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Monomorphic Ornamentation Related to Oxidative Damage and Assortative Mating in the Black Guillemot (*Cepphus grylle*)

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Abstract.—The Black Guillemot (*Cepphus grylle*) is a monomorphic seabird characterized by its muted plumage and bright red feet, which it prominently displays during courtship. Foot color and oxidative stress were analyzed in a Black Guillemot colony at the Bay of Fundy during the 2006 and 2007 breeding seasons. While no relationship between red intensity of feet and carotenoids was uncovered, the level of plasma oxidative damage was negatively correlated with foot color. Additionally, red intensity of male feet was significantly correlated with the red intensity of their mates' feet, suggesting the possibility of assortative mating by foot color in this species. Further experimental work is necessary to determine whether foot color is used in this species as an honest signal to relay information on the ability of an individual to manage oxidative stress. *Received 11 April 2014, accepted 31 July 2014.*

Key words.—assortative mating, Black Guillemot, carotenoids, *Cepphus grylle*, color, oxidative stress, visual signals.

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Exaggerated phenotypic traits, such as brightly colored plumage and elaborately shaped antlers, are often the result of sexual selection. Sexually selected colored ornaments, and the information that such traits convey, have been widely studied in birds (Hill and McGraw 2006). These ornamental features have been shown to relay reliable information to potential mates across several avian taxa, and extravagant displays have been linked to body condition (Velando *et al.* 2006), reproduction (Torres and Velando 2007), and survival (Freeman-Gallant *et al.* 2011).

One cellular mechanism that has recently received a great deal of interest from evolutionary ecologists is oxidative stress, as it appears to be an important mediator of life history trade-offs (Vleck *et al.* 2007). Oxidative stress results from an imbalance that arises when the production of damaging free radicals exceeds the ability of antioxidant defense and repair mechanisms to neutralize the free radical threat. The oxidative damage that ensues has been linked to cellular dysfunction, disease, and aging (Vleck *et al.* 2007). However, antioxidants can play other physiological roles as well, and, specifically, dietary carotenoid antioxidants are impor-

tant for the maintenance of many brightly colored traits (Hill and McGraw 2006). This has led to the hypothesis that carotenoid-based sexual ornaments are targets of sexual selection and can act as reliable signals of quality, based on an allocation trade-off between their use as pigments vs. their use as antioxidant defense (von Schantz *et al.* 1999; Freeman-Gallant *et al.* 2011).

Here we examine the relationship between oxidative stress and the bright red feet displayed by male and female members of a monomorphic seabird, the Black Guillemot (*Cepphus grylle*). Black Guillemots prominently display their bright red feet when courting (Winn 1950). We tested the hypothesis that foot coloration in this species acts as an honest signal of individual quality, as defined by oxidative damage levels. In addition, it is possible that foot color is used by one or both sexes in mate choice. In species with mutual ornamentation, like the Black Guillemot, traits are available to both sexes as a true signal of potential mate quality. This may drive assortative mating favoring pairs expressing similar phenotypes (Kraaijeveld *et al.* 2007), and therefore we also predicted assortative mate choice with respect to foot color.

METHODS

Study Population

We sampled the Black Guillemot population at the Bowdoin Scientific Station on Kent Island, New Brunswick, Canada (44° 34' 59" N, 66° 45' 21" W) in June and July of 2006 and 2007. In 2006, we wanted to determine if carotenoids were associated with foot color, and we captured 35 incubating adults, but only both members of three breeding pairs. In 2007, we focused on whether oxidative damage was associated with foot color, and whether there was any evidence of assortative mating by foot color in this species. We captured both members of 16 breeding pairs ($n = 32$ individuals) during incubation.

In both years, blood samples were collected within 3 min of capture. Blood was stored briefly on ice and then centrifuged to separate plasma and erythrocytes; both were stored at -80 °C. In addition, we measured mass (g) and wing length (mm). On Kent Island, average incubation length is 29 ± 2 days (Butler and Buckley 2002). While we were unable to determine the precise time in the incubation period at the time of capture, we estimated the phase of incubation to allow us to control for the time of breeding. Incubation phase was calculated based upon the interval between the date we first located a nest and the period of time that passed until detection of the first egg, second egg, or chick in the nest. This allowed us to categorize each bird into one of three incubation phases (early: 0-9 days; middle: 10-20 days; late: 21+ days).

Photographs and Color Analysis

We took at least two photographs of the right foot of each bird against a color chart (Gretag MacBeth) using a Nikon Coolpix 5200 5.1 Megapixel (Nikon, Inc.) in a light-tight box with a camera mount that allowed consistent and repeatable foot placement.

Using Adobe Photoshop (Adobe, Inc.), we analyzed foot color for each bird by selecting the photograph in which the Red-Green-Blue of the standard gray was closest to standard gray values (red = 122, green = 122, blue = 121), and then adjusting the photograph to the neutral gray on the color chart. We selected the webbing on either side of the middle toe of the foot and recorded average red, green, and blue values.

We calculated red intensity with the following formula:

$$\text{Red Intensity} = \frac{R}{R + G + B}$$

This measure makes no assumptions about color perception in the visible spectrum and provides a single metric that has been used to describe color-related patterns in birds (Mougeot *et al.* 2009).

Lab Analyses

We determined the sex of all individuals by amplifying areas of DNA as described by Griffiths *et al.* (1998), using primers P2 and P8. We adjusted our thermocycler conditions to 94°/90 sec (50°/45 sec, 72°/45 sec,

94°/30 sec) x 35, 48°/1 min, 72°/5 min. We used high performance liquid chromatography to determine carotenoid concentration for each plasma sample, as reported in McGraw *et al.* (2006), except that elution time was reduced to 14 min because no carotenes or other non-polar carotenoids were detected. We measured oxidative damage in plasma samples by assessing reactive oxygen metabolites (ROMs) using an assay that measures the levels of hydroperoxides as an indicator of lipid and protein oxidative damage (Hausmann *et al.* 2012). All analyses were run in duplicate and the intra-assay coefficients of variation were 4.6%.

Statistical Analysis

In 2006, we assessed whether variation in foot color was related to carotenoids by constructing general linear models of foot color including three uncorrelated fixed effects (carotenoids, phase of incubation, and body condition [residuals of the regression of wing length on body mass]; all possible interaction terms were included). In 2007, we assessed whether variation in foot color was related to oxidative damage by constructing mixed-effects models of foot color with oxidative damage and sex as fixed effects and breed pair as a random effect. Due to the small sample size of individuals for which we had enough plasma to assess oxidative damage in 2007 ($n = 21$) and the fact that capture date, incubation phase, and body condition were not significantly related to color in 2006, these variables were not included in the 2007 analyses.

Because assortative mating is an ordering process, we used Spearman rank correlations to evaluate the relationship between red intensity of mated pairs in 2007. For presentation of the assortative mating data in the figure only, we combined color data from both years, and thus normalized values for birds from each year to compare relative foot color across years. However, because few pairs were captured in 2006, analyses were run only on the 2007 birds. We used JMP Professional (SAS Institute, Inc. 2013) for all tests.

RESULTS

In 2006, there was no effect of carotenoids, body condition, or incubation phase on foot color ($F_{4,26} = 0.34$, $P = 0.95$), and this remained true when including all possible interactions ($F_{7,26} = 0.77$, $P = 0.62$). There was interesting variation in the types of carotenoids found in Black Guillemot plasma (Table 1), with red forms significantly more abundant than yellow forms ($t = -7.98$, $P < 0.001$).

In 2007, reactive oxygen metabolites were negatively correlated with foot color ($F = 7.11$, $P < 0.05$; Fig. 1), but not with sex ($F = 0.71$, $P > 0.8$). In addition, red intensities of

Table 1. Percentage of red-form and yellow-form carotenoids identified in circulating plasma of Black Guillemots ($n = 27$).

Variable	Carotenoid Form	Percent of Total Carotenoids
Astaxanthin	Red form	73%
Adonixanthin	Red form	10%
Canthaxanthin	Red form	9%
Iscryptoxanthin	Yellow form	< 5%
Canary xanthophylls	Yellow form	< 5%
Lutein	Yellow form	< 5%
β -cryptoxanthin	Yellow form	< 5%
Isozeaxanthin	Yellow form	< 5%
Zeaxanthin	Yellow form	< 5%

male feet (0.80 ± 0.03 SD) were significantly correlated (Spearman $r = 0.56$, $P = 0.02$) with those of their mates (0.80 ± 0.03 SD; Fig. 2).

DISCUSSION

Foot color in Black Guillemots during the incubation period was negatively correlated with our measure of oxidative damage and not correlated with circulating carotenoid levels. However, it was unexpected that circulating carotenoids were unrelated to foot color as carotenoids affect integument color across many avian taxa (Perez-Rodriguez *et al.* 2008). Experimental work in Blue-footed Boobies (*Sula nebouxi*) has shown that a carotenoid rich diet can quickly increase

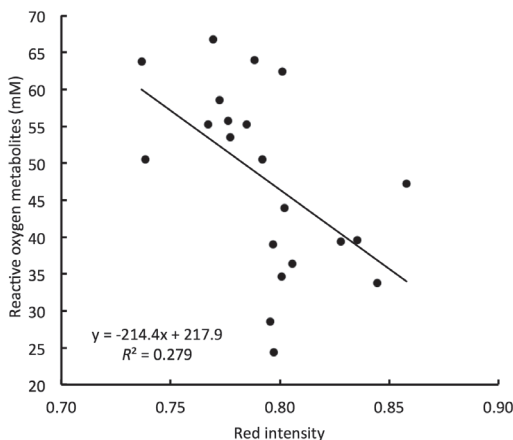


Figure 1. The relationship between oxidative damage, as measured by plasma reactive oxygen metabolites (mM of H_2O_2 equivalents), and red intensity of foot color in Black Guillemots ($n = 21$) in 2007.

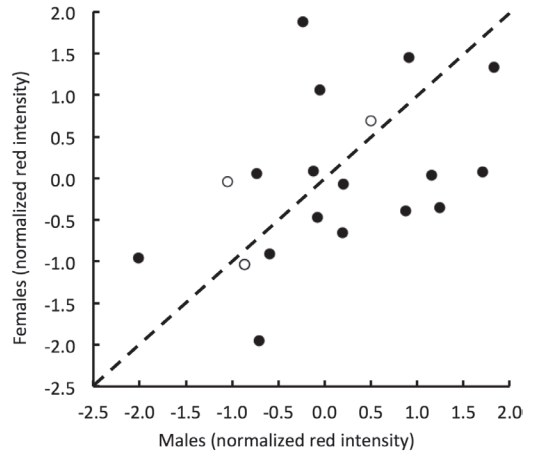


Figure 2. Red intensity of male and female foot color for breeding Black Guillemot pairs ($n = 16$) photographed on Kent Island in 2007 (closed circles). Pairs ($n = 3$) from 2006 were included for reference (open circles), but were left out of the statistical analysis. Dotted line represents hypothetical perfect assortative mating.

foot brightness (Velando *et al.* 2006). To our knowledge, no previous work has explained the nature of foot color in alcid feet and, thus, we expected that carotenoids would be involved in red intensity of Black Guillemot feet.

Regardless of the source of variation in Black Guillemot foot color, a separate interesting question is the underlying reasons for the maintenance of this extravagant trait. Selection for such a trait usually requires that it provide useful information to a potential mate (von Schantz *et al.* 1999), and one possibility is that the negative correlation between foot color and oxidative stress could suggest foot coloration is indicative of an individual's ability to cope with oxidative stress. However, testing this prediction appropriately requires further experimental work. Another possibility is that the negative correlation between foot coloration and oxidative stress could be explained by an increased susceptibility to oxidative stress as the breeding season progresses. In this case, we would expect to observe a positive relationship between oxidative stress and date, or between oxidative stress and breeding phase. However, in our sample, neither phase of incubation nor body condition explained variation in foot color.

In species with mutual ornamentation, like the Black Guillemot, exaggerated traits are available to both sexes as signals of potential mate quality, and we predicted their presence could drive assortative mating between pairs expressing similar phenotypes (Kraaijeveld *et al.* 2007). We found evidence of assortative mating on foot color with red intensity significantly correlated between mates, suggesting that the bright red feet of the Black Guillemot have a role in mate choice in at least one and possibly both sexes. The Blue-footed Booby is a species similar to the Black Guillemot in that it is a monomorphic seabird with rather muted plumage and brightly colored feet, which it prominently displays during courtship (Torres and Velando 2005). Experimental evidence suggests that both male (Torres and Velando 2003) and female (Velando *et al.* 2006) Blue-footed Boobies use foot color to make both pre- and post-copulatory reproductive decisions, though assortative mating has not been reported with respect to this signal.

Although our results are consistent with the idea of assortative mating through mutual mate choice, there exist several more proximate explanations. While Black Guillemots do not mate assortatively based on six different measures of body size (Berzins *et al.* 2009), for continuous traits, such as plumage color in European Starlings (*Sturnus vulgaris*), assortative mating has been attributed to signaling for age or parental quality (Komdeur *et al.* 2005). In Common Terns (*Sterna hirundo*), individuals tend to choose similarly aged mates that arrive at the breeding grounds at about the same time (Ludwig and Becker 2008), or choose them with respect to molt strategy (Bridge and Nisbet 2004). It is possible that Black Guillemots choose mates of similar age and that age is related to foot color and oxidative stress; however, we did not know the age of the birds in this study. Kraaijeveld *et al.* (2007) suggests that mutual ornamentation and mate choice can also arise because pairs share a common physical environment. If mates tend to forage together, then we would expect them to consume similar foods. Though we did not

assess foraging behavior directly, one prediction from this proximate hypothesis is that the longer mates forage together, the more similar should be the red-intensity of their feet. However, in our study we found no relationship between phase of incubation, which reflects the length of time mates may forage together, and foot color.

We found that foot color in Black Guillemots during the incubation period was negatively correlated with oxidative damage. Although many studies have linked carotenoids to coloration (von Schantz *et al.* 1999), fewer have linked colorful ornamentation to measures of oxidative stress (Torres and Velando 2007; Mougeot *et al.* 2009; Freeman-Gallant *et al.* 2011). Additionally, red intensity of male feet was significantly correlated with red intensity of their mates', suggesting the possibility of assortative mating by foot color in this species. Future experimental work is needed to establish whether foot color is used in Black Guillemots as an honest signal to relay information on the ability of an individual to manage oxidative stress.

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LITERATURE CITED

- Berzins, L. L., H. G. Gilchrist and G. Burness. 2009. No assortative mating based on size in Black Guillemots breeding in the Canadian Arctic. *Waterbirds* 32: 459-463.
- Bridge, E. and I. C. T. Nisbet. 2004. Wing molt and assortative mating in Common Terns: a test of the molt-signaling hypothesis. *Condor* 106: 336-343.
- Butler, R. G. and D. E. Buckley. 2002. Black Guillemot (*Cepphus grylle*). No. 675 in *The Birds of North America Online* (A. Poole, Ed.). Cornell Lab of Ornithology, Ithaca, New York. <http://bna.birds.cornell.edu/bna/species/675>, accessed 9 April 2014.

- Freeman-Gallant, C. R., J. Amidon, B. Berdy, S. Wein, C. C. Taff and M. F. Haussmann. 2011. Oxidative damage to DNA related to survivorship and carotenoid-based sexual ornamentation in the Common Yellowthroat. *Biology Letters* 7: 429-432.
- Griffiths, R., M. Double, K. Orr and R. Dawson. 1998. A DNA test to sex most birds. *Molecular Ecology* 7: 1071-1075.
- Haussmann, M. F., A. S. Longenecker, N. M. Marchetto, S. A. Juliano and R. M. Bowden. 2012. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. *Proceedings of the Royal Society B-Biological Sciences* 279: 1447-1456.
- Hill, G. E. and K. J. McGraw (eds.). 2006. *Bird coloration, vol. 1: mechanisms and measurements*. Harvard University Press, Cambridge, Massachusetts.
- Komdeur, J., M. Oorebeek, T. van Overveld and I. Cuthill. 2005. Mutual ornamentation, age, and reproductive performance in the European Starling. *Behavioral Ecology* 16: 805-817.
- Kraaijeveld, K., F. J. L. Kraaijeveld-Smit and J. Komdeur. 2007. The evolution of mutual ornamentation. *Animal Behaviour* 74: 657-677.
- Ludwig, S. C. and P. H. Becker. 2008. Supply and demand: causes and consequences of assortative mating in Common Terns *Sterna hirundo*. *Behavioral Ecology and Sociobiology* 62: 1601-1611.
- McGraw, K. J., P. M. Nolan and O. L. Crino. 2006. Carotenoid accumulation strategies for becoming a colourful house finch: analyses of plasma and liver pigments in wild moulting birds. *Functional Ecology* 20: 678-688.
- Mougeot, F., J. Martinez-Padilla, L. M. I. Webster, J. D. Blount, L. Perez-Rodriguez and S. B. Pieltney. 2009. Honest sexual signaling mediated by parasite and testosterone effects on oxidative balance. *Proceedings of the Royal Society B-Biological Sciences* 276: 1093-1100.
- Perez-Rodriguez, L., F. Mougeot, C. Alonso-Alvarez, J. Blas, J. Vinuela and G. R. Bortolotti. 2008. Cell-mediated immune activation rapidly decreases plasma carotenoids but does not affect oxidative stress in Red-legged Partridges (*Alectoris rufa*). *Journal of Experimental Biology* 211: 2155-2161.
- SAS Institute, Inc. 2013. JMP Professional v. 11.1.1. SAS Institute, Inc., Cary, North Carolina.
- Torres, R. and A. Velando. 2003. A dynamic trait affects continuous pair assessment in the Blue-footed Booby, *Sula nebouxii*. *Behavioral Ecology and Sociobiology* 55: 65-72.
- Torres, R. and A. Velando. 2005. Male preference for female foot colour in the socially monogamous blue-footed booby, *Sula nebouxii*. *Animal Behaviour* 69: 59-65.
- Torres, R. and A. Velando. 2007. Male reproductive senescence: the price of immune-induced oxidative damage on sexual attractiveness in the Blue-footed Booby. *Journal of Animal Ecology* 76: 1161-1168.
- Velando, A., R. Beamonte-Barrientos and R. Torres. 2006. Pigment-based skin colour in the Blue-footed Booby: an honest signal of current condition used by females to adjust reproductive investment. *Oecologia* 149: 535-542.
- Vleck, C. M., M. F. Haussmann and D. Vleck. 2007. Avian senescence: underlying mechanisms. *Journal of Ornithology* 148: 611-624.
- von Schantz, T., S. Bensch, M. Grahn, D. Hasselquist and H. Wittzell. 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society B-Biological Sciences* 266: 1-12.
- Winn, H. E. 1950. The Black Guillemots of Kent Island, Bay of Fundy. *Auk* 67: 477-485.