correction is commonly used to avoid this problem, but it has also been criticized for increasing the risk of a Type II error (Moran 2003; Nakagawa 2004). An alternative to the Bonferroni correction is to show effect sizes, because they allow evaluation of the relative magnitude and hence the biological importance of the results (Nakagawa 2004). Therefore, standardized effect sizes (Pearson's *r*), in addition to *P* values, were calculated for all statistical tests. Wald statistics, which are Chi-square distributed, and *t* and *z* statistics were transformed into *r* following Rosenthal (1994). According to Cohen (1988), effect sizes can be classified as small ( $r = 0.1$ ), medium ( $r = 0.3$ ) or large ( $r = 0.5$ ). Although there are no fixed rules, in general medium effect sizes may be considered biologically meaningful. In fact, the average variance explained in ecological and evolutionary studies is no more than 7% (Møller and Jennions 2002), i.e. slightly smaller than a medium effect size.

## Results

When males and females in breeding pairs of gentoo penguins were compared, males were heavier than females, but the sexes did not differ significantly in body mass relative to structural body size (Table 1). The only beak colour variable that differed significantly between sexes was the UV hue, which was lower in males than in females (Table 1; Fig. 2). This means that the beak spot was more UV (less violet) in males than in females. Males and females did not mate assortatively for body mass (absolute or relative to body size) or for any of the five colour variables ( $n = 18$  for body mass relative to body size and  $n = 19$  for the other



**Fig. 2** Mean red beak spot reflectance spectra for UV wavelengths (360–400 nm) in 19 male (solid circles, solid line) and 19 female (open circles, dashed line) gentoo penguins. Y-axis representation is limited to 8–10% reflectance to make sexual differences clearer

variables,  $-0.406 \leq r \leq 0.337$ ,  $P \geq 0.085$  in the seven cases). All colour variables except UV hue were significantly related to one another in males, but the relationships were not so clear-cut in females (Table 2). UV hue was negatively related to red chroma and red hue, but only in females (Table 2).

The best model explaining body mass variation in males only included red chroma, and the best model explaining body mass relative to body size in males included brightness and red hue (Table 3). Both estimates of body condition were significantly related to beak spot colouration in males. Body mass was positively related to red chroma, and body mass relative to body size was positively related to brightness and red hue (Table 4; Fig. 3). This means that the beak spot in males in good condition was mainly redder (less orange), and also brighter, than in males in poor condition. Regarding females, the best model explaining absolute or relative body mass variation included only red hue in both cases, but red hue was not significantly related to

**Table 2** Correlations among five colour variables of the beak spot in male ( $n = 19$ ) and female ( $n = 19$ ) gentoo penguins

	Brightness	Red chroma	Red hue	UV chroma
<b>Males</b>				
Red chroma	-0.598*	-	-	-
Red hue	-0.624*	0.799*	-	-
UV chroma	0.574*	-0.810*	-0.591*	-
UV hue	-0.239	-0.139	0.056	-0.259
<b>Females</b>				
Red chroma	-0.428	-	-	-
Red hue	-0.514*	0.754*	-	-
UV chroma	0.326	-0.801*	-0.349	-
UV hue	0.102	-0.631*	-0.653*	0.285

Pearson and Spearman rank order correlations were used for normally (brightness, red chroma, UV chroma) and non-normally (red hue and UV hue) distributed variables, respectively

\* Correlations associated with a  $P$ -value smaller than 0.05

body mass [estimate (SE) = 0.012 (0.014), Wald = 0.80,  $P = 0.37$ ,  $r = 0.205$ ] or body mass relative to body size [estimate (SE) = 0.010 (0.011), Wald = 0.91,  $P = 0.34$ ,  $r = 0.225$ ]. Beak spot colouration did not reflect body condition in female gentoo penguins.

**Table 3** Best models of beak colour variables explaining body mass and body mass relative to structural body size in male gentoo penguins

Dependent variable	Model	$df$	AIC	L.R. $\chi^2$	$P$
Body mass	Brightness + red chroma	2	26.34	8.91	0.012
	Brightness + red chroma + UV hue	3	26.45	10.80	0.013
	Brightness + red hue + UV chroma	3	26.89	10.35	0.016
	Brightness + red chroma + UV chroma	3	27.09	10.16	0.017
	Red chroma	1	27.42	5.83	0.016
	Brightness + UV chroma	2	27.59	7.66	0.022
	UV chroma	1	27.93	5.32	0.021
	Brightness + red chroma + UV chroma + UV hue	4	28.22	11.03	0.026
	Brightness + red chroma + red hue	3	28.28	8.97	0.030
	Body mass relative to body size	Brightness + red chroma + UV chroma + UV hue	4	15.29	15.27
Brightness + red chroma + red hue + UV hue		4	15.50	15.06	0.0046
Brightness + red chroma + UV hue		3	15.65	12.91	0.0048
Brightness + red chroma + red hue + UV chroma + UV hue		5	16.81	15.75	0.0076
Brightness + red hue + UV hue		3	16.96	11.60	0.0089
Brightness + red hue		2	17.19	9.37	0.0092

Models were found using AIC. Only models differing by less than two AIC units from the best model are shown

**Table 4** Relationship between body condition estimates (body mass and body mass relative to structural body size) and beak colour variables in 19 male gentoo penguins using best models found with AIC

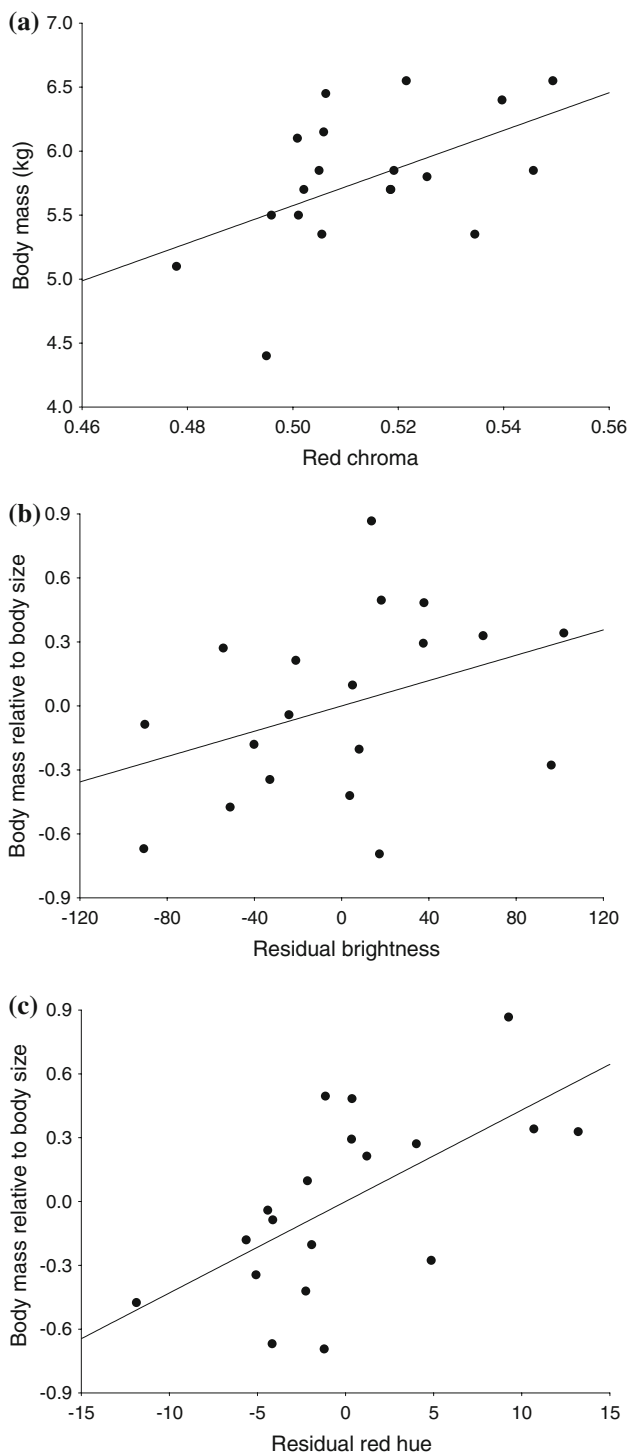
Dependent variable	Independent variable	Estimate (SE)	Wald statistic	$P$	$r$
Body mass	Red chroma	14.701 (5.630)	6.82	0.0090	0.599
Body mass relative to body size	Brightness	0.003 (0.001)	4.45	0.035	0.484
	Red hue	0.043 (0.012)	12.00	0.0005	0.795

Effect sizes (Pearson's  $r$ ) of these tests are also shown. See text for definition and calculation of variables

All statistical tests with  $P$  values less than 0.05 had effect sizes larger than 0.4 (see Tables 1, 2 and 4). These medium or large effect sizes suggest that relationships were relatively strong and differences relatively clear, thus allowing us to reject the null hypotheses.

## Discussion

We hypothesized that the red beak spot of gentoo penguins might be a sexual ornament and investigated sexual dichromatism and assortative mating for this trait and the possible relationship between beak spot colouration and body condition. Males and females did not mate assortatively in relation to beak spot colouration, but the sexes differed in the UV wavelengths, with beak spots reflecting deeper in the UV in males than in females. This is one of the few studies describing sexual dichromatism in penguins and, to our knowledge, the first one describing sexual differences not based on the size of a colour patch (e.g. Forero et al. 2001), but on the colour itself. On the other hand, a strong positive relationship between body mass relative to structural body size and red hue was found in male gentoo penguins. Red hue is the colour variable that determines the redness of the



**Fig. 3** Body condition estimates in relation to beak spot colour variables in 19 male gentoo penguins. **a** Body mass in relation to red chroma; **b** body mass relative to body size in relation to residual brightness (residuals from a GLZ of brightness on red hue); **c** body mass relative to body size in relation to residual red hue (residuals from a GLZ of red hue on brightness)

beak spot, so males in good condition tended to show redder beaks and males in poor condition more orange beaks. Other colour variables were also related to male body

condition and are discussed below. Beak spot colouration in females was not significantly related to body condition.

The relationship between body mass relative to body size and beak spot redness (red hue) may be mediated by carotenoids, because these pigments are responsible for the red colour of the beak (Jouventin et al. 2007; Barbosa et al., unpublished data). For example, individuals in good condition might also have good hunting skills (e.g. swimming, spotting prey), enabling them to catch more or better-quality prey. Since carotenoids are acquired exclusively through diet, and other things being equal, an increase in carotenoid intake would result in a larger amount of these pigments available for colouration. Another non-exclusive explanation is that individuals in good condition are also healthy, and, therefore, carotenoids that are needed for the immune system in diseased individuals can be used for colouration in healthy individuals (Møller et al. 2000). Whatever the mechanism responsible for the relationship between body condition and beak spot redness, this study supports the high potential of the beak spot for providing information on the phenotypic quality of males. Obviously, beak spot potential as a signal does not prove that the information it contains is really used by conspecifics, and only experimental manipulation could confirm it. However, given the conspicuousness of the red spot and the strong relationship with male body condition, it is plausible that gentoo penguins use this trait to assess the quality of potential mates or competitors. If this were so, the red colour (the long-wave portion of the spectrum) of the beak spot would be a sexual ornament in males. In contrast, this study does not support that beak spot colouration is a sexual ornament in females. The expression of this character in females might be the consequence of genetic correlation between the sexes, even if the trait is only selected in males (Lande 1980; Muma and Weatherhead 1989).

In addition to the strong association with red hue, body mass relative to body size in males was also positively related to total brightness. Males in good condition showed brighter (lighter) beak spots than individuals in poor condition. The relationship between brightness and relative body mass agrees with previous studies, which have found an association between brightness of carotenoid-based colouration and such important components of fitness as health (e.g. Figuerola et al. 2003; Saks et al. 2003). As for the red hue, the relationship between condition and brightness is probably also mediated by carotenoids, because an increase of carotenoids in the diet generally produces brighter colours (Hill 2002). Our second estimate of the condition, absolute body mass, was significantly related to beak spot colouration in males; namely, it was positively related to red chroma. Red chroma indicates the purity of the red colour, and so it is another way (together with red hue) of measuring the redness of the beak spot. In fact, red hue and red

chroma are strongly correlated (Table 2). Consequently, males in good condition tended to show redder beaks than males in poor condition, a similar result to that found when the relative body mass was used as an estimate of condition (see above).

There is evidence that UV can reflect individual quality in birds [including penguins (Nicolaus et al. 2007; Dobson et al. 2008)], and is of great importance in social status signalling and reproductive performance (e.g. Sheldon et al. 1999; Siefferman and Hill 2005). However, we found no significant relationship between relative or absolute body mass and UV beak spot colouration in the gentoo penguin. Instead, we found sexual dichromatism for this trait, with males showing more UV (less violet) beak spots than females. This is the first time that beak spot colouration in gentoo penguins has been studied with spectroradiometry in the field. Spectroradiometry was essential to finding previously “hidden” sexual dichromatism in this species because of human inability to see UV wavelengths. A recent field study on the possible role of UV signalling in penguins using standard photography and visible light filters found that the beak spot of adult gentoo penguins strongly absorbs UV light and concluded that UV reflectivity in penguins is probably an “associative phenomenon” without biological importance (Meyer-Rochow and Shimoyama 2008; but see Jouventin et al. 2009). We also found very low reflectance of the beak spot in UV wavelengths (Fig. 1), but spectroradiometry, which is by far more accurate than other techniques, enabled us to find a small UV peak and sexual differences that were overlooked in that study. Penguins can probably see wavelengths longer than 315 nm (see “Introduction”), although gentoo penguin’s response to slight differences in UV hue, or even whether they can detect them, has never been studied. One possible explanation for sexual dichromatism in beak colouration is sexual selection, i.e. the role of beak UV reflectance as a sexual ornament. An alternative explanation might be the association between age and UV reflectance (Nicolaus et al. 2007), and within-pair sexual differences in age, but the age of the sampled individuals was unknown. Sexual dichromatism in UV beak spot colouration has also been found in a sample of moulting gentoo penguins in the same population (Cuervo et al., unpublished data), but the generality of this dichromatism should be verified in other populations. Given the small UV reflectance peak, which is much smaller than in other penguin species (Jouventin et al. 2005), the small (although statistically significant) difference in UV between the sexes (Fig. 2) and our current lack of knowledge of the ability of gentoo penguins to see UV light, we must be cautious in the interpretation of this sexual dichromatism.

The fact that this study took place in the field is probably important, because colouration may be different in captivity

due to diet, health or other factors. Our own experience tells that gentoo penguin beak spots are generally paler and more orange in captivity than in the wild. In contrast, a weakness of this study was that the role of a trait as a sexual ornament would have to be studied mainly during mating, when the signal is presumably working. However, beak colouration was measured more than 1 month later, when breeding penguins had chicks in the nest. We do not know whether beak colouration may have changed during the breeding season, but in case it did, it might have implications for the interpretation of the results. In any case, there are numerous examples of post-mating sexually selected traits in birds (e.g. Soler et al. 1998, 2008), so the patterns found here might be relevant to sexual selection even in case they do not represent the situation at mating.

In general, our results suggest that beak spot colouration in gentoo penguins has the potential to be a secondary sexual character in males. Long (carotenoid-based) wavelengths may indicate individual quality, whereby beaks are redder in males in good condition and less red (more orange) in males in poor condition. Beak colouration is sexually monochromatic for the red portion of the spectra, and females might show red beak spots because of genetic correlation with male character. On the other hand, short (UV) wavelengths are sexually dichromatic, with beaks more UV in males and more violet in females, and thus have the potential to indicate sex. However, it is currently unknown whether gentoo penguins can discriminate small differences in UV reflectance. Experimental manipulation of beak colourations will be needed to ascertain evolutionary influences on these traits.

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## References

- Amundsen T, Forsgren E, Hansen LTT (1997) On the function of female ornaments: male bluethroats prefer colourful females. *Proc R Soc Lond B Biol Sci* 264:1579–1586. doi:10.1098/rspb.1997.0221
- Andersson M (1994) *Sexual selection*. Princeton University Press, Princeton
- Barbosa A, Moreno J, Potti J, Merino S (1997) Breeding group size, nest position and breeding success in the chinstrap penguin. *Polar Biol* 18:410–414. doi:10.1007/s003000050207



- Bortolotti GR (2006) Natural selection and coloration: protection, concealment, advertisement, or deception? In: Hill GE, McGraw KJ (eds) Bird coloration. Function and evolution, vol 2. Harvard University Press, Cambridge, pp 3–35
- Bortolotti GR, Iko W (1992) Non-random pairing in American kestrels: mate choice versus intra-sexual competition. *Anim Behav* 44:811–821. doi:10.1016/S0003-3472(05)80577-9
- Bost CA, Clobert J (1992) Gentoo Penguin *Pygoscelis papua*: factors affecting the process of laying a replacement clutch. *Acta Ecol* 13:593–605
- Brooks R, Endler JA (2001) Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evol Int J Org Evol* 55:1002–1015. doi:10.1554/0014-3820(2001)055[1002:DAISSA]2.0.CO;2
- Brown ME (1996) Assessing body condition in birds. In: Nolan V Jr, Ketterson ED (eds) Current ornithology, vol 13. Plenum Press, New York, pp 67–135
- Burley N (1983) The meaning of assortative mating. *Ethol Sociobiol* 4:191–293. doi:10.1016/0162-3095(83)90009-2
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York
- Cohen J (1988) Statistical power analysis for the behavioural sciences, 2nd edn. Lawrence Erlbaum, Hillsdale
- Darwin C (1871) The descent of man and selection in relation to sex. John Murray, London
- Dobson FS, Nolan PM, Nicolaus M, Bajzak C, Coquel AS, Jouventin P (2008) Comparison of color and body condition between early and late breeding king penguins. *Ethology* 114:925–933. doi:10.1111/j.1439-0310.2008.01545.x
- Dresp B, Jouventin P, Langley K (2005) Ultraviolet reflecting photonic microstructures in the king penguin beak. *Biol Lett* 1:310–313. doi:10.1098/rsbl.2005.0322
- Figuerola J, Domènech J, Senar JC (2003) Plumage color is related to ectosymbiont load during moult in the serin, *Serinus serinus*: an experimental study. *Anim Behav* 65:551–557. doi:10.1006/anbe.2003.2072
- Forero MG, Tella JL, Donazar JA, Blanco G, Bertellotti M, Ceballos O (2001) Phenotypic assortative mating and within-pair sexual dimorphism and its influence on breeding success and offspring quality in Magellanic penguins. *Can J Zool* 79:1414–1422. doi:10.1139/cjz-79-8-1414
- Freeman S, Jackson WM (1990) Univariate metrics are not adequate to measure avian body size. *Auk* 107:69–74
- Green AJ (2001) Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* 82:1473–1483
- Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. *Mol Ecol* 7:1071–1075. doi:10.1046/j.1365-294x.1998.00389.x
- Hart NS, Partridge JC, Cuthill IC (1999) Visual pigments, cone oil droplets, ocular media and predicted spectral sensitivity in the domestic turkey (*Meleagris gallopavo*). *Vision Res* 39:3321–3328. doi:10.1016/S0042-6989(99)00071-1
- Hill GE (2002) A red bird in a brown bag: the function and evolution of colorful plumage in the house finch. Oxford University Press, New York
- Hill GE (2006) Female mate choice for ornamental coloration. In: Hill GE, McGraw KJ (eds) Bird coloration: function and evolution, vol 2. Harvard University Press, Cambridge, pp 137–200
- Hill GE, McGraw KJ (eds) (2006a) Bird coloration. Mechanisms and measurements, vol 1. Harvard University Press, Cambridge
- Hill GE, McGraw KJ (eds) (2006b) Bird coloration. Function and evolution, vol 2. Harvard University Press, Cambridge
- Höglund J, Sillén-Tullberg B (1994) Does lekking promote the evolution of male-biased size dimorphism in birds? On the use of comparative approaches. *Am Nat* 144:881–889. doi:10.1086/285716
- Iwasa Y, Pomiankowski A, Nee S (1991) The evolution of costly mates preferences. II. The “handicap” principle. *Evol Int J Org Evol* 45:1431–1442. doi:10.2307/2409890
- Jones IL, Hunter FM (1993) Mutual sexual selection in a monogamous seabird. *Nature* 362:238–239. doi:10.1038/362238a0
- Jouventin P, Lequette B, Dobson FS (1999) Age-related mate choice in the wandering albatross. *Anim Behav* 57:1099–1106. doi:10.1006/anbe.1999.1083
- Jouventin P, Nolan PM, Ornborg J, Dobson FS (2005) Ultraviolet beak spots in king and emperor penguins. *Condor* 107:144–150. doi:10.1650/7512
- Jouventin P, McGraw KJ, Morel M, Célerier A (2007) Dietary carotenoid supplementation affects orange beak but not foot coloration in gentoo penguins *Pygoscelis papua*. *Waterbirds* 30:573–578. doi:10.1675/1524-4695(2007)030[0573:DCSAOB]2.0.CO;2
- Jouventin P, Nolan PM, Dobson FS, Nicolaus M (2008) Coloured patches influence pairing rate in king penguins. *Ibis* 150:193–196
- Jouventin P, Couchoux C, Dobson FS (2009) UV signals in penguins. *Polar Biol* 32:513–514. doi:10.1007/s00300-008-0564-3
- Lande R (1980) Sexual dimorphism, sexual selection and adaptation in polygenic characters. *Evol Int J Org Evol* 34:292–305. doi:10.2307/2407393
- Lande R, Arnold SJ (1985) Evolution of mating preferences and sexual dimorphism. *J Theor Biol* 117:651–664. doi:10.1016/S0022-5193(85)80245-9
- Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121
- Massaro M, Davis LS, Darby JT (2003) Carotenoid-derived ornaments reflect parental quality in male and female yellow-eyed penguins (*Megadyptes antipodes*). *Behav Ecol Sociobiol* 55:169–175. doi:10.1007/s00265-003-0683-3
- McGraw KJ (2006) Mechanics of carotenoid-based coloration. In: Hill GE, McGraw KJ (eds) Bird coloration: mechanisms and measurements, vol 1. Harvard University Press, Cambridge, pp 177–242
- McGraw KJ, Wakamatsu K, Ito S, Nolan PM, Jouventin P, Dobson FS, Austic RE, Safran RJ, Siefferman LM, Hill GE, Parker R (2004) You can’t judge a pigment by its color: carotenoid and melanin content of yellow and brown feathers in swallows, bluebirds, penguins, and domestic chickens. *Condor* 106:390–395. doi:10.1650/7384
- 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. doi:10.1111/j.1600-0749.2007.00386.x
- Meyer-Rochow VB, Shimoyama A (2008) UV-reflecting and absorbing body regions in gentoo and king penguin: can they really be used by the penguins as signals for conspecific recognition? *Polar Biol* 31:557–560. doi:10.1007/s00300-007-0387-7
- Møller AP (1993) Sexual selection in the barn swallow *Hirundo rustica*. III. Female tail ornaments. *Evol Int J Org Evol* 47:417–431. doi:10.2307/2410061
- Møller AP, Jennions MD (2002) How much variance can be explained by ecologists and evolutionary biologists? *Oecologia* 132:492–500. doi:10.1007/s00442-002-0952-2
- Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF (2000) Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence, or detoxification ability? *Avian Poult Biol Rev* 11:137–159
- Montgomerie R (2006) Analyzing colors. In: Hill GE, McGraw KJ (eds) Bird coloration: mechanisms and measurements, vol 1. Harvard University Press, Cambridge, pp 90–147
- Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* 100:403–405. doi:10.1034/j.1600-0706.2003.12010.x
- Muma KE, Weatherhead PJ (1989) Male traits expressed in females: direct or indirect sexual selection? *Behav Ecol Sociobiol* 25:23–31. doi:10.1007/BF00299707

- Nakagawa S (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav Ecol* 15:1044–1045. doi:10.1093/beheco/arh107
- Nicolaus M, Le Bohec C, Nolan PM, Gauthier-Clerc M, Le maho Y, Komdeur J, Jouventin P (2007) Ornamental colors reveal age in the king penguin. *Polar Biol* 31:53–61. doi:10.1007/s00300-007-0332-9
- Oakes EJ (1992) Lekking and the evolution of sexual dimorphism in birds: comparative approaches. *Am Nat* 140:665–684. doi:10.1086/285434
- Ödeen A, Håstad O (2003) Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. *Mol Biol Evol* 20:855–861. doi:10.1093/molbev/msg108
- Olsson O (1996) Seasonal effects of timing and reproduction in the king penguin: a unique breeding cycle. *J Avian Biol* 27:7–14. doi:10.2307/3676955
- Owens IPF, Hartley IR (1998) Sexual dimorphism in birds: why are there so many different forms of dimorphism? *Proc R Soc Lond B Biol Sci* 265:397–407. doi:10.1098/rspb.1998.0308
- Prum RO (2006) Anatomy, physics, and evolution of structural colors. In: Hill GE, McGraw KJ (eds) *Bird coloration: mechanisms and measurements*, vol 1. Harvard University Press, Cambridge, pp 295–353
- Quintana RD, Cirelli V (2000) Breeding dynamics of a gentoo penguin *Pygoscelis papua* population at Cierva Point, Antarctica Peninsula. *Mar Ornithol* 28:29–35
- Rand AS, Williams EE (1970) An estimation of redundancy and information content of anole dewlaps. *Am Nat* 104:99–103. doi:10.1086/282643
- Reid WV (1988) Age correlations within pairs of breeding birds. *Auk* 105:278–285
- Rosenthal R (1994) Parametric measures of effect size. In: Cooper H, Hedges LV (eds) *The handbook of research synthesis*. Russell Sage Foundation, New York, pp 231–244
- Saks L, Ots I, Hõrak P (2003) Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia* 134:301–307
- Schamber JL, Esler D, Flint PL (2009) Evaluating the validity of using unverified indices of body condition. *J Avian Biol* 40:49–56. doi:10.1111/j.1600-048X.2008.04462.x
- Schulte-Hostedde AI, Zimmer B, Millar JS, Hickling GJ (2005) Restitution of mass-size residuals: validating body condition indices. *Ecology* 86:155–163. doi:10.1890/04-0232
- Senar JC (2006) Color displays as intrasexual signals of aggression and dominance. In: Hill GE, McGraw KJ (eds) *Bird coloration: function and evolution*, vol 2. Harvard University Press, Cambridge, pp 87–136
- Setchell JM, Dixson AF (2001) Changes in the secondary sexual adornments of male mandrills (*Mandrillus sphinx*) are associated with gain and loss of alpha status. *Horm Behav* 39:177–184. doi:10.1006/hbeh.2000.1628
- Sheldon BC, Andersson S, Griffith SC, Örnborg J, Sendecka J (1999) Ultraviolet colour variation influences blue tit sex ratios. *Nature* 402:874–877. doi:10.1038/47239
- Siefferman L, Hill GE (2005) UV-blue structural coloration and competition for nest boxes in male eastern bluebirds. *Anim Behav* 69:67–72. doi:10.1016/j.anbehav.2003.12.026
- Soler JJ, Cuervo JJ, Møller AP, de Lope F (1998) Nest building is a sexually selected behaviour in the barn swallow. *Anim Behav* 56:1435–1442. doi:10.1006/anbe.1998.0938
- Soler JJ, Navarro C, Pérez-Contreras T, Avilés JM, Cuervo JJ (2008) Sexually selected egg coloration in spotless starlings. *Am Nat* 171:183–194. doi:10.1086/524958
- StatSoft Inc. (2007) Statistica (data analysis software system). Version 8.0. <http://www.statsoft.com>
- Storer RW (1966) Sexual dimorphism and food habits in three North American accipiters. *Auk* 83:423–436
- Viera VM, Nolan PM, Côté SD, Jouventin P, Groscolas R (2008) Is territory defence related to plumage ornaments in the king penguin *Aptenodytes patagonicus*? *Ethology* 114:146–153
- Williams TD (1995) *The penguins*. Oxford University Press, Oxford
- Yamaguchi K, Miki W, Toriu N, Kondo Y, Murakami M, Konosu S, Satake M, Fujita T (1983) The composition of carotenoid pigments in Antarctic krill *Euphausia superba*. *Bull Jpn Soc Sci Fish* 49:1411–1415
- Zahavi A (1975) Mate selection: a selection for a handicap. *J Theor Biol* 53:205–214. doi:10.1016/0022-5193(75)90111-3