Dietary Carotenoid Supplementation Affects Orange Beak but not Foot Coloration in Gentoo Penguins *Pygoscelis papua*

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Abstract.—Red, orange, and yellow carotenoid-based coloration abounds in birds, with over half of all avian orders known to display it in some form. Penguins (Order Sphenisciformes), however, are one order of birds for which the proximal causation of ornaments is unclear, i.e., whether such colors in plumage or bare-parts are carotenoid-based. We experimentally supplemented the diet of captive Gentoo Penguins *Pygoscelis papua* for two months with extracts of krill, a common carotenoid-rich food source for these animals in the wild, to determine whether orange coloration in the beak and feet is influenced by carotenoid content of the diet. We found using UV-Vis reflectance spectrophotometry that dietary carotenoid enrichment elevated beak but not foot brightness. This suggests that the crustacean part of the diet is at least in part responsible for orange beak coloration but not feet in Gentoo Penguins and that, like other carotenoid signals, these traits have the potential to reveal important aspects of mate quality (e.g., nutrition, health). *Received 21 March 2007, accepted 9 July 2007.*

Key words.—carotenoids, determinism of beak color, Gentoo, penguins, pigmentation, ornamental coloration.

Waterbirds 30(4): 573-578, 2007

Many birds use intense coloration of the integument as a sexual signal to attract mates or to combat rivals (reviewed in Hill and McGraw 2006). There has been much interest among behavioral ecologists in the mechanisms underlying different sources of color (e.g., pigmentary, structural). Thus, it is important to determine the forms of coloration that different species use so that proper conclusions can be drawn about their production.

Compared to research on most other groups of birds, studies of bright seabird coloration are in their infancy, both at the proximate and ultimate levels (Jones and Hunter 1993). A first book comparing sexual ornaments in all penguins including Gentoos *Pygoscelis papua* demonstrated that head ornaments have a biological function in mating (Jouventin 1982). Recently some investigations showed that orange head coloration (plumage and beak) of King Penguins *Aptenodytes patagonicus* (Jouventin *et al.* in press) and of Yellow-eyed Penguins *Megadyptes antipodes* (Massaro *et al.* 2003), play important roles in mate choice.

Red, orange, and yellow colors are traditionally thought to result from the presence of carotenoid pigments in birds. However in most cases, especially among seabirds, proper biochemical or nutritional studies have not been conducted to confirm the proximate basis for color expression. In fact, a study investigating the nature of pigmentation in two species of penguins found that orange and yellow plumage colors were not carotenoid-based (McGraw *et al.* 2004).

We studied experimentally the proximate basis for orange beak and foot coloration in Gentoo Penguins *Pygoscelis papua*. These animals inhabit the southern Ocean and have a diet rich (ca. 80% of all food) in krill (Croxall and Lishman 1987), which contains a high concentration of carotenoid pigments (Yamaguchi *et al.* 1983; Breithaupt 2004). Thus, we hypothesized that Gentoo Penguins derive their beak coloration from this dietary source of carotenoids. However, we supposed that foot coloration, not involved in mate choice, will not be influenced by supplementation. To test this, we supplemented the diet of a group of Gentoo Penguins in captivity with extracts of krill (a well-known source of carotenoids) for two months and examined chromatic properties of the beak and feet using UV-Vis reflectance spectrophotometry. We compared color

changes in supplemented birds with controls as well as at three time points during the experiment (prior to supplementation, one-month and two-months after the initiation of supplementation) to see if these tissues are colored by carotenoids.

METHODS

Background on Study Animals

The study was carried out from March-May 2005 in the Discovery Park of Oceanopolis (Brest, France) on a group of Gentoo Penguins that were donated by the Zoo of Zurich in 2001. Nine seven-year-old healthy adults (four males, five females) were kept in temperature and light conditions that simulate subantarctic seasons. As there were no significant differences between sexes before and during experiments, data from males and females were pooled. Each bird was identified by a color ring that was attached to one foot.

Carotenoid Supplementation

Throughout the study, all birds were hand-fed twice a day with a base diet of fishes (Sprat, *Sprattus sprattus*; Atlantic Caplan, *Mallotus villosus*). Starting in March, the food of five birds (two males, three females) was supplemented with carotenoids daily (SUP birds), while the food of four control birds (two males, two females) was not supplemented (Ct birds). A 100 mg dose of carotenoid beadlets (Polaris; Quimper, France) originally extracted from krill *Pandalus borealis* and containing 0.11% (±0.01%) astaxanthin was added to the food of supplemented birds once a day during the two-month experiment. To ensure that supplemented birds did not receive more base food than controls, the amount of fish fed to each bird on each day of the experiment was weighed; in fact, food intake did not differ between treatment groups (Mann-Whitney U-test; Z = - 0.857, P = 0.38).

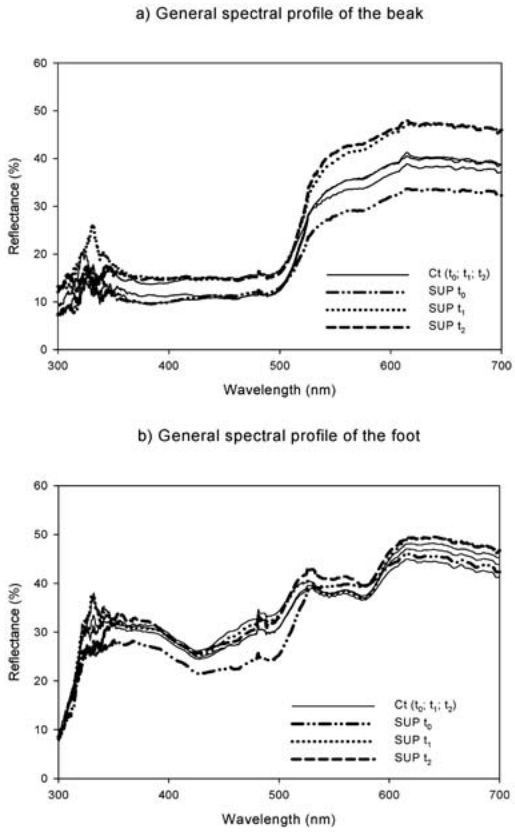

Figure 1. General spectral profile (% of reflectance as a function of wavelength) of the (a) beak and (b) foot of Carotenoid-supplemented (SUP) and control (Ct) Gentoo Penguins before carotenoid supplementation (t_0), after one month of carotenoid supplementation (t_1), and after two months of carotenoid supplementation (t_2).

Color Analysis

Reflectance Spectrometry. Spectral measurements were obtained with a S2000 spectrophotometer (Ocean Optics Inc., Dunedin, Florida, USA) that was attached to a tungsten-halogen light source (Ocean Optics LS-1) with a 200 µm fiber-optic cable. The spectrophotometer was calibrated against a white reference tile (WS-2) as well as a black standard before each series of measurements. Reflectance measurements were taken with the probe perpendicular to the surface of the beak (middle of the lower mandible) or foot (upper part, three cm from feathers) at the same location on all birds. Only two measurements were taken on each spot (diameter = one mm) because variations were small and were averaged for statistical analyses (see below for color variables used) using OOI Base Software (Ocean Optics). Spectral measurements of beak and foot were performed on supplemented and control birds before the beginning of experiment (t_0) and one month (t_1) and two months (t_2) after the beginning of experiment (Fig. 1). The comparisons between treatment groups and the change in coloration over time were included in order to evaluate possible seasonal effects. The measurement frequency was one month because preliminary studies showed that a color change was obvious after several weeks.

Color Variables. Indices of the main perceptual dimensions of color vision were calculated: *hue*, *chroma* and *brightness* between 320 and 700 nanometres (nm) and *maximal reflectance* (R_{max}) between 500 and 700 nm. *Hue* is the everyday meaning of color (i.e. orange or yellow for example) and was calculated according to the following equation: Hue = Arc tan (Ry-Rb/Rt)/(Rr-Rg/Rt). Ry represents the sum of reflectance from 550 nm to 625 nm, Rb represents the sum of reflectance from 400 nm to 475 nm, Rr represents the sum of reflectance from 625 nm to 700 nm, Rg represents the sum of reflectance from 475 nm to 550 nm and Rt represents the sum of reflectance from 320 nm to 700 nm. *Chroma* is a measure of saturation or tone of a color and is calculated as the difference between the values of maximum and minimum reflectance, relative to the average reflectance across the curve ($(Rmax\text{-}Rmin)/Raverage_{320\text{-}700}$). *Brightness*, the spectral intensity, is a measure of luminosity or lustre of a color and was estimated by the sum of reflectance from 320 to 700 nm ($R_{320\text{-}700}$). R_{max} is the *maximal*

reflectance measured between 500 and 700 nm, corresponding to the yellow-orange-red range of wavelength.

Statistical Analysis

Results are reported as means ± standard error (SE). Statview software was used to perform statistical analysis. Non-parametric tests were performed because data were not normally distributed and could not be transformed to fit a normal distribution. Comparisons of the color parameters between the two experimental groups (SUP v. Ct) were conducted using non-parametric Mann-Whitney U-tests. Comparisons between color measures at different time points were conducted using Kruskal-Wallis tests, followed by non-parametric *post hoc* Dunn tests.

RESULTS

General spectral profiles of the beak and the foot are presented in Figure 1.

Beak Coloration in Relation to Carotenoid Supplementation

Comparison Between Treatment Groups. Color parameters did not differ significantly between supplemented and control groups prior to supplementation (Brightness: Z = -0.98, P = 0.32; Chroma: Z = -0.98, P = 0.32; Hue: Z = 0; P > 0.99; R_{max}: Z = -0.735; P = 0.46; Table 1). One month after carotenoid supplementation began, supplemented penguins had brighter (Z = -2.205; P = 0.03, Fig. 2) and more chromatic (Z = -2.5; P = 0.01) beaks than the control group. Groups did not differ in hue (Z = -1.47; P = 0.14) or R_{max} (Z = -1.47; P = 0.14) at t_1. At t_2, the significant group difference in brightness remained (Z = -2.45; P = 0.01; Fig. 2), and supplemented birds also reflected more yellow-orange light than controls (R_{max}: Z = 2.45; P = 0.01; Fig. 3). No significant differences were observed between the two groups for Hue (Z = -0.245; P = 0.8) and Chroma (Z = -0.735; P = 0.46) two months after the initiation of-supplementation.

Change in Coloration Over Time. A significant increase in beak brightness (Kruskal Wallis H = 9.38; P = 0.009; Dunn t_0 vs t_1: P = 0.0005; t_0 vs t_2: P = 0.0005; t_1 vs t_2: n.s.) and R_{max} (H = 9.5; P = 0.009; Dunn t_0 vs t_1: P = 0.001; t_0 vs t_2: P = 0.0008; t_1 vs t_2: n.s.) was found in the supplemented group at t_1 and t_2 compared to t_0. There were no significant

Table 1. Average measures (SE = standard error) of color parameters as a function of body region tested (beak and feet) in carotenoid-supplemented and control gentoo penguins at three time points: before experiment (t_0), one month later (t_1) and two months later (t_2).

		t_0 (before carotenoid supplementation)		t_1 (after 1 month of carotenoid supplementation)		t_2 (after 1 month of carotenoid supplementation)	
Body region	Color Parameter	Controls (N = 4)	Carotenoid supplemented (N = 5)	Controls (N = 4)	Carotenoid supplemented (N = 5)	Controls (N = 4)	Carotenoid supplemented (N = 5)
Beak	Brightness	28213.60 ± 4008.18	23417.56 ± 2191.43	26940.47 ± 2452.10	33049.68 ± 756.61	26034.53 ± 1642.32	32918.05 ± 857.89
	Chroma	1.08 ± 0.11	1.21 ± 0.07	1.35 ± 0.05	1.12 ± 0.06	1.34 ± 0.09	1.22 ± 0.01
	Hue	51.61 ± 1.07	52.28 ± 1.53	53.56 ± 0.97	51.50 ± 0.78	52.66 ± 2.052	53.00 ± 0.99
	Rmax	38.87 ± 5.22	33.80 ± 3.46	41.28 ± 3.58	47.48 ± 1.15	39.41 ± 2.35	48.07 ± 1.5
Foot	Brightness	39110.75 ± 5446.2	37377.69 ± 4659.47	41473.15 ± 1505.36	41419.25 ± 1065.85	36066.09 ± 6867.33	41699.50 ± 2046.94
	Chroma	0.62 ± 0.08	0.86 ± 0.18	0.62 ± 0.03	0.66 ± 0.03	0.65 ± 0.03	0.77 ± 0.02
	Hue	53.03 ± 2.32	53.95 ± 2.15	49.17 ± 3.15	47.16 ± 1.49	53.30 ± 2.12	55.25 ± 2.43
	Rmax	44.94 ± 5.04	46.15 ± 3.79	48.23 ± 1.18	49.50 ± 0.65	41.76 ± 8.01	49.56 ± 1.90

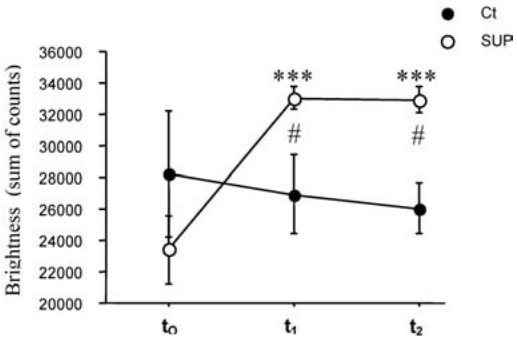

Figure 2. Temporal changes in beak brightness of supplemented (SUP) and control (Ct) Gentoo Penguins during the study. Results are presented as mean ± SE; "*" indicates significant differences from measurement at t_0 (P < 0.001); "#" indicate significant differences from control group (P < 0.05).**

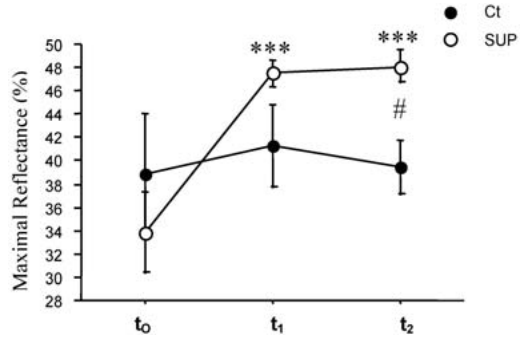

Figure 3. Temporal changes in R_{max} of the beak of supplemented (SUP) and control (Ct) Gentoo Penguins during the study. Results are presented as mean ± SE; "*" indicates significant differences from measurement at t_0 (P < 0.001); "#" indicate significant differences from control group (P < 0.05).**

changes in the control group for these two color parameters (Brightness: H = 0.27; P = 0.9 and R_{max}: H = 0.154; P = 0.9). There were no differences over time in Chroma or Hue for either the supplemented group (Chroma: H = 1.46; P = 0.48 and Hue: H = 1.52; P = 0.47) or the control group (Chroma: H = 3.5; P = 0.17 and Hue: H = 1.38; P = 0.5).

Foot Coloration in Relation to Carotenoid Supplementation

Comparison Between Treatment Groups. Foot color parameters did not differ significantly between supplemented and control groups at t_0 (Brightness: Z = 0, P > 0.99; Chroma: Z = -0.98, P = 0.32; Hue: Z = -0.49, P = 0.62; R_{max}: Z = -0.245, P = 0.8) or at t_1 (Brightness: Z = 0, P > 0.99; Chroma: Z = -0.73, P = 0,.6; Hue: Z = -0.5, P = 0.6; R_{max}: Z = -0.3, P = 0.7). However, at t_2 supplemented penguins had more chromatic feet than did the control group (Z = -2.45; P = 0.01; Fig. 4). But one month later ('t_3': data not shown), this difference in feet Chroma between supplemented penguins and controls disappeared (P > 0.1). No significant differences were observed between the two groups for Brightness (Z = -0.245, P = 0.8), Hue (Z = -0.49, P = 0.62), or R_{max} (Z = -0.735, P = 0.46).

Change in Coloration Over Time. There were no significant changes in coloration during the course of the experiment in either the control group (Brightness: H = 0.27, P = 0.87; Chroma: H = 0.154, P = 0.9; Hue: H = 2.6, P = 0.27; R_{max}: H = 0.73, P = 0.69) or the supplemented group (Brightness: H = 0.8, P = 0.9; Chroma: H = 3.38, P = 0.18; Hue: H = 5.04, P = 0.08; R_{max}: H = 0.26, P = 0.88).

DISCUSSION

On the one hand, this study provides some evidence that Gentoo Penguins use carotenoids to color their bare parts orange. First, the reflectance spectra generated for both beak and feet tissue show a carotenoid-typical pattern (e.g., MacDougall and Montgomerie 2003; Bleiweiss 2004). Second, de-

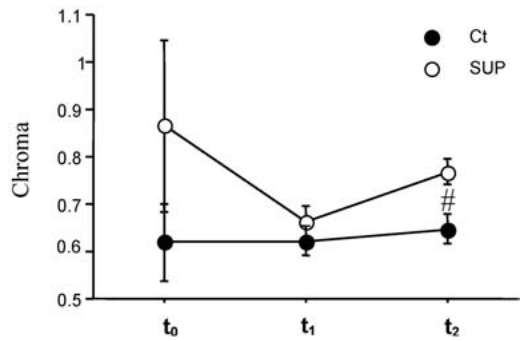

Figure 4. Temporal change in foot chroma of supplemented (SUP) and control (Ct) Gentoo Penguins during the study. Results are presented as mean ± SE. "#" indicate significant differences from control group (P < 0.05).

spite a small sample size, our dietary carotenoid supplementation experiment had significant effects on beak coloration. Among studies on both avian plumage and bareparts, including those using krill with flamingos (Fox *et al.* 1967), our experiment demonstrates a response of yellow/orange/red coloration to dietary carotenoid content (reviewed in Hill 2006). Although previous studies have argued for a carotenoid basis of yellow-orange features in penguins (Massaro *et al.* 2003), this is the first empirical evidence suggesting that carotenoids confer bright colors on seabirds.

However, carotenoid supplementation affected differently the orange bareparts: the tissues of beak in gentoos were modified by dietary carotenoid enrichment, inducing the development of brighter beaks, but not the feet. The sample of studied penguins was small according to our available captive penguins but it was enough to demonstrate that dietary supplementation affects the beak. For the orange integumentary coloration in feet, comparing t_O to t_1 and t_2, it seemed that there was a significant increase in chroma due to supplementation. Continuing to measure the feet reflectance several weeks later to confirm this trend (t_3 in results), we found a decrease to the t_1 level when supplementation continues: in fact, our sample was small and exhibited great individual variation. A larger sample would be necessary to show whether this result was an artefact. The difference between the actions of carotenoids on two apparently similar tissues suggests that beak coloration linked to sexual selection could be endocrinologically induced by breeding, while foot coloration might be endocrinologically induced by sexual maturity. New experiments are necessary to test this hypothesis.

What are the proximal and ultimate causes of this difference in coloration of beaks and feet? Concerning proximate causation, chroma is most commonly sensitive to carotenoid content across carotenoid colors in birds (Saks *et al.* 2004; Andersson and Prager 2006), with higher concentrations of pigment imparting purer colors. The fact that the beaks of Gentoo Penguins reflected more light when birds were enriched with carotenoids may be a function of how the carotenoids interacted with the reflective tissue microstructure that accompanies the colorful beaks of penguins (see Shawkey and Hill 2005 for comparable evidence in carotenoid-colored bird feathers). Although for ethical reasons we could not chemically examine the tissues of Gentoo Penguins for carotenoid content, it is likely that astaxanthin is the primary colorant of orange integumentary features in these birds. Astaxanthin is an orange-red pigment that comprises 90% of carotenoids in krill (Yamaguchi *et al.* 1983; Negro and Garrido-Fernandez 2000; Breithaupt 2004), and birds generally are not known to convert dietary astaxanthin into other types of carotenoids (but do transform other dietary components like xanthophylls into astaxanthin; McGraw 2006). In future studies, it will be interesting to track dietary consumption of krill astaxanthin in relation to integumentary coloration in free-ranging Gentoo Penguins to determine whether the results shown in captive birds remain in the wild.

Concerning ultimate causation, the role of these bright carotenoid colors as mating signals is not experimentally demonstrated in this species, but it was assumed previously comparing sexual signals in all penguins (Jouventin 1982) that head colorations were used for mate choice, whereas other bare parts of the body such as the feet of Gentoos, were not. For three reasons, we suggest that head ornaments contain valuable information about individual quality in wild birds. First, interindividual beak color variability is particularly high in this species within colonies (Reilly and Kerle 1981). Comparing penguins, differences in immunoglobulin levels were recently found showing the Gentoo Penguin the highest level (Barbosa *et al.* 2007), the immune function being supposed to be correlated with sexual selection (Nunn 2002). Second, their diet (especially the fish-to-crustacean ratio) is also highly variable across individuals (Bost and Jouventin 1990), such that birds with better foraging skills might be able to select the best food and thus advertise their nutritional state and foraging prowess with their colors (e.g., as in House Finches *Carpodacus mexicanus*; Hill

et al. 2002). Third, other penguins like King Penguins use their orange beak, which also appears to be carotenoid-based (McGraw and Jouventin, unpubl. data), to acquire mates (Jouventin *et al.* in press). Future studies should be aimed at elucidating both the signal content of these colors, including information such as health (Lozano 1994; Hill 2006), as well as the sexual-selection mechanism (e.g., intrasexual competition or intersexual mate-choice) that maintains this exaggerated trait.

ACKNOWLEDGMENTS

For their support in this project and for their friendly help, we thank Sami Hassani of the Marine Mammal Laboratory of Oceanopolis (Brest-France), Patrick Rep, Jean-Marc Menegaz, Jean-Yves Leclech and Christine Dumas. For useful comments, we thank the Editor Keith Hobson, Geoffrey Hill, Joël Bried and Stephen Dobson. The spectrophotometer was funded by the Institut Polaire P. E. Victor (IPEV-France). This work conforms to the legal requirements of France where experiments were carried out.

LITERATURE CITED

Andersson, S. and M. Prager. 2006.Quantifying coloration. Pages 41-89 *in* Bird Coloration. Volume I. Measurements and Mechanisms (G. E. Hill and K. J. McGraw, Eds.). Harvard University Press, Cambridge, Massachusetts.

Barbosa, A., S.Merino, J. Benzal, J. Martinez and S. Garcia-Fraile. 2007.Geographic variation in the immunoglobulin levels in pygoscelid penguins. Polar Biology 30: 219-225.

Bleiweiss, R. 2004. Variation in ultraviolet reflectance by carotenoid-bearing feathers of tanagers (Thraupini: Emberizinae: Passeriformes). Biological Journal of the Linnean Society 84: 243-257.

Bost, C. A. and P. Jouventin. 1990. Evolutionary Ecology of Gentoo Penguin *Pygoscelis papua*. Pages 85-112 *in* Penguin Biology (L. S. Davis and J. Darby, Eds.). Academic Press, Orlando, Florida.

Breithaupt, D. E. 2004. Identification and quantification of astaxanthin esters in shrimp *Pandalus borealis* and in a microalga *Haematococcus pluvialis* by liquid chromatography-mass spectrometry using negative ion atmospheric pressure chemical ionization. Journal of Agricultural and Food Chemistry 52: 3870-3875.

Croxall, J. P. and G. S. Lishman. 1987. The food and feeding ecology of penguins. Pages 101-134 *in* Feeding Ecology and Role in Marine Ecosystems (J. Croxall, Ed.). Cambridge University Press, Cambridge.

Endler, J. A. 1990. On the measurement and classification of color in studies of animal color patterns. Biological Journal of the Linnean Society 41: 315-351.

Fox, D. L., V. E. Smith and A. A. Wolfson. 1967. Carotenoid selectivity in blood and feathers of lesser (African). Chilean and greater (European) flamingos. Comparative Biochemistry and Physiology 23: 225-232.

Hill, G. E. 2006. Environmental regulation of ornamental coloration. Pages 507-560 *in* Bird Coloration. Volume I. Mechanisms and Measurements (G. E. Hill and K. J. McGraw, Eds.). Harvard University Press, Cambridge, Massachusetts.

Hill G. E. and K. J. McGraw. 2006. Bird Coloration. Volume II. Function and Evolution. Harvard University Press, Cambridge, Massachusetts.

Hill, G. E., C. Y. Inouye and R. Montgomerie. 2002. Dietary carotenoids predict plumage coloration in wild house finches. Proceedings of the Royal Society London B 269: 1119-1124.

Jones, I. H. and F. M. Hunter. 1993. Mutual selection in a monogamous seabird. Nature 362: 238-239.

Jouventin, P. 1982. Visual and vocal signals in penguins, their evolution and adaptive characters. Paul Parey Ed, Advances in Ethology, Berlin.

Jouventin, P., P. M. Nolan, F. S. Dobson and M. Nicolaus. In press. Colored patches influence pairing in King Penguins. Ibis.

Lozano, G. A. 1994. Carotenoids, parasites and sexual selection. Oikos 70: 309-311.

MacDougall, A. K. and R. Montgomerie. 2003. Assortative mating by carotenoid-based plumage color: a quality indicator in American goldfinches *Carduelis tristis*. Naturwissenschaften 90: 464-467.

Massaro, M., S. D. Lloyd and J. T. Darby. 2003. Carotenoid-derived ornaments reflect parental quality in male and female yellow-eyed penguins *Megadyptes antipodes*. Behavioral Ecology and Sociobiology 55: 169-175.

McGraw, K. J. 2006. The mechanics of carotenoid coloration. Pages 177-242 *in* Bird Coloration. Volume I. Measurements and Mechanisms (G. E. Hill and K. J. McGraw, Eds.). Harvard University Press, Cambridge, Massachusetts.

McGraw, K. J., K. Wakamatsu, S. Ito, P. M. Nolan, P. Jouventin, F. S. Dobson, R. E. Austic, R. J. Safran, L. M. Siefferman, G. E. Hill and R. S. Parker. 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.

Negro, J. J. and J. Garrido-Fernandez. 2000. Astaxanthin is the major carotenoid in tissue of white storks *Ciconia ciconia* feeding on introduced crayfish *Procambarus clarkia*. Comparative Biochemistry Physiology B-Biochemistry and Molecular Biology 126: 347-352.

Nunn, C. L. 2002. Spleen size, disease risk and sexual selection: a comparative study in primates. Evolutionary Ecological Research 4: 91-107.

Reilly, P. N. and J. A. Kerle. 1981.A study of the gentoo penguin. Notornis 28: 189-202.

Saks, L., K. McGraw and P. Horak. 2004. How feather color reflects its carotenoid content. Functional Ecology 17: 555-561.

Shawkey, M. D. and G. E. Hill. 2005. Carotenoids need nanostructures to shine. Biology Letter 1: 121-124.

Yamaguchi, K., W. Miki, N. Toriu, Y. Kondo, M. Murakami, S. Konosu, M. Satake and T. Fujita. 1983. Chemistry and utilization of plankton. 1. The composition of carotenoid-pigments in the Antarctic krill *Euphausia superba*. Bulletin of the Japanese Society of Scientific Fishing 49: 1411-1415.