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# Immunocompetence is signalled by ornamental colour in king penguins, *Aptenodytes patagonicus*

Paul M. Nolan,[1]* F. Stephen Dobson,[1] Birgitta Dresp[2] and Pierre Jouventin[2]

[1]*Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA and*
[2]*CNRS – CEFE, Equipe Ecologie Comportementale, F-34293 Montpellier, France*

## ABSTRACT

**Hypothesis:** Colourful ornaments reveal an individual's immunocompetence, allowing birds to choose mates on the basis of these characters.

**Organism:** King penguins, *Aptenodytes patagonicus*.

**Field site:** Colony of approximately 16,000 breeding pairs on Possession Island in the Crozet Archipelago, southern Indian Ocean.

**Methods:** We measured foot-web swelling in males resulting from experimental injection of a novel mitogen (phytohaemagglutinin, PHA) and compared that measure of immunocompetence with colours of the beak spot and plumage.

**Conclusions:** Breast plumage colour is a reliable indicator of immunocompetence in king penguins. Breast plumage colour appears to rely on the presence of pterin pigments, and the fact that pterins are implicated in immune function may underlie the honesty of this signal.

*Keywords*: *Aptenodytes patagonicus*, condition-dependent signal, immunocompetence, PHA, phytohaemagglutinin, sexual selection.

## INTRODUCTION

Individual fitness depends on the way in which an organism's traits respond to a variety of circumstances both internal and external (Darwin, 1859). Darwin (1871) championed the idea that behavioural or ornamental traits, such as sexual displays and colour patterns, could provide information within the context of the social environment that facilitates the choice of a partner for mating and raising offspring. Such traits are thought to evolve through the process of sexual selection, and they may be particularly potent influences on individual fitness (Fisher, 1930).

Animals rely on a wide variety of sexually selected signal types to advertise their quality and to select mates (Andersson, 1994; Espmark *et al.*, 2000; Smith, 2003). For example, vocalizations of some ungulates reveal their body size (Reby and McComb, 2003), singing by male birds reflects

* Address all correspondence to Paul M. Nolan, Department of Biology, The Citadel, 171 Moultrie Street, Charleston, SC 29409, USA. e-mail: paul.nolan@citadel.edu

their age and body condition (Loffredo and Borgia, 1986; Saino *et al.*, 1997), their dominance status (Møller, 1987), or their territory quality (Galeottie, 1998), and integumentary colour in some fish reflects their parasite load (Houde and Torio, 1992). Similarly, in many species, immune strength is reflected by the colour of ornamental characters such as the bill (Blount *et al.*, 2003; Faivre *et al.*, 2003), feet (Torres and Velando, 2003), or feathers (Saks *et al.*, 2003; Hill and Farmer, 2005), traits that are important in mate choice (Andersson, 1994; Hill, 2002).

Colourful ornaments might be especially revealing in penguins because the birds have no opportunity to feed during moult. So ornamental coloration of the beak and plumage depends entirely on fat and nutrient reserves accumulated before the beginning of moult (Cherel *et al.*, 1994). A bird that forages well and builds large nutrient stores before moult should suffer fewer immunocompetence problems related to poor nutrition. It should be in a better position to resist any parasite- or immune-mediated influences on its plumage colour (Hamilton and Zuk, 1982; Folstad and Karter, 1992).

We tested the hypothesis that ornamental colour in the king penguin (*Aptenodytes patagonicus* Miller) reflects an individual's immunocompetence by presenting birds with an immune challenge from a novel mitogen and measuring their cellular immune response (in the form of foot-web swelling) 24 h later. Higher levels of immunocompetence are reflected by increased swelling (Smits *et al.*, 1999). Male and female king penguins have coloured plumage patches on the auricular region on each side of the head and on the upper breast. Each patch can range in colour from yellow to dull orange to a rusty-brown. There is also a coloured spot on each side of the beak at the base of the lower mandible, which ranges from pink to orange.

The pigmentary or structural origin(s) of these colours remains unknown. Thus we cannot make directional predictions regarding which colour extreme should be associated with the highest levels of immunocompetence. Instead, we hypothesize here only that ornamental coloration honestly reflects immune function in king penguins. McGraw *et al.* (2004) suggested that pterins, a type of pigment never before identified in avian integumentary colour, are responsible for at least some of the coloration in these penguins. Therefore, our study represents the first attempt to define which pterin-based plumage colours represent the highest-quality individuals.

## METHODS

### Study site and species

From November 2001 to January 2002, we worked in a large colony [~16,000 pairs (Delord *et al.*, 2004)] of king penguins on Possession Island, Crozet Archipelago (46°25′S, 51°45′E). In November 2001, we captured 53 unpaired adult males after observing each individual perform courtship calls for several minutes. Watching them call allowed us to discriminate between male and female individuals because male and female calls are distinct from each other (Jouventin, 1982). We are confident that our selection of solitary, calling males gave us a sample of unpaired birds of the desired sex. In this species, a male and a female maintain close proximity during the later stages of the courtship process and during the subsequent selection of a site for egg-laying. Paired birds do not often make courtship vocalizations (Jouventin, 1982). After capturing an unpaired male, we measured its mass and the length of its right flipper. Then we attached temporary plastic flipper bands to allow us to identify individuals during the experiment.

## Measuring colour

We used a hand-held Colortron spectrophotometer (Hill, 1998) to measure the hue, saturation, and brightness of colours at three points on the upper breast, three points on the left auricular patch, and two points on the beak spot. Hue values are represented by the Colortron as angles on a 360° colour wheel (for details, see Hill, 1998), with orange/yellow colours being arbitrarily assigned lower numbers and rusty brown colours being assigned higher numbers. Saturation describes the spectral purity of a colour – that is, its 'peakedness' around the wavelength of maximal reflectance. Brightness simply describes the total amount of light reflected back across all the wavelengths being studied.

We averaged the values within each measured region to give colour scores for the plumage patches on the breast and on the auricular region, and for the beak spot. The spectra generated from king penguin beaks clearly showed an increase in reflectance as the spectra neared the ultraviolet (UV) wavelengths. Therefore, we also measured the percentage reflectance at 400 nm for each bird, using this percentage as an index of the bird's UV reflectance. We compared this reflectance with the degree of footweb swelling. The Colortron spectrophotometer has 10-nm resolution, and is sensitive only in the range of wavelengths visible to humans (390–700 nm) and a few nanometres into the UV range visible to birds (typically defined as 320–400 nm). However, the correlation between percentage reflectance at 400 nm and peak UV reflectance is strong ($r = 0.87$, $P = 0.026$), based on king penguins measured in captivity (for details, see Jouventin *et al.*, 2005). Therefore, percentage reflectance at 400 nm is a good index of the peak UV reflectance from king penguin beaks.

## Measuring auricular patch size

Using a Canon G2 4.0 mega pixel digital camera, we took high-resolution pictures of a subset of males, and included a size standard (a ruler) held next to the bird in the same plane as the feathers. This size standard allowed us to control for any between-photo differences in distance-to-subject. Then, using the public domain NIH Image J program (developed at the US National Institutes of Health; http://rsb.info.nih.gov/ij/), we quantified natural variation in the auricular patch size of all birds.

## Assessing immunocompetence and body condition

To assess immune strength, we first marked a spot on the right footweb with a non-toxic permanent marker. Then we measured the thickness of the footweb at that spot using a micrometer (Mitutoyo, Inc.) capable of measuring to 0.001 mm. After the initial measurement, we injected the footweb with distilled water (500 $\mu$l) containing 500 $\mu$g of phytohaemagglutinin (PHA). We placed the bird into an isolated enclosure ($3 \times 3$ m) for 24 h and again measured footweb thickness at the marked spot. We defined the strongest innate immune response as the largest difference between the two measurements (Smits *et al.*, 1999). In our birds, mass and flipper length were significantly and positively correlated ($r^2 = 0.15$, $P < 0.001$) [for a discussion of the merits of ordinary least squares versus reduced major axis regression, see Schulte-Hostedde *et al.* (2005)]. So to estimate current body condition, we used the residuals from an ordinary least squares regression of mass on flipper length.

### Statistical analyses

We used forward, stepwise regression to identify which of the colour variables we measured (i.e. hue, saturation, and brightness for each of the three coloured patches) explained significant amounts of the variation in footweb swelling. After log-transforming the footweb swelling data to fit them to a normal distribution, we used linear regression to compare the degree of footweb swelling with auricular patch size, body condition, and UV reflectance from the beak. We used JMP software (SAS Institute, Cary, NC, USA) to conduct all analyses.

## RESULTS

All males showed a response to the PHA challenge with a mean (± standard error) swelling of 2.27 (± 0.12) mm. All colour measures showed substantial variation (Table 1).

Breast hue was the only variable to enter the forward stepwise regression model (Table 2). It showed a significant negative relationship with footweb swelling (our measure of immune strength). Breast hue varies from a yellow/orange colour (lower hue values) to a rusty brown colour (higher hue values), so this result indicates that birds with less darkly coloured breast patches showed the strongest response to challenge with the novel mitogen PHA.

Footweb swelling was unrelated to current body condition ($r^2 = -0.01$, $P = 0.47$). Similarly, footweb swelling did not correlate with auricular patch size among the subset of birds ($n = 23$) for which we had patch size data available ($r^2 = -0.04$, $P = 0.79$); that subset of birds did not differ significantly from the remaining birds in their degree of footweb swelling (Wilcoxon $z = 0.53$, $P = 0.60$). Finally, footweb swelling was not significantly related to the degree of UV reflectance from the beak ($r^2 = 0.02$, $P = 0.87$).

## DISCUSSION

We tested the hypothesis that coloured ornaments of male king penguins convey honest information about immune strength, an aspect of health. In support of this hypothesis, we found that the breast plumage hue of males reflected a measure of their innate immune response.

Acquiring information about the quality of potential mates is particularly important in slow-breeding species, because a poor choice of mate cannot be corrected until the next breeding attempt (which may be a year or more away). In contrast, individuals of species that lay multiple clutches each year can quickly re-nest after a failed attempt or can respond to changing conditions by adjusting clutch size or by seeking extra-pair fertilizations. King

**Table 1.** Variation in colour scores of three ornamental patches in the king penguin (*Aptenodytes patagonicus*) (mean ± standard error with range in parentheses)

| | Hue | Saturation | Brightness |
|---|---|---|---|
| Breast patch | 41.6 ± 0.76 (19 to 52) | 79.0 ± 0.86 (62.5 to 88.1) | 52.7 ± 1.27 (31.0 to 74.5) |
| Beak spot | 25.6 ± 1.13 (−5 to 37) | 64.4 ± 0.98 (48.6 to 79.8) | 66.2 ± 0.61 (51.0 to 72.9) |
| Auricular parch | 45.7 ± 0.36 (40 to 53) | 83.4 ± 0.43 (75.7 to 88.1) | 59.6 ± 0.93 (44.7 to 72.2) |

*Note*: Hue values represent angles on a 360° colour wheel, generated by a hand-held Colortron spectrophotometer (for details, see Hill, 1998). $N = 53$ males for all categories.

**Table 2.** Results of forward stepwise regression investigating the relationship between immune strength, as measured by footweb swelling in response to a phytohaemaglutinin challenge, and ornamental colour in king penguins (*Aptenodytes patagonicus*)

| | Breast patch | | | Beak spot | | | | Auricular patch | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Hue | Saturation | Brightness | Hue | Saturation | Brightness | UV | Hue | Saturation | Brightness |
| Partial correlation | −0.32 | 0.18 | 0.03 | −0.05 | 0.18 | 0.12 | −0.04 | 0.04 | −0.01 | 0.01 |
| *P*-value | <0.01 | 0.20 | 0.46 | 0.74 | 0.38 | 0.38 | 0.85 | 0.87 | 0.67 | 0.67 |

*Note*: Hue values represent angles on a 360° colour wheel, generated by a hand-held Colortron spectrophotometer (for details, see Hill, 1998). $N = 53$ males for all categories. UV = reflectance at 400 nm.

penguins exhibit obligate bi-parental care of the single egg and chick for 12–13 months. So they breed only twice per each 3-year period, at best (Jouventin and Lagarde, 1995). In most years, only 25% of king penguin pairs fledge a chick and mortality takes a high toll during the chick's first year (Weimerskirch *et al.*, 1992). Thus, it is important that breeding king penguins are healthy at the beginning of the breeding cycle because variation in chick survival depends on the degree of parental investment, which in turn will vary with the parent's health. It seems reasonable that selection should favour those individuals that are best able to assess the quality of potential mates, and thus should also favour elaboration of condition-dependent traits used to advertise quality.

Immune response appeared to be associated with breast colour. Many red, orange, and yellow ornamental colours in birds and fish are carotenoid-based and can be directly tied to health due to the antioxidant and immunomodulatory properties of carotenoids (Blount *et al.*, 2003; Grether *et al.*, 2004; McGraw and Ardia, 2005). However, McGraw *et al.* (2004) reported that a mix of melanins and an unknown pigment, rather than carotenoids, is responsible for the colour of plumage patches of king penguins. McGraw *et al.* (2004) also indicated that the predominant pigment found in the coloured feathers of penguins is most likely a type of pterin. Like carotenoids, pterins have also been implicated as having a role in the immune system (Oettl and Reibnegger, 2002). Confirming the use of pterins in king penguin feathers would provide a logical mechanism for the association that we found between immune strength and plumage colour because it would suggest a trade-off between their use in an ornamental trait and in the immune system. Only the highest-quality birds could produce sufficient amounts of pterin to meet immune-system demands and mobilize the pigment that colours their plumage.

Neither the colours of the beak spot nor those of the auricular plumage are associated with innate immune strength. Møller and Pomiankowski (1993) suggestted that those colours have roles that differ from that of the breast patch. For example, auricular patch size and UV reflectance from the beak may mediate social interactions, correlate with age, or reflect hormonal state rather than innate immune strength. The size of plumage patches in particular is important in determining dominance status among house sparrows [*Passer domesticus* (Møller, 1987)] and great tits [*Parus major* (Norris, 1993)]. This means that the honesty of these plumage patches is maintained by social costs rather than by production costs and is determined at the time of moult (typically weeks or months before the breeding season).

In contrast, the role of more dynamic signals such as behaviours or skin colours that can change quickly may be to reflect current condition (Zuk *et al.*, 1990; Martin-Vivaldi *et al.*, 1998). The beak spot of king penguins would appear to be a good candidate: it is a fleshy structure that is probably able to respond to changing environmental conditions or to changes in the individual's physiology (Birkhead *et al.*, 1998; Faivre *et al.*, 2003) more quickly than is possible for plumage. Thus, the colours of the beak spot and auricular patch in king penguins may respond differently to immune challenge than the breast patch because they signal competitive ability, short-term condition, or some other information other than innate immune strength.

We showed previously that auricular patch size and UV reflectance from the beak play a role in pairing (Jouventin *et al.*, 2005, in press). That is consistent with the idea that they are useful cues in pairing, although their signal functions differ from that of the breast patch. In one experiment, we reduced the size of the auricular patch in unpaired birds and found that those birds had a significantly lower probability of pairing than did control birds. Similarly, in a second experiment, we reduced UV reflectance from the beak of other unpaired birds and again found that the manipulated birds suffered a reduced probability of pairing. These

results agree with those found in other species where pairing is mediated by colour signals through mechanisms other than their hue, and with our results here showing auricular and beak colour fail to signal immunocompetence.

We conclude that if immunocompetence is an important criterion for female king penguins during mate choice, then they are able to assess that quality by examining the breast coloration of potential mates. The definitive test of the relationship between immunocompetence and ornament colour in king penguins will require presentation of an immune challenge and/or immune boost near the time of moult. Experiments requiring activation of the immune response while an individual is also facing the energetic expenses of moult will force a trade-off in pigment use if one exists. Such experiments will hopefully reveal the relative abilities of colour ornaments to signal immunocompetence in the king penguin.

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## REFERENCES

Andersson, M. 1994. *Sexual Selection*. Princeton, NJ: Princeton University Press.

Birkhead, T.R., Fletcher, F. and Pellatt, E.J. 1998. Sexual selection in the zebra finch *Taenopygia guttata*: condition, sex traits, and immune capacity. *Behav. Ecol. Sociobiol.*, **44**: 179–191.

Blount, J.D., Metcalfe, N.B., Birkhead, T.R. and Surai, P. 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science*, **300**: 125–127.

Cherel, Y., Charrassin, J.B. and Challet, E. 1994. Energy and protein requirements for molt in the king penguin *Aptenodytes patagonicus*. *Am. J. Physiol.*, **266**: 1182–1188.

Darwin, C. 1859. *On the Origin of Species by Natural Selection, or the Preservation of Favored Races in the Struggle for Life*. London: John Murray.

Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. London: John Murray.

Delord, K., Barbraud, C. and Weimerskirch, H. 2004. Long-term trends in the population size of king penguins at Crozet archipelago: environmental variability and density dependence? *Polar Biol.*, **27**: 793–800.

Espmark, Y., Amundsen, T. and Rosenqvist G. 2000. *Animal Signals: Signaling and Signal Design in Animal Communication*. Trondheim, Norway: Tapir Academic Press.

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

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. New York: Dover.

Folstad, I. and Karter, A.J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.*, **139**: 603–622.

Galeottie, P. 1998. Correlates of hoot rate and structure in male Tawny Owls *Strix aluco*: implications for male rivalry and female mate choice. *J. Avian Biol.*, **29**: 25–32.

Grether, G., Kasahara, S., Kolluru, G.R. and Cooper, E.L. 2004. Sex-specific effects of carotenoid intake on the immunological response to allografts in guppies (Poecilia reticulata). *Proc. R. Soc. Lond. B*, **271**: 45–49.

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

Hill, G.E. 1998. An easy, inexpensive method to quantify plumage coloration. *J. Field Ornithol.*, **69**: 353–363.

Hill, G.E. 2002. *A Red Bird in a Brown Bag: The Function and Evolution of Colorful Plumage in the House Finch*. New York: Oxford University Press.

Hill, G.E. and Farmer, K.L. 2005. Carotenoid-based plumage coloration predicts resistance to a novel parasite in the house finch. *Naturwissenschaften*, **92**: 30–34.

Houde, A.E. and Torio, A.J. 1992. Effect of parasitic infection on male color pattern and female choice in guppies. *Behav. Ecol.*, **3**: 346–351.

Jouventin, P. 1982. *Visual and Vocal Signals in Penguins, Their Evolution and Adaptive Characters*. Berlin: Paul Parey.

Jouventin, P. and Lagarde, F. 1995 Evolutionary ecology of the king penguin (*Aptenodytes patagonicus*): the self-regulation of the breeding cycle. In *The Penguins: Ecology and Management* (P. Dann, I. Norman and P. Reilly, eds.), pp. 80–95. Chipping Norton, NSW: Surrey Beatty & Sons.

Jouventin, P., Nolan, P.M., Örnborg, J. and Dobson, F.S. 2005. Ultraviolet beak markings in large penguins. *Condor*, **107**: 144–150.

Jouventin, P., Nolan, P.M., Dobson, F.S. and Nicolaus, M. in press. Coloured patches influence pairing in King Penguins, *Aptenodytes patagonicus. Ibis*.

Loffredo, C.A. and Borgia, G. 1986. Male courtship vocalizations as cues for mate choice in the satin bowerbird (*Ptilorhynchus violaceus*). *Auk*, **103**: 189–195.

Martin-Vivaldi, M., Palomino, J.J. and Soler. M. 1998. Song structure in the Hoopoe (*Upupa epops*) – strophe length reflects male condition. *J. Ornithol.*, **139**: 287–296.

McGraw, K.J. and Ardia, D.R. 2005. Sex differences in carotenoid status and immune performance in zebra finches. *Evol. Ecol. Res.*, **7**: 251–262.

McGraw, K.J., Kazumasa, W., Shosuke, I., Nolan, P.M., Jouventin, P., Dobson, F.S. *et al.* 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.

Møller, A.P. 1987. Variation in badge size in male house sparrows *Passer domesticus*: evidence for status signaling. *Anim. Behav.*, **35**: 1637–1644.

Møller, A.P. and Pomiankowski A. 1993. Why have birds got multiple sexual ornaments? *Behav. Ecol. Sociobiol.*, **32**: 167–176.

Norris, K. 1993. Heritable variation in a plumage indicator of viability in male great tits *Parus major. Nature*, **362**: 537–539.

Oettl, K. and Reibnegger, G. 2002. Pteridine derivatives as modulators of oxidative stress. *Curr. Drug Metab.*, **3**: 203–209.

Reby, D. and McComb, K. 2003. Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Anim. Behav.*, **65**: 519–530.

Saino, N., Galeotti, P., Sacchi, R. and Møller, A. 1997. Song and immunological condition in male barn swallows (*Hirundo rustica*). *Behav. Ecol.*, **8**: 364–371.

Saks, L., Orts, I. and Horak, P. 2003. Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. *Oecologia*, **134**: 301–307.

Schulte-Hostedde, A.I., Zinner, B., Millar, J.S. and Hickling, G.J. 2005. Restitution of mass/size residuals: validating body condition indices. *Ecology*, **86**: 155–163.

Smith, J.S. 2003. *Animal Signals*. New York: Oxford University Press.

Smits, J.E., Bortolotti, G.R. and Tella, J.L. 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct. Ecol.*, **13**: 567–572.

Torres, R. and Velando, A. 2003. A dynamic trait affects continuous pair assessment in the blue-footed booby, *Sula nebouxii. Behav. Ecol. Sociobiol.*, **55**: 65–72.

Weimerskirch, H., Stahl, J.C. and Jouventin, P. 1992. The breeding biology and population dynamics of king penguins *Aptenodytes patagonicus* on the Crozet Islands. *Ibis*, **134**: 107–117.

Zuk, M., Johnson, K., Thornhill, R. and Ligon, J.D. 1990. Parasites and male ornaments in free-ranging and captive red jungle fowl. *Behaviour*, **114**: 232–248.